Annex A

Preliminary remarks

The following Annex represents the scientific basis for the drafting of this Technical Standard. The guidelines given in the standard regarding the acoustic impact and the limits specified are in accordance with the data reported in the bibliography studied. The above mentioned Standard is the result of the PhD in Mediterranean Biodiversity XXXII cycle (International) – PhD Innovative with Industrial Characterization PON 2014-2020 carried out at the University of Palermo in collaboration with the National Research Council (CNR) of Capo Granitola (Campobello di Mazara), The National Institution of Italy for Standardization Research and Promotion (ENR) based in Palermo and HR Wallingford Ltd based in Oxford (United Kingdom). The following annex summarizes the scientific literature used with reference to different types of marine maritime activities in relation to the possible acoustic impact. The suggested acoustic limits and the recommended operating methods are therefore applicable to various types of human activities carried out at sea. In particular, the standard drawn up, for the first time, has the aim of regulating the acoustic impact possibly produced by mining activities in the ocean depths (Deep Sea Mining, DSM).

A "sound" is the result of a mechanical propagation of acoustic waves in a medium that could be water or air (Wartzok et al., 1999) while a "noise" is an undesired or disturbing sound. Human noise has become widespread in terrestrial and aquatic ecosystems (Andrew, 2002; World Health Organization, 2011) and is now considered as a real and actual contaminant in accordance with the Marine Strategy Framework Directive of the European Union (Directive 2008/56/EC of 17 June 2008) and the World Health Organization (Kunc et al., 2016). The increase in anthropogenic noise originated in the industrial revolution (McDonald et al., 2006; Normandeau Associates I., 2012) and different activities determine it: shipping, offshore development, urbanization, military and non-military sonars, recreational and non-recreational naval activities, resource extraction, transport and energy production and seismic exploration (Richardson et al., 1995; Hildebrand et al., 2009; Slabbekoorn et al., 2010; Radford et al., 2014; Kunc et al., 2016; Hawkins & Popper, 2017; Kuşku et al., 2018). The types of sound produced by anthropogenic activities can be of different types: high intensity acute sounds from military exercises (Dolman et al., 2009), oil and gas exploration (McCauley et al., 2000) and pile driving (Bailey et al., 2010), or lower level sounds produced by fishing, commercial and recreational activities (Codarin et al., 2009; Malakoff, 2010). In recent years, the attention has been focused in particular on the noise generated by the marine and maritime industries, oil and gas exploration and extraction, sonar systems, dredging and construction of offshore renewable energy devices and deep-water mining activities (Hawkins et al., 2017). Popper & Hastings (2009 a, b) analyze the effects of noise by describing the different sources of noise pollution.

The noise produced by most human activities is low-frequency (<1 kHz) (Thomsen et al., 2009; Robinson et al., 2011) and the noise from navigation, for example, makes an important contribution to environmental noise levels (<300 Hz). Ocean noise levels have increased in 40 years to 12 dB in the North-East Pacific areas (Hildebrand, 2009) and by at least 3-10 dB between 20 and 300 Hz in the North-East Pacific since the 1960s (Andrew et al., 2002). Although anthropogenic noise is increasing rapidly especially in the marine environment (Andrew et al., 2002; Hildebrand, 2009; Popper & Hastings, 2009 a, b), it remains one of the least studied sources of pollution (Hawkins et al., 2015).

In water the propagation of sound is different from that of air (Wartzok et al., 1999) and since its attenuation coefficient is lower than the one of air (Wartzok et al., 1999) it travels at a higher speed (almost 4.5 times faster) (Urick, 1983) and for much greater distances (Williams et al., 2015). Noise pollution in aquatic environments could therefore affect much larger areas than terrestrial environments and during its long-distance propagation could undergo, depending on environmental conditions, variations in its characteristics (Rogers & Cox, 1988). Sound propagation in water is characterized by pressure, particle movement (Popper et al., 2001) and scalar pressure (Ceraulo et al., 2016). The knowledge of the physical properties and propagation of an acoustic wave are therefore considered essential in the performance of human activities at sea.

Going into the details of Deep Sea Mining activities, most of the scientific work analyses and describes the sites, the possible impacts such as fragmentation and loss of habitat (following mechanical removal) and the possible formation of plumes of sediment probably toxic to living organisms (Petersen et al, 2016; Rakhyun, 2017; Kaikkonen et al., 2018; Gillard et al., 2019; Monserrat et al., 2019; Drazen et al., 2019; Lopes et al., 2019; Rzeznik et al., 2019; Ma et al., 2018). None of this work takes into account the possible acoustic impact if not marginally. In addition, the determination of the scale of noise impacts from mining activities may depend on the type of site (Peukert et al., 2018).

The International Seabed Authority (ISA, https://www.isa.org.jm/), which aims at managing the activities concerned, is trying to achieve the writing of a mining code while continuing to neglect the problem of noise impact (Art.137(2); UNCLOS 1982; Boschen et al., 2013; Mengerink et al., 2014; Jaeckel et al., 2017; Durden et al., 2018; Van Dover et al., 2018). The recommendations provided by ISA LTC in ISBA/19/LTC/8 (ISA, 2018) describe the procedures to be followed for the data acquisition phase and for the monitoring to be performed during and after activities potentially harmful to the environment. Concession holders are required to prepare annual reports, as set out in ISBA/21/LTC/15 (ISA, 2018), providing general information on biological communities, biodiversity studies and information on the functioning of ecosystems (ISA, 2018). Christiansen et al., (2019) and Jaeckel et al., (2019) describe this recommendation by highlighting its shortcomings and providing advice for possible improvements. These recommendations require an environmental baseline study, monitoring of possible environmental effects and monitoring during and after system testing. Community "Pelagic Assessment" in the water column and benthic boundary layer is required; sighting of marine mammals, turtles or other groups of fish; and identification of at least one station within each habitat for assessment of community time variations in the water column and seabed. No trophic or other process studies are required (Christiansen et al., 2019). Methodological aspects are not addressed although they are essential for data quality and comparability. The analysis of spatial variation in the biological community is considered but the variability between benthic and pelagic communities is not considered (Christiansen et al., 2019). The information in this recommendation is still scarce, demersal fish are not considered. Work to be done on plankton communities is indicated but not considered micronekton, nekton, vertical migration, structure and dynamics of the food network (Christiansen et al., 2019). The recommendation deals with trace metals and potentially toxic elements in demersal fish and invertebrates, analyses assessments of potential ecotoxicological impacts on phytoplankton and zooplankton considered necessary only if the discharge plume is released to the surface or water column (Christiansen et al., 2019). Ecotoxicological measures are planned for benthic organisms and not for pelagic fauna (Christiansen et al., 2019). The need for an EIA is also expressed, which will have to consider impacts not only in the areas directly affected but also in the regions affected by the plumes and discharge materials. EIA is required for discharge plumes that have the potential to: alter food chains; disturb vertical and other migrations; lead to changes in the geochemistry of an area. However, no specific requirements are given for this EIA (Christiansen et al., 2019). Indications on observations and measures to be carried out do not include biological information. Direct biological measurements are only required after the activity but are unclear and incomplete. Additional requirements are provided for Seafloor Massive Sulfide (SMS) deposits and Iron-manganese crusts but are almost exclusively addressed to benthic communities. The work of Christiansen et al., (2019) is one of the few that best analyses the possible impacts of the DSM. It highlights that most studies address the expected effects on benthic communities, identify different mining processes that may affect the pelagic environment and provide an in-depth description of possible impacts. According to these authors, the actual scale of impacts is not yet known and the recommendations provided in 2018 by the International Seabed Authority are not very specific, as the consequences of many impacts are not considered. Lethal impacts and impacts capable of damaging essential processes such as food, growth and reproduction are assumed with the loss of biodiversity as a possible consequence. Although the authors provide advice to improve the recommendations, the problem of the noise impact produced by the DSM remains neglected. They simply consider that information on the generation and propagation of sound from activities is not available and that knowledge about the perception of sound in animals is scarce, which is why the acoustic impacts that could be generated by DSM cannot be predicted at present. There are also many problems with how to carry out an Environmental Impact Assessment (EIA). Clark et al., (2019) highlight scientific shortcomings and make recommendations to improve them. Poor consideration of noise impacts continues to be highlighted but not dealt with in detail. Anthropogenic sounds propagating in water overlap with biologically important sounds produced by animals for their vital functions (Hastings & Popper, 2005; Slabbekoorn et al., 2010), posing a real threat to the life of deep ecosystems. Marine organisms live in an acoustically complex world that is the result of a mixture of biotic and abiotic sounds (De Jong et al., 2011). All animals evaluate the environment by analyzing the sound landscape or the "acoustic scene" that surrounds them (Popper & Fay, 1997; Fay, 2009). Many marine species use aquatic noise to acquire various types of information about their survival: auditory information, habitat selection, identification of predator or prey positions and for communication. In recent years, many research programs have been developed to study the effect of noise on aquatic life (Erbe, 2012a). Political organizations are very interested in the problem of noise in the sea because the noise produced by commercial ships is between 0.1 and 1 kHz (Hildebrand, 2009). This is all the more important given the low frequency bands between 10 Hz and 10 kHz (Monitoring Guidance for Underwater Noise in European Seas-PartII), which are likely to affect mining activities (Kaikkonen et al., 2018). These are the frequency ranges used by many species for communication. Many authors have attempted to predict future impacts of noise pollution for species using sound for their vital functions (Codarin et al., 2009; Picciulin et al., 2010; Purser & Radford, 2011; Bracciali et al., 2012; Voellmy et al., 2014 a,b; Shannon et al., 2016; Simpson et al., 2016) but there are still many gaps to be filled.

