Mauro, M. (2020). Mining extraction in the ocean depths: a baseline to understand and reduce acoustic impact on biodiversity [Tesis doctoral]. Universitat Politècnica de València. https://doi.org/10.4995/Thesis/10251/144131

Tesis por compendio

UNIVERSITÀ
DEGLI STUDI
DI PALERMO

UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

Biodiversità Mediterranea – Internazionale
Scienze Agrarie, Alimentari e Forestali
BIO/06

Mining extraction in the ocean depths: a baseline to understand and reduce acoustic impact on biodiversity

PhD CANDIDATE	PhD COORDINATOR
MANUELA MAURO	**PROF.SSA MARIA ANTONIETTA GERMANA'**
SUPERVISOR	CO-SUPERVISOR
PROF.SSA MIRELLA VAZZANA	**PROF.SSA ISABEL PÉREZ ARJONA**
PROF. EDUARDO JORGE BELDA PÉREZ	**DOTT.SALVATORE MAZZOLA**

CICLO XXXII
2020

To my mom,
to her dream began reality

Abstract

Throughout history, man has exploited the earth's mineral resources for its survival and for technological development without regard for their regeneration. Given the growth of the world population and given the fall in resources, man started looking for new deposits, which were found in 1960s in the ocean depths. Humankind then began to consider extracting minerals from these deposits and this gave origin to Deep Sea Mining (DSM). The consequences of mining activities in the deep sea are not entirely known and the effects can be varied: noise pollution, light pollution, chemical pollution, habitat destruction, habitat fragmentation and the loss of species which we consider the basis of many life systems. The acoustic impact of these activities could have significant consequences on marine species; nevertheless, this has been the most overlooked issue to date. The aim of this PhD project was to provide baseline knowledge of possible acoustic impacts of DSM on biodiversity before mining begins. In order to do this, the PhD project was organised into 3 different stages. First, during an indoor experiment, the biochemical responses of invertebrates *Arbacia lixula* and *Mytilus galloprovincialis* subjected to acoustic stress were analysed. The results showed significant changes in cytotoxicity activity, expression of heat shock proteins (HSPs), and enzyme activities (esterases, alkaline phosphatases, peroxidases) in the coelomic fluid of sea urchins subjected to acoustic stress. Significant effects were also observed in glucose levels, cytotoxicity and enzyme activities (esterase, alkaline phosphatase, peroxidase) in the digestive gland of the mussel. Second, the biochemical responses of vertebrates and invertebrates subjected *in-situ* to watergun emission were analysed: *Chromis chromis*, *Holothuria tubulosa* and *Arbacia lixula*. Significant effects on fish cortisol levels and on enzyme activities in sea urchin peristomes were found. Furthermore, the enzyme biochemical responses analysed in the coelomic fluids of echinoderms showed significant effects only in *A. lixula* sea urchin and only in peroxidase activity. Third, behavioural changes in experimental conditions were studied in juveniles of *Sparus aurata* subjected to 4 different acoustic emission frequencies. This experiment showed that only low frequencies had effects on all the behavioural responses analysed: swimming height, motility and dispersion of the group. Based on behavioural data obtained *in vivo* on juvenile fish, a numerical model was created to predict the impacts of different acoustic emission frequencies. Using the results obtained and literature, a first technical standard useful for mining activities was drawn up.

Keywords: biochemical parameters; behavioural responses; Deep Sea Mining; noise pollution; physiological responses

Resumen

A lo largo de la historia, el hombre ha explotado los recursos minerales de la tierra para su supervivencia y desarrollo tecnológico sin un equilibrio con su regeneración. Dado el crecimiento de la población mundial y la reducción de recursos, el hombre comenzó a buscar nuevos depósitos que se encontraron en la década de 1960 en las profundidades de los océanos. Con estos, la humanidad empezò a pensar en extraer los minerales de estos depósitos y esto llevò al nacimiento de Deep Sea Mining (DSM). Las consecuencias de las actividades mineras en las profundidades del mar no se conocen realmente y los efectos pueden ser diferentes: contaminación acústica, contaminación lumínica, contaminación química, destrucción del hábitat, fragmentación del hábitat y pérdida de especies que son la base de muchos sistemas vitales. El impacto acústico de estas actividades puede tener importantes consecuencias en las especies marinas, aunque este es el tema más ignorado. El propósito de este proyecto de doctorado fue proporcionar una comprensión básica de los posibles impactos acústicos del DSM en la biodiversidad antes de que comiencen estas actividades. Para hacer esto, el proyecto de doctorado se organizó en varios pasos. Primero, durante un experimento indoor, se analizaron las respuestas bioquímicas en invertebrados sometidos a estrés acústico, *Arbacia lixula* y *Mytilus galloprovincialis*. Los resultados demostraron efectos significativos en la actividad de citotoxicidad, expresión de heat shock protein (HSPs) y actividades enzimáticas (esterasas, fosfatasas alcalinas, peroxidasas) en el líquido celomático de los erizos de mar sometidos a estrés acústico. También se observaron efectos significativos en el nivel de glucosa, la citotoxicidad y las actividades enzimáticas (esterasa, fosfatasa alcalina, peroxidasa) de la glándula digestiva del mejillón. En segundo lugar, se analizaron las respuestas

bioquímicas de vertebrados e invertebrados sometidos *in-situ* a la emisión de watergun: *Chromis chromis, Holothuria tubulosa* y *Arbacia lixula*. Se encontraron efectos significativos sobre los niveles de cortisol en peces y las actividades enzimáticas (esterasas, fosfatasas alcalinas, peroxidasas y superoxide dismutasas) en membrana peristomial de erizo de mar. Además, las respuestas bioquímicas enzimáticas analizadas en los fluidos celómicos de los equinodermos fue significativa solo por *A. lixula* y solo en la activade peroxidasica. Tercero se estudiaron los cambios de comportamiento en las condiciones experimentales en juveniles de *Sparus aurata* sometidos a 4 frecuencias de emisión acústica diferentes. Este experimento demostró que solo las bajas frecuencias tuvieron efectos en todas las respuestas comportamental: altura de natación, motilidad y dispersión del grupo. Sobre la base de los datos de comportamiento obtenidos *in vivo* en peces jóvenes, se creó un modelo numérico para predecir los impactos de diferentes frecuencias de emisión acústica. Utilizando los resultados obtenidos y la bibliografía científica, se propuso un primer estándar técnico que es útil para la minería.

Resumé

A lo llarc de l'historia, l'home ha explotat els recursos minerals de la terra per a la seua supervivencia i desenroll tecnologic sense un equilibri en la seua regeneracio. Donat el creiximent de la poblacio mundial i la reduccio de recursos, l'home escomençà a buscar nous deposits que se trobaren en la decada de 1960 en les fondaries dels oceans. En estos, l'humanitat empezò a pensar en extraure els minerals d'estos deposits i aço llevò al naiximent de Deep Sea Mining (DSM). Les conseqüencies de les activitats mineres en les fondaries del mar no se coneixen realment i els efectes poden ser diferents: contaminacio acustica, contaminacio lluminica, contaminacio quimica, destruccio de l'habitat, fragmentacio de l'habitat i perdua d'especies que son la base de molts sistemes vitals. L'impacte acustic d'estes activitats pot tindre importants conseqüencies en les especies marines, encara que este es el tema mes ignorat. El proposit d'este proyecte de doctorat fon proporcionar una comprensio basica dels possibles impactes acustics del DSM en la biodiversidad abans de que escomencen estes activitats. Per a fer aço, el proyecte de doctorat s'organisà en varis passos. Primer, durant un experiment indoor, s'analisaren les respostes bioquimiques en invertebrats somesos a estrés acustic, *Arbacia lixula* i *Mytilus galloprovincialis*. Els resultats demostraren efectes significatius en l'activitat de citotoxicidad, expressio d'heat shock protein (HSPS) i activitats enzimáticas (esterasa, fosfatasas alcalina, peroxidasas) en el liquit celomático dels capellanets de mar somesos a estrés acustic. Tambe s'observaren efectes significatius en el nivell de glucosa, la citotoxicidad i les activitats enzimáticas (esterasa, fosfatasa alcalina, peroxidasas) de la glandula digestiva de la clochina. En segon lloc, s'analisaren les respostes bioquimiques de vertebrats i invertebrats somesos *in-situ* a l'emissio de watergun: *Chromis chromis*, *Holothuria* *tubulosa* i *Arbacia lixula*. se trobaren efectes significatius sobre els nivells de cortisol en peixos i les activitats enzimáticas (esterasas, fosfatasas alcalines, peroxidasas y superoxide dimutases) en peristomes de capellanet de mar. Ademes, les respostes bioquimiques enzimáticas analisades en els decorreguts celómicos dels equinoderms fon significativa nomes per *A. lixula* i nomes en l'activade peroxidasica. Tercer s'estudiaren els canvis de comportament en les condicions experimentals en jovenils de *Sparus aurata* somesos a 4 freqüencies d'emissio acustica diferents. Este experiment demostrà que nomes les baixes freqüencies tingueren efectes en totes les respostes comportamental: alçada de natacio, motilidad i escampada del grup. Sobre la base de les senyes de comportament obtinguts *in vivo* en peixos jovens, se creó un model numeric per a predecir els impactes de diferents freqüencies d'emissio acustica. Utilisant els resultats obtinguts i la bibliografia cientifica, se propongue un primer estandart tecnic que es util per a la mineria.

Index

1. Introduction

1.1 General view about Deep Sea Mining (DSM)

The world population is growing and with it the demand for metals and minerals necessary for essential technologies for human life. In the last ten years, global consumption of these materials has increased together with market prices (United States, 2015). Mining deposits on earth represent less than a third of the earth's surface and their resources are running out (Petersen et al., 2016; Calvo et al., 2016). These factors, coupled with improvements in technologies, have led several countries and industries to explore the environment in deep waters in search of new mineral resources (Hein & Koschinsky, 2013). Scientists discovered the first manganese nodules during the HMS Challenger expedition (1872-76) and, with the publication of Mineral Resources of the Sea (Mero, 1977), the possibility of extracting minerals from the depths of the ocean began. The discovery of underwater hydrothermal vents at the Galapagos Rift in 1977 triggered a period of intense exploration of the seabed that continues today (Corliss et al., 1979). In 1977, Emery & Skinner (1977) analysed the first assessments of mineral deposits and, in the early 1990s, over 150 sites were discovered (Rona & Scott, 1993). The first explorations and feasibility studies date back to the 1980s in the East Pacific Rise and in the Red Sea (Crawford et al., 1984; Amann, 1985). The ocean floor has an enormous amount of minerals and the estimated value is $ 2 trillion (Lodge, 2015). The recovery of metallic resources from seabed mining has been identified as one of the five sectors with high development potential in the European Commission's Blue Growth Strategy (European Commission, 2017a), which estimates that by 2020, 5% of minerals of the world could come from the bottom of the ocean (potentially reaching 10% by 2030). The global annual turnover of the maritime mining sector will increase from almost nothing to ~ $ 10 billion by 2030 (CE, 2012; Ehlers, 2016). Mineral exctraction is likely to start in 2020 in the southwest of the Pacific Ocean (Baker & Beaudoin, 2013) and nothing is known about the real impacts on biodiversity.

1.2 Mineral deposits and their distribution

The deep mineral deposits are distributed over different geological sites: mid-ocean ridges (65%), along the volcanic arcs (12%) and back-arc spreading centers (22%) (Hannington et al., 2011) (Fig.1). There are potentially three types of mineral deposits: Seafloor Massive Sulfide (SMS), cobalt-rich ferromanganic crusts (Schulz & Zabel, 2006; Grigoriev et al., 2013; Zhamoida et al., 2017; Miller et al., 2018) and manganese nodules (Cronan, 1980; Rona, 2008; Peukert et al., 2018; Miller et al.,2018). Studies on their composition and structure began many years ago, and have continued over time (Krasnov et al., 1995; Boschen et al., 2013; Petersen et al., 2016; Kaikkon et al., 2018; Takaya et al., 2018).

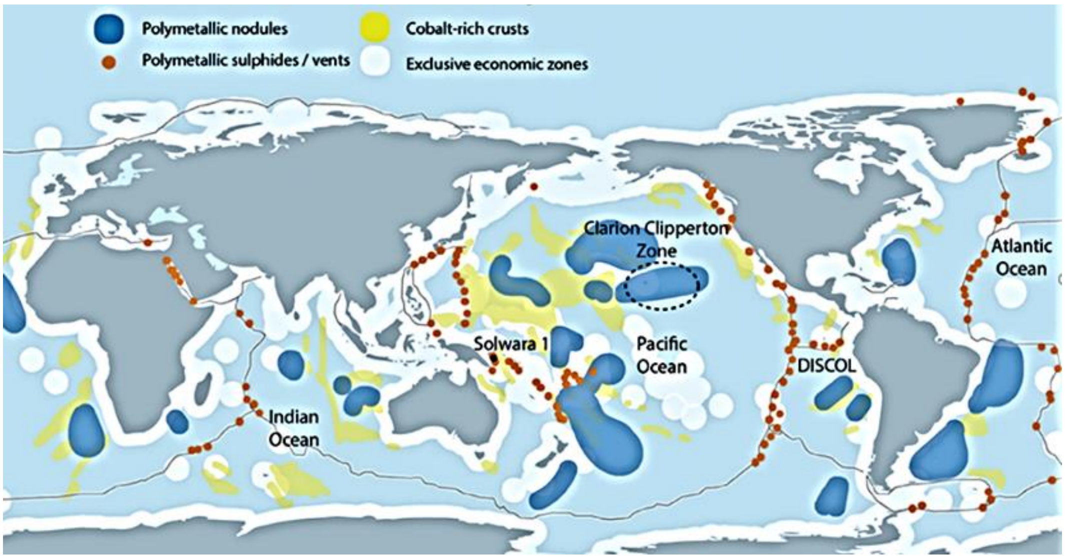


Fig.1 Taken by Miller et al., (2018). The geographical distribution of three main mineral deposits.

Seafloor Massive Sulfide (SMS) deposits are formed by hydrothermal fluids and are mainly concentrated in the Pacific, Atlantic, Arctic and Indian Oceans (Van Dover et al., 2002; Juliani et al., 2018) (Fig.2). The composition of these deposits depends on the depth, which is between 2-3 km and 1500 m (Hannington et al., 2005). They are formed by contact between the hot magma of active volcanic centers and the cold water of the oceanic depths (Yang et al., 1996; Hannington et al., 2005; Seewald et al., 2015). Are rich in different minerals, in particular, copper and zinc, but also gold and silver (Ahnert & Borowski, 2000; Baker & German, 2009; Ascension Holdings, 2016; Nautilus Minerals, 2016a; b). Their chemical composition is highly variable (Monecke et al., 2014; Jamieson et al., 2014); based on the site and geological process, their growth can differ by mms per year (Hannington et al., 2005; McCaig et al., 2007; Andersen et al., 2015) and they are formed over tens or hundreds of thousands of years (Fouquet et al., 2010; Cherkashev et al., 2013) (Fig. 2 Up). A quantitative assessment is only available for some of the Solwara sites of Papua New Guinea EEZ (Nautilus Minerals, 2008; 2011; Jankowski et al., 2012; Nautilus Minerals, 2017) and one of the most important deposits is located in the Atlantis II Deep of the Red Sea (Nawab, 1984; Hannington et al., 2010; 2011). Based on formation processes, these deposits are characterized by different areas which differ in the richness of metals (Humphris et al., 1995; Petersen et al., 2014). Given their history and geological dimensions, probably no more than ten deposits would be of sufficient metallic size and grade to support multi-year exploitation on a single mining license (Hannington et al., 2009). Information on the distribution and quantity of deposits is constantly increasing (Baker & German, 2004; Beaulieu, 2010; Hannington et al., 2011). The biological communities of these sites were described for the first time in 1977, however, to date, information is poor (Ramirez-Llodra et al., 2010). It is important to know more about these communities, given that there are endemic species and chemosynthetic bacteria that are the main organisms for the ecosystems of these deposits living in these sites and for life on Earth (Van Dover et al., 2002; Ramirez-Llodra et al., 2007; Martin et al., 2008; Thurber et al., 2011; Thatje et al., 2015; Rouse et al., 2016; Goffredi et al., 2017).

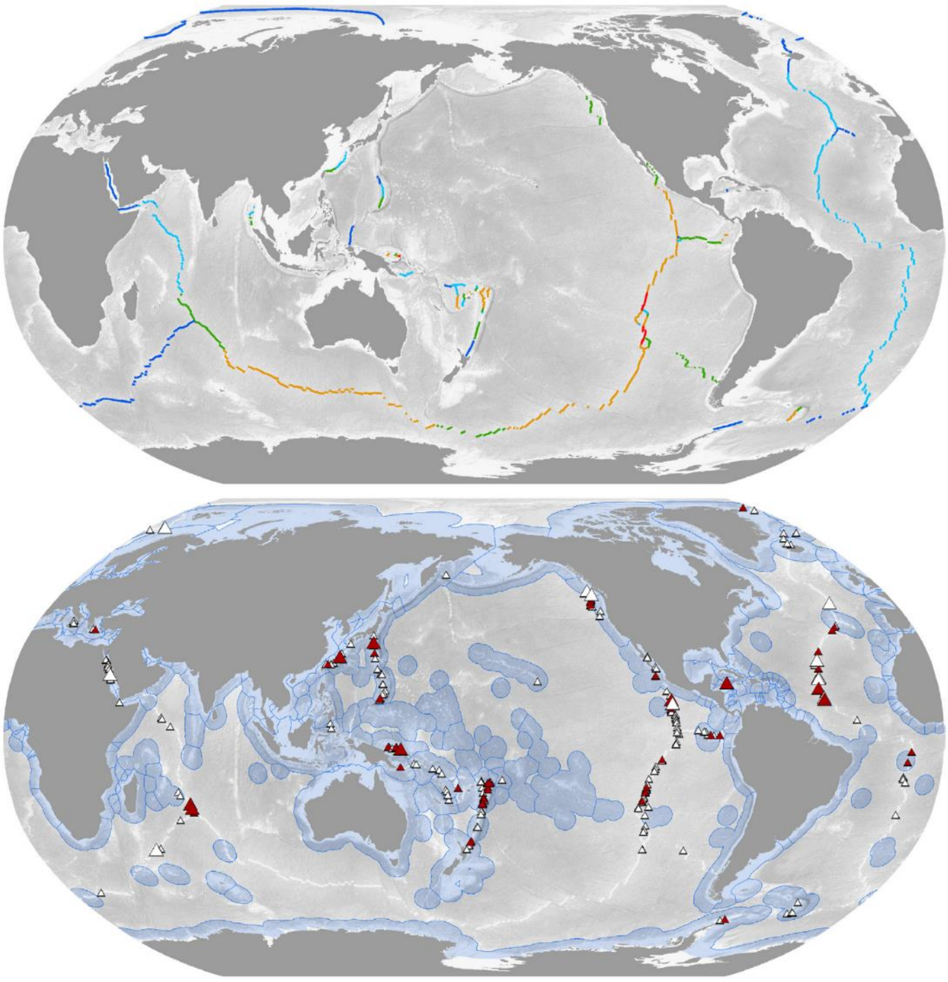


Fig. 2 Taken by Petersen et al., (2016). (Up) Geographical positions and growth rates of SMS. Dark blue <20 mm/yr; light blue between 20-40 mm / yr; green between 40-60 mm/yr; orange between 60-140 mm/yr; red> 140 mm/yr. (Down) The triangles in red indicate the presence of economically interesting concentrations of metal and those in white estimates of sizes greater than one million tons. With the blue color, exclusive economic zones are indicated.

The cobalt-rich ferromanganic crusts (Fig.3) are formed by the precipitation of minerals. Their thickness varies from less than 1 mm to approximately 260 mm and are formed at depths ranging from 400 to 7000 m (Hein et al., 2013). These deposits have a simple mineralogy and contain cobalt, nickel, traces of rare earth elements and mineral debris (Hein et al., 2013; 2014). The deposits of greatest economic interest are located at a depth of approximately 800-2500 m (Hein et al., 2013) and the central equatorial Pacific offers the best potential for extracting these deposits with the Prime Crust Zone (PCZ) in the western Pacific (Miller et al., 2018). These deposits can provide 20% of global cobalt demand (Ramirez-Llodra et al., 2010). Extraction from ferromanganese crusts was carried out for the first time in 2006-2008 in the eastern Baltic Sea (Zhamoida et al., 2017); information on this type of deposit, however, is not yet complete (Hein, 2008). The importance for the different communities of organisms was already studied in previous literature. The physics of the currents creates an oceanographic flood that transports nutrients to surface waters favouring corals, anemones, featherstars and sponges (Koslow et al., 2001; Yesson et al., 2011).The cobalt-rich ferromanganic crusts are oases of epibentic species, essential for migration and foraging of fish and marine mammals (Rowden et al., 2010; Yesson et al., 2011; Garrigue et al., 2015; Morato et al., 2016; Reisinger et al., 2015).

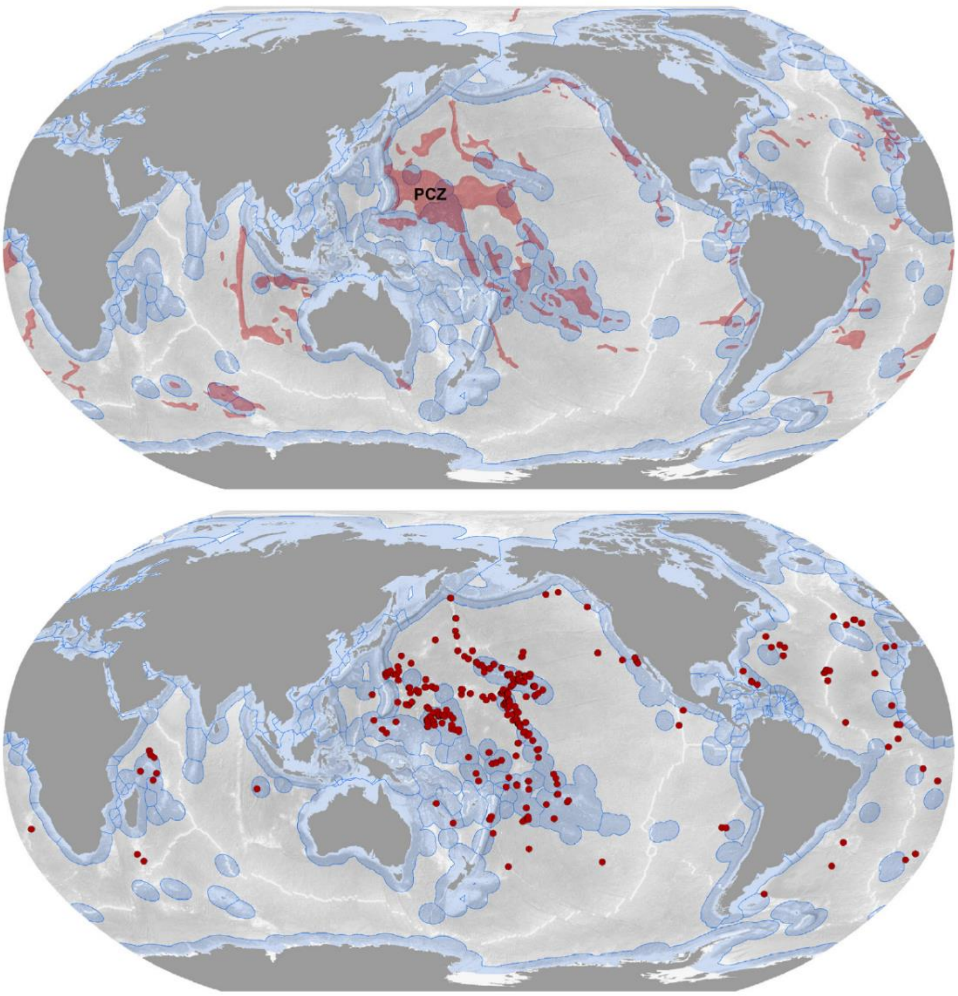


Fig. 3 Taken by Petersen et al., (2016). (Up) Area with highest ferromanganese crust potential and light blue areas indicate the EEZs. (Down) Ferromanganese crust with Co concentrations above 0.5 wt%.

Manganese nodules are composed of manganese, iron oxides, rare earth metals, platinum, tellurium, and other elements (Hein et al., 2013; Antoni et al., 2017; Ojo & Dharmadasa, 2017). The nodules vary between one and 12 cm in diameter and are found at a depth of approximately 3000-6000 m (Schulz & Zabel, 2006; Hein et al., 2013, 2014). They are distributed in the Peruvian basin and in the Indian, Atlantic and Pacific Oceans (Hein et al., 2014; 2015) (Fig.4). They are formed by sedimentation processes (20 mm per thousand years) (Hein et al., 2014; Blöthe et al., 2015; Vanreusel et al., 2016) of metals dissolved in sea water from the erosion of the continents (Bruland et al., 2014) or given by hydrothermal sources. The highest concentrations of metal-rich nodules occur in the Clarion-Clipperton Zone (CCZ) in the eastern Pacific Ocean (Halbach & Fellerer, 1980; ISA, 2010a; Hein et al., 2014; Wedding et al., 2015). Information for this type of deposit is not yet complete and the nodules are distributed unevenly (ISA, 2010a). Of great interest are also the deposits of the Cook Islands (Hein et al., 2015). In August 2015, the Cook Islands decided to make available 10 exploration blocks of 10,000 km^2. Few studies have analysed the fauna as these sites are not easy to reach; however, it is known that sponges and molluscs, nematode worms and crustacean larvae have been found inside fissures (Thiel et al., 1993). These deposits are key habitats for benthic communities (Kaiser et al., 2017) and more than half of the megafauna (Annelida, Arthropoda, Bryozoa, Chordata, Ctenophora, Mollusca) live around these nodules with hard substrates (Amon et al., 2016). These types of habitats, like those of other deposits, are included in the EU Habitats Directive (92/43/CEE). Although this is only valid for EU water deposits, it mentions sub-marine structures caused by gas emissions. The geological processes of deposit formation may be different and may also include this type of habitat.

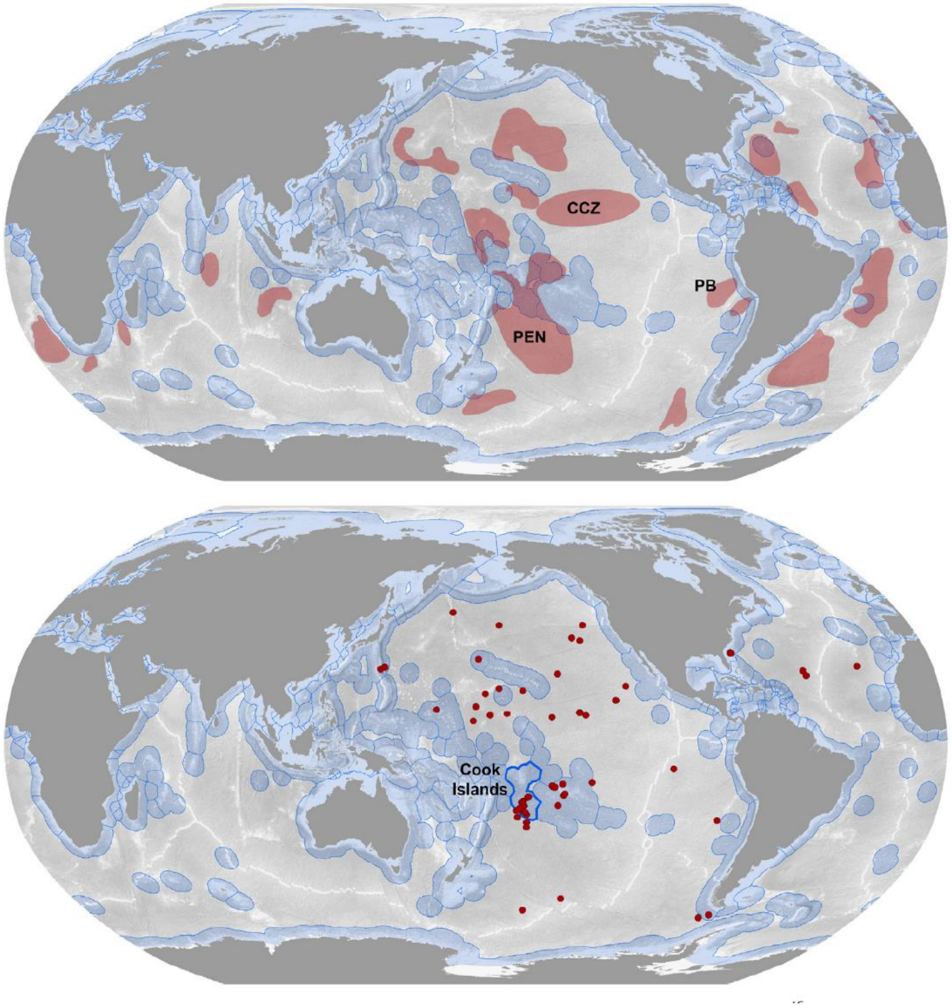


Fig. 4 Taken by Petersen et al., (2016). (Up) Areas with highest Mn-nodule potential and light blue areas indicate the EEZs. (Down) Manganese nodule with Co concentrations above 0.5 wt%.

1.3 General rules and guidelines about DSM

In view of the exploitation of deep mineral resources, it is necessary to analyse the laws and the guidelines that govern them to understand how and if the environment is adequately considered and protected. The United Nations Convention on the Law of the Sea (UNCLOS) is at the base of the management of these resources. It establishes effective protection against the effects of extraction of the seabed together with a legal obligation to avoid serious damage (Levin et al., 2016). According to UNCLOS, the only authority that can approve the exploitation and exploration of these sites is the International Seabed Authority (ISA) (Mengerink et al., 2014). The ISA is an autonomous intergovernmental body with 168 members and was founded in 1982 by UNCLOS. As far as the territorial division of deposits between different States is concerned, starting from the coast of each state, in legal terms, the seas have been divided into different areas. Up to a distance of 200-350 nautical miles from the coast, each country enjoys decision-making power over that area of water, the possibility of using it or exploiting it, and exercises jurisdiction over the area. This part is called the Exclusive Economic Zone (EEZ) (Mengerink et al., 2014). The extension of EEZs may vary for different countries. For example, if the territorial waters wide are 12 miles, the EEZ can have a maximum extension of 188 miles. Moreover, the EEZ must also be formally proclaimed towards the international community. Areas outside national jurisdiction are called "Area" (UNCLOS) and cover approximatively 54% of the oceans (Fig.5).

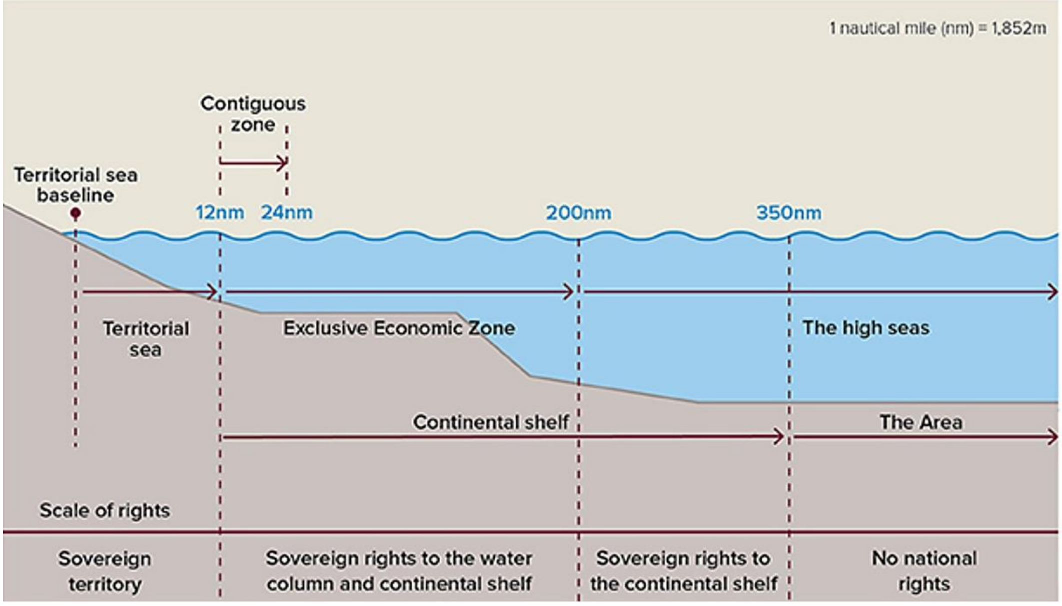


Fig. 5 Taken by Miller et al., (2018). A graphical representation of jurisdictional zones from a nation’s coast.

In this context, the ISA is responsible for mineral resources and the marine environment in the Area. In 1970, the United Nations General Assembly declared Resolution 25/2749 which specifies that the seabed and its resources were "common heritage of humanity" (CHM) and that the benefits obtained must be shared and conserved for present and future generations (UNGA, 1970; UNCLOS, 1982, Art. 136; Art. 157 (1), Art. 140 (1); Tiele et al., 2016; Jaeckel et al., 2017a). The "Area" (due to the fact that it "belongs to all and does not belong to anyone") legally becomes part of the CHM and cannot be subject to direct claims by sovereign States (UNCLOS Art. 136; UNCLOS Art. 137.2; Wolfrum et al., 2009). It is crucial to guarantee effective protection of the marine environment from the harmful effects of mineral extraction (UNCLOS Art. 145) and of the interests of future generations (Wolfrum, 2009). The ISA has the obligation to make the CHM principle operational and is a judicial officer for the law of the sea (Jaeckel et al., 2017a). In order to manage DSM activities, the ISA is creating a mining code

(Durden et al., 2018) and States that implement DSM in EEZs under their jurisdiction must guarantee effective national as well as international standards incorporating the approaches adopted by the ISA (Jaeckel et al., 2015). The mining code is essential since resource management requires the creation of a valid legislative framework to achieve important objectives, such as management, conservation, protection and greater knowledge of ecosystems and biological communities in these areas (Durden et al., 2018). However, too few biological and geological data are available for impact assessment and for the creation of a satisfactory mining code (Gjerde, 2006; Wright & Heyman, 2008). Rules and guidelines have been written over the years (Boschen et al., 2013; Van Dover et al., 2018) and the ISA issued a series of regulations to help manage the activities of those who have obtained exploration licenses (International Seabed Authoruty, 2010c; International Seabed Authority 2014; 2013a, b). These form the "Mining Code", which is still being developed by ISA Council experts on the Legal and Technical Commission (LTC). An essential requirement for ISA is the precautionary approach (ISA, 2010b), on which the various existing international standards are based (Wang et al., 2011; Lallier & Maes, 2016; Jaeckel et al., 2017; Kim et al., 2017). Although the mining code is not yet published, the ISA has already established what needs to be done before commencing activities, in order to consider possible impacts on the environment (Durden et al., 2018). The regulations for the exploitation of deposits were discussed during the 23rd session of the ISA, highlighting the need to consider the presence of vulnerable marine ecosystems (VME), biologically significant marine areas (EBSA) and how the ISA would justify biodiversity loss (ENB, 2017). In recent years, a number of projects have been published regarding management of the contractual conditions for the exploitation of mineral resources (www.isa.org); regional legislative and regulatory frameworks have been produced (http://dsm.gsd.spc.Int/ public/files/2014/RLRF2014.pdf) and currently others are under development (for example, the MIN-Guide, European Union initiative: http://www.min-guide.eu/mineral-policy). Despite these developments, work is not yet complete and all the details on how the ISA will manage environmental aspects during exploitation have not been finalized. There are few published environmental data and too many unanswered questions (Miller et al., 2018). In a reductive way, to manage these activities, we often refer to the different conventions (Boschen et al., 2013) and environmental codes (Devey et al., 2007; International Marine Minerals Society, 2011), which aim to implement the 'precautionary principle' using an ecosystem approach and with the possible creation of networks of protected marine areas (Boschen et al., 2013).

In addition to these approaches, a number of laws have been developed in different areas of exploration and exploitation: The mining industry Act (1992), Environmental Act (2000), Crown Minerals Act 1991 and Exclusive Economic Zone and Continental Shelf (Environmental Effects) Act (2012) (Boschen et al., 2013). Together with the precautionary approach, environmental impact assessment (EIA) plays an essential role in managing DSM activities (Treweek, 2009; ISA 2013; 2014; Ma et al., 2016; Pérez, 2017) to ensure effective protection of the marine environment, as required by UNCLOS (Art. XI part XI 145, United Nation 1982). The Department of Environment and Conservation (http://www.dec.gov.pg/legislation.html) requires an EIA before the start of activities, together with management plans, basic studies and continuous monitoring of the areas. However, to date, only regulations in the Area specify the use of EIA and in a somewhat inefficient manner, due to the numerous scientific shortcomings (Clark et al., 2019). There are a number of EIA proposals (Holling, 1978; Jaeckel, 2017b; Durden et al., 2017; 2018) but no real impact mitigation techniques (Van Dover, 2011b; 2014; Van Dover et al., 2017). Some countries have established marine protected areas (Van Dover, 2014), and in 2015, a proposal was made to the ISA to suspend approval of new exploration and exploitation contracts until marine protected areas are fully designed and their advantages are known (Wedding et al., 2015). Despite the fact that, in recent years, the potential of biological and mineral resources in the various deposits (International Marine Minerals Society, 2011) has been amply considered, advanced knowledge, research and long-term monitoring are required before authorizing any extraction activity (Jones et al., 2019). To reduce impacts, specific environmental management, EIA, monitoring, mitigation and environmental management planning are essential (Ekstrom et al., 2013; Jones et al., 2019). The current ISA mining code is applicable only to prospecting and exploration, and not to exploitation (Seabed International Authority, 2012; 2013; Vanreusel et al., 2016). The International Marine Mining Society (IMMS) has created a voluntary code for environmental management of marine extractions (International Marine Minerals Society, Code for Environmental Management of Marine Mining, 2011) and ISA has encouraged its contractors to use it (ISA, 2011, Section VII B, page 12; ISBA/17/LTC/7;ISBA/16/LTC/2). Only Germany and the United Kingdom have adopted DSM legislation with the Seabed Mining Act of 1995 and the Deep-Sea Mining Act 2014. The United States, however, is not part of UNCLOS and has a specific jurisdiction: Outer Continental Shelf Lands Act, 1953 (OCSLA) (American Law Institute, Vol.2). To date, the ISA is pushing to complete definition of its requirements and the ISA council met in 2019 in Kingston, Jamaica, to work on a draft of the still evolving mining code (Heffernan, 2019). It is evident that clarification is still needed on standards and guidelines; however, what is also evident is that current laws and regulations exists mainly for political, social and economic needs. For environmental problems, we only find references to keywords such as “prevention”, “EIA”, “SEA”, “precaution” or to various agreements with incomplete or inadequate regulations.

1.4 Exploration contracts

Mining companies and national governments have contracts to explore the seabed (ISA, 2014; UNEP, 2014), but few or none with exploitation authorization. From 2002 to 2016, the ISA granted approximately 27 licenses (29 to date, Nature, 2019) (Fig. 6), which cover an area of over 1.4 million km^2 of the Pacific, the Atlantic and the Indian Ocean, including the “Areas” (ISA, 2013a,c; UNEP, 2014; Earthworks, 2015; Petersen et al., 2016). These licenses are valid for 15 years, some renewed for another five years. It is not easy to establish the amount of exploration in the EEZ as the contracts are not made public. Some explorations within the EEZ concern the western Pacific (SPC, 2013; Masuda et al., 2014; Fouquet et al., 2014). Miller et al. (2018) report the list of contracts approved by the ISA in June 2017 (with the start and end dates) to explore mineral deposits and the list of some seabed mining operations implemented. The precious and substantial resources have attracted the international mining industry and several countries, including China, Korea, Russia, France, Germany (Hannington et al., 2011) and Japan, which has become the first nation to extract a hydrothermal vent in deep water (Japan Times, 2017). Following huge investments in research and development, a number of companies are trying to overcome the challenges of deep-water extraction by developing a series of technologies (Ma et al., 2017a, b; Schoening et al., 2017). The most important mining companies are Nautilus Minerals, Diamond Fields International and Neptune Minerals. Nautilus Minerals and Diamond Fields International (the Atlantis II project, Diamond Fields International, 2016) are the two companies that today have permission to exploit the sea. Nautilus Minerals won the first license in the world (20 years released in January 2011) and is the owner of the "Solwara Project 1" (Ellefmo et al., 2017) in Papua New Guinea, where the first mining activities should begin. Similar projects were carried out from 2008 to 2010 at the AMOR Arctic Mid-Ocean ridge. However, neither of them had started commercial operations until now (Miller et al., 2018; Jones et al., 2019), despite the fact that different extractive activities in shallow waters have already started. Interest is concentrated in the Papua New Guinea EEZ (with contracts signed in 1997 and 2011 http: //www.nautilusminerals.com/) and in New Zealand, where deposits were evaluated approximately 20 years ago and where most of the resources available in the world's oceans are concenrated (Glasby & Wright, 1990; Gleason, 2008; Sharma, 2011). Several areas have been explored (http://www.nzpam.govt.nz/cms/online-services/current-permits/) and others are preparing for exploitation. In 2019, a start-up called DeepGreen began in Vancouver, Canada, which is raising $150 million to start exploring part of the Pacific Ocean, and aims to open a deep mine by 2027. A prototype harvester nodule is due to be tested in the near future in shallow Mediterranean waters (Heffernan, 2019); Nautilus Minerals, in fact, has shown interest in potential deposits in the Tyrrhenian Sea. In way of contrast, in June 2017, 35 organizations participated in the United Nations Conference on the Ocean in an attempt to interrupt these activities; due to the fact that, as mentioned in 2016 during the "Sydney Sustainable Futures Institute", there are other ways for the recovery of minerals. Metals can be replaced by other more abundant minerals with similar properties (United States Department; Energy, 2010; Department of Environment, Food and Rural Affairs, 2012; United Nations Environment Program, 2013b), or we can turn to collecting, recycling, recovering rare metals from sea water (Hoshino, 2015) or exploiting landfill mines (Wagner & Raymond, 2015). The European Commission supports the transition to a circular economy that promotes recycling and re-use of materials (European Commission, 2017b), although the strategy may not meet current consumption-based needs. Metal recycling involves the potential release of toxic substances and not all components are recovered (United Nations Environment Program, 2013a). All metals are recyclable, but a satisfactory system has not yet been developed (Reck & Graedel, 2012).