More than two-thirds of our planet is covered with water, and given the current activities in the marine maritime and mining sectors planned, the problem becomes even more urgent. Techniques that will be used for mineral extraction, such as hydraulic dredging or other related activities (McKenna et al., 2012) are likely to cause noise levels that will inevitably affect the marine habitat.

To date, it is essential to know about its effects on deep ecosystems. There are few studies on this subject (Robinson et al., 2011), but although information on its impact and containment is scarce, we believe that the time is ripe to propose a guideline and/or technical standard to be followed. It is estimated that human activities will cause a non-homogeneous increase in sound levels in the depths of the ocean (OSPAR Commission, 2009). For this reason, we are currently trying to improve the legislation on seismic surveys for the characterization of the ocean floor. Several countries apply only the "precautionary principle", limiting the time and duration of explorations (Lewandowski, 2015). Recently, Popper et al., (2019) analyzed the problem of the acoustic impact of different human activities, the guidelines written to date and the difficulties in establishing acoustic limits.

In Europe, despite efforts to reduce and regulate noise pollution (Pottering & Lenarcic, 2008) many countries do not have adequate regulation or management.

To date, there is no single, well-defined protocol for measuring marine noise levels (André et al., 2011). The methods for carrying out these measurements are very variable and the data are heterogeneous (André et al., 2010). Some documents provide indications but are not yet sufficient (Guide to the monitoring of underwater noise in European seas - Part II; Jones et al., 2019) to indicate adequate prevention and protection measures to be implemented. It is necessary to further study the issue in order to identify the correct precautionary policies. For example, the Italian Environmental Impact Assessment Commission requested seismic operators, in 2015, to try to reduce the impact of noise on marine organisms, to use a scientific protocol for the assessment, by visual and acoustic methods, of the presence of marine organisms before, during and after the onset of noise pollution. This monitoring method could implement knowledge on noise at the international level (Fossati et al., 2017). Problems about the difficulties of determining acoustic limits that protect marine biodiversity are certainly caused by the variability of species present in the sea, the variability of their acoustic ranges, their anatomy, physiology (Hildebrand, 2009) and the complexity of the marine ecosystem as a whole. In fact, most of the scientific works present in the literature reproduce the environmental conditions in the laboratory. We are aware that the laboratory conditions and the results obtained do not reflect the real conditions of the deep environments in which the variables involved are more. However, these data may contribute to the establishment of guidelines, rules or technical standards.

This Technical Standard provides a first useful document from which to derive minimum requirements and/or recommendations for containing the possible acoustic impact of this activity. This document contributes to the identification of a baseline that could be followed for DSM activities until the acoustic frequencies emitted during the performance of the same activities are made known. It is believed that starting these activities (DSM) with guidance to be followed is in accordance with the precautionary principle. All the works consulted for the drafting of this standard highlight the acoustic impact on biodiversity from invertebrates to mammals at physical, physiological, anatomical and behavioral levels. On the basis of the effects of sounds with known acoustic intensities and frequencies, typical of other anthropic activities, it was possible to hypothesize the possible effects of DSM activities if the noise emitted coincides with some of these frequencies and/or intensities. Although we do not know the acoustic frequencies produced by mining (much information is still missing or not available), in our opinion using the knowledge of the effects of different types of anthropogenic noise is certainly a good starting point to mitigate the impacts.

Notes on acoustics

The sound pressure p represents the average variation of pressure with respect to the pressure of the medium and is defined as the mean square value RMS (Root-Mean-Square) of the differences between the total pressure and the pressure of the medium:

$$p_{rms} = \sqrt{\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} p(t)^2 dt}$$

Conventionally, we prefer to refer to the *SPL* (Sound Pressure Level) sound level, which is linked to the square of the sound pressure and therefore to its intensity. The sound level is therefore defined as follows:

$$SPL = 10log_{10} \frac{p_{rms}^2}{p_0^2} = 20log_{10} \frac{p_{rms}}{p_0}$$

where p_0 is the reference pressure whose value is 20 µPa if the medium is the atmosphere and 1 µPa if the medium is water. Sound level measurements are expressed in decibels (dB).

The sound level is typically variable over time. In many cases, therefore, it may be useful to define an equivalent continuous sound level (*Leq*) which, if replaced with a sound level that varies over time for the same time interval *T*, would produce the same total amount of sound energy. The continuous equivalent sound level is determined by the following expression:

$$L_{eq} = 10log_{10} \left(\frac{1}{T} \int_0^T \frac{p(t)^2}{p_0^2} dt \right)$$

The continuous equivalent sound level is expressed in dB and is a parameter widely used as a measure of acoustic noise because it allows to evaluate the average exposure to a sound in a given time interval. In the presence of transient or impulsive noise, however, it does not provide an exhaustive measure of noise exposure. Parameters such as peak-to-peak ($SPL_{peak\text{-}to\text{-}peak}$), 0-peak ($SPL_{0\text{-}peak}$) and *SEL* (Sound Exposure Level) offer more information in such cases. *SEL*, in particular, represents the constant sound level that contains the same amount in one second of the original sound event. The expression for determining *SEL* is as follows:

$$SEL = 10log_{10} \left(\frac{1}{T_0} \int_0^{\Delta T} 10^{\frac{L_{eq}}{10}} dt \right) = L_{eq} + 10log_{10} \frac{T}{T_0}$$

with $T_0 = 1$ s.

In the case of repetitive impulsive noises, it is also possible to evaluate the cumulative *SEL* (SEL_{cum}) whose expression is shown below:

$$SEL_{cum} = SEL + 10log_{10}N$$

with *N* number of impulsive events.

Noise pollution and marine biodiversity

Recently, Weilgart, (2017) reviewed 114 studies dealing with acoustic impacts on 61 species of fish and 26 species of invertebrates. From this review and from other scientific works it can be deduced that anthropic noise influences physiology (Santulli et al., 1999; Buscaino et al., 2010; Celi et al., 2016; Filiciotto et al., 2017; Vazzana et al., 2017); behaviour (Popper, 2003a; ; Popper & Hastings, 2009a,b; Slabbekoorn et al., 2010; Radford et al., 2014; Wong et al., 2015; Kunc et al., 2016; Hawkins and Popper, 2017); foraging (Wale et al., 2013; Voellmy et al., 2014a; Magnhagen et al., 2017) compromising the ability to distinguish edible from non-edible foods (Purser & Radford, 2011); parental care (Picciulin et al., 2010; Bruintjes & Radford, 2013; Kunc et al., 2016; Nedelec et al., 2017a), the avoidance of predators (Wale et al., 2013; Simpson et al., 2015, 2016; Bruintjes et al., 2016; La Manna et al., 2016); egg laying (Montie et al., 2017) and reproduction (Amoser & Ladich 2003; Kight et al., 2011; De Jong et al, 2016; Krahforst, 2017); acoustic communication (Myrberg & Lugli, 2006; Thomsen et al.,2006; Vasconcelos et al. 2007; ; Shannon et al., 2016; Alves et al., 2016, 2017); habitat orientation and selection (Holles et al., 2013; Lecchini et al., 2018); conservation (Francis et al, 2013); alarm reactions (Webb,1986); courtship calls (Picciulin et al., 2012; Montie et al., 2017); prey perception (Amoser & Ladich 2003; Kunc et al.,2016); larval survival (Nedelec et al., 2017a); larval development (Aguilar de Soto, 2013; Nedelec et al., 2014,2015). Acoustic stress can adversely affect species capture rates, abundance and distribution (Løkkeborg, 1991; Skalski et al., 1992; Engås et al., 1996; Løkkeborg et al., 2012). It may also cause internal lesions (Sverdrup et al., 1994), temporary or permanent hearing loss (McCauley et al., 2003; Popper et al., 2005; Codarin et al., 2009; Halvorsen et al., 2012a) due to hearing loss, cell damage to statocysts and neurons. It may even reduce growth, weight, food consumption, immune response and DNA integrity with irreversible damage (Kight & Swaddle, 2011). High mortality rates have even been observed in zooplankton (McCauley et al., 2017).

Fish, for example, take information about movements and positions through lateral and visual systems (Partridge et al., 1980; Faucher et al., 2010) and anthropogenic noise could influence the individual's ability to process information by compromising the dynamics of schooling and thus of the group (Halfwerk et al., 2015). Noise pollution can affect the acoustic communication of many species (Popper & Fay, 2011), masking their auditory signals (Pollack, 1975; Brungart, 2001; Slabbekoorn et al., 2008). This would compromise the ability of marine organisms to communicate. It can cause body malformations, mortality, developmental delays, delays in metamorphosis and stabilization and slower growth rates (Weilgart et al., 2017). Increased noise levels may lead to alterations in activity and patterns of locomotion and motility (Mendl, 1999; Buscaino et al., 2010) and may have implications for the energy budget (Buscaino et al., 2010; Shannon et al., 2016) by changing the vigilance capabilities of organisms.