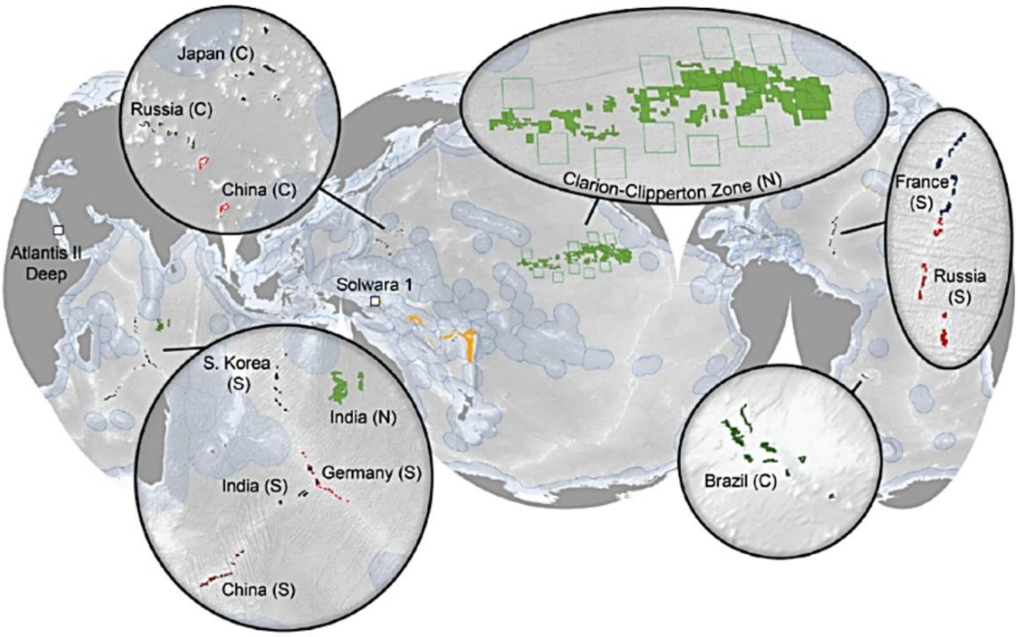


Fig. 6 Taken by Petersen et al., (2016). Graphic representation of the distribution of exploration licenses. Areas of particular environmental interest are indicated in the CCZ by squares with a green outline. Locations of mining licenses of Atlantis II Deep in the Red Sea and Solwara 1 in Papua New Guinea are indicated by white squares.

1.5 Economic, political and environmental aspects of Deep Sea Mining

Various authors have reviewed the impacts of Deep Sea Mining on a legal, economic and social level (Armstrong et al., 2012; Wakefield et al., 2016; Koschinsky et al., 2018; Folkersen et al., 2018a; b). Technological, political and economic problems have slowed down the development of this activity and the problems related to DSM are neither few nor simple: technologies are difficult to implement, costs are unsustainable and environmental problems are at once important and unknown (Mengerink et al., 2014). Information on the technologies that will be used is poor (Thiel et al., 2001; Volkmann & Lehnen, 2017; Jones et al., 2017; Miller et al., 2018; Peukert et al., 2018; Kaikkonen et al., 2018; Teague et al., 2018) and extraction processes will probably differ depending on the site of interest (Fig. 7). To date, situations are constantly changing and evolving. Any technological problems and costs of extraction/exploration (Hein et al., 2013; Beaulieu et al., 2017) are offset by high demand and prices for potentially extractable metals, and new technological developments are emerging continuously for this new sector of the mining industry (Deep Sea Mining Summit, 2016; Vidal et al., 2017). As a result, DSM has become economically viable. From an economic and political point of view, the ISA must ensure that activities promote the development of the world economy together with balanced growth in trade and the promotion of international cooperation that encourages the development of developing states (UNCLOS, Art. 150). However, to do this, a better understanding of the basic conditions, types of disturbance and environmental impact is needed (ISA, 2011b; Collins et al., 2013).

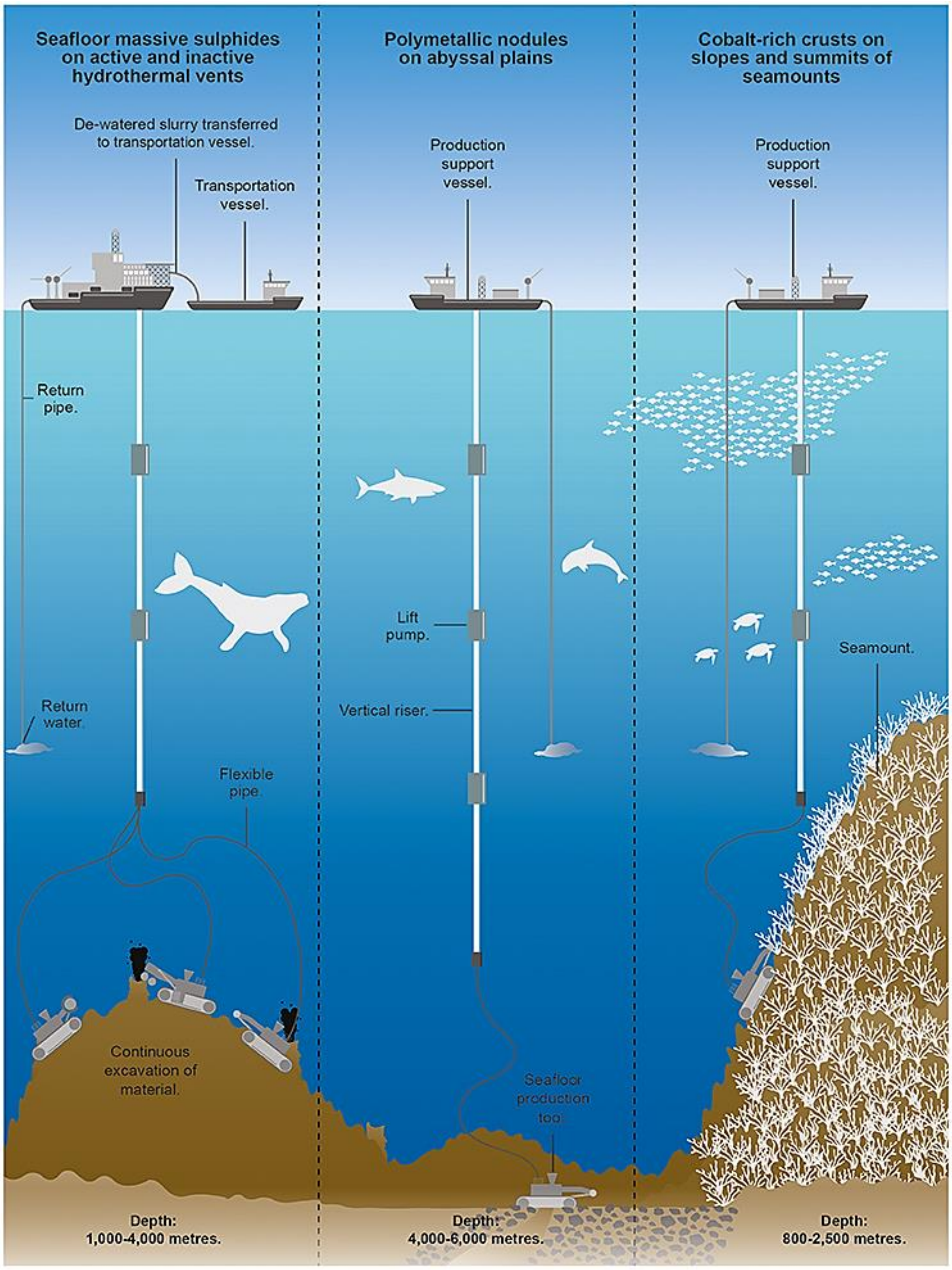


Fig. 7 Taken by Miller et al., (2018). The processes involved in deep-sea mining for the three main types of mineral deposit.

In addition to the economic and political aspects of DSM activities, it is necessary to consider the possible environmental impacts. The depths of the sea represent 95% of the global biosphere (Thistle, 2003; Smith et al., 2009; Danovaro et al., 2014) and, given the nature, extent and location of seabed mining activities, the negative effects on biodiversity will be inevitable and probably irreversible (Van Dover et al., 2017). There are many concerns about mining activities for which the biota disorder has not yet been described (Miller et al., 2018). These deep deposits contain the systems that regulate life on earth and are a probable energy base for most of life (Corliss et al., 1981; Galkin, 1997; Van Dover et al., 2000; Moalic et al., 2012; Collins et al., 2012; Boschen et al., 2015). Due to the depths involved, since it is very difficult to reach the deposits, the biology on the seabed is not well known. The deposits are home to vulnerable species which reproduce slowly and live in unhurriedly changing environments (Miller et al., 2018). Various different studies have highlighted the risks derived from DSM activities (Fig. 8) and most scientific research describes the sites and possible impacts, such as fragmentation, habitat loss due to the action of mechanical removal, biodiversity loss (Ellis, 2001; Boschen et al., 2013; Van Dover, 2014; Vanreusel et al., 2016; Jones et al., 2017) and the possible formation and distribution of plumes, which will influence the structure of ecosystems and are probably toxic to living organisms (Amos & Roels, 1977; Rolinski et al., 2001; Oebius et al., 2001; Thiel & Tiefsee- Umweltschutz, 2001; Glover & Smith, 2003; Gwyther, 2008; Ramirez-Llodra et al., 2011; Petersen et al., 2016; Rakhyun, 2017; Phillips et al., 2017; Ma et al., 2018; Kaikkonen et al., 2018; Lindh et al., 2018; Peacock & Alford, 2018; Gillard et al., 2019; Monserrat et al., 2019; Drazen et al., 2019; Lopes et al., 2019; Rzeznik et al., 2019).

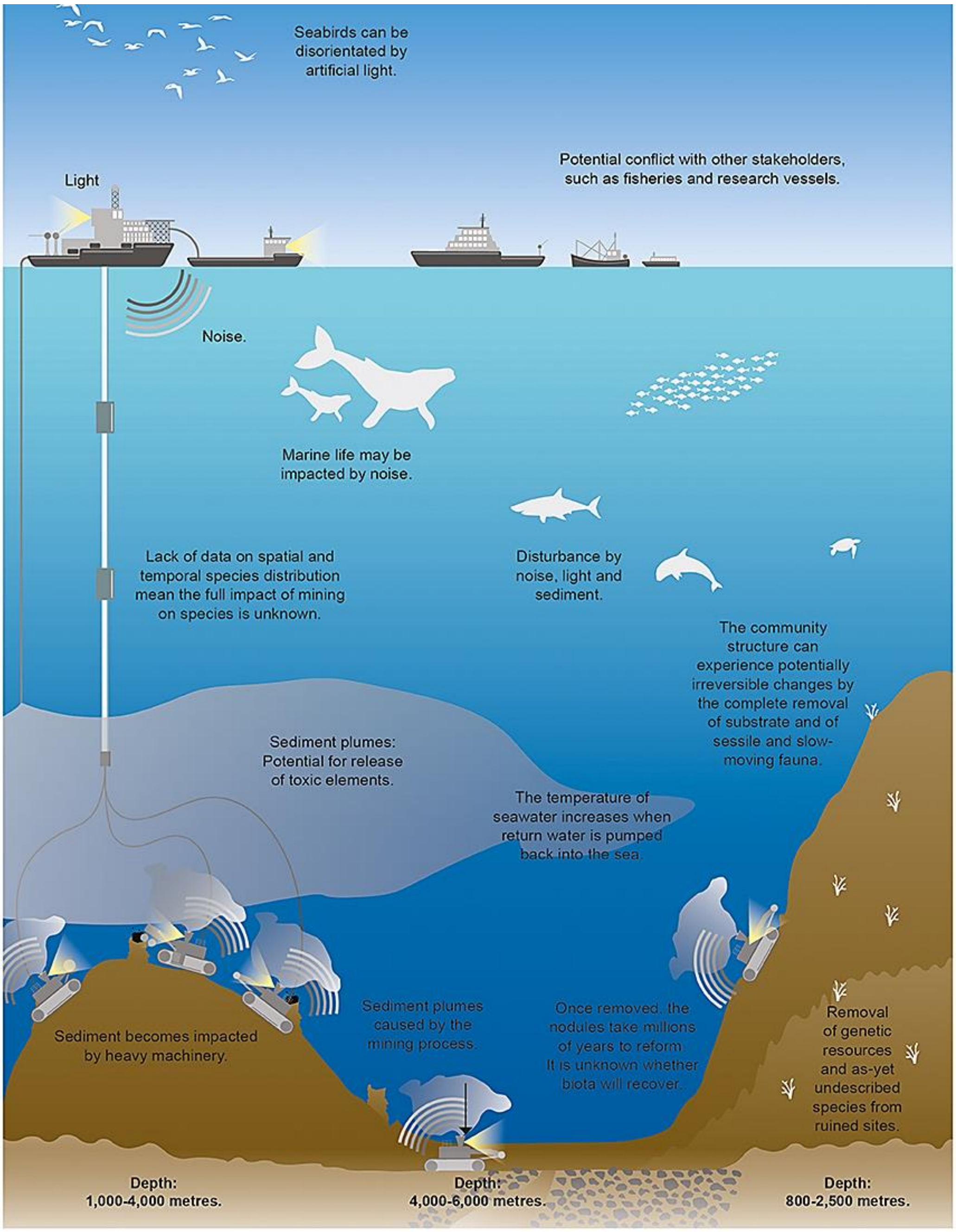


Fig. 8 Taken by Miller et al., (2018). Potential impacts of deep-sea mining on marine ecosystems.

The ecology and biodiversity of these habitats are poorly understood and, therefore, it is difficult to fully understand the impacts (Veillette et al., 2007; Vanreusel et al., 2016). Some authors propose evaluation of the genetics of the species (to understand connectivity, larval transport and genetic exchange), and monitoring of the impacts due to extraction operations (Boschen et al., 2016; Taboada et al., 2017). This would allow impact mitigation by creating biological rest areas similar to the disturbed sites, which could then recover (Coffey Natural Systems, 2008; Smith et al., 2008b; ISA, 2010c; Collins et al., 2012; Boschen et al., 2016). Impacts could be contained thanks to conservation models (Van Dover et al., 2010), using an approach which includes ecosystem services for better management of deep resources (Le et al., 2017) and creates models to evaluate effects on the population (Nabe-Nielsen et al., 2018). Despite the lack of information to date, many studies try to provide significant and useful large-scale guiding principles for EIA (Faulkner et al., 2018). However, different types of extraction will have different types of impact on the marine environment (De Groot, 1979; Newell et al., 1998; 2004; Desprez, 2000; Cooper et al., 2007a, b; Makogon et al., 2007; Waye-Barker et al., 2015; blue Noduls, 2016; Chong et al., 2016; Jones et al., 2017; Miller et al., 2018; Kaikkonen et al., 2018) and it is not possible to have a non net loss (NNL) of biodiversity during these activities due to the vulnerability of ecosystems and poor knowledge of technologies (Niner et al., 2018). Given that mining activities destroy habitats, it is essential to know the abundance and structure of the community and, for this reason, several authors have characterized the megafauna of some sites highlighting their importance in biomonitoring; according to these studies, endemics are the organisms most at risk (Lambshead et al., 2003; Boschen et al., 2016; Amon et al., 2017; Leitner et al., 2017; Kersten et al., 2017). Furthermore, the communities that inhabit these sites are very different in terms of abundance, composition and diversity; there is, therefore, urgent need to understand spatial and temporal variability (Pape et al., 2017) in order to contain impact.

However, limited sampling due to sea depth complicates the study of effects on deep species. A valid alternative would be to study species that are easily found which share genetic links to deep species. In recent years, it has been demonstrated that, after years of extraction activities, biodiversity and the structure of the sites do not recover (Jones et al., 2017). As the hard substrate is a key factor in the structuring of these habitats (Buhl-Mortensen et al., 2010; Bell et al., 2016), every change can also influence larval settlement processes (Van Dover et al., 1988; Roberts et al 2006), exercising a certain control on life in deep waters (Levin et al., 2001; Smith & Demopoulos 2003; Phillips et al., 2017; Simon-Lledó et al., 2019), modifying the communities of these sites for geological periods (Gollner et al., 2017). Disturbance could extend over extremely large seabeds (Aleynik et al., 2017), creating a change in the resident fauna (Jones et al., 2017). To date, the real extent of the impact is not yet known and the recommendations provided by the ISA in 2018 are not adequate, as the consequences of many impacts are not taken into consideration (Christiansen et al., 2019). Lethal impacts are defined as those capable of damaging essential processes such as nutrition, growth and reproduction, with loss of biodiversity as a possible consequence. To promote research and development, the EU has funded projects such as: MIDAS (2013-2016) (MIDAS recommendations, 2016; MIDAS Research Highlights, 2016; Managing Impacts of Deep Sea Exploitation Resource Exploitation; MIDAS, 2016), Blue Mining (2014-2018), Blue Nodules (2016-2020) (Volkmann & Lehnen, 2017), DISCOL (German project) and OMCO (1978). The results of these projects have confirmed that even after years, traces left by vehicles on the substrate were still visible (2004) and reduced biodiversity (Miljutin et al., 2011; Vanreusel et al., 2016; Heffernan, 2019).

Although the different impacts of DSM activities on biodiversity have been investigated, problems related to the acoustic impact have not been studied. Several scientific papers analyse the effects of acoustic impact from other marine-maritime activities on biodiversity but not from DSM, as the frequencies and acoustic intensities emitted are not known. However, this fact cannot and must not limit intervention before it is too late.

1.6 Noise pollution: what about it?

A sound is the result of a mechanical propagation of acoustic waves (Wartzok et al., 1999) while a noise is an unwanted "sound". Nautilus Minerals will carry out extractions on the Solwara1 site 24 hours a day/ 365 days a year for 30 months (Nautilus Minerals, 2008; 2016b), and for this reason it is important to gain knowledge immediately on the possible acoustic impact of these activities. The different mineral extraction techniques will increase environmental noise. Moreover, the work done by Nautilus Minerals will further increase this (McKenna et al., 2012). Despite this, noise characteristics of the equipment are not known and, in their environmental impact statement (EIS), Nautilus Minerals did not measure environmental noise or assess sound attenuation (Miller et al., 2018); no mitigation strategies were suggested either. It appears that noise produced by dredging vessels is of low frequency (<1 kHz) similar to a noisy merchant ship (Thomsen et al., 2009; Robinson et al., 2011). In the aquatic world, sound propagation differs from the terrestrial world, in water is attenuated to a lesser extent than in the air (Wartzok et al., 1999), traveling at a faster speed (Urick, 1983) and for much greater distances (Williams et al., 2015). Low frequency sounds have long wavelengths and suffer less absorption than higher frequencies. They can travel for miles and can change depending on environmental conditions (Rogers & Cox, 1988; McCormick et al., 2018a). Anthropic noise in water is refracted or reflected (Fig. 9) and these properties can influence the propagation of submarine sound.

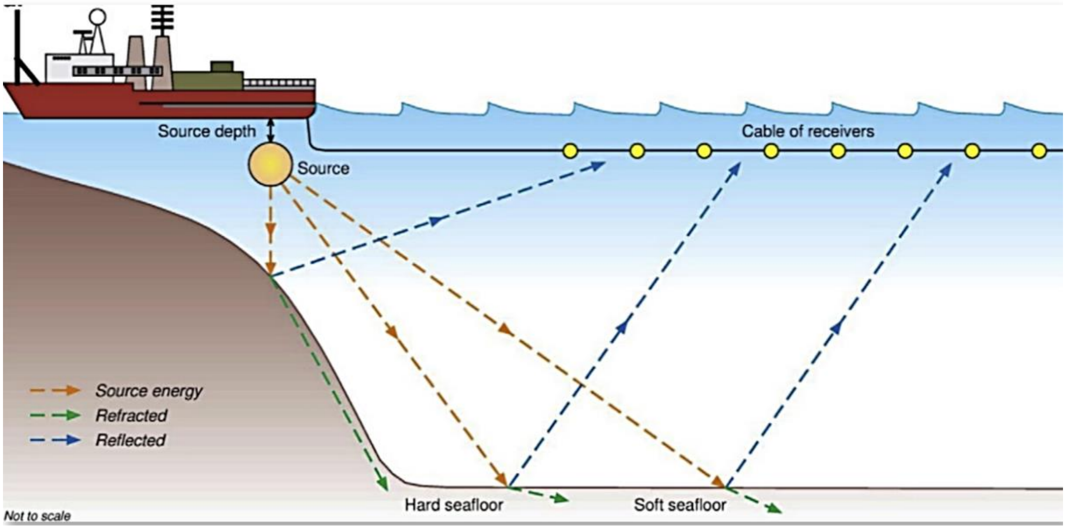


Fig. 9 Taken by Carroll et al., (2017). Representation of physical characteristics regarding sound propagation through the water column and seabed.

For all these reasons, noise pollution in aquatic environments could implicate much more extensive areas than terrestrial environments and the possible impacts on biodiversity depend on many factors that must be considered. Marine organisms live in this acoustically complex world, the result of a mixture of biotic and abiotic sounds (De Jong et al., 2011). Many marine species contribute to the soundscape with their vocalizations and use aquatic noise for auditory information, habitat selection, predator/prey interaction and communication. All animals evaluate the environment by analyzing the soundscape or the "acoustic scene" (Popper & Fay, 1997; Fay, 2009). Anthropogenic sound then propagates in the water and overlaps with the sounds of biological importance produced by animals for their vital functions and, for this reason, it becomes a real threat to life in deep ecosystems (Hastings & Popper, 2005; Slabbekoorn et al., 2010). In aquatic ecosystems, fish are key components and impacts on this group are of global interest (FAO, 2016). The sensitivity of organisms living in water, their hearing ability (Smith et al., 2004a; Davidson et al., 2009; Voellmy et al., 2014), sound propagation, frequency and duration of sound (Slabbekoorn et al., 2010; Popper & Hawkins, 2012), noise level, duration and spectral characteristics of the noise (Erbe, 2012b), all contribute to modifying acoustic stress in animals (Fig. 10). Effects on marine species depend on whether the frequencies of the sound disturbance exceed the auditory frequencies of the organisms themselves or not (Popper & Hastings, 2009a).

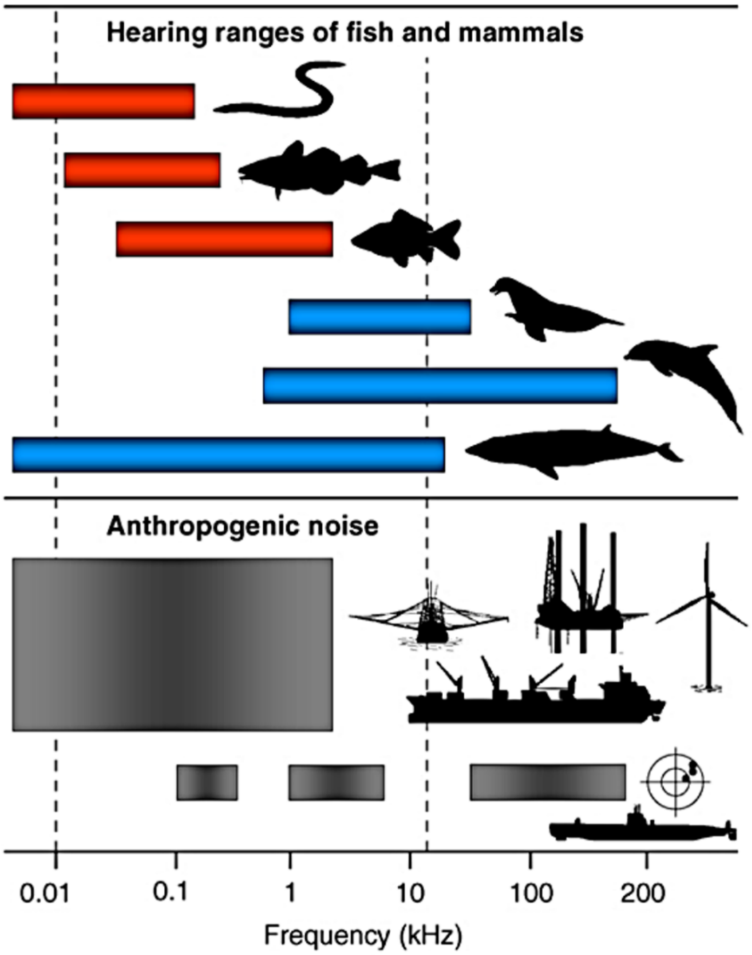


Fig. 10 Taken by Slabbekoorn et al., (2010). Representation of the auditory intervals of some fish and mammals, from top to bottom: European eel, Atlantic cod, goldfish, Californian sea lion, bottlenose dolphin and fin whale (Southall, et al., 2007; Fay & Popper, 2000). The human auditory area is indicated by the vertical dotted lines. In constast, noise ranges of human activities are represented in the lower part of the figure.

A number of authors have analysed the effects of different sources of noise pollution (Popper et al., 2009b); however, studies were poor in previous years, especially regarding commercially important species such as fish and invertebrates (Gordon et al., 2003; Williams et al., 2015; Hawkins & Popper, 2017), and many studies concerned marine mammals (Hatch et al., 2008; Brandt et al., 2011; Erbe et al., 2012; Melcón et al., 2012; Tsujii et al., 2018). Cetaceans have long been considered engineers of the marine ecosystem and are very sensitive to noise pollution in their habitats (Bossart, 2011; Rolland et al., 2012,2014; Williams et al., 2013; Gordon et al., 2018). It has been shown, for example in whales, that the nearer the acoustic emission source (e.g a ship), the more communication capacity is reduced and increases in anthropogenic noise have caused a loss of the communication interval (Slabbekoorn et al., 2010). Underwater noise overlaps with the auditory sensitivity of many fish species and can lead to the masking of their auditory signals (Pollack, 1975; Brungart, 2001; McDonald et al., 2006; Myrberg & Lugli, 2006; Popper & Fay, 2011; Normandeau Inc., 2012). Noise pollution influences the use of sound in marine organisms (Popper & Hastings, 2009a). In recent years, various research programs have studied the impacts of noise on aquatic life, which can cause effects both at the individual organism level and at an ecological level (Erbe, 2012b). For example, some ecological services performed by invertebrates, such as water filtration, are negatively affected. Several reviews talk about noise in aquatic environments produced by different human activities and its effects on marine organisms (Gordon et al., 2003; Popper & Hastings, 2009a; Slabbekoorn et al., 2010; Kight et al., 2011; Radford et al., 2014; Morley et al., 2014; Peng et al., 2015; Kunc et al., 2016; Edmonds et al., 2016; Erbe et al., 2016;

Shannon et al., 2016; Carroll et al., 2017; Weilgart et al., 2018; Kuşku et al., 2018). However, there are still many gaps in knowledge and more information regarding the response of species to noise emissions, is needed (Parsons et al., 2009; Prideaux & Prideaux, 2016). There are several suggestions to mitigate the effects of anthropogenic noise (Underwood, 1992; Andrè et al., 2011a; Boyd et al., 2011; Hawkins et al., 2015) and, in the case of DSM activities for example, impact could be mitigated using drilling or by reducing work times, thereby allowing species to rest between sessions (Broudic et al., 2014; Spiga et al., 2017) . However, all this depends on acoustic conditions, exposure to the sound, sound pressure, particle movement and the sound source. It would be more useful to develop field studies, but these are made particularly complicated by the absence of controls (Slabbekoorn, 2016). This would make any data obtained of difficult and dubious interpretation (La Bella et al., 1996; Christian et al., 2003; Andriguetto-Filho et al., 2005). Although papers on the acoustic impact of human activities are increasing in number, no one has examined the possible acoustic impacts of DSM. To date, there are no studies on the influence of sound frequencies produced by mining on organisms (acoustic emissions are not known and a great deal of information is still missing). However, knowing the effects of the noise produced by different types of anthropogenic activities is certainly a good starting point. Anthropogenic noise can disturb behavior and generate physiological effects on individuals with negative consequences on the population as showed in Fig. 11 (Sun, et al., 2001; Slabbekoorn et al., 2010; Buscaino et al., 2010; Wale et al., 2013; Voellmy et al., 2014; Radford et al., 2014; Popper et al., 2014; Kunc et al., 2016; Magnhagen et al., 2017; Hawkins and Popper, 2017; Weilgart, 2018).

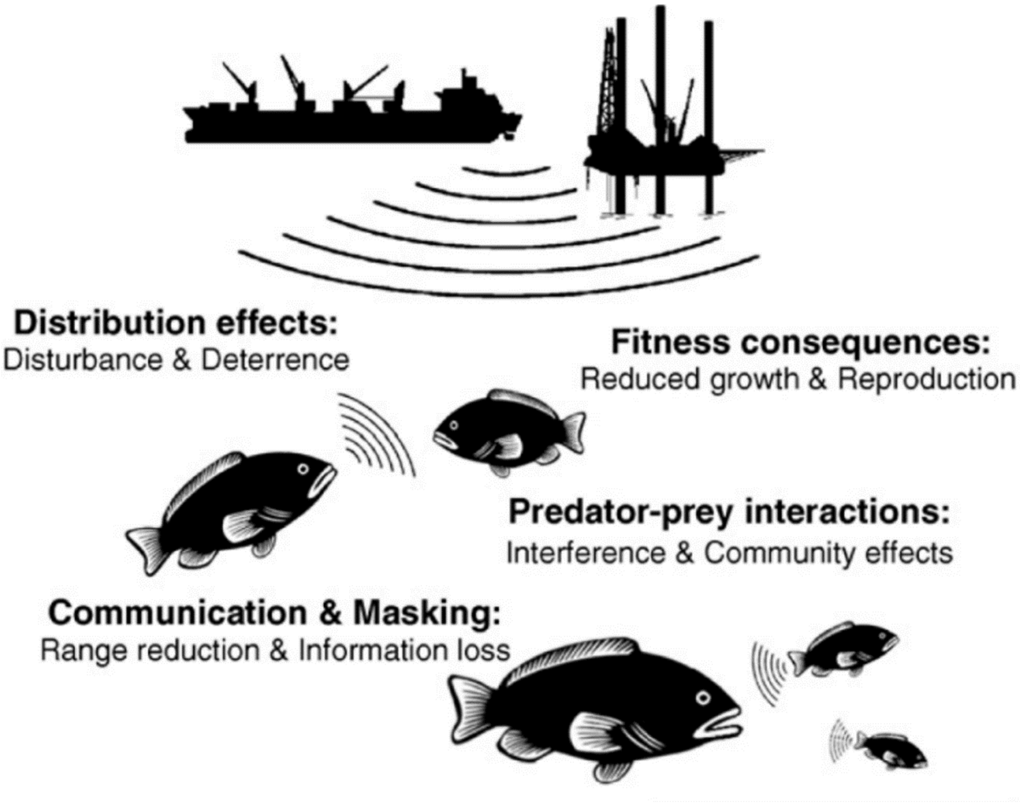


Fig. 11 Taken by Slabbekoorn et al., (2010). A representation of possible effect of noise pollution on marine organisms

Noise pollution can cause stress in marine organisms, which react by trying to restore homeostasis through three main mechanisms, with primary, secondary and tertiary responses. The primary response concerns the sympathetic nervous system, the hypothalamic-pituitary-interrenal axis, catecholamines and the release of glucocorticoids (Barton, 2002; Schulte, 2014). The secondary response concerns physiological metabolism (hematological and immune characteristics and changes in respiratory rate) (Pickering, 1981; Rotllant & Tort, 1997; Iwama, 1998; Simontacchi et al., 2008). The tertiary response comes into play if the former fails to establish homeostasis, and relates for example to growth, behaviour, reproduction and survival (Wedemeyer et al., 1990; Pavlidis et al., 2011).

Behavioural changes have very close links with physiological changes, in fact, changes in the movements or in the structure of group cohesion leads to changes in metabolic rates, stress, immune response, reproduction and predation (Hawkins et al., 2015). Physical and physiological responses are quite similar within a species and are preserved. On the other hand, behavioural responses are more complex (Aguilar de Soto & Kight, 2016). Many marine species live in particularly noisy environments; however, this is not an adequate reason to think that they do so because they are fine. It is most likely that they live in these places for more important reasons, linked to the vital function that the site provides, in their lives. Further below is a detailed description of scientific research that studied the effects of anthropogenic noise at different levels on marine organisms.

1.6.1 Physical and physiological effects of noise pollution

Noise can cause physical and physiological stress on marine organisms, triggering, in particular, (Popper et al., 2003a; Weilgart et al., 2018; Cox et al., 2018) changes in metabolism and immune responses, hormonal changes, changes in the amount of heat shock proteins, in oxygen consumption, cardiac output and excretion rates (Carroll et al., 2017). Noise can also cause states of irritation and anxiety, increased parasites and disease, a decline in body conditions, growth rates, weight and food consumption, and irreversible damage at the DNA level (Kight & Swaddle, 2011). Several effects have also been found at the anatomical level, with auditory lesions, hearing loss, changes in the auditory threshold, internal lesions and cell damage to the statocyst and neurons, all of which cause disorientation and even death. Following acoustic stress, the animal, through immune responses, can avoid lifethreatening situations by restoring homeostasis. Physiological responses are not as immediately obvious as physical and behavioural responses (Carroll et al., 2017), however, understanding these responses is of particular importance as they are probably the basis of the other two. In fact, a study of this type of response is essential as effects of noise pollution can be predicted thanks to the study of genetic, physiological and cellular processes (Aguilar de Soto & Kight, 2016). A summary of the scientific literature that focus on the acoustic impacts of various human activities, ranging from invertebrates to mammals, at the physical and physiological level, is put forward.

Starting from invertebrates, it has been shown that different type of anthropogenic noise can cause bodily malformations on these organisms, increased mortality of the egg or immaturity, developmental delays, metamorphoses and stabilization delays, and slower growth rates. High mortality events have been reported in zooplankton (McCauley et al., 2017) and delays in larval development with possible malformations have been found in scallops (Aguilar de Soto et al., 2013), in crab larvae (Christian et al., 2003; Pine et al., 2012) and in barnacle larvae. In the latter, a reduction in the percentage of metamorphoses until 13 days was found (Branscomb & Rittschof, 1984). In molluscs, noise has been shown to reduce embryo development and increase mortality levels (Nedelec et al., 2014). However, data on invertebrate larvae in literature are conflicting; in fact, several studies have not observed significant changes in embryonic development, mortality levels or larval abundance (Pearson et al., 1994; Parry et al., 2002; Day et al., 2016a). The presence or absence of effects could depend on the distance from the acoustic source, as it has been shown that larvae closest to the acoustic source are more likely to show mortality events or developmental anomalies (Booman et al., 1996; Aguilar de Soto et al., 2013). Furthermore, in the study of acoustic impacts on invertebrates, it is important to consider also vibrations transmitted in the seabed (Roberts & Elliot, 2017). Moreover, some invertebrates, such as squid, detect sound through the use of the statocyst (Mooney et al., 2010), which detects the particle motion of a sound field that is produced by other animals or by human activities. The study of statocysts is of considerable importance and cephalopod damage, such as *Cotylorhiza tuberculata* and *Rhizostoma pulmo* (Solé et al., 2016), has been observed through Scanning Electron Microscopy (SEM). Following an acoustic stress event, damage was identified in the statocyst sensory epithelium, resulting in acoustic trauma. Damage of this kind could cause changes in swimming depth, in blood chemistry, in oxygen availability, and can also cause death (Guerra et al., 2004). Harmful effects on invertebrate statocysts could explain the strandings of squid, which occurred in Spain, caused by seismic investigations with air guns (Guerra et al., 2004). Low-frequency emissions can cause obvious damage not only at the level of invertebrate statocysts but also at neuronal level, with enormous acoustic trauma incompatible with life due to permanent alterations of sensory hair cells (André et al., 2011b; Solé et al., 2013a, b; Day et al., 2016b). Moreover, crabs have demonstrated anatomical damage evident when exposed to acoustic emissions (Christian et al., 2003; 2004; Lee-Dadswell, 2009). Beyond the structural damages, several scientific studies have observed sublethal effects in the serum biochemistry of some invertebrates, even weeks and months after acoustic exposure (Payne et al., 2007). Biochemical responses can differ between species and obvious effects have been found at THC, electrolyte content, minerals and metabolites level. Acoustic stress also affects the pH of hemolymph and can cause mortality events in scallops (Day et al., 2016b). Low-frequency sound can affect respiratory rates (Kaifu et al., 2007) thereby reducing growth rates and increasing mortality and disease rates (Lagardère, 1982). Changes in metabolic rates, in oxygen consumption and in ammonia excretion have been observed (Régnault & Lagardère, 1983; Wale et al., 2013) and acoustic impact on the biochemical parameters of invertebrates, such as THC, can also occur as much as 365 days after acoustic stress with chronic impact up to 120 days after exposure (Fitzgibbon et al., 2017). To confirm this, Celi et al., (2013) observed significant changes in different immunological parameters including THC levels, differential blood counts (DHC) and Hsp70 in *Procambarus clarki* exposed to acoustic stimulus. Lovell et al., (2005) report sensitivity of the statocyst in *Palaemon serratus* to particle movement in water and Filiciotto et al. (2016), in the same species, observed variations in total protein concentrations in hemolymph and the brain, variations in DNA integrity, and in HSP27 and HSP70 expression levels in brain tissues. Biochemical variations following acoustic stress were confirmed in different species of invertebrates, with significant effects on glucose levels, total protein, HSP70 expression, THC, blood count, lactate concentrations, hydrocortisone levels, AChE activity (La Bella et al., 1996; Filiciotto et al., 2014; Vazzana et al., 2016; Filiciotto et al., 2018; Zhou et al., 2018) and on clearance rates due to increases in filtering levels and, therefore, in metabolic rates (Spiga et al., 2016). Acoustic stress negatively influences DNA structure in haemocytes and gills of mussels (Wale et al., 2016), Ca^{2+}/Mg^{2+} -ATPase activity in feed tissues and O:N ratios or gene expression related to metabolism via glycolysis, biosynthesis of fatty acids, tryptophan metabolism and the tricarboxylic acid cycle

(Peng et al., 2016). Moreover, Shi et al., (2019) recently observed adverse effects of noise on the metabolism and ATP synthesis in *Tegillarca granosa,* and Solan et al., (2016) observed changes in the transport of fluids and particles in invertebrates, considered an essential element of the nutrient cycle at the bottom of the sea. Given the commercial importance of these organisms, Harrington et al., (2010) analysed the quality of the meat and eggs of scallops following acoustic stress, and, fortunately did not detect any significant changes. The various studies produce somewhat conflicting data; however, differences may be due to different types of sound exposure, to differences in taxa, to the physical conditions of organisms or to studies conducted in the laboratory. Invertebrates have significant levels of intraspecific variability in the physiological response.

As regards fish, starting once again from the larval level, results concerning larvae are contrasting. Kostyuchenko, (1973) analysed survival and lesions in the eggs of some fish species, showing that those closest to the acoustic source had higher mortality. In other cases, no changes were found in the survival of eggs or larvae of different fish species of *Gadus morhua* (Dalen & Knutsen, 1987; Payne et al., 2009; Bolle et al., 2012), while Nedelec et al., (2015) showed in the same species that noise caused reduced growth, and Dalen et al., (2007) showed that the duration of acoustic disturbances can have an impact on survival and development. A reduction in offspring survival was also observed in *Acanthochromis polyacanthus*, caused by changes in the behaviour of parents stressed by acoustic noise (Nedelec et al., 2017a). Noise lead to increased defensive actions and reduced interactions between feeding and offspring. Following exposure to noise, Fakan et al., (2019) observed adverse effects on the embryogenesis of *Amphiprion* *melanopus* and *Acanthochromis polyacanthus*, with even an increase in heart rate. Young fish are often subject to noise pollution, for example from exploration and construction projects (Maragos et al., 1993), which can have negative effects on hearing ability (Caiger et al., 2012). Seahorse also showed weight loss, deterioration of body conditions, high levels of cortisol and parasites in the kidney following exposure to acoustic stress (Anderson et al., 2011). These differences can be caused by the differing capacity of fish to respond to diverse stresses (Akinrotimi et al., 2009). Seismic noise or noise reproduced in the laboratory can create endocrinological effects with variations in levels of adrenaline, cortisol, glucose, lactate, AMP levels, ADP, ATP, cAMP and HSP70 (Sverdrup et al., 1994; Santulli et al., 1999; Buscaino et al., 2010; Celi et al., 2016; Vazzana et al., 2017; Lin et al., 2019). Of particular importance is the ability to recover biochemical parameters, which in some cases returned to normal physiological within 72 hours (Santulli et al., 1999), while in others within 24 hours (Wei et al., 2017). In contrast to blood parameters, McCauley et al., (2013) observed considerable damage to hair cells caused by acoustic exposure and no repair or improvement up to 58 days after exposure to noise emission. This type of response can change according to the type of acoustic emission and species considered. Noise also influences the expression of those genes involved in cortisol synthesis by influencing energy distribution during stress (Wei et al., 2017). Despite this, cortisol levels and other parameters seem to be more influenced by intermittent noise exposure rather than continuous noise (Nichols et al., 2015; Radford et al., 2016) and the effects of piling are different to driving (Spiga e al., 2017). Exposure to acoustic stress has negative effects on oxygen consumption, lactate levels (Debusschere et al., 2016), total oxidant levels, lysozyme activity, antiprotease activity and white blood cells, as well as on the albumin/globulin ratio (Filiciotto et al., 2017). Acoustic stress cause mortality events (Debusschere et al., 2014) and changes in ventilation rates as soon as 30 minutes (Bruintjes et al., 2017) and up to two weeks after sound exposure (Nedelec et al., 2016a). At the genetic level, of particular importance is the work of Andrews et al., (2014), who conducted genomic studies on the inner ear of salmon. They performed microarray analyses that identified 42 up-regulated and 37 down-regulated transcripts with marked effects in terms of cellular energy and cellular respiration. Furthermore, transcript coding for hemoglobin was upregulated, similar to coding for nicotinamide riboside kinase 2, which is important in nerve cell damage, demonstrating the presence of neuronal damage to the ear. Protein transcriptional changes confirm damage to ear tissues and their altered expression may suggest changes even in the repair or regeneration of hair cells. As in the case of invertebrates, tolerance levels can change over time and, for this reason, long-term studies are essential (Nedelec et al., 2016a). Picciulin et al., (2012) observed increases in heart rate following numerous boat passages and several studies confirm that low-frequency sounds can cause barotrauma or physical damage, detected histologically or morphologically, with effects also at the cellular level/structural, such as temporary threshold shift (TTS) (Popper et al., 2005; Popper & Hastings, 2009a; McCauley & Kent, 2012; Popper et al., 2014). Furthermore, with higher sound levels and longer exposure time, TTS is more likely to occur (Weilgart, 2007). High intensity sound can even create permanent threshold shift (PTS) and various factors can influence these effects: number and frequency of repetitions, SPL, duration and physiological state of the organisms

(Popper & Hastings, 2009a). Acoustic effects vary among species. In fact, in *Oncorhynchus tshawytscha*, lesions that did not influence the survival of the organisms were present and no mortality events were observed (Casper et al., 2012). On the other hand, when the same species was exposed to impulsive sounds, Halvorsen et al., (2012a) found various lesions, from mild hematomas at the lowest levels of sound exposure to bleeding at the highest exposure levels. Considering possible variations in ventilation rates, Poulton et al., (2017) observed no adverse effects on the ventilation rate of *Dicentrarcus labrax* following an interaction between CO_2 and the noise produced. Nevertheless, further studies need to be carried out in view of climate change and ocean acidification, a process that could change the physics of sound propagation in water.