Many marine organisms, including fish and invertebrates, have sensory systems to perceive even particle motion (Fay, 1984; Popper & Fay, 2011; Popper et al., 2018) and are equipped with pressure-sensitive organs (Wysocki et al., 2009). The physics of sound propagation also plays an important role in assessing seismic impact (Carroll et al., 2017). The vibrations produced by various human activities, and probably by mining, propagate on the seabed and have effects on different species of invertebrates, especially on benthic organisms (Roberts et al., 2016a, 2016b; Roberts & Elliot, 2017). In this context, the study of marine invertebrates becomes indispensable as they play important roles in deep-sea biology. Some ecological services performed by these organisms (such as water filtration) could be negatively affected. Activities such as those of the DSM could cause significant vibrations of the seabed and, therefore, significant impacts on invertebrates.

Among the anthropic activities, the airgun produces sounds that allow us to understand the deep structure of the seabed by building images of it. The frequencies emitted by this type of analysis fall within the frequency range of the sounds detected by many marine species (McCauley et al., 2000; Popper et al., 2003b; Gausland, 2003; Popper & Fay, 2011; Ladich & Fay, 2013a). A better understanding of the species' responses to low frequency sound exposure levels (Parsons et al., 2009; Prideaux & Prideaux, 2016) may be needed. Several reviews talk about noise in aquatic environments produced by human activities (Gordon et al., 2003; Popper & Hastings, 2009 a,b; Slabbekoorn et al., 2010; Kight et al., 2011; Morley et al., 2014; Peng et al., 2015; Kunc et al., 2016; Edmonds et al., 2016; Erbe et al., 2016; Radford et al., 2014; Shannon et al., 2016; Carroll et al., 2017; Weilgart et al., 2017; Kuşku et al., 2018). Cetaceans are considered the "engineers" of the marine ecosystem (Bossart, 2011; Roman et al., 2014) and are very sensitive to noise pollution in their habitats (Williams et al., 2013; Gordon et al., 2018). While most of the studies so far concern cetaceans (Hatch et al., 2008; Brandt et al., 2011; Erbe et al., 2012b; Melcón et al., 2012; Tsujii et al., 2018) few deal with commercially important species such as fish and invertebrates (Engås & Løkkeborg, 1996; Gordon et al., 2003; Sarà et al., 2007; Celi et al., 2015; Filiciotto et al., 2016; Vazzana et al., 2016). As a result of stress, such as acoustic stress, organisms react by trying to restore homeostasis through three main mechanisms. The primary response (sympathetic nervous system, hypothalamus-pituitary-interrenal axis, catecholamines and glucocorticoid release) (Barton, 2002; Schulte, 2014). The secondary answer, concerning physiological metabolism (haematological and immune characteristics and changes in breathing rates) (Pickering, 1981; Rotllant and Tort, 1997; Iwama, 1998; Simontacchi et al., 2008). The tertiary response (which comes into play if the former failed to establish homeostasis), related to growth, behavior, reproduction, survival (Wedemeyer et al., 1990; Pavlidis et al., 2011). With regard to the latter, there is very little data on the effects of noise on reproduction and behavior in aquatic animals. Behavioral effects are most likely, especially at low sound levels (Hawkins et al., 2015), although more difficult to study and control. They also have very close links with physiological changes that are not immediately obvious, such as physical and behavioral responses (Carroll et al., 2017). Changes in movement or structure in group cohesion lead to changes in metabolic rates, stress, reproduction and predation (Hawkins et al., 2015). For all these reasons, potential impacts of noise could have important ecological and evolutionary implications for marine species.

In recent decades, the traffic related to naval activities recreational not, has increased and is considerable in surface and coastal habitats. The stress produced by noise pollution affects the larvae of various species of invertebrates (Branscomb & Rittschof, 1984; Jeffs et al., 2003; Vermeij et al., 2010; Nedelec et al., 2014) and beyond. This type of noise can influence the settlement behaviour of coral planulae (*P. damicornis* and *A. cytherea*) (Lecchini et al., 2018). Low frequency sounds (30 Hz) reduce metamorphosis in *B. amphitrite* (Branscomb & Rittschof, 1984). As confirmation of the fact that acoustic impact plays an important role in the settlement behavior of many coastal organisms, Wilkens et al., (2012) have shown a significantly faster settlement of *P. canaliculus* larvae when exposed to the noise produced by a ferry. Probably the decrease in settlement time is related to the intensity of the noise generated by the ship. On the other hand, Holles et al., (2013) report a reduction in the settlement phases of the larvae of *A. doryssa*. They would lose more time to swim before settling at a defined point, thus increasing the risk of predation and energy costs with significant consequences on population dynamics. Fakan et al., (2019) observed the presence of physiological responses in fish during embryogenesis in the presence of this type of anthropogenic noise with negative effects on heart rate. Effects on morphological development also differed between species. Jain-Schlaepfer et al., (2018) studied the heart rate of *A. curacao* embryos to learn about the effects of noise generated by in situ motorboats. The heart rate of the embryos increased in the presence of this anthropogenic noise. 2-stroke engines have a more pronounced effect on the heartbeat of the embryo than 4-stroke engines.

Jellyfish play an important role in the oceans as a food source for different taxa and as predators of fish larvae and planktonic organisms. In *C. tuberculata* and *R. pulmo* with low frequency sound exposure through scanning electron microscopy (SEM), lesions to statocysts consistent with the manifestation of massive acoustic trauma were detected (Solé et al., 2016). Damage detected in *C. tuberculata* and *R. pulmo* confirm that anthropogenic noises have caused negative effects on cnidarians (Solé et al., 2016). Filiciotto et al., (2016) observed changes in locomotion and the refuge strategy of *P. serratus*. Variations in total protein concentrations in the hemolymph and brain, DNA integrity and Hsp27 and 70 protein expression levels in brain tissue were observed

(Filiciotto et al., 2016). In *P. elephas*, Filiciotto et al., (2014) showed behavioral changes at the locomotor level and changes in hemolymphatic parameters with increases in glucose levels, total protein, Hsp70 expression and total blood cell count (THC), following exposure to noise. Celi et al., (2015) confirm these biochemical and immunological changes in *P. elephas* following noise pollution. The animals were exposed to a mixture of noise produced by different types of motorboats. Assessments were made for THC, protein concentration, phenoloxidase (PO) activity and Hsp27 protein expression. The results showed that acoustic stress influences the cellular and biochemical parameters of this species. Anthropogenic sounds can distract prey and make them more vulnerable to predation as demonstrated in *C. clypeatus* (Chan et al., 2010). Chronic exposure to the noise of merchant ships reduces vital functions in oysters, the volume of water flowing through their gills, the opening of valves and thus the absorption of metals, food absorption and growth (Charifi et al., 2018). Acoustic stimulation has also been studied in individuals of *M. galloprovincialis* (Vazzana et al., 2016). The frequency ranges were different: low (0.1-5 kHz), medium low (5-10 kHz), medium (10-20 kHz), medium high (20-40 kHz) and high (40-60 kHz). Although the behavior in this case did not change, at low frequencies high levels of glucose, total protein, THC, Hsp70 expression and acetylcholinesterase (AChE) activity in plasma and tissues were observed. Also, for invertebrates, distance influences effects. Decapods only showed alarm behavior when they were 10 cm away from the sound source (Goodall et al., 1990). Peng et al., (2016) analyze the effects of different types of frequencies and intensities of anthropogenic sounds on the behavior of *S. constricta*. The variations in the intensity of the sound modified the behavior relative to the depth of the excavation with subsequent reactions, especially at a genetic level. In addition, exposure to sound altered the O:N ratios and the expression of ten genes related to glycolysis metabolism, fatty acid biosynthesis, tryptophan metabolism and the TCA cycle (tricarboxylic acids). Even the activity of Ca^{2+}/Mg^{2+}-ATPase in foot tissues, related to musculature, contraction and digging behavior, has been negatively modified. Bivalves therefore perceive sound primarily as a change in the movement of particles in water. In *C. maenas* the ability to find food, to find shelter from predators and to increase the speed of correct repositioning in space is reduced (Wale et al., 2013). At the behavioral level, Lagardère, (1982) observed in *C. crangon* a reduction in growth rates, reproduction and food consumption, with an increase in mortality rates, disease and cannibalism. Metabolic rates increase (Régnault & Lagardère, 1983) along with oxygen consumption and ammonia excretion. Invertebrates can get used to this type of stress and this is confirmed by the reduction of alarm responses in squid and cuttlefish (Samson et al., 2014; Mooney et al., 2016), as well as in crabs (Roberts et al., 2016a). Noise causes increased levels of glucose, total proteins, thermal shock proteins and total lobster haemocyte counts (Filiciotto et al., 2014). Noise generated by a ship also creates disruptions in the DNA structure (Wale et al., 2016) with increased oxygen consumption and reduced clearance rates. Exposure to sound fields typical of navigation and construction activities at sea may alter the well-being of sediments due to induced changes in invertebrates (e.g. *R. philippinarum*) in the transport of fluids and particles essential to the nutrient cycle at the bottom of the sea (Solan et al., 2016). Some vital choices, such as the choice of shell in *P. bernhardus* (Walsh et al., 2017) can be influenced. This type of anthropic noise can also have effects on cephalopods, particularly in cuttlefish and octopus, effects have been observed on the function and physiology of statocysts, the organs responsible for their balance and movement. Solé et al., (2013a) analyzed the acoustic impacts on *I. coindetii* and *L. vulgaris* through scanning electron microscopy (SEM) and detected lesions in the internal structure of statocysts. Several behavioral changes were also observed in octopus, cuttlefish and squid concerning ink ejection as an alarm response, lack of mobility, foraging and mating (Solé et al., 2013b). André et al., (2011) demonstrate the presence of massive acoustic traumas, such as damage to the sensory hair cells of statocysts in four species of cephalopods (*L. vulgaris*, *S. officinalis*, *O. vulgaris*, *I. coindetii*) subjected to low-frequency acoustic emissions characteristic of different types of marine and maritime anthropogenic activities.

Noise from a motorboat affects the behavior of the juvenile *P. amboinensis*. Behavioral observations made before, 1, 10 and 20 minutes after the start of the boat noise showed an immediate decrease in the boldness and relative distance of the fish displacement in response to the noise and a recovery time of 20 minutes (Holmes et al., 2017). The effects of boat noise have also been tested by McCormick et al., (2018) on the behaviour, use of space and escape response of *P. wardi.* The results of their work have shown that this type of noise affects the way the juvenile fish assesses the risk. Obviously, this may have consequences on their physical form and survival as immediate changes in behavior can even alter short-term mortality rates. Low frequency sounds can affect the respiratory rate of *O. ocellatus* (Kaifu et al., 2007). Hastings et al., (1996) analyzed the effects of noise emission on hair cells and the lateral line of the same species. The results of their work showed that the lesions could develop slowly after acoustic exposure. Physically and anatomically, in some species the effects may be delayed and not immediate, especially at the level of hair cells (Hastings et al., 1996). Several works refer to the anthropic noise produced by boat engines that can influence fish behavior (La Manna et al., 2016). The effects of noise may depend on both the species and the intensity of the sound emitted. Noise from speedboats may have effects on prey-predator responses (Voellmy et al., 2014b). Noise from motorboats severely reduces the acoustic space of *H. dydactilus* (Alves et al., 2017). Tidau & Briffa, (2019) have shown that exposure to anthropogenic noise in hermit crabs can alter not only individual behavior but also social behavior. Shi et al., (2019) observed negative effects of noise on *T. granosa* on feeding activity, metabolism and ATP synthesis. The noise of speedboats damages the hearing ability of fish (Scholik & Yan, 2002) and also affects the interspecific mutualism of cleaning for reef fish (Nedelec et al., 2017b). Noisy cleaners inspect guests longer and less collaboratively. This confirms that cognitive deficits due to distraction are likely to reduce the quality of service and energy use of cleaners (Nedelec et al., 2017b) as observed in *L. dimidiatus* (Nedelec et al., 2017b). Noises create cognitive damage and distraction phenomena, greatly influencing this type of behavior, which is essential for many of the coral reef processes. Unlike other types of pollutants, acoustic noise is generally short-lived. Immediately after the source is switched off, the noise is dissipated. Short and long-term effects of increased ambient sound on the stress and hearing of *C. auratus* exposed to noise conditions have been assessed in plasma (cortisol and glucose levels) and on hearing ability through analysis of brain stem responses (Smith et al., 2004). No long-term physiological responses were found but a transient peak in plasma cortisol within 10 minutes of the onset of the noise returning to normal levels after one hour. Hearing thresholds had significant changes after only 10 min of noise exposure with maximum hearing loss within 24 hours. After 21 days of exposure, it took 14 days to fully recover hearing levels (Smith et al., 2004). Significant shifts were detected in the hearing threshold, which increased linearly to about 28 dB after 24 hours. This may be due to acclimation or perception of noise with less stress (Smith et al., 2004). Tolerance levels may change over time and, for this reason, long-term studies are of considerable importance (Nedelec et al., 2016). When exposed to motorboat noise, individuals of *D. trimaculatus* had concealment responses and high ventilation rates only after two days, the same effects were not found after one and two weeks of exposure (Nedelec et al., 2016).

No changes in plasma cortisol levels and body growth were detected after three weeks (Nedelec et al., 2016). This shows the importance of the recovery time of the different species and the variability of the effects that we can find, some organisms can recover quickly (La Manna et al., 2016). Recovery in some cases is rapid in behavior and swimming, but not in breathing speed, which increases in the presence of noise (Bruintjes et al., 2016). Bruintjes et al., (2016) analyze effects during and immediately after emission on *A. anguilla* and *D. labrax*. They analyzed the anti-predatory response and the rate of ventilation. Exposure to noise increased ventilation rates, reduced responses to predators and influenced startle responses. However, their results show possible recovery rates following short-lived sounds. Time responses may change, animals may respond to such stresses more slowly and less frequently (Simpson et al., 2016). Wysocki et al., (2006) studied the effects of noise generated by ships in three fish species with different hearing capacities for 30 minutes while observing equal significant increases in cortisol levels. Significant hearing loss was recorded in hearing specialists such as *C. auratus* and *P. pictus* (Amoser & Ladich, 2003). The recovery times of the two species were different (shorter for goldfish), highlighting that they are differently affected by noise exposure. Their reporting according to these data could be reduced in particularly noisy habitats. Hearing loss, even if temporary, could compromise the reproductive level of the species, communication distances and prey/predator relationships (Amoser & Ladich, 2003). Scholik & Yan, (2001) also found significant effects in hearing specialists with obvious hearing reductions. However, this may vary depending on the frequency analyzed. Frequency and duration of exposure affect species recovery (Scholik & Yan, 2001). Nichols et al., (2015) analyzed the effects of increased boat noise on *H. rostratus* by measuring stress responses by assessing cortisol levels. When exposed to intermittent noise, fish show acute stress responses unlike exposure to continuous noise (such as environmental noise). European sea bass and sea bream exposed to vessel traffic for 10 minutes showed increases in movement, metabolic levels and changes in blood glucose and lactate content (Buscaino et al., 2010). In *C. chromis*, significant increases in plasma glucose, lactate, total protein, and heat shock protein 70 (Hsp70) were observed after exposure to noise levels at 200 and 300 Hz (Vazzana et al., 2017). Lin et al., (2019) observed a change in Hsp70 expression in fish liver tissue cells. Significant biochemical changes in blood or plasma (cortisol, ACTH, glucose, lactate, hematocrit, etc.) were found in *S. aurata* after 10 days of exposure to such anthropogenic noise (Celi et al., 2016). This demonstrated the activation of a primary and secondary response to acoustic stress. Graham & Cooke, (2008) studied the effects of noise produced by canoes, internal combustion engines and combustion engines by observing a change in cardiac output levels in animals with a recovery time that varies according to species and source type. The work of McCormick et al., (2019) confirms these findings. In the case of *M. asiaticus*, exposed to a ship's noise, changes in hearing thresholds were detected but temporary: Temporary Threshold Shift (TTS) (Liu et al., 2013). Temporal changes in hearing can be very frequent (temporary threshold shift, TTS) (Popper et al., 2005; Popper et al., 2014). Today we do not know the defined levels that create these temporary changes. Nevertheless, the factors that influence them are different: number and frequency of repetitions, SPL, duration, physiological state of the organisms (Popper & Hastings, 2009a). The noise of the speedboat influences the shape and structure of the group in *T. thynnus* (Sarà et al., 2007). Tuna change their swimming directions (moving to the surface or depths), aggregations, aggressive behavior and migrations based on the presence of noise from a ferry or small boat (Sará et al., 2007). The group loses its aggregate structure and becomes uncoordinated. Hydrofoils caused a similar reaction but for shorter periods (Sará et al., 2007). This creates problems with anti-predatory benefits, individuals in larger, more cohesive groups have less risk than individuals in smaller, less cohesive groups (Hamilton et al, 1971; Ioannou et al., 2017; Correa et al., 2018). Predation rates in the presence of anthropogenic noise change (Bruintjes et al., 2016). Noise from boats influences fish orientation (Neo et al., 2016). Low-frequency noise (100 and 1000 Hz) can affect the time of the group used to reach the seabed and their feeding rates (Bracciali et al., 2012). Effects on foraging that cause low absorption and higher metabolism may cause reductions in growth rates (Kuşku et al., 2018). Boat noise favors predators by giving them greater success on prey (Simpson et al., 2016) influencing the structure of the community habitat. Some species, in the presence of this type of noise, show greater inactivity and greater social behavior as a result of fear and stress. However, depending on the species observed, the effects change. *G. aculeatus* compared to *P. phoxinus* maintains high levels of foraging but with a high number of errors (Voellmy et al., 2014a). Sempere et al., (2018) analyze the impact of vessel traffic in the Western Mediterranean, confirming what has been said so far: the noise of motorboats has a negative effect on fish aggregation. Communications and calls between species are higher at uncontaminated sites (Sempere et al., 2018). Noise from small speedboats also has the potential to cause latent learning effects long after the stressor has disappeared (Ferrari et al., 2018). Exposure to anthropogenic noise by influencing communication (Naguib, 2013) can influence individual suitability and information at the level of conspecific networks and thus the community (Francis & Barber, 2013). Vessel traffic noise can reduce the communication range of cod and haddock (Stanley et al., 2017), species that vocalize during spawning. In fact, cod already reacts at very low sound levels (Engås et al., 1998). Vocalization masking may reduce the ability to attract companions and reproductive success (Rowen et al., 2008; Stanley et al., 2017). In toadfish, communication distances are reduced by several meters (Alves et al., 2017) and the noise generated by ferries influences them (Vasconcelos et al., 2007). However, fish are limited in changing the frequency or volume of their calls (Amorim, 2006). Different species change the number of vocalizations and depending on whether they are at noisy or noisy sites (Krahforst et al., 2017), they vocalize more in moments of silence (without passing ships). This behavior causes a greater energy expenditure at the muscle level. If the vocalizations depend on the intensity of the passage of ships, these species may reproduce less at the busiest sites (Krahforst et al., 2017). *P. pictus* uses visual and acoustic signals for courtship phases. The presence of noise influences the behavior of male courtship and female deposition. Females make greater use of visual courtship (De Jong, et al., 2018) and depending on the noise conditions change the nesting or number of embryos (Krahforst, 2017). Fish may respond to different sources of stress in different ways depending on their characteristics, although the reaction to the stimulus is similar (Akinrotimi et al., 2009). The species' hearing sensitivity depends on their hearing ability and is different from their behavioral reactivity. Responses are either similar among species or specific, depending on their hearing ability. *D. labrax* has a higher sensitivity to low frequencies (100-1000 Hz) (Lovell, 2003), typical of many anthropogenic noises (Götz et al., 2009). Through the use of audiograms, it has been possible to observe that *S. melanostictus* presents levels of sensitivity at higher frequencies than other fish (Akamatsu et al., 2003). Their swim bladder plays an important role in the perception of sounds. Brehmer et al., (2019) observe behavioral variations in different fish depending on the noise level of the vessel. In the study of sound effects, it is also important to include particle movement and pressure. Kojima et al., (2010), studied these two factors in the red sea bream *P. major*. The fish probably detects the movement of the particles in a frequency range of 50-200 Hz. Startle responses also occur after exposure to low frequency sounds and each species reacts differently to different types of sound (Kastelein et al., 2008). Several factors can influence reactions: temperature, animal physiology, age, individual and group size (Kastelein et al., 2008). Field studies confirm that noise influences the amount of time that fish spend in their shelters and that care for nests decreases (Picciulin et al., 2010). Reproduction of noise generated by a 40 hp outboard engine may cause adverse effects on the behavior of *G. cruentatus* and *C. chromis*, in particular the time spent in their nests or shelters (Picciulin et al., 2010). Codarin et al., (2009) also show that the noise produced by a ship influences the auditory sensitivity in *C. chromis*, *S. umbra* and *G. cruentatus* and, therefore, the communication of these organisms. The effects are also found in an increase in defensive acts, a reduction in diet and a change in parental behavior. The survival of the offspring and therefore the fitness in *A. polyacanthus* is also reduced (Nedelec et al., 2017a). It is also possible that the animal can get used to, compensating or moving away from the noise (Bejder et al., 2009; Morley et al., 2014; Radford et al., 2016). Noise pollution can affect the ability of organisms to maintain their territory as observed in *G. cruentatus* (Sebastianutto et al., 2011).