Physical and physiological effects have also been observed in freshwater fish. Changes in egg mortality levels were observed (Banner & Hyatt, 1973) with changes based on the life stage (Bruintjes & Radford, 2014). Even in freshwater fish, noise pollution can affect ventilation rates (Bruintjes et al., 2016), metabolic rates (Simpson et al., 2015; Purser et al., 2016) and growth rates (Sun et al., 2001), with very serious effects on the survival of the population (Bruintjes & Radford, 2013). At the biochemical parameter level, effects on hormone levels have been found with different responses according to the species analysed (Wysocki et al., 2006). Effects were reported as soon as within the first ten minutes of exposure to acoustic stress and included significant changes in the hearing threshold (Smith et al., 2004a). In particular, Smith et al., (2004a) showed that after 21 days of noise exposure, it took 14 days to restore hearing levels and the damage was recovered only after 28-35 days. Cardiac output levels are also adversely affected in the presence of different types of noise and in different species (Graham & Cooke, 2008); as regards saltwater fish, effects have been found on a cellular and structural level with changes, albeit temporary, in auditory thresholds (TTS) (Song et al., 2008; Liu et al., 2013). Popper et., (2005) analysed effects on different species by studying auditory brainstem response (ABR). Hearing threshold changes were recovered within 24 hours of exposure, although not in all species studied. Popper et al., (2007), observed a shift in the auditory threshold in *Oncorhynchus mykiss*; however, the results varied with different groups of trout. They observed no mortality during or after exposure and the inner ear tissue did not show any morphological damage, even days after acoustic exposure. Anatomical damage may depend on exposure to single or multiple impulses (Casper et al., 2012; Popper et al., 2016) and the structure of the swim bladder may influence the type of damage (Casper et al., 2012; Casper et al., 2013a; Halvorsen et al., 2012 a,b); fish with physoclistosis suffer greater damage than others (Casper et al., 2013a; Halvorsen et al., 2012 a,b). Differences in the extent or lack of damage found in the different species could depend on the fact that, in some species, effects may not be immediate, especially at the hair cell level (Hastings et al., 1996). Consequently, susceptibility of the species may depend on genetics, development and seasonal variations (Halvorsen et al., 2013). Hearing specialists, for example, suffer significant effects on hearing sensitivity, with obvious reductions but this may vary based on the acoustic frequency analysed (Scholik & Yan, 2001; Amoser & Ladich, 2003). The frequency and duration of exposure also influenced the recovery of a species (Scholik & Yan, 2001). Scholik & Yan, (2002) have shown that a noise emitted for two hours can significantly increase the auditory threshold of the fish; subsequent hearing loss, even if temporary, can compromise reproductive levels of the species, communication distances and prey/predator relations leading to significant effects on the survival of the species (Amoser & Ladich, 2003). Smith et al., (2006) reported that fish exposed to noise for 48 hours showed a significant change in TTS up to 7 days after exposure with considerable damage to the auditory cells. However, functional recovery that preceded morphological recovery was observed.

Regarding mammals, Dalen et al., (2007) did not find any mammalian mortality or injury events. However, changes in physiological parameters such as aldosterone, noradrenaline, adrenaline, dopamine have been detected in *Delphinapterus leucas* and *Tursiops troncati* (Romano et al., 2004) subjected to acoustic stress. In Beluga, changes at the cardiorespiratory level have been observed at low emission intensities (Lyamin et al., 2011) and several studies indicated that maintaining noise levels around 120 dBre 1µPa could be a good standard for the physiological integrity of whales (IUCN, 2006; Weir et al., 2007). Gordon et al., (2003; 2018) reviewed the effects of anthropogenic activity, on the physiology of marine mammals, which are complex, variable and conflicting. Gordon et al., (2018) reviewed the impacts of several sources of anthropogenic noise and concluded that these resulted in temporary or permanent hearing loss. They reported that recent studies showed increases in whale hormone levels in the presence of anthropogenic noise; in particular, the glucocorticoid stress hormone. Thus, anthropogenic noise has harmful impacts also on mammalian physiology, in cetaceans in particular (Gordon et al., 2018). Recently, De Quirós et al., (2019) reviewed the information about the impacts of anti-submarine sonar on whales. It has been observed that whales can even show pathological decompression effects.

However, the effects of these medium-frequency acoustic emissions vary between individuals and populations. Many other scientific studies analysed the effects of the acoustic impact on mammals at physical and physiological level. However, this Section only refers describes more in detail vertebrates and invertebrates that are the object of study of the experimental plans described later. However, the indicated studies and reviews allow to have a complete amount of information to understand that the acoustic emission can have effects also at physical and physiological level in marine mammals.

1.6.2 Behavioural effects of noise pollution in marine organisms

As previously mentioned, all aquatic species can hear sounds (Slabbekoorn et al., 2010; Simpson et al., 2011; Filiciotto et al., 2014; Celi et al., 2015; Nedelec et al., 2016a; Hawkins & Popper 2017) and use them to carry out vital activities such as foraging, predation, mating and habitat selection (Fay & Popper, 1998; Popper 2003a; Eggleston et al., 2016). Behavioural changes play a key role in the fate of the species (Wong et al., 2015) and anthropogenic noises can influence them (Popper, 2003a; Slabbekoorn et al., 2010; Cox et al., 2018) particularly at low sound levels (Hawkins et al., 2015). Juanes et al., (2017) through a meta-analysis studied how anthropic or biological noises can influence fish behaviour and, although some species may be more sensitive to noise pollution than others, most fish species show negative effects. Behavioural responses provide very important information, as acoustic communication plays a crucial role in the life of marine organisms. A summary of scientific papers that studied the acoustic impacts of different anthropic activities on the behaviour of different marine organisms is put forward.

Invertebrates possess sensory systems that allow them to perceive particle motion (Fay, 1984; Roberts et al., 2015) and, therefore, sounds. As in the case of physical and physiological effects, different acoustic impacts have also been observed at the behavioural level. Acoustic noise can influence settlement behaviour of coral planulas (Lecchini et al., 2018) and effects on the late larval stages may depend also on the type of motor used (McCormick et al., 2019). Information concerning the effects of noise on larvae is very important, since underwater sound can play an important role in the orientation of pelagic stages (Jeffs et al., 2003; Montgomery et al., 2006; Vermeij et al., 2010). Noise pollution in adult invertebrates can cause reductions in reproduction rates, increases in cannibalism levels and alarm reactions (Lagardère, 1982; Goodall et al., 1990; McCauley et al., 2000). Different invertebrate species change and adapt their behaviour according to the type of sound received (McCauley et al., 2000), and the types of response depend on the frequency and level of the sound (Mooney et al., 2016). In the presence of acoustic stress, squid, for example, react by inking and jolting, responses which occur more frequently with increasing sound levels (Fewtrell & McCauley, 2012). At high levels of exposure, the number and intensity of alarm responses increased. Based on the type of acoustic emission, different behavioural responses have been found in octopus, cuttlefish and squid, such as inking, rapid color change, position inside the tank, absence of foraging or mating (Solé et al., 2013a; Samson et al., 2014). Low frequency sound may even influence ability to detect predators (Kaifu et al., 2007). Despite this, invertebrates are able to get used to this type of stress, reducing their alarm responses (Fewtrell and McCauley, 2012; Samson et al., 2014; Mooney et al.,

2016) and capture rates do not seem to be affected (Andriguetto-Filho et al., 2005). However, information is too limited (Christian et al., 2003; Parry & Gason, 2006) and variations in abundance estimates have been observed (Courtenay et al., 2009). Noise pollution influences the feeding behaviour of lobsters and scallops even long after acoustic exposure (Payne et al., 2007); lobsters often respond with extension or straightening of the tail, which can influence the predator-prey relationship (Day et al., 2016b). Even in shrimps, reductions in agonistic behaviour have been observed (Celi et al., 2013) and further experimental evidence shows negative effects of noise on the foraging capacity of crab (Hubert et al., 2018). If anthropogenic noise can influence the foraging interactions of a species, it could also create problems underlying the marine food chain (Hubert et al., 2018). Anthropic noise can influence grouping behaviour in hermit crabs (Tidau & Briffa, 2019) and the speed of choice of shell (Walsh et al., 2017). Noises change predation levels (Chan et al., 2010), locomotion, shelter strategies (Filiciotto et al., 2014; 2016; 2018; Roberts et al., 2016b; Zhou et al., 2018), foraging capacity and the ability to find shelter (Wale et al., 2013; Shi et al., 2019) in different species. Behavioural changes in invertebrates are also caused by vibrations that are transmitted to the bottom of the sea and that can cause, for example, valve closure responses, deeper excavation and changes in locomotion and the movement of the antennas (Roberts et al., 2015; Peng et al., 2016; Roberts et al., 2016a). This type of response could affect health, reproduction, energy costs, respiratory rate and heart rate of these organisms (Roberts et al., 2015) as it can change the volume of water flowing through the gills and therefore the absorption of food metals and growth (Charifi et al., 2018). It has also been shown that some species respond with avoidance behaviour and vary swimming depth (Solan et al., 2016). In general, the higher the sound intensity and the lower the water depth, the greater the risk and invertebrates seem to be more at risk than pelagic fish (Webster et al., 2018).

Moving from invertebrates to seawater fish, the effects of noise have been observed even in sharks, which showed a surprising reaction in response to sudden sounds (Myrberg et al., 1978). However, information regarding acoustic impact on elasmobranchs remains poor. As in the case of invertebrates, at the larval level in fish it has been shown that noise pollution can cause confusion and disruption of orientation behaviour (Simpson et al., 2010). Considering that sound plays an important role in the orientation of fish larvae, acoustic impact could have important consequences on their physical form (Montgomery et al., 2006; Leis et al., 2011). Furthermore, larvae with acoustic stress may spend more time swimming before settling at a defined point, with negative consequences on the well-being of the population and on connectivity. Noise has the ability to influence the behaviour of newborn fish by triggering alarm responses (Nedelec et al., 2015) and the ability to recognise predation threats (Spiga et al., 2017) causing anxiety states. Noise can distract fish and prevent them from responding to the threat of predation (Chan et al., 2010; Simpson et al., 2015). The behavioural effects in fish are visible even long after the stress factor has ended and this suggests that the effects are present for long periods of time (Nedelec et al., 2016a; Ferrari et al., 2018). In juvenile individuals of some fish species, it has been shown that these can respond to acoustic stress by reducing the audacity and relative distance, with recovery times of approximately 20 minutes (Holmes et al., 2017). Response to chronic acoustic stress was also observed in seahorse (Anderson et al., 2011) with anxiety and irritation behaviour. Noise pollution compromises communication in fish (Popper & Fay, 2011; Alves et al., 2016) and masks their own acoustic signals (Pollack, 1975; Brungart, 2001; Codarin et al., 2009). However, changes in communication and alarm reactions may depend on distance from the source (Santulli et al., 1999; Hassel et al., 2004) and communication distances can be reduced by up to several meters (Vasconcelos et al., 2007; Alves et al., 2017). Fish, like invertebrates, have sensory systems to perceive particle movement (Partridge et al., 1980; Faucher et al., 2010; Mueller-Blenkle et al., 2010; Popper et al., 2018) and compromising this ability means losing information on their movements, on positions through the use of the lateral line (Kojima et al., 2010; Halfwerk et al., 2015) and on ability to care for nests and offspring (Picciulin et al., 2010). Acoustic stress can, therefore, negatively affect the conservation of a species (Slabbekoorn et al., 2008), growth rates (Terhune et al., 1990), speed and aggregation states (Mueller-Blenkle et al., 2010), capture rates, abundance and distribution (Løkkeborg, 1991; Skalski et al., 1992; Engås et al., 1996; 1998; Hassel et al., 2004; Løkkeborg 2012; Miller & Cripps, 2013; Peña et al., 2013; Thomson et al., 2014; Paxton et al., 2017), with long-term effects (Slotte et al., 2004) that differ based on the species analysed. Elevated noise levels can increase behavioural responses in some fish species (Hawkins et al., 2015) and have implications for the energy balance, changing the supervision capacity of marine organisms (Read, Jones & Radford, 2014; Shannon et al., 2016). Anthropic noise also negatively influences the number of calls between individuals (Amorim, 2006; Krahforst et al., 2017; Correa et al., 2019), causing an energy expenditure at the muscular level and a reduction in the possibility of reproduction (Rowe, et al., 2008; Krahforst et al., 2017; Stanley et al., 2017) thereby compromising courtship (Amorim et al., 2016; De Jong et al., 2017; Krahforst, 2017), parental behaviour (Picciulin et al., 2010; Nedelec et al., 2017a; Blom et al., 2019), the release of gametes (Hawkins & Amorim, 2000; Rowe & Hutchings, 2006; Casaretto et al., 2014) and spawning success (Sierra- Flores et al., 2015; Krahforst, 2017). Bruintjes et al., (2014) for example, developed a model to study the behaviour of *Dicentrarcus labrax* exposed to noise, showing that it took longer for the fish to reach the site where it had to spawn. Furthermore, the times and duration of sound emission are important factors in the type of response from the animal and some authors have observed differences in recovery between intermittent and continuous emissions (Neo et al., 2014, 2015). Both emissions trigger alarming reactions, increases in the speed and cohesion of swimming and increases in swimming depth (Neo et al., 2014; 2015; Buscaino et al., 2010; Herbert-Read et al., 2017) but recovery was slower in the presence of intermittent noise (Neo et al., 2014; 2015). Behavioural responses can be influenced by several factors, such as temperature, animal physiology, age, individual, group dimensions (Kastelein et al., 2008) or the state of the environment, whether closed or open (Mesinger et al., 2018). Among the alarm reactions, C-start reactions were also observed (Hirst & Rodhouse, 2000; McCauley et al., 2000; Wardle et al., 2001; Popper & Hastings, 2009a; b). These consist in a bending of the body that takes the form of the letter C and can change schooling patterns, the positions in water columns and swimming speeds (Pearson et al., 1992; Santulli et al., 1999; Wardle et al., 2001; Hassel et al., 2004; Boeger et al., 2006; Fewtrell & McCauley, 2012). Anthropic noise changes the structure, swimming dynamics, speed and cohesion of the groups, and also affects swimming height of the fish, which become less ordered and less correlated in speed (Chapman & Hawkins, 1969; Slotte et al., 2004; Sarà et al., 2007; Bracciali et al., 2012; Herbert-Read et al., 2017; Correa et al., 2018). Moreover, it has been shown that impacts are greater during the night (Neo et al., 2018). All of this has effects on anti-predator benefits; in fact, individuals in larger more cohesive groups run fewer risks than individuals in smaller, less cohesive groups (Hamilton et al, 1971; Ioannou et al., 2017). Changes in cohesion can increase the risk of predation and change the stability of food webs (Holles et al., 2013), favoring predators that have greater success on prey (Simpson et al., 2016). Noise can, therefore, negatively affect foraging or fish orientation (Løkkeborg et al., 2012; Bracciali et al., 2012; Holles et al., 2013; Neo et al., 2016), influencing the level of attention: some species are not able to distinguish edible from inedible foods (Purser & Radford, 2011). Animals can respond to stress with loss of appetite (Wendelaar Bonga, 1997), and foraging effects with low absorption feed and higher metabolism can cause growth reductions (Kusku et al., 2018). However, we must not forget that the sensitivity of the species depends on their hearing ability (Pearson et al., 1992; Akamatsu et al., 2003; Lovell, 2003; Götz et al., 2009) and that temporal response can differ; animals can respond more slowly and less frequently (Simpson et al., 2016). Real understanding of acoustic impacts on fish depends on relating the behavioural reactions of organisms to their hearing capabilities. Each species reacts differently to different types of sound; responses may be similar between species or specific, but they ultlimately depend on their hearing ability (Kastelein et al., 2008). It also appears that some fish may become accustomed to acoustic disturbance, reducing jolt responses especially in the case of repeated exposure (Pearson et al., 1992; Boeger et al., 2006; Fewtrell & McCauley, 2012). It seems that animals can get used to, compensate for or move away from noise (Bejder et al., 2009; Normandeau Associates, 2012; Morley et al., 2014; Radford et al., 2016). It all depends also on environmental conditions. Herring, for example, has been shown to be more sensitive to engine noise during the winter period (Doksæter et al., 2012). Noise pollution also influences intraspecific and interspecific relationships, such as cleaning activities in coral reef fish, due to cognitive impairments from distraction (Nedelec et al., 2017b). Exposure to anthropogenic noise, therefore, has effects from the individual to the community level, influencing the structure of the habitat and the community (Francis & Barber, 2013; Naguib, 2013). Recent studies continue to confirm negative effects of anthropogenic noise on fish behaviour (Brehmer et al., 2019) with sublethal effects based on the noise level of human activities (Mickle et al., 2019). The anthropic noise is not "music" for "fish ears" and Papoutsoglou et al., (2008; 2010) showed an improvement in the growth, quality and production of some species exposed to the music of Mozart. Moreover, in light of the environmental changes taking place, it is reasonable to assume that other environmental factors will be able to influence anthropogenic noise in ocean waters, such as water acidification, which is likely to reduce the ability to absorb sounds. Studies on this should increase even if Poulton et al., (2017), who studied the effects of noise pollution combined with carbon dioxide for the first time, observed that ocean acidification is unlikely to affect response behaviour of fish.

Even in freshwater fish, behavioural changes due to noise pollution have been observed at larval stage (Vetter et al., 2017). Also in this case, observed changes were in the use of space and escape reactions (Simpson et al., 2015; McCormick et al., 2018b), in anti-predator responses (Voellmy et al., 2014b; Simpson et al., 2015; Bruintjes et al., 2016; Purser et al., 2016; La Manna et al., 2016) and in social behaviour, fear, stress and foraging levels, with different effects between species (Voellmy et al., 2014; Xinhai et al., 2016; Magnhagen et al., 2017). Changes in alarm reactions (Webb, 1986; La Manna et al., 2016; Sabet et al., 2016), in swimming speed (Sabet et al., 2016), in vocalizations (Picciulin et al., 2012; Holt & Johnston, 2015) in calling and laying eggs (Montie et al., 2017) and in swimming depth (Sabet et al., 2016) have been observed. Noise can affect sound detection and acoustic orientation differently within a single family of freshwater fish (Ladich et al., 2013b) and can have negative effects on growth, survival, disease resistance (Wysocki et al., 2007; Bruintjes & Radford, 2013) calls and reproduction (Bruintjes & Radford, 2013; De Jong et al., 2016). The impacts of anthropogenic noise may depend on the context, such as the sex of the fish, the role of fish in the group and the presence or absence of eggs (Bruintjes & Radford, 2013). Even freshwater fish responded to acoustic stress by changing intensity of excavation, reacting less to prey or with changes in behaviour of aggression and submission (Bruintjes & Radford, 2013).

As far as mammals are concerned, noise pollution can affect species-specific acoustic detection distances (Thomsen et al., 2006). Noise frequencies produced by ships fall within the 20 to 200 Hz band (Tyack, 2008): the frequency band used by communication whales. Anthropic noise reduces the communication space of mammals and has chronic effects on different species (Putland et al., 2017), causing acoustic masking (Lucke & Siebert, 2009) up to 2000 m away from the acoustic source (Lossent et al., 2018). Noise levels that have negative effects on mammals seem to be those higher than 160 dB 1µPa (Weir et al., 2007; Department of Fisheries and Oceans. Canadian Practice Statement: Mitigation of Seismic Noise in the Marine Environment, 2005). Several studies demonstrate negative impacts from airguns on mammals at behavioural level (Dalen et al., 2007), concluding that these responses are complex, variable and conflicting (Gordon et al., 2003; 2018). Sonars, for example, create echolocation problems for toothed whales (Gordon et al., 2018). Anthropic noise also interferes with the communication and acoustic functions of cetaceans, triggering behavioural responses that have negative effects on individuals and population. Regarding mammals in literature there are several studies that analyse the effects of acoustic emissions on their behaviour. However, since the species analysed in several PhD experimental plans were invertebrates and vertebrates, this Section analysed these species in more detail and leave the information on mammals to the cited reviews that contain the most important information available, such as De Quirós et al., (2019). Also, in marine mammals, the acoustic emission has important effects at behavioural level.