Important effects can also be observed in freshwater species. Behavior, respiration and metabolism in the presence of ship noise change in juvenile eels (Simpson et al., 2015). *P. clarkii* presents a reduction in levels of competitive behavior and significant changes in haematological parameters at certain emission frequencies (Celi et al., 2013). Further effects were found in *H. nobilis* (Vetter et al., 2017), *D. rerio* (Sabet et al., 2016) and also in *A. japonica* (Xinhai et al., 2016). Behavioral effects were observed in *O. mykiss* (Davidson et al., 2009) and *C. venusta* (Holt & Johnston, 2015). Sabet et al., (2015) studied the effects of sounds on prey-predator interactions of *D. rerio* and *D. magna*, testing different sound conditions that varied according to time model: continuous, fast, slow, regular and irregular intermittent. Their results showed that high sound levels, and in particular intermittent conditions, can affect prey-predator interactions. *D. rerio* was compared with *H. piceatus*, sensitive to lower thresholds and wider spectral ranges (Sabet et al., 2016). They are species sensitive to different sound thresholds. They showed reductions in swimming in the first minute of exposure and in particular, the zebrafish, unlike cichlids, showed a startle reaction. The two species did not show differences in horizontal movements but in vertical movements changing the depth of swimming. The cichlids moved downwards, while the zebrafish maintained their swimming height. Responses in these two species differed according to their hearing ability (Sabet et al., 2016). Magnhagen et al., (2017) studied this type of impact on *R. rutilus* and *P. fluviatilis*. This study has an important value because it also considers sound pressure and particle acceleration. Noise exposure affects foraging in a species-specific way. The habitat and the presence of other species affect the final effects (Magnhagen et al., 2017). Noise produced by boats, because they have effects on masking and communication (Codarin et al., 2009), can change the detection distances in a species-specific way even in cichlids (Ladich et al., 2013b). In cichlids, reactions even depend on the sex of the organism, the role of the fish in the group and the presence/absence of eggs (Bruintjes & Radford, 2013). Animals respond by digging less, reacting less to prey, with more aggressiveness and submission and consequent increase in metabolic rates, the effects depend on the context (Bruintjes & Radford, 2013). If the environmental noise increases, Goby males reduce courtship calls and females lay fewer eggs with important effects on the genetic heritage of the population. Female choices change according to noise pollution conditions. Mickle et al., (2019) analysed the effects of boat noise on *A. melas* and demonstrated the presence of sublethal effects on the behavior of these fish.

Other studies refer to noise emitted by aquaculture equipment. High levels of oxidation status, lysozyme activity, antiprotease activity and white blood cells together with a lower albumin/globulin ratio were observed in the juveniles of *S. aurata* exposed to offshore aquaculture conditions (Filiciotto et al., 2017). Noise along the offshore coast even affects the growth performance of young *S. aurata* fish (Filiciotto et al., 2013). These can affect the hearing ability of fish such as *P. aurata* (Caiger et al., 2012). Anderson et al., (2011) examined stress responses to chronic noise exposure in 32 animals of *H. erectus* for one month. Behavioral changes, weight changes (ΔWt), change in Fulton condition factor (ΔK), hepatosomatic index, gonadosomatic index, differential and non-leukocyte count, cell volume, heterophilic/lymphocyte ratio (H: L), glycemic concentration, plasma cortisol concentration, presence/absence of parasites and number of infected organs, presence/absence of bacterial infection were evaluated. Seahorses, exposed to strong environmental noises, present a primary, secondary and tertiary stress and therefore responses to behavioral and physiological levels.

The main noise frequencies produced by ships fall in the band between 20 and 200 Hz (Tyack, 2008). Such low frequencies propagate efficiently at sea. Whales for communication use this frequency band. The tendency of marine mammals to avoid certain anthropogenic noises (even at kilometer intervals) has been demonstrated and this increases the concern for the displacement of their habitats. Slowing down ships could reduce communication damage, especially in noisy conditions (Pine et al., 2018), however there are too many differences in hearing sensitivity and anatomical characteristics of species to be sure. Noise from ships reduces communication space and can have chronic effects on *B. edeni* and *P. adspersa* (Putland et al., 2017). The most significant risk to marine mammals may arise from chronic exposure effects (Tyack, 2008). Maintaining noise levels around 120 dB re 1μPa could be a good reference standard for not endangering the physiological integrity of whales (Weir et al., 2007; IUCN, 2006) and levels above 160 dB re 1μPa would appear to have negative effects on mammals also at behavioral level (Department of Fisheries and Oceans. Statement of Canadian Practice: Mitigation of Seismic Noise in the Marine Environment, 2005; Weir et al., 2007). Changes in physiological parameters such as aldosterone, norepinephrine, adrenaline, dopamine have also been detected in *D. leucas* and *T. truncatus* when exposed to levels above 100 kPa (Romano et al., 2004).

Some authors have studied the effects of pile driving at larval level in some species of crabs for which noise can delay metamorphosis (Pine et al., 2012). It is known that many offshore anthropogenic activities produce high noise levels (<1000 Hz), levels that pose a threat to crustaceans due to their acoustic sensitivity bands. Kostyuchenko, (1973) analyzed the survival and lesions in eggs of some fish species. The closer they were to the source of the noise, the higher the mortality. No change was found in the survival of *G. morhua* eggs (Dalen & Knutsen, 1987), and in mortality in the larvae of *S. solea* (Bolle et al., 2012).