1.7 Standards and guidelines about noise pollution

It is known that due to different human activities, anthropic noise has become omnipresent in terrestrial and aquatic ecosystems (Andrew, 2002; World Health Organization, 2011) and, for this reason, it is considered a significant pollutant under the framework directive on the European Union navy strategy (Directive 2008/56/EC of 17 June 2008) and the World Health Organization (Kunc et al., 2016). Noise has increased since the industrial revolution (McDonald et al., 2006; Normandeau Associates I. 2012) and is rapidly increasing in the marine environment (Andrew et al., 2002; Hildebrand, 2009; Popper & Hastings, 2009a). Human activities in the seas are various: navigation, 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). These activities emit high-intensity, high-pitched sounds when produced by military exercises, oil and gas exploration and driving in piles (Dolman et al., 2009; McCauley et al., 2000; Bailey et al., 2010), or lower-level sounds by fishing, commercial and recreational activities (Codarin et al., 2009; Malakoff, 2010). Despite the increasing attention paid to noise generated by maritime industries in recent years (Hawkins et al., 2017), anthropogenic noise is one of the least studied sources of pollution (Hawkins et al., 2015) and nothing is known on the noise produced by DSM activities. The descriptor 11.2 on "continuous low frequency sound" of Marine Strategy Framework Directive (MSFD) aims to monitor trends in the ambient noise level within the 1/3 octave bands of 63 and 125 Hz (centre frequencies) (Marine Strategy Framework Directive; Tasker et al., 2010; CE, 2010a). However, to date noise has become the eleventh descriptor in guidelines on underwater noise pollution (Tasker et al., 2010; CE, 2010a), there are no regulations for noise pollution relating to DSM activities. Bibliographic research highlighted, beyond the MSFD, the existence of different laws or regulations concerning noise produced by anthropogenic activities, such as the international agreements ACCOBAMS, ASCOBANS and EUROBATS, the French law Grenelle II n. 2010-788 of 12 July 2010 and the United States Environmental Policy Law. Most of these originated in an interest by political organizations in the problem of acoustic noise as noise from commercial ships is between 0.1 and 1 kHz (Hildebrand, 2009): the frequency range used by many species for communication. The “Monitoring guidance for underwater noise in European seas - Part II” (Dekeling et al., 2014) for example, suggests monitoring trends in ambient noise levels (annual average values measured in RMS re 1μPa) emitted within 1/3 octave bands with central frequencies of 63 and 125 Hz. The National Marine Fisheries Service (NMFS), based on mortality data of species exposed to explosives (Popper & Hastings 2009a), developed further intermediate criteria (FHWG 2008; Woodbury & Stadler 2008; Stadler & Woodbury 2009; Caltrans, 2009) and further guidelines were written by the Marine Strategy FrameWork MSFD 2008/56/CE. The overall aim is to protect the marine environment in the European area more effectively and to achieve a "good environmental status (GES) by 2020". Currently in Europe, despite efforts to reduce and regulate noise pollution (Pottering & Lenarcic, 2008), many countries do not have adequate regulation and management, and there are several criteria for exposure to noise (Stöber et al., 2019). In an attempt to reduce noise impact on marine animals, the Italian Environmental Impact Assessment Commission, for example, asked seismic operators in 2015 to use a scientific protocol to study the presence of the marine organism before, during and after noise pollution with visual and acoustic methods. This monitoring method is likely to help and improve the study of noise on an international level (Fossati et al., 2017). However, one of the problems is that to date there is no well-defined protocol for measuring sea noise pollution (André et al., 2011a, b; Dekeling et al., 2014; Das, 2019). This is because there are no long-term studies and the parameters to perform these measurements are highly variable, causing a large quantity of heterogeneous data (André et al., 2010). Among possible mitigation measures, the slowing down of ships has been proposed (Pine et al., 2018) which, however, could have even worse consequences.

Several authors such as Popper et al., 2014, 2019, have tried to contribute to the writing of guidelines for noise pollution. These will be reviewed later in Chapter 5. Several documents provide indications but none which are sufficient (Dekeling et al., 2014) and the different data available do not allow the implementation of adequate prevention and protection measures. To date, the problem of noise pollution should not be overlooked, especially in light of information from the Commission on marine mammals, (2007). This is compounded by the fact that future human activities are bound to lead to further increases in sound levels in the depths of the ocean and not in a homogeneous manner (OSPAR Commission, 2009). Despite various attempts to regulate the impact of noise from human activities at sea, this type of impact has not been taken into consideration for mining activities. This is confirmed by reading the recommendations provided by the ISC

LTC in ISBA/19/LTC/8 (ISA, 2018), which describe procedures to be followed during data acquisition and for monitoring during and after potentially harmful activities for the environment. These recommendations require concession managers to prepare annual reports providing very general information on biological communities, biodiversity studies and information on the functioning of ecosystems (ISA, 2018). Within the recommendation, different environmental aspects are treated, but in a superficial way and several authors recommend improvements (Christiansen et al., 2019; Jaeckel et al., 2019). The impact of noise from DSM has not yet been mentioned in any regulation, since information on the generation and propagation of sound of activities is not available and knowledge on sound perception in animals is still scarce. This seems to be justified by the fact that the noise impacts that could be generated by DSM cannot be predicted.

2. Aim of PhD project

The PhD project performed is part of two PhD calls: the PhD program on the XXXII cicle of Mediterranean Biodiversity (International) of the University of Palermo and the Innovative PhD with industrial characterization PON 2014-2020 funded by the Ministry of Education, University and Research (MIUR). The research project involved a number of different organizations, such as the University of Palermo (UNIPA), the Universitat Politècnica de València (UPV), the Italian National Research Council (CNR) of Capogranitola (Campobello di Mazara), The Italian Institution for research and promotion of standardization (ENR) based in Palermo and HR Wallingford Ltd based in Oxford (United Kingdom, Wallingford). The PhD project is also based on an agreement between the two PhD programs of UNIPA and UPV (Agreement for the International PhD Program in Mediterranean Biodiversity) within which the PhD course it took place and the thesis was written. The research period lasted three years and the aim of the PhD project was to contribute to the definition of a "baseline" for use when carrying out extractive activities in deep seawater in order to ensure that the biodiversity of our seas is respected to as great a degree as possible. Scientific literature in recent years has begun to predict possible impacts to be expected from DSM activities in the near future: habitat fragmentation, species loss, sediment plume production, temperature variations and light/acoustic pollution. Scientific research and data are not yet available for all types of impact envisaged; in fact, to date no research has been carried out on noise impact generated by Deep Sea Mining (DSM) activities.

This gap in scientific data, mainly due to the lack of knowledge on frequencies and acoustic intensities that will be emitted by mining activities, has made the production of guidelines and regulations on limiting impact more complex to date. This is in net contrast to various other human activities carried out at sea for which scientific data on the acoustic impact of those activities on marine biodiversity at different levels is widely available.

The aim of the PhD project was to create a baseline in order to understand the acoustic impact of DSM activities on marine biodiversity, using knowledge on the acoustic impacts caused by other human activities as a starting point. Given the complexity of the topic, the research project was carried out in a modular way and different research activities were carried out within each module.

In particular, the PhD Project was structured as follows:

A) Biological-environmental module

B) Technical-technological module

C) Standard and standardization module

A) The biological-environmental module was implemented at the University of Palermo (UNIPA), the Universitat Politècnica de València (UPV) and the National Research Council (CNR). In this module, aspects relating to marine biodiversity, underwater bioacoustics, ocean noise pollution, and the effects of underwater noise on invertebrate and vertebrate organisms were studied. Behavioural and biochemical analyses were conducted on marine organisms during experiments carried out in Palermo, Gandia (Spain) and Campobello di Mazara (CNR). During this module, indoor and outdoor experiments were conducted to evaluate and describe biochemical and behavioural parameters of invertebrates and fish in the presence of anthropogenic noise.

B) The technical-technological module included a period of six months at HR Wallingford Ltd. During this period, the technical and technological aspects relating to the energy balance of subsea industrial activities were examined. In particular, the data obtained from the biological-environmental module concerning the behavioural responses of juvenile fish exposed to acoustic stress was used for the creation of a numerical model that could contribute to the prediction and reduction of the acoustic impacts of human activities on marine organisms.

C) The standards and the standardization module included six months carried out at The Italian Institution for research and promotion of standardization (ENR) in Palermo. Aspects relating to the regulation and standardization of the acoustic impact of human activities at sea were analysed, starting from current national and international regulations. This module led to the drafting of the first technical standard on the acoustic impacts of DMS activities regulating each phase of mining in the ocean depths. The aim is to ensure that the exploitation of mineral resources is entirely eco-sustainable and respectful of biodiversity and marine ecosystems. The technical standard in question was formulated based on a bibliographic study of the acoustic impact of other marine-maritime activities on biodiversity and by using the data obtained during the biological-environmental module.

3. Biological-environmental module

The Biological-environmental module used three different experimental plans with three specific objectives:

a) Objectve 1: Study of the effect of high frequency noise on invertabrates (Section 3.1 and 3.2). Experiments carried out at the University of Palermo, Department of Biological Chemical and Pharmaceutical Sciences and Technologies (Italy). The experiments were carried out indoor: two species of invertebrates, *Arbacia lixula* and *Mytilus galloprovincialis,* were stressed with underwater high frequency noise. The aim of these experiments were to understand, for the first time, the effects of these frequencies on new invertebrate species (echinoderms) and new matrix (mussels digestive gland). Regarding echinoderms no studies are available in literature about the acoustic stress, but our hypothesis was to find significant effects in coelomic fluid at biochemical level. Regarding mussels in literature there are different studies about the acoustic impact analysed in hemolymph. In this case our hypothesis was to find significant effects in other matrices such as digestive gland especially on enzyme activities of which this tissue is the main site.

b) Objectve 2: Study of the effect of impulsive noise on vertebrates and invertabrates. Experiments carried out at the National Research Council (CNR) at Campobello di Mazara (Italy). The experiments were carried out outdoor and were part of a watergun research project led by the CNR bioacoustic group. The experimental animals were vertebrates and invertebrates: *Chromis chromis, Holothuria tubulosa*, *Arbacia lixula*. The purpose of this experiment was to evaluate the impacts of these activities, used to analyse the seabed, on invertebrate and vertebrate species. Having demonstrated with the previous experiment's significant effects on echinoderms, it has been hypothesised that also activities like watergun (which could precede DSM to study the structure of seabed) could have important effects at biochemical level on marine species. Biochemical responses in sea urchins and sea cucumbers coelomic fluid were analysed to compare the effects respect to the acoustic emissions used in experiments done in part (a). Moreover, new matrices have been used (the peristomial membrane of sea urchins) and new protocols developed (extraction of cortisol from juvenile fish body).

c) Objectve 3: Study the effect of different acoustic frequencies on juvenile fish. Experiments carried out at the Campus of Gandia, Universitat Politècnica de València (Spain). The experiment was carried out indoor and the aim was to study behavioural responses in vertebrates (juveniles of *Sparus aurata*) stressed with acoustic stimulus. The hypothesis was that different behavioural responses (swimming height, motility, group dispersion) depending on emission frequencies would have been found. This would help to understand what frequencies would have the most impact on juvenile fish for which studies are still poor and that are important for the species conservation. Furthermore, a species of considerable commercial importance was chosen. The results obtained have completed the information necessary to carry out the numerical model of Chapter 4 and the technical standard of Chapter 5.

3.1 Underwater high frequency noise: biological responses in sea urchin *Arbacia lixula* (Linnaeus, 1758)

Every marine ecosystem is characterized by its own, very individual soundscape (Radford et al., 2010; Ceraulo et al., 2018), made up of sounds generated by abiotic components (water currents, geophysical processes, waves, rain, ice), biotic components (sounds emitted voluntarily or involuntarily by marine species) and by human activities (Kennedy et al., 2010; Piercy et al., 2014; Buscaino et al., 2016). In recent decades, anthropogenic activities have increased underwater acoustic energy globally (Ross, 2005; Slabbekoorn et al., 2010; Hildebrand, 2009), thereby changing the acoustic characteristics (signature) of marine ecosystems at coastal, pelagic, and deepwater levels. Noise pollution, generated by commercial traffic, offshore and inshore construction, seismic explorations and sonar, has been recognised as a pollutant for aquatic ecosystems and a threat to marine fauna (World Health Organization, 2011; European Framework Directive 2008/56/EC - Marine Strategy (MSFD)).

All aquatic species, both vertebrates and invertebrates, are able to hear sounds (Slabbekoorn et al., 2010; Simpson et al., 2011; Filiciotto et al., 2014; Celi et al., 2015; Nedelec et al., 2016b; Hawkins and Popper, 2017) and use this ability to carry out vital biological activities, such as foraging, predation, mating and habitat selection (Fay and Popper, 1998; Popper 2003a,b; Eggleston et al., 2016).

In this context, anthropogenic noise can disturb animal behaviour and physiology with potential consequences at population level (Sun et al., 2001; Slabbekoorn et al., 2010; Buscaino et al., 2010; Wale et al., 2013; Voellmy et al., 2014; Radford et al., 2014; Popper et al., 2014; Kunc et al., 2016; Magnhagen et al., 2017; Hawkins and Popper, 2017; Weilgart, 2018).

Reactions to boat or to seismic noise (Wardle et al., 2001; Engås and Løkkeborg, 2002; Slotte et al., 2004; Parry and Gason, 2006) were the most explored, given the fact that low frequencies can influence species at greater distances from the source than higher frequencies. However, echo sounders, fishing net control sonar, CHIRP sonar, side-scan and multi-beam sonar, deterrent devices and small, fast boats produce high-frequency sounds up to 800 kHz (Bonanno et al., 2006; Hildebrand, 2009; Buscaino et al., 2009; Hawkins et al., 2015).

Despite the fact that absorption of high-frequency sounds is greater than that of low frequencies (resulting in an attenuation over shorter distances), their use is so extensive that they can contribute to increasing environmental noise (Hawkins et al., 2015), determining potential impact, especially on sessile species that cannot move quickly to avoid a noisy area. In particular, many sonars are designed to explore the sea bottom and, therefore, to reach the bottom at high intensity in order to receive backscattering. The upper hearing limit for fish and marine mammals is variable and can be as high as 160 kHz, suggesting possible effects of high frequency systems on animals at behavioural and physiological level (Dunning et al., 1992; Mann et al., 1997; Deng et al., 2014). However, effects of anthropogenic noise differ from species to species due to individual characteristics, behavioural responses, disturbance duration and emission characteristics. Furthermore, some animals can recover over time, while others suffer irreversible damage (Kight and Swaddle, 2011). A number of scientific studies describe the occurrence of behavioural and physiological changes in the presence of noise (Fewtrell and McCauley, 2012; Aguilar de Soto et al., 2013), including effects on immune response (Filiciotto et al., 2014; Celi et al., 2016; Vazzana et al., 2016) and on growth and development rates (Wysocki et al., 2007; Davidson et al., 2009) in marine organisms. For example, mussels exposed to low-frequency band treatment showed significantly higher values for cellular and biochemical stress parameters, such as glucose, total proteins, total number of haemocytes (THC), expression of heat shock proteins 70 (HSP70) and acetylcholinesterase (AChE) activity, measured in plasma and tissues (Vazzana et al., 2016). It is known that cellular stress response (in addition to molecular chaperone synthesis and the activation of the DNA repair system) also includes the enhancement of enzyme activities, which play an important role in environmental stress resistance (Sørensen et al., 2003). Alkaline phosphatase (AKP) is a metalloenzyme which catalyses the nonspecific hydrolysis of phosphate monoesters (Zhang, 2004). When exposed to different environmental stresses, lysosomal enzymes, in addition to AKP, are involved in the degradation of foreign proteins, lipids and carbohydrates (Ottaviani, 1984; Pipe et al., 1993; Xue and Renault, 2000), and can be used to assess immune status in invertebrates (Mou et al., 1999; Liu et al., 2000, 2004; Sarlin and Philip, 2011; Parisi et al., 2017). Esterase is one of the most common biomarkers of environmental exposure used in aquatic organisms (Galloway et al., 2002; Forget et al., 2003; Rickwood and Galloway, 2004; Barata et al., 2004; Hannam et al., 2008; Ren et al., 2015; Parisi et al., 2017). Another enzyme, peroxidase, is also involved in disease resistance and stress response, and changes in peroxidase levels can due to contaminants and other environmental stressors (Mydlarz and Harvell, 2007).

Moreover, cytotoxicity can be an efficient natural defence system in vertebrates and invertebrates; lysins can be secreted in the body fluids or act at the membrane level of the effector cell. Cytotoxicity was reported as a rapid and effective response to the K562 tumour cell line and rabbit erythrocytes in the fish *Dicentrarchus labrax* (Cammarata et al., 2000) and in the sea urchin *P. lividus* (Arizza et al., 2007), and against rabbit and sheep blood cells in sea cucumber *H. tubulosa* (Vazzana et al., 2018).

Although boat noise has been reported to influence behaviour and haemato-immunological parameters, such as THC, total protein concentration (PC), phenoloxidase (PO) activity, DNA integrity and HSPs protein expression (Celi et al., 2013, 2015; Filiciotto et al., 2014,2016) in crustaceans, nothing is known about the influence of acoustic stimulus on different enzymes and on cytotoxic activity in invertebrates.

Therefore, knowledge on acoustic perception in invertebrates is little or entirely lacking (even more so in echinoderms) and more information is necessary to understand the potential negative effects on these marine organisms.

During the first year of this study an indoor experiment was carried out at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo. The experiment was carried out with the collaboration of the CNR bioacoustics group. The aim of this study was to evaluate the effects of high frequency noise, from 100 to 200 kHz, on *Arbacia lixula* sea urchin in order to better understand the effects of this frequency-band noise on invertebrates. More specifically, the biochemical effect at cell-free coelomic fluid level was evaluated for the first time, including total protein concentrations, levels of alkaline phosphatase, esterase and peroxidase, degree of hemolysis (cytotoxicity), and the gene and protein expression of heat shock proteins 70 (HSP70).

3.1.1 Materials and methods

Animals

The experiment was carried out in the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) at the University of Palermo (Italy) according to current regulations regarding animal experimentation in Italy. Sea urchins, of the species *Arbacia lixula*, (weight ranging between 16 ± 1 gr) were collected from Terrasini, Gulf of Palermo, from environments characterized by rocky coves and steep cliffs hanging over the sea. The animals were acclimatized for one week in square tanks (1 m x 1 m, depth: 1 m, water height: 80 cm, volume: 800 L at 18 °C) with: re-circulated, filtered seawater and a flow-through of dissolved oxygen at 8 mg l^{-1} at 38 ‰ salinity. The organisms were fed commercially available invertebrate food (Azoo, Taikong Corp. Taiwan). For the experimental plan, the animals were chosen randomly from those available.

Experimental plan

Two rectangular tanks (85 cm x 43 cm, depth: 49 cm, water height: 27 cm, volume: 250 L) were used for the experiment: a test tank and a control tank (Fig. 12). A projector and a calibrated hydrophone were placed both in the control and the experimental tanks. 16 individuals of *A. lixula* were randomly divided in the two tanks, eight specimens for each. Only the individuals in the experimental group were exposed to acoustic stimulus for three hours, while no stimulus was applied to the control group. The control and experimental treatments were conducted at the same time.

The two tanks were located in two different cabinets without any contact points and no noise could be transmitted through vibration of the walls. Moreover, the different acoustic conditions (with and without acoustic stimulus) of the two tanks were monitored before trials, using the calibrated hydrophones located inside each tank (see “Acoustic stimulus, recording and analysis” Section for details).

The experiment was repeated three times, under the same environmental conditions using a total of 48 animals (24 control and 24 experimental). The number of the animals was chosen to guarantee an equitable distance of the individuals from each other, with respect to the walls of the tank and with respect to the acoustic emission considering any reflections or refraction of the acoustic wave. The animals were not fed in the 24 hours prior to the experiment. All sea urchins, treated and not, were then taken to extract cell-free coelomic fluid for subsequent biological assays.

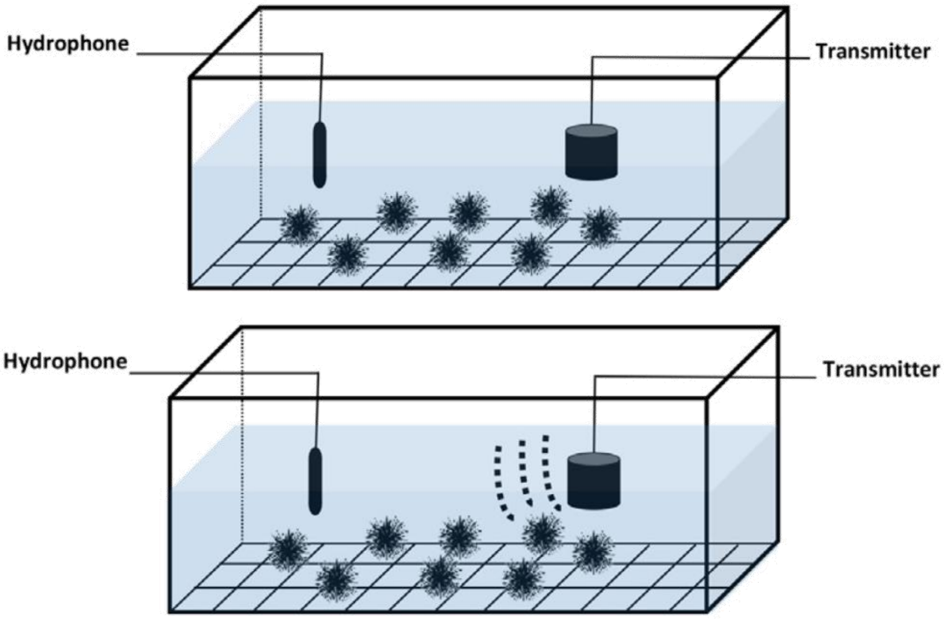


Fig. 12 Schematic drawing of the control and experimental tanks. Each tank was equipped with one hydrophone and one transmitter.

Acoustic stimulus, recording and analysis

A linear sweep ranging from 100 to 200 kHz for the duration of 1 second and emitted in continuous mode was used as acoustic stimulus. To generate the acoustic stimulus in the experimental tank, the Agilent 33210A signal generator was used. This was coupled with a projector as shown in Fig. 12. The characterization of background noise in both tanks and of acoustic stimulus in the experimental tank, were performed using the hydrophone (ResonTC 4034-3, Denmark; receiving sensitivity of -218 dB re 1V/µPa +2dB and -4dB in the range 1Hz - 250 kHz) located in each tank (Fig. 12) connected to an analogical/digital card (Avisoft USGH416b, Germany; sampling frequency 500 kHz, Gain 20 dB). This acquisition system was handled using specific software (Avisoft recorder USGH software, Germany). During the trials, the acoustic stimulus was monitored for 30 seconds every 30 minutes.

The background noise sound pressure level in the tanks was 140±1 dB (re 1µPa^{2}rms). Sound pressure during stimulus in the test tank ranged between 145 and 160 dB. The Power Spectrum and the spectrogram of the recorded acoustic stimulus are shown in Fig. 13.

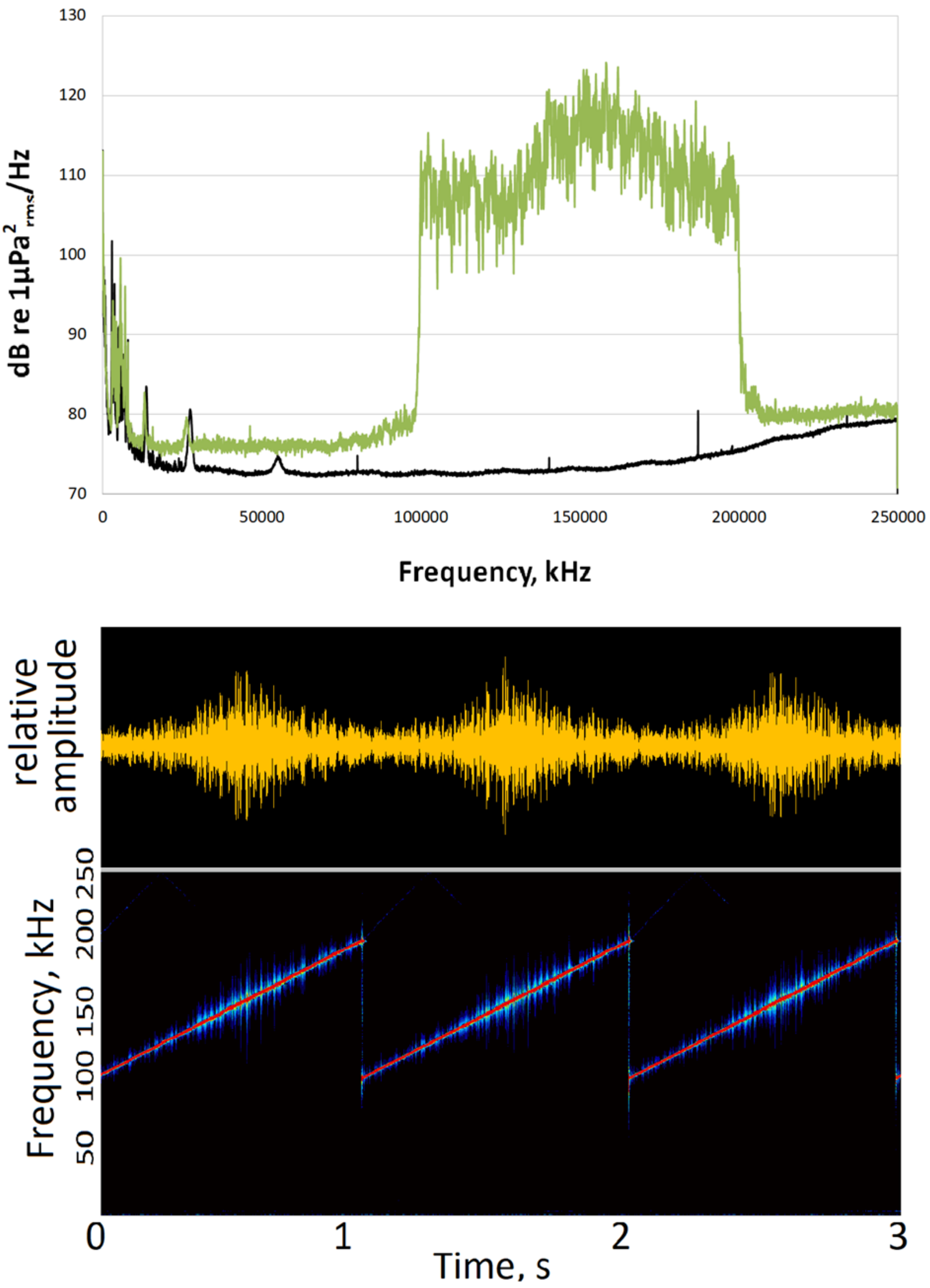


Fig. 13 Top: Power Spectrums of 3 consecutive sweeps and of the median background noise in the tanks (FFT size 16384, Resolution 30.5 Hz); below: oscillogram and spectrogram (FFT 1024, window Hamming), sampling frequency 500 kHz.

Coelomic fluid withdrawal

The sampling of coelomic fluid was carried out with isosmotic anticoagulant medium (ISO-EDTA: 20 mM Tris, 0.5 M NaCl, 30 mM EDTA, pH 7.5) in a centrifuge tube. Cell counts were performed with Neubauer chamber and each sample was centrifuged at 1650 rpm for 10 minutes at 4°C. The supernatant (cell-free coelomic fluid) was removed and stored at – 20 °C until the time of assaying. The pellets were separated into two aliquots (one for RT-PCR and one for western blot analysis) and stored at - 80 °C for subsequent analysis.

Protein concentration measure

Protein concentration was measured using the Bradford method (1976), both in the cell-free coelomic fluid and the pellet samples at 595 nm.

Alkaline phosphatase activity

Alkaline phosphatase activity was measured according to Ross et al., (2000), incubating 50 µl of *A. lixula* cell-free coelomic fluid with 50 µl of buffer (4 mM p-nitrophenyl liquid phosphate in 100 mM ammonium bicarbonate containing 1 mM $MgCl_2$, pH 7.8, 30 °C). Optical density (OD) was read at 405 nm for 1 hour.

Enzyme activity is expressed in U/µg and calculated as:

$$\{(Abs/min) \times (1000/Eb) \times (Vf/Vi)\}, \text{ with } Eb=18.4.$$

Where:

Abs/min was the Absorbance value obtained for each sample divided for the time of the measurement, in this case 1 hour and then 60 minutes.

Vf indicated final volume of well

Vi indicated inizial volume of well

Eb was a costant value related only for alkaline phosphatase enzymatic assay (18.4). One unit of activity was defined as the amount of enzyme required to release 1 μmol of p-nitrophenol produced in 1 minute.

Esterase activity

Esterase activity was evaluated by incubating 50 μl of *A. lixula* cell-free coelomic fluid with 50 μl of buffer (0.4 mM p-nitrophenyl-myristate substrate in 100 mM ammonium bicarbonate buffer containing 0.5 % Triton X-100, pH 7.8, 30 °C) (Ross et al., 2000). OD was read at 405 nm for 1 hour.

Enzyme activity is expressed in U/µg and calculated as:

$$\{(\mathrm{Abs/min}) \times (1000/\mathrm{Eb}) \times (\mathrm{Vf/Vi})\}$$

Where:

Abs/min was the Absorbance value obtained for each sample divided for the time of the measurement, in this case 1 hour and then 60 minutes.

Vf indicated final volume of well

Vi indicated inizial volume of well

Eb was a costant value related only for esterase enzymatic assay (16.4).

One unit of activity was defined as the amount of enzyme required to release 1 μmol of p-nitrophenol produced in 1 minute.

Peroxidase activity

Peroxidase activity was measured modifying the Quade and Roth (1997) method. Cell-free coelomic fluid (50 μl) was incubated for 30 minutes with 100 μl of TMB

(3.3', 5.5' tetramethylbenzidine) (Sigma, Italy). The reaction was stopped with 50 µl of 2M sulfuric acid (H_2SO_4). OD was read at 450 nm. Peroxidase activity was expressed as unit U/µg. One unit of activity was defined as the amount of enzyme required to release 1 µmol of substrate produced in 1 minute.

Cytotoxicity assay

To analyse the cytotoxic activity of cell-free coelomic fluid, rabbit and sheep erythrocytes, provided by the Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri (IZS, Palermo), were used as target cells. Erythrocytes were washed three times in PBS and suspended at 1% (8×10^6 fresh erythrocytes) in ISO–Ca^{2+} (0.5 M NaCl, 20 mM Tris-HCl, 10 mM $CaCl_2$; pH 7.4). The 100 µl samples were incubated with 100 µl of erythrocyte solution for 1hour at 37 °C. At the end of the incubation period, the samples were centrifuged for 10 minutes at 1500 rpm at 4 °C and the amount of released haemoglobin in the supernatant was measured at 540 nm using a microplate reader (GloMax; Promega Corporation, USA).

To obtain the degree of hemolysis, the following formula was used:

$$\frac{\text{O.D. measured release} - \text{O.D.T. spontaneous release}}{\text{O.D. complete release} - \text{O.D.T. spontaneous release}} \times 100$$

Where:

O.D. measured release was the Optical Density value obtained from each sample.

O.D.T. spontaneous release was the Optical Density value of blood cell control obtained incubating erythrocyte only in ISO–Ca^{2+}

O.D. complete release was the Optical Density of total hemolysis obtained incubating only the erythrocyte then crushed with distilled water.

Complete haemoglobin release values were obtained by preparing an erythrocyte in distilled water at room temperature; a control erythrocyte suspension was prepared under identical experimental conditions using the same medium and incubation in order to measure haemoglobin release values.

RNA Extraction from Arbacia lixula coelomocyte

The HSP70 gene expression was evaluated using one aliquot of cellular pellet. RNA extraction was performed using an RNA MiniPrep kit (Direct-zol™). To lyse the samples, 5 x 10^6 cells were resuspended in 600 µl of trizol. The samples were then centrifuged at 10,000g for 30 seconds to remove particulate debris and the supernatant transferred to a new tube. An equal volume of ethanol (95%) was added to the supernatant and mixed thoroughly. This was transferred into a Zymo-Spin IIC Column within a Collection Tube before centrifugation; the flow-through was discarded. Subsequently, 400 µl of RNA wash buffer was added to samples and centrifuged. In a new RNase-free tube, 5 µl of DNaseI and 75 µl of DNA digestion buffer were mixed. This solution was added directly to the column. Samples were incubated at room temperature (20 °C) for 15 minutes. 400 µl of Direct-zol™ RNA Pre Wash was then added to the column and centrifuged. Flow-through was discarded before repeating this step and discarding flow-through once again. At this point, 700 µl RNA Wash Buffer was added to the column and centrifuged with an extended time of two minutes (to ensure total removal of wash buffer). The column was transferred into a new RNase-free tube. RNA was eluted by adding 50 µl DNase/RNase-Free Water to the column and centrifuging. At the end, 1 µl of RNA was extracted from *A. lixula* ceolomatic liquid cell samples and used in real-time polymerase chain reaction.

Real Time PCR of Arbacia lixula RNA for HSP70

HSP70 gene expression was detected by Real-Time PCR (RT-PCR). This was performed using the Rotor-gene Q system (Qiagen) with QuantiTechSYBR Green RT-PCR kit. The QuantiTechSYBR Green RT-PCR kit provides accurate, real-time quantification of RNA targets in an easy to handle format. Use of 2xQuantiTechSYBR Green RTPCR master mix together with QuantiTechSYBR Green RT PCR mix allowed both reverse transcription and PCR to take place in a single tube; converting extracted RNA into cDNA. The products supplied for this Real-Time included 1µl of each RNA sample, 0.5 µl of forward primer and 0.5 µl of reverse primer, 2 x 12.5 µl of QuantiTechSYBR Green RT-PCR Master Mix, 0.5 µl of QuantiTectRT mix and 10.75µl of distilled water added to complete the amounts. Specific primers set for HSP70 (accession no. X61379), according to Marrone et al., (2012), were used. RT-PCR was run as follows: reverse transcription 1× cycle at 50 °C for 30 minutes, PCR initial activation step at 95 °C for 15 minutes and 40 cycles, denaturation 15 seconds 95 °C, annealing at 60 °C and 30 seconds at 72 °C for extension.

SDS-PAGE and western blot

The remaining aliquot of the cellular pellet was homogenized for eight minutes in a Potter-Elvehjem tissue grinder using 1 ml of trizma base (20 mM, pH 7.5) with 300 mM NaCl and 10% SDS; this was followed by sonication for 60 seconds.

Samples were centrifuged at 9000 rpm for 10 minutes at 4 °C and protein concentrations in the cell lysate supernatants (CLS) were evaluated.

Using western blot analyses (Towbin et al., 1979) we performed HSP70 protein expression. The equivalent of 20 μg/ml of *Arbacia lixula* cellular lysate supernatants were separated on 7.5 % SDS-PAGE gels at 60 V for the first 10 minutes and at 120V for 1hour. The separated proteins were subsequently transferred to polyvinylidenedifluoride (PVDF) membranes (Bio-Rad) at 15 V for 1hour in a transfer buffer (48 mM tris, 39 mM glycine, 20% v/v methanol, pH 8.3) and a wet transfer apparatus (Bio-Rad, Mini-Protean II Cell); correct transfer was confirmed by Ponceau red-staining. After blocking PVDF blots, HSP70 was detected by overnight incubation at 4 °C with antimouse HSP70 (primary antibody dilution was 1:7500, Sigma-Aldrich). After incubation, PVDF blots were washed twice with TBS-T 1X and incubated with secondary antibody alkaline phosphatase-conjugated goat antimouse IgG (1: 3000 dilution, Sigma-Aldrich). Development was finally done with BCiP-NBT.

Densitometric analysis of the immunoblotted bands was performed using ImageJ software. Densitometry data were expressed as the mean values of different experiments and reported as a percentage of the integrated density value. The integrated density value (I.D.V.) of the relevant bands was normalized to the I.D.V. of the beta-actin bands (data not shown).

Statistical Analysis

All the data were statistically analysed to assess possible differences between the control and experimental groups. To determine statistical differences, the data were analysed using a non-parametric test (Mann-Whitney U test) as our data did not fall into a normal distribution (verified by a Shapiro-Wilk tests p< .05). Statistical differences between control and experiment are shown on the error bars for each graph as ***p<.001, **p<.01, *p<.05.

3.1.2 Results

No statistical differences were found between the control and experimental values (p=.657533; Z=0.44) for total protein levels in cell-free coelomic fluid. Fig. 14 shows approximately the same values in the control and experimental samples, 54.51 ± 4.6 (mean ± SD) µg/ml and 53.95 ± 4.6 µg/ml respectively.

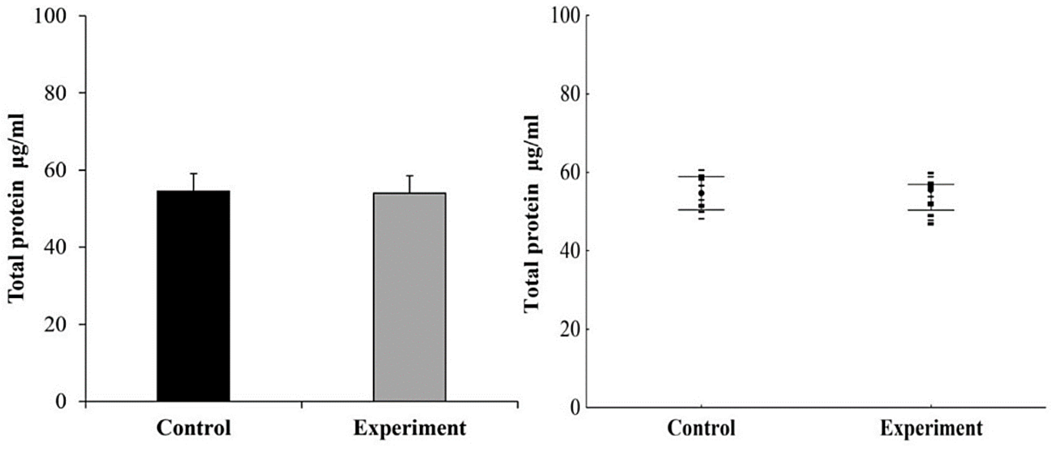


Fig. 14 Bar-graph (left) and dot-plot (right) of protein concentration (µg/ml) of cell-free coelomic fluid expressed as means±SD, (−) raw data, (•) median values and (⌶) percentiles values.

Our experimental plan also included an evaluation of the different enzyme activity involved in immune responses. The alkaline phosphatase activity of experimental cell-free coelomic fluid was significantly influenced (p=.000012; Z=-4.38) by high frequency noise, showing higher values (7.6 ± 4.84 U/µg) compared to the control (2.38 ± 1.90 U/µg) (Fig.15).

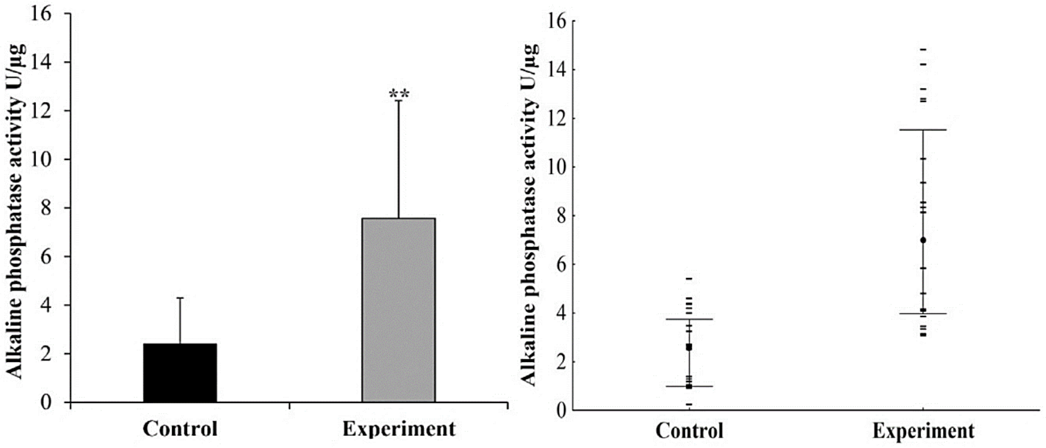


Fig. 15 Bar-graph (left) and dot-plot (right) of alkaline phosphatase activity (U/µg) of cell-free coelomic fluid expressed as means±SD. The asterisks indicate the significant difference (**p < .01), (−) raw data, (•) median values and (⌶) percentiles values.

As shown in Fig. 16, the esterase activity in cell-free coelomic fluid was significantly higher (p=.000000; Z=-5.57) in the experimental samples (71.96± 50.61 U/µg) compared to the control (21.68± 12.31 U/µg).