Zhou et al., (2018) simulated the main frequency bands related to anthropic activities of this type, studying possible effects on behavior and physiological response (Hsp70), on juvenile individuals of *S. paramamosain*. The results of their work showed significant increases in locomotor activity and gene expression of Hsp70. The effects are evident in the functioning of the antennae of hermit crabs (Roberts et al., 2016a). This type of frequency may also have effects on invertebrate filtration, which is higher in blue mussels in the presence of pile driving noise (Spiga et al., 2016). *M. edulis* mussels respond to acoustic stimulation and vibration with immediate valve closure (Roberts et al., 2015). Probably the greater stress pushes these organisms to filter more for an increase in their metabolic rate. Spiga et al., (2017) analyze the effects of two types of noise pollution ("piling" and "drilling noise") on the behavior of juveniles of *D. labrax* in captivity. Exposure to high noise levels influences the anti-predatory behavior and physiology of these species more intensively for piling (Spiga et al., 2017). In the presence of pile driving, it is also possible to observe mortality events in juvenile individuals of *D. labrax* (Debusschere et al., 2014). Debusschere et al. (2016) studied the response in the juveniles of *D. labrax*. Levels of oxygen and lactate consumption were reduced. Bruintjes et al., (2017) analyze the impacts of this type of noise emission on oxygen consumption of *S. cantharus* and *P. platessa* showing that this type of activity has effects on biodiversity in a species-specific manner. After only 30 minutes, only the gilthead seabreams increased the rates of oxygen consumption. Emissions of this type affect the variations in the trajectories of *D. labrax* with changes in the structure and dynamics of the fish group (Herbert-Read et al., 2017). These were less cohesive, less tidy and less correlated in speed and directional changes. The groups of animals use the rules of interaction to coordinate their movements and consequently to obtain the benefits of group life (reduction of the risk of predation and exchange of social information). Noise may change the way individuals interact (Herbert-Read et al., 2017). In the context of physiological effects, in some cases the effect has been found at the endocrinological level. Seismic noise reproduced in the laboratory can create variations in adrenaline and cortisol levels in *S. salar* (Sverdrup et al., 1994). In *D. labrax*, the response to this type of acoustic stress (impulsive noise) led to increased ventilation levels (Radford et al., 2016). Low frequency and high intensity sounds, such as those generated by pile driving, can cause barotrauma or histologically or morphologically detectable physical damage. Evident damages have also been found in the swim bladder, liver, kidney and gonads of different aquatic organisms (Casper et al., 2012; Halvorsen et al., 2012). The nature of noise influences behavioral responses (Neo et al., 2014), in particular impulsive sounds (Neo et al., 2015). In the study of the effects of acoustic noise in the marine environment, it is also relevant to evaluate the difference in impact between day and night. Neo et al., (2018) studied for the first time the acoustic effects on *D. labrax* in the day/night period at repeated and impulsive exposures. Exposure to acoustic stimulus changed swimming speed, cohesion and swimming depth within the water column. In the presence of this type of stress, the cohesion, speed of swimming and depth of swimming have increased. Noise emissions could be higher at night (Neo et al., 2018). De Jong et al., (2017) tested the effect of continuous noise on courtship behavior in *G. flavescens* and *P. pictus*, demonstrating that reproductive success can be sensitive to noise pollution. In particular, the male of *P. pictu*s showed less ability in visual courtship, while the female showed less chance of laying eggs. To confirm this, the sound production synchronizes the release of the gametes in *M. aeglefinus* (Hawkins & Amorim, 2000; Casaretto et al., 2014) and in *G. morhua* (Rowe and Hutchings, 2006). Also, in *H. didactylus*, the success of the coupling depends on the acoustic courtship performance of the males (Amorim et al., 2016). Juanes et al., (2017) conducted a meta-analysis to understand how anthropogenic or biological noises can influence fish behavior and physiology and most fish species exhibit negative effects. The noise of pile driving influences the movements, speed and states of aggregation of sole and cod (Mueller-Blenkle et al., 2010).

Sound pressure levels and particle movement have effects on fish behavioral responses (Mueller-Blenkle et al., 2010; Popper et al., 2018). There is a difference in recovery between intermittent and continuous emissions into the seabass. The latter has more evident effects (Neo et al., 2014). Wei et al., (2018) expose *C. chanos* to noise for 24 hours, 3 days and 1 week. Fish exposed to noisy conditions had higher plasma cortisol levels in the first 24 hours. These returned to normal levels quickly. In addition, fish exposed to acoustic stress had high levels of acute steroidogenic regulation and high levels of mRNA hsd11ß2 (11-ß-hydroxysteroid dehydrogenase 2). Weak but continuous noise is a potential stressor. However, impacts may differ depending on sound levels and exposure time and it is always good to consider that intraspecific and interspecific variations affect the responses of organisms (Solan et al., 2016). Continuous noise can regulate genes related to cortisol synthesis. This makes fish more sensitive to stress by influencing the distribution of energy resources during long-term exposures. This type of stress affects both secondary and primary responses. The effects of acoustic stress also occur at the cellular/structural level, and in TTS variations. This happens when the hair cells of the inner ear are tired. With the highest sound level and the longest duration of exposure, TTS are most likely to occur (Weilgart, 2007). The permanent threshold shift, Permanent Threshold Shift (PTS), is different and occurs when the hearing does not return to normal. Depending on the distance from the source, impacts may change, in fact only behavioral changes for mammals within 1 km have been detected between 100m and 2400m (Lossent et al., 2018).

In freshwater fish, too, obvious damage was found in the swim bladder, liver, kidney and gonads (Casper et al., 2013a, b; Halvorsen et al., 2013). The structure of the swim bladder influences the type of damage; physioclist fish have more damage. In addition, larger fish are more sensitive than smaller fish, perhaps because of the size of the bladder and its "resonance box" effect (Casper et al., 2013a). Impulsive sounds can cause barotrauma in *O. tshawytscha* with different types of lesions, from small hematomas to intense bleeding depending on exposure levels. A Response-Weighted Index (RWI) was used to assess the physiological impact of different lesions. Higher exposure noise levels led to higher levels of RWI. Tissue damage and physiological damage correspond to RWI levels above 2. Halvorsen et al., (2012b) analyzed swim bladder damage in several species: *A. fulvescens*, *O. niloticus* and *T. maculatus*. These species differed according to the presence or absence of the swim bladder and its structure. The damage after stress was evident. This was particularly the case for species such as *A. fulvescens* and *O.niloticus* (Halvorsen et al., 2012b). The extent of the lesions and the effects on the organisms depend on the type of swim bladder and the sound levels received.

Kastelein et al., (2013) seek to understand the effects of pile driving on porpoise behavior by exposing porpoises to typical noise emissions from these activities and highlighting an increase in their breathing rate. With higher emission levels, the animal jumped out of the water. This has shown that the noises emitted by this type of human activity have effects on the behavior of these organisms that tend to respond by moving from the source. The sounds of different time models (intermittent and continuous) affect the behavior of *P. phocena* with different response patterns depending on the noise levels emitted (Kok et al., 2017). The work of Bailey et al., (2010) may be useful in determining and establishing possible distances at which to monitor the emitted sound. In this work, the impact of noise on bottlenose dolphins caused by pile driving of wind turbines present at a depth of more than 40 m was analysed. The noise was measured at distances of 0.1 to 80 km. For these organisms, auditory lesions would be detected at 100 m from the disturbance while behavioral variations up to 50 km away. Through controlled experiments, foraging changes in whales at distances of 1.4 and 12.6 km were also observed (Jochens et al., 2008) and increases in avoidance behaviour (Weir et al., 2008a, 2008b). Grey whales *E. robustus* depending on emission levels have behavioral reactions to continuous broadband noise and intermittent noise (Moore et al., 2002). Noise can interfere with communication and navigation, and thus with their migration (Evans et al., 1998; Parsons et al., 2007). A report on the noise perceived by cetaceans has been treated and reviewed by Wilson et al., (2010).

Another type of anthropic activity studied is the airgun. Several works show that these noise emissions can have impacts on embryos and scallop larvae (Aguilar de Soto et al., 2013). Field studies have shown delays in development and increases in larval mortality in bivalves and decapods (Pearson et al., 1994). Pearson et al., (1994) study its impacts in *C. magister*, observing its survival and development. Field experiments revealed no statistically significant effects (> 0.05) at a distance of 1 m from the emission. Payne et al., (2009) also found no significant effects on fish embryo survival while two other studies indicate that exposure to this type of human activity within 1m increased mortality in fish larvae (Kostyuchenko, 1973; Booman et al., 1996). In the light of various data, even regarding coral reef larvae, it is reasonable to think that noise pollution could cause confusion and disruption of orientation behavior (Simpson et al., 2010). Banner & Hyatt, (1973) observed increased mortality in eggs and McCauley et al., (2017) showed that airgun affects and kills even the zooplankton that forms the basis of the food chain.
Real estate invertebrates on the ocean floor, such as mollusks, seem to be more at risk (Webster et al., 2018) but also in cephalopods (Mooney et al., 2010) and decapods (Lovell et al., 2005) negative effects of airgun have been found. Behavioural changes were observed in squid up to 2-5 km from emission (McCauley et al., 2000). Cuttlefish respond to this type of acoustic stress by releasing ink (Samson et al., 2014) as well as squid (Fewtrell & McCauley, 2012). Day et al., (2016) tested the effect of airguns on lobsters and scallops. Lobsters did not present mortality events but variations in tail size. The data are of particular importance as this characteristic influences the possibility of escape from predators (Day et al., 2016). These changes also depended on possible damage to hair cells and organisms were even more susceptible to disease and infection. Unlike lobsters, scallops showed mortality events due to immunosuppression events (Day et al., 2016). Changes in the behavior of such invertebrates may influence the prey-predator ratio (Day et al., 2016). Exposure to airgun may reduce the count of blood cells in the scallops and alter the biochemistry of the haemolymph. This type of noise pollution influences the behavior and blood parameters (total hemoglobin, haemocytes count, glucose, lactate concentrations and total proteins) of *N. granulate* (Filiciotto et al., 2018). Under noisy conditions, crabs have been shown to choose the shell faster without wasting time analyzing it (Walsh et al., 2017). As with fish, some invertebrates may become accustomed to sound (Fewtrell & McCauley, 2012; Samson et al., 2014; Mooney et al., 2016) and may suffer impacts on reproductive speed. Fitzgibbon et al., (2017) analyse the effects of the airgun on the physiology of the lobster *J. edwardsii*. The effects were evident in increasing THC up to 365 days after stress, showing a chronic impact up to 120 days after exposure. Anatomically, no evident effects were found in crabs (Lee-Dadswell, 2009). Emissions from airguns cause damages to statocysts, changes in blood chemistry, changes in oxygen availability and therefore in the subsequent death in squids (Guerra et al., 2004). Further experimental evidence highlights the negative effects of this type of noise on *C. maenas* crabs, although the same does not apply to *C. crangon* shrimps (Hubert et al., 2018). The crabs move away from the food source due to the presence of noise and this could influence the reduction of competition. Anthropic noise influences, therefore, the foraging interactions of the species in question (Hubert et al., 2018). The effects on invertebrates are contrasting, the low frequency sound had no effect on the bio-indicators of stress in lobsters (Payne et al., 2007) or on snow crabs (Christian et al., 2004), unlike *P. aurea* which showed high levels of glucose, hydrocortisone and lactate after airgun noise exposure (La Bella et al., 1996). A sudden onset of sound can cause an alarming reaction in sharks (Myrberg et al., 1978), although information on the low frequency sound response of elasmobranchs is scarce today. The explosion of airgun can cause alarm reactions in teleost fish (Hirst & Rodhouse, 2000; McCauley et al., 2000). This includes the C-starts reaction and changes in schooling, water column positions and swimming speed (Pearson et al., 1992; Wardle et al., 2001; Hassel et al., 2004; Boeger et al., 2006; Fewtrell & McCauley, 2012). The reaction of C-starts consists in a bending of the body that takes the form of the letter "C". It seems that some fish may become accustomed to this type of disorder by also reducing startle responses (Pearson et al., 1992; Boeger et al., 2006; Fewtrell & McCauley, 2012), especially after continuous exposure to compressed air. Seabass and sandeel in captivity

show "alarm" reactions at certain distances from the source (2.5 and 5 km) (Santulli et al., 1999; Hassel et al., 2004). The airgun affects fish in different ways between species, in fact, based on emission levels, startle responses are found in *S. serranoids* and *S. melanops*, unlike *S. miniatus* and *S. auriculatus* (Pearson et al., 1992). The alarm responses are located several kilometers from the sound source in the European bass and in the sand eel (Santulli et al., 1999; Hassel et al., 2004). In *M. bilinearis*, the group of fish responded to the noise by moving downwards at greater depths in a more compact manner (Chapman & Hawkins, 1969). In other species, there is an increase in their swimming speed and a change in movement patterns. Blue whiting and mesopelagic species were in deeper waters during seismic exposure (Slotte et al., 2004). However, the effects are not important in terms of horizontal distribution (Slotte et al., 2004). The most likely short-term response to seismic sound is vertical displacement. Fish may show potential addiction to repeated exposure of the airgun, as demonstrated in captive scorpion fish that have returned to pre-exposure behavioral patterns (Pearson et al., 1992). *L. synagris*, *L. apodus*, *C. faber* demonstrated that repeated exposure produced less and less obvious startle reactions (Boeger et al., 2006). Temporary addiction to discharges of airgun can be observed in schooling (Boeger et al., 2006). Terhune et al., (1990) showed low growth rates in *S. salar* subjected to high acoustic noise. However, Peña et al., (2013) found no effect on swimming speed and direction following 3D seismic surveys. Analysis of stomach content shows in some cases a reduction in the feeding rate (Løkkeborg et al., 2012). The abundance of herring, blue whiting and other mesopelagic fish also changes according to seismic detection areas with long-term effects (Slotte et al., 2004). These fish, found at greater depths to avoid noise, move vertically and non-horizontally (Slotte et al., 2004). Also along the coral reef, abundance has been reduced during seismic surveys (Paxton et al., 2017). In general, the greater the intensity of the sound, the less the depth of the water, the greater the risk (Webster et al., 2018). For marine organisms, impacts in waters deeper than 250m seem acceptable, while in waters less than 250m deep impacts may also be severe depending on depth and seismic intensity (Webster et al., 2018). Santulli et al., (1999) analyze the effects of airguns on *D. labrax*, analyzing their biochemical response. Variations in cortisol, glucose, lactate, AMP, ADP, ATP and cAMP levels were observed in different animal tissues confirming the presence of an acoustic stress response. Nevertheless, no damage to the skeletal systems of the animals was detected. In an interval of 72 hours the biochemical parameters returned to physiological values with a rapid recovery of homeostasis. Further effects of sound levels seem to depend on depth. The Economic Exclusive Zone (EEZ) where mitigation measures are established for an array of 20 guns at a sound level of 160 dB re 1μPa can be about 2.5 km in deep water (~ 3200 m) but can extend for over 12 km in a shallow area (~ 30 m) (Weir et al., 2007). *P aurata*, after exposure to airgun, showed extensive damage to the hair cells of the ear without evidence of recovery (McCauley et al., 2003). Andrews et al., (2014) conducted genomic studies on the inner ear of salmon exposed to airgun noise emissions. They performed microarray analyses that identified 42 up-regulated and 37 down-regulated transcripts. The effects were significant in terms of cellular energy and cellular respiration. In addition, transcripts coding for hemoglobin were overregulated as those coding for nicotinamide riboside kinase 2, important in nerve cell damage. It was clear that the noise had created neuronal damage to the ear. Transcriptional changes in proteins confirmed damage to ear tissues, as in the case of transcription of cytoskeleton proteins. The set of results obtained from the work of Andrews et al., (2014) allows us to understand the potential of molecular biomarkers in assessing the effects of noise pollution on fish. Emissions from airgun affect catch rates as demonstrated for cod and haddock (Løkkeborg et al., 2012). The reduction in capture rates could depend on an avoidance reaction in *G. morhua* and *M. aeglefinus* (Engås et al., 1996) or in redfish (Skalski et al., 1992). Some studies have revealed no damage to the auditory system (Popper et al., 2005; Song et al., 2008; McCauley & Kent, 2012) as in *S. albus* and *P. spathula* (Popper et al., 2016). Wysocki et al., (2007) observed that hearing, growth, survival and resistance to *O. mykiss* diseases were unaffected. Lucke & Siebert, (2009) observed that *P. phocena* in the presence of airgun emissions presented adverse behavioral reactions indicating hidden acoustic thresholds.

Dunlop et al., (2017) showed that humpback whales were more likely to emit airguns within 3 km of the source. Both the proximity to the source of the emission and the sound level received were important factors. Gordon et al., (2003; 2018) review the effects of airgun on the behavior and physiology of marine mammals, which are complex, variable and conflicting.

Another source of anthropogenic noise emission are sonars that are divided into three categories according to their operating frequency; low frequency (LF) for 1 kHz and less, mid frequency (MF) from 1 kHz to 10 kHz and high frequency (HF) from 10 kHz onwards. Although several studies have been carried out on the effects of high frequency emissions on marine organisms (Richardson et al., 1995; Southall et al., 2007), there are few studies on sonars such as Popper et al., (2007) and Kane et al., (2010) which do not show negative impacts at the auditory level. Low-frequency sonars and possibly mid-frequency sonars are the most relevant for fish and sea turtles because of the low-frequency auditory ranges of these animals (Halvorsen et al., 2012c). Hearing loss due to low frequency sonar exposures have been observed in catfish (Halvorsen et al.,2006). Sonar may have contrasting effects on schooling of some fish species (Schwarz & Greer, 1984; Sivle et al., 2012). Reactions not only depend on species, but also on environmental conditions. Herring, for example, is more sensitive to engine noise during the winter period (Doksæter et al., 2012). Despite this, Doksæter et al., (2012) confirm that sonars do not create particular reactions in this species. Activities of this type have caused changes in orientation, swimming direction of individuals, collective movement, horizontal or vertical movement (Pitcher et al., 1996; Nøttestad & Axelsen, 1999; Wilson & Dill, 2002). Halvorsen et al., (2013) found no hearing effects in some freshwater species. However, this may depend on a different susceptibility influenced by genetic characteristics, developmental conditions or seasonal variation. Popper et al., (2007) observed temporary hearing loss and differences between different groups of the same trout species in rainbow trout (Popper et al., 2007).

Conclusions and work carried out

The study of the bibliography taken into consideration in this work, concerning the acoustic impact, has allowed the extrapolation of the limits and indications reported in the above-mentioned technical standard.

Most of the published works concern laboratory experiments and not *in situ* experiments. This constitutes a possible limit in the establishment of noise emission thresholds. In this respect, there is a full awareness that the two experimental situations are not overlapping.

However, it was appropriate to make a first attempt to provide these indications on the acoustic limits to be respected considering: the type of emission of the various marine maritime activities, the possibility that the activities of DSM will concern low frequency emissions and the variability of the auditory capacity of marine species.

We remain aware of the fact that there are many scientific limitations, but at the same time, we remain even more aware of the fact that the activities of the marine maritime sector and future mining activities in the ocean depths cannot begin without indications or basic limits to contain the noise impact. From this, our initiative to propose for the first time possible noise emission limits to be considered during these activities for a containment (as far as possible) of environmental impacts takes its cue. We are still in time to give indications before DSM's activities begin, "prevention is better than cure" and it is in this context that we hope to make our contribution. Not to stop development, but to find the right balance between development and life in the depths of the ocean.

It is true that many animal species so far live in particularly noisy environments, but this is not a good reason to think that they do so because they "feel good". Probably the reasons that push these organisms to live in noisy environments are more important and related to the vital function that the site plays in their lives.

The multidisciplinary approach has been evaluated as the only type of useful and meaningful approach. The large number of factors involved and discussed in the annex gives rise to a number of difficulties, such as establishing fixed and unambiguous parameters and distances for safe noise monitoring. Emission frequencies have also been indicated considering that for most species, sound sensitivity occurs from below 100 Hz to several hundred hertz, or several thousand hertz in a few species (Mann et al., 1997, 2001). The “Monitoring Guidance for Underwater Noise in European Seas - Part II” suggests to monitor the trends of the noise levels of environmental noise (annual average values measured in RMS re 1µPa) emitted within the bands at 1/3 octave with central frequencies at 63 and 125 Hz. The "Guidelines for the management of the impact of anthropogenic noise on cetaceans in the ACCOBAMS area have been useful to indicate a possible time interval of the monitoring, acoustic and visual activities that should be carried out throughout the duration of the noise emission. From this document have been extracted also the information about the professional figures of the MMOs (Marine Mammals Observer). The Guidelines of Germany - BfN 2013: 1 were useful for making suggestions about minimum distances for monitoring and measuring noise emissions. These are works for which consultation is strongly recommended in the context of human activities with possible noise impact.

The bibliographic works showed in most cases emissions of dBrms re 1µPa and this allowed us to extrapolate the limit values. Levels causing severe animal injury appear to be much higher than 180 dB rms re 1µPa (OGP-IAGC). However, most of the work with impacts on physiology, physics and animal behavior concerns emissions ≥ 130 dBrms re 1µPa. Some works are outliers but in insignificant percentages. This has allowed us to establish that emissions above 130 dBrms re 1µPa cause "serious impacts" on biodiversity, in many cases not reversible. Given the lower percentage of works with an impact below 90 dBrms re 1µPa, it was decided to recommend this limit as a "low impact" emission on marine biodiversity. Intermediate levels, between 90 dBrms re 1µPa and 130 dBrms re 1µPa, cover a type of impact defined as "average impact". The data for determining distances are insufficient but in general the closer the animal is to the source, the more likely it is that the high energy will have a resulting effect (Popper et al., 2019). In this context, regulators need to consider the levels of origin and noise reception by animals. Efforts towards standardization inherent in particle movement have been made by the International Organization for Standards in ISO/DIS 1683 (2013). This standard recommends the following 1pm (picometer) for the dislocation of sound particles, 1 nm/s for the speed of sound particles and 1 $\mu m/s^2$ for the acceleration of sound particles. In addition, the levels chosen were selected with the ultimate aim of ensuring that the technical standard is not too restrictive and therefore unenforceable. The purpose of the standard is to be transposed and to contribute to the reduction of noise impact. At present there are no national or international standards for the exposure of fish to impulsive sounds. The National Marine Fisheries Service (NMFS), based on data on mortality of species exposed to explosives (Popper & Hastings 2009), developed intermediate criteria for pile driving (FHWG 2008, Woodbury and Stadler 2008; Stadler and Woodbury 2009; Caltrans, 2009) and specified a maximum SPL_{peak} of 206 dB re 1µPa and a maximum SEL_{cum} of 187 dB re 1µPa2 s^{-1} for fish ≥2 grams and 183 dB re 1 µPa2 s^{-1} for fish <2 grams (Carlson et al., 2007). In the case of impulsive emissions, the works of Popper et al., (2014; 2019) were used to indicate the emission levels. Popper et al., (2019) organize the scientific data of further work according to species, type of damage detected and type of source or sound exposure. Halvorsen et al. (2011, 2012a, c) and Casper et al., (2012, 2013a, b) describe the effects of impulsive sounds on different species by formulating the Response Severity Index (RSI), determining the maximum sound pressure levels associated with different RSI levels. Tissue damage increases with increasing SEL_{cum} and SEL_{ss}.

In the bibliography, however, there are other more appropriate metrics for pulsed sounds: Sound Exposure Level (*SEL*) for single and cumulative sounds; Peak sound pressure level; Peak-to-peak sound pressure level. However, the available data did not allow us to identify reliable sound levels. In the case of Sound Exposure Level (*SEL*), significant negative effects already occur at levels above 120 dB re: μPa^2 s (183 dB re: $\mu Pa^2 s$) (Southall et al. 2007; Borsani & Franchi, 2011). The threshold values at which physical/physiological damage to marine mammals can be observed are in most cases equal to or greater than 120 dB re: $\mu Pa^2 s$ (Malme et al., 1983; Ljungblad et al., 1988;

Todd et al., 1996; McCauley et al., 1998; Southall et al. 2007; Borsani & Franchi, 2011). Minor effects can also be observed below 120 dB re: $\mu Pa^2 s$, for which it is important to implement the measures reported in the technical standard (Madsen & Mohl, 2000; Madsen et al., 2002). In the context of drilling and piling activities, within the frequency bands between 10 Hz and 20 kHz, noise levels above 120 dB re 1 $\mu Pa^2/Hz$ have been shown to cause adverse effects on biodiversity (Sabet et al., 2016; Spiga et al., 2017; Nedelec et al., 2017; Weilgart et al., 2017; McCormick et al., 2018). The use of drilling also seems to be recommended compared to piling (Broudic et al., 2014). It may also be useful to calculate peak compressional sound pressure level and peak rarefactional sound pressure level, pulse duration and pulse repetition frequency. The *SEL* can be considered as a measure of the energy content of the impulse (Good Practice Guide No.133-Underwater Noise Measurement). The impact of these activities could be contained by using the "soft start" technique, which would ensure a possible possibility of removal of marine organisms. To minimize additional noise, the soft start should not last longer than 40 minutes (Joint Nature Conservation Committee). The power increase due to the soft start technique should not exceed 6 dB every 5 minutes (Guidelines for the management of anthropogenic noise impact on cetaceans in the ACCOBAMS area). The reduction of drilling times, taking advantage of any resting sessions, could contribute to the reduction of the acoustic impact. All this, bearing in mind that the acoustic conditions and pressure levels depending on the type of environment may change (modified by Jasny et al., 2005; modified by Borsani & Franchi, 2011; Spiga et al., 2017). The creation of "Areas of Particular Environmental Interest" (APEI) (Dunn et al., 2018) could be useful.

From this bibliographic study it emerged that a good monitoring of noise and the possible presence of mammals might be allowed through the consultation of documents such as Monitoring Guidance for Underwater Noise in European Seas - Part II; Good Practice Guide No.133- Underwater Noise Measurement; Guidelines for the management of the impact of anthropogenic noise on cetaceans in the ACCOBAMS area; Guidelines for the study of anthropogenic noise introduced into the sea and inland waters (ISPRA, part one and part two). These documents have allowed us to extract some of the information contained in this standard. One way to minimize sound impacts would be to minimize activities in the SOFAR channel (sound fixation and alignment) (typically at depths of ~1000 m). This recommendation is in line with the precautionary principle given the poor and varied understanding of the effects of noise on marine animals (Drazen et al., 2019). Despite this considerable amount of variables at stake, providing information on possible limits of exposure to noise is of great importance in order to direct all marine and maritime activities, towards greater environmental friendliness and eco-sustainability raising the awareness among all stakeholders to a greater protection of the marine environment also from the point of view of noise impact.

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