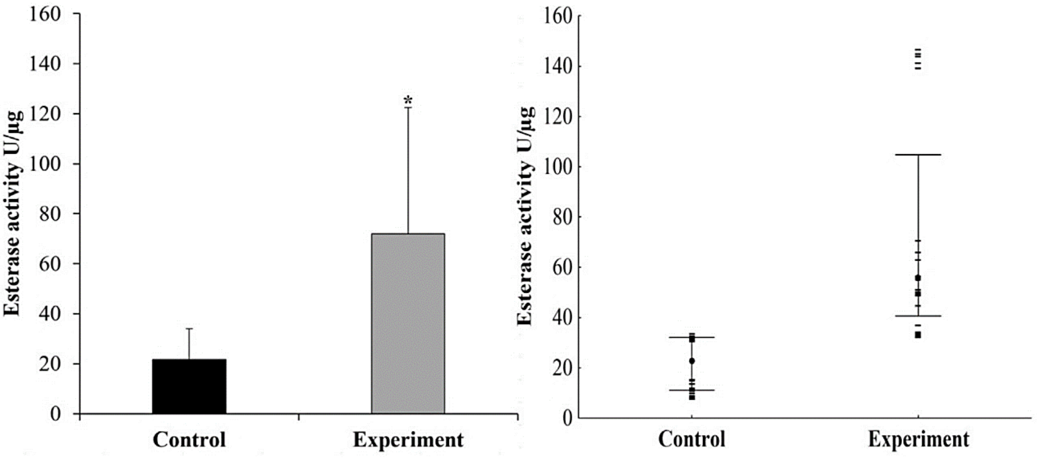


Fig. 16 Bar-graph (left) and dot-plot (right) of esterase activity (U/µg) of cell-free coelomic fluid expressed as means±SD. The asterisk indicates the significant difference (*p < .05), (−) raw data, (•) median values and (⌶) percentiles values.

Cell-free coelomic fluid of the experimental groups had a significantly (p=.000553; Z=-3.45) higher amount (6 ± 3.76 U/µg) of peroxidase activity compared to the control (2.70± 1.85U/µg, Fig.17).

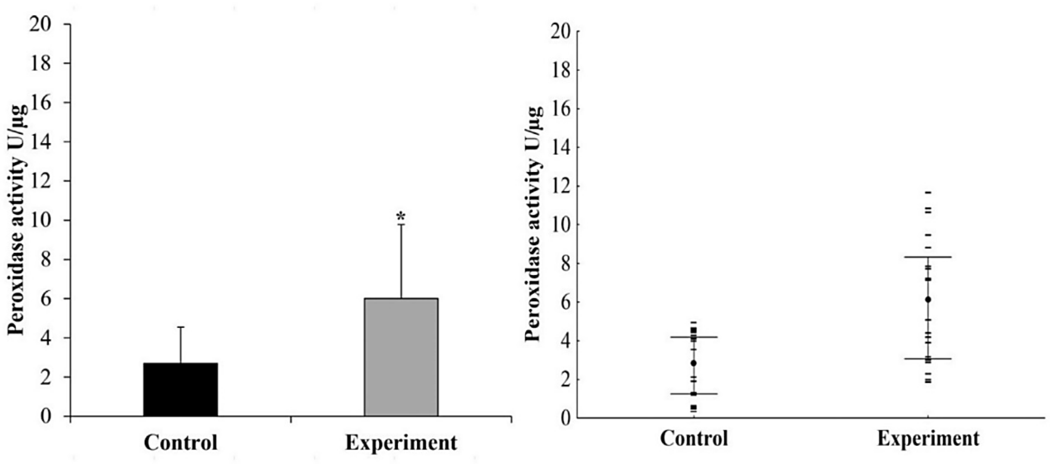


Fig. 17 Left: bar graph of peroxidase activity (U/µg) of cell-free coelomic fluid is expressed as means±SD. The asterisk indicates the significant difference (*p < .05) calculated using Mann-Whitney U test to compare the means values. Right: dot plot of peroxidase activity (U/µg) represented like raw data (−), median values (•) and percentiles values (⌶).

Regarding cytotoxicity (Fig.18) in the control samples, the degree of hemolysis was found to be 4.99 ± 1.91 % against sheep erythrocytes and 3.93 ± 1.72 % against rabbit erythrocytes. Significant increases were observed in the degree of hemolysis of experimental samples against rabbit erythrocytes with values of 11.91 ± 2.68 % (p =.000037; Z=-4.12) and against sheep erythrocytes with values of 9.92 ± 4.46 % (p =.001652; Z=-3.14).

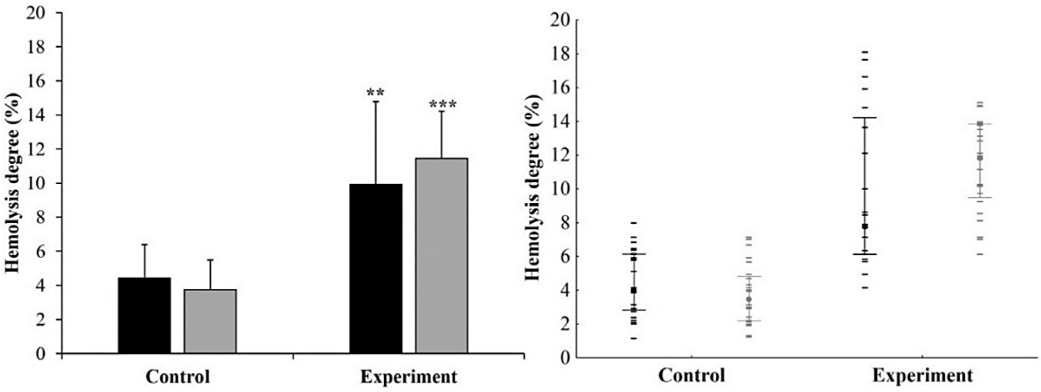


Fig. 18 Bar-graph (left) and dot-plot (right) of hemolysis degree (%) of cell-free coelomic fluid expressed as means±SD. Cell-free coelomic fluid tested against sheep erythrocytes (■); Cell-free coelomic fluid tested against rabbit erythrocytes (▨). The asterisks indicate the significant differences between control and experimental (**p < .01; ***p < .001), (−) raw data, (•) median values and (⌶) percentiles values.

Evaluation of the gene expression of HSP70 (Fig.19) in *A. lixula* with primers of *P. lividus* highlighted that the experimental group had significantly higher (p=.000037; Z=-4.12) relative quantification (7.18) compared to the control. Relative HSP70 expression was calculated by dividing the normalised value of HSP70 by the normalised value obtained from the control sample. Densitometric analysis showed significant lower (p=.008616; Z=2.62) values of HSP70 in the experimental group.

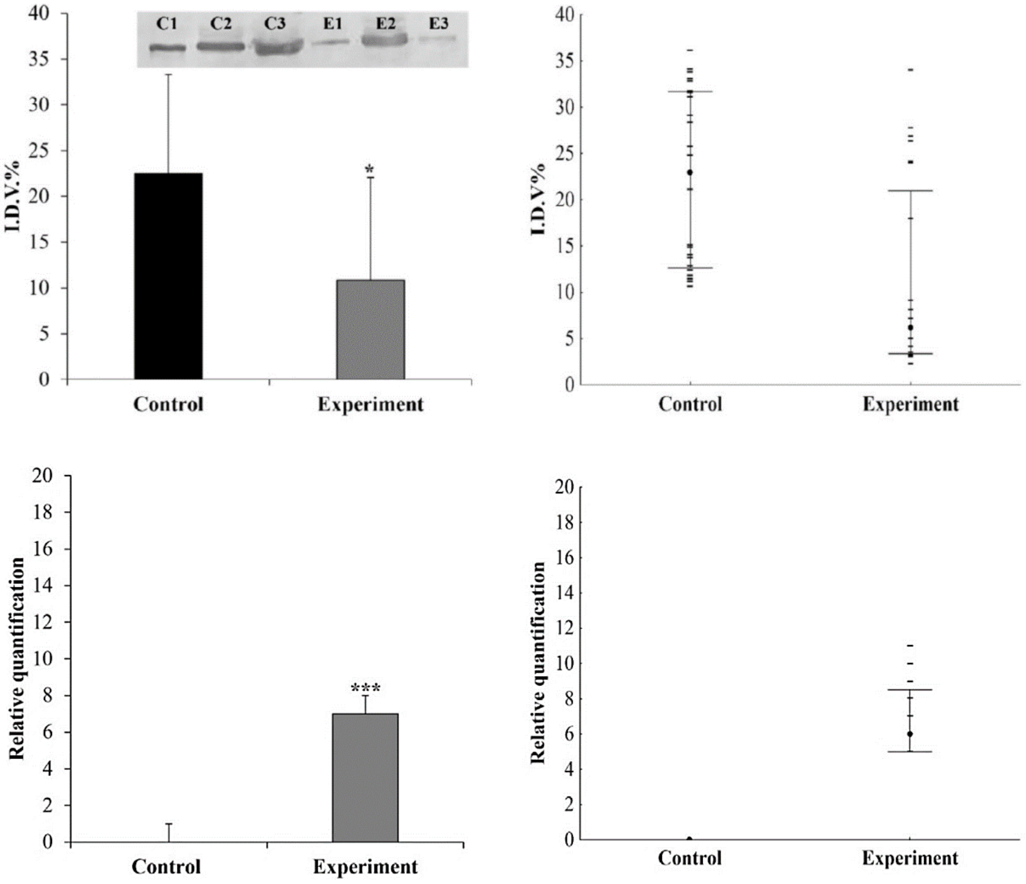


Fig. 19 Bar-graph (left up) and dot-plot (right up) of I.D.V. of the western blot HSP70 protein bands (inset). C1, C2, C3 were control samples; E1, E2, E3 were experimental samples. Bar-graph (left down) and dot-plot (right down) of real-time PCR analysis of HSP70 expression. The statistical differences are indicated with the asterisks (*p<.05, ***p < .001), (−) raw data, (•) median values and (⌶) percentiles values.

3.1.3 Discussion

In coastal environments, background noise at high frequencies is influenced by human-generated noise, such as sonars, CHIRP, multi-beam devices, etc. The use of this instrumentation is widespread, mostly in coastal areas and is linked to fishing activities, recreational boats and large vessels using sonars for safe navigation in shallow waters. Impact, in terms of low and mid-frequency noise, was investigated and noise presence could be heard continuously in the area, reaching at least 30% of daytime presence (Buscaino et al., 2016; Ceraulo et al., 2018). Source levels of sonars can reach high values, even greater than 220 dB (re 1µPa @1m), and acoustic stimulus, tested with an SPL of 160 dB re 1µPa, represents a realistic sound that an animal attached to the bottom could receive. However, despite the fact that the vast majority of animals, including aquatic species, rely on sound (Bregman, 1990) for communicative and survival purposes, no studies have reported the impacts of high frequency sound and potential effects on benthic species which are unable to move rapidly to get away from noise. In this study, the *A. lixula* sea urchin was used for the first time to investigate the impact of high frequency noise at cellular, enzyme, cytotoxic and gene/protein expression levels.

Total protein concentrations in cell-free coelomic fluid samples did not show significative differences between control and treated specimens. This result is in accord with data reported by Celi et al. (2013, 2016) on haemolymph of *Procambarus clarkii* and on plasma of *Sparus aurata* exposed to boat noise recordings.

In our study, the reason for this lack of response could be attributed to the acoustic frequencies of the stimulus used. It has been demonstrated, in fact, that the modulation of total protein concentration could depend on the type of frequency and the duration of acoustic stress (Coeurdacier et al., 2011). Individuals of *Chromis chromis* exposed to acoustic stimuli at 300 Hz showed significantly higher plasma total protein levels compared to untreated fish; however, when exposed to 200 Hz, no significant differences were found (Vazzana et al., 2017). As an immune response indicator, a number of scientific studies have considered endogenous and antioxidant enzyme activities in haemolymph and in tissues of many bivalve species (Beckmann et al., 1992; Hine and Wesney, 1994; Carballal et al., 1997; Toreilles et al., 1997; Chen et al., 2007; Parisi et al., 2017). Esterase activity, for example, was chosen as a biomarker in mollusca exposed to organophosphorus and carbamate pesticides (Galloway et al., 2002; Valbonesi et al., 2003; Bolton-Warberg et al., 2007; Wheelock et al., 2008; Solé et al., 2010) and recently it has been used also in toxicological studies performed on echinoderm embryos and larvae (Torres-Duarte et al., 2019). The hydrolase alkaline phosphatase (AKP) was used to evaluate the state of health of the sea cucumber (Wang et al., 2008a, b; Mazorra et al., 2002; Jing et al., 2006b) and increased AKP activity was reported in sea urchin *Strongylocentrotus intermedius* gametes following exposure to environmental pollutants. This suggests a correlation between phosphatase concentration and environmental stress (Seitkalieva et al., 2016). In addition to hydrolase activities, cells have various mechanisms to reduce and repair damage; antioxidant enzymes are an example of this: the first line of defence against free radicals (Chainy et al., 2016).

Regarding echinoderms, Rabeh et al. (2019) showed an up-regulation of glutathione peroxidase (GPx) in *Holothuria forskali* intestine treated with mercury, and

Telahigue et al. (2019) confirmed this result in the body wall of the same species.

Our results showed significant increases in esterase, alkaline phosphatase and peroxidase activity in cell-free coelomic fluid of treatment animals, confirming that even noise modulates enzymes involved in immunity.

Many marine species exposed to stress conditions or potentially pathogenic microorganisms have defence responses based on immunocytes and humoral factors contained in coelomic fluid (Coffaro and Hinegardner, 1977; Smith, 1981; Chia and Xing, 1996; Smith et al., 1996; Pancer et al., 1999; Gross et al., 2000; Pancer, 2000; Kudriavtsev and Polevshchikov, 2004). Focusing on invertebrates, in addition to the defense activity performed by enzymes in stress conditions, cytotoxic activity acts through the release of lysines in body fluids. In echinoids, in particular, it is known that amebocytes and spherulocytes are the main coelomocyte populations that perform cytotoxic activity (Bertheussen, 1979; Lin et al., 2001); coelomic fluid of *Paracentrotus lividus* sea urchin contains different types of celomocytes (including amebocytes and colourless spherulocytes) involved in immune defence. Furthermore, the cytoplasmic granules of this species show a calcium-dependent cytolytic activity (Canicattì, 1991; Pagliara and Canicattì, 1993; Arizza et al., 2007).

In our study, significant increases in toxicity levels against rabbit and sheep erythrocytes were detected. The significant increase in cytotoxic activity of cell-free coelomic fluid against mammalian erythrocytes could be due to the release of lysins by competent cells circulating in the coelomic fluid as a response to acoustic stress. Another good biomarker to study environmental pollution in invertebrates is the HSP protein family, given their cytoprotective role (Hamer et al., 2004; Anestis et al., 2007; Vazzana et al., 2016). In particular, the heat shock proteins HSP70 protect cells from apoptosis by interacting directly with cellular signalling pathways and apoptotic factors (Lanneau et al., 2008). Both HSP70 genes and proteins are inducible and involved in immune response. Changes in pH, exposure to metals and UV-B radiation increase the expression levels of HSP70 in celomocytes of *P. lividus* (Matranga et al., 2000, 2002) and the use of these as a model to test whether environmental stress can affect HSP70 expression was evaluated (Matranga et al., 2000). Individuals exposed at 4 °C showed an HSP70 gene expression five times greater than the control group, while at 35 °C, a 2-fold expression was detected. The up-regulation of this gene is indicative of a link between expression and stress in the body, providing evidence for the use of HSP70 as a molecular marker in the study of stress factors in sea urchin. Pinsino et al., (2008) used *P. lividus* coelomocytes to assess environmental stress by evaluating the production of HSC70 proteins. Increased production of HSC70 is a defense mechanism of *P. lividus* coelomocytes used to cope with metal pollution. HSC70 is a protein part of the multi-gene family used as a molecular marker in long-term stress exposure, while HSP70 in short-term stress exposure (Ryan and Hightower, 1996). This validates the use of HSP70 in our study as a molecular marker in *A. lixula* exposed to short-term acoustic stimulus. Hamer et al. (2004) showed that the expression of HSP70 reached highest levels in the gills of *M. galloprovincialis* following exposure to environmental pollution, while Vazzana et al., (2016) showed how acoustic noise up-regulates HSP70 protein expression in the gills and mantle of *M. galloprovincialis*. The results of real-time PCR in this study showed a significant increase in HSP70 gene expression in coelomocytes of individuals treated with acoustic emission at 100-200 kHz. At high levels of gene expression, there is a decrease in the protein available in circulation in the experimental samples, probably due to the use of the protein produced in response to three hours of acoustic emission.

There are still very few studies in literature to describe the effects of noise on invertebrates and even fewer concerning echinoderms. In this study, we showed, for the first time, that acoustic emission between 100 and 200 kHz, lasting three hours, significantly influences some immune responses in *A. lixula* sea urchin, such as enzyme activity of cell-free coelomic fluid and HSP70 gene/protein expression levels, demostrating a perturbation of homeostasis. This highlights the fact that sea urchin could be an important species to study the effects of noise on marine organisms and that cell-free coelomic fluid could be considered a good matrix to evaluate animal welfare. This is reinforced by its wide distribution and by its unusual anatomical structure (an ovoid calcareous skeleton) which acts as a Helmholtz resonator (Radford et al., 2008). In conclusion, this study contributes to knowledge on the effects of high frequency noise on marine invertebrates. This is of considerable importance since negative impacts on marine animals could have considerable consequences, even at population level.

The results of this research were presented at the 79° National Congress Of Italian Zoological Union (UZI). Lecce, 25 - 28 September 2018 (see Chapter 7)

3.2 Effects of acoustic stimulus on biochemical parameters in digestive gland of *Mytilus galloprovincialis* (Lamark, 1819)

A number of underwater species rely on sound for key life activities: crustaceans use sound during mating (Buscaino et al., 2015), fish orientate themselves by the reception of environmental sounds (Simpson, 2005) and also produce sounds during mating periods (Casaretto et al., 2014), and dolphins use sound for navigation and orientation, for catch, for communication, etc. (Au, 1993; Lammers et al., 2003).

Low frequency (LF) noise, continuous or impulse, generated by boat and seismic surveys has recently been categorized as a threat to marine fauna by the Marine Strategy Framework Directive (Directive 2008/56/EC). The ability to propagate over long distances makes it harmful to several of marine species. Blood and faecal analyses carried out on samples from marine mammals demonstrated that LF noise causes an increase in levels of norepinephrine, epinephrine, and dopamine in the white whale (Romano et al., 2004) and an increase in glucocorticoids levels in the North Atlantic right whale (Rolland et al., 2012). In the Section 1.6.1 it has been said that that also for fish species the acoustic stimuli from ships cause significant changes in blood parameters and increased cortisol plasmatic concentration (Wysocki et al., 2006). Moreover, also noise propagated from recreational boating activities can generate sublethal physiological disturbance in fish species (Graham et al., 2008). The persistence over time of this noise condition, characterized by amplitude and frequency fluctuations, could compromise the acquisition of food, the migration, the reproduction, and the intraspecific communication (Slabbekoorn et al., 2010; Stanley et al., 2017; Buscaino et al., 2019).

Unfortunately, a lot of human activities in the sea are carried out with the fundamental support of acoustic instrumentation, producing loud sound at very high frequency (i.e. fishing, military and scientific sonars, acoustic deterrent devices, underwater acoustic positioning systems, acoustic modems, etc) even as great as 200 kHz (Hildebrand 2009; Bonanno et al., 2006; Buscaino et al., 2009). This kind of noise consists of extremely loud tones or broadband-frequency modulated signals, typically used for navigation, detection, localization, communication, mapping and surveillance (Hildebrand, 2009). These sounds are considered ubiquitous given their widespread use for marine and civilian purposes.

The hearing range of marine mammals largely covers those frequencies and effects ranging from temporary threshold shift to death have been documented (Wright et al., 2007). Beaked whale mass strandings have been associated with the use of mid-frequency naval sonars (Fernandez et al., 2005). Although exposure to these kinds of sonar (2-10 kHz) might not have acute effects on fish tissue (Kane et al., 2010), temporary threshold shifts have been detected for catfish (Halvorsen et al., 2012). Regarding invertebrates, Celi et al. (2013) observed aggressive behavioural patterns and changes in components of the haemato-immunological system in red swamp crayfish when exposed to an acoustic stimulus within a frequency band of 0.1–25 kHz, clearly reflecting a stress condition.

Roberts et al. (2015) quantified behavioural changes of the bivalve *Mytilus edulis* exposed to substrate-borne vibration of tonal signals at frequencies in the range of 5 to 410 Hz. Vazzana et al. (2016) recently evaluated physiological sensitivity to underwater noise in the Mediterranean mussel, *Mytilus galloprovincialis*. This species has considerable commercial value and is considered a key sentinel organism in the biomonitoring of environmental pollution. Results showed significant increases in plasma protein levels and glucose levels in response to acoustic stimulus, especially at the frequency band 0.1-5 kHz.

During the first year of this study a second indoor experiment was carried out at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) with the collaboration of the CNR bioacoustics group. The aim of this experiment was to gain a better understanding of the effects of very high-frequency (100-200 kHz) noise pollution on *M. galloprovincialis*. In order to test if acoustic stimulus triggered a stress status in the animals, total haemocyte count (THC) was determined in haemolymph (Vazzana et al., 2016). Furthermore, due to the role played by the mussel’s digestive gland in intracellular and extracellular digestion, storage of nutritive substances (Bayne et al., 1976), and in the reproductive activities (Robledo and Cajaraville, 1997), the digestive gland was used as a biological matrix for the first time. Glucose levels, cytotoxic activity and enzyme activity (alkaline phosphatase, esterase and peroxidase) were evaluated at the digestive gland level.

3.2.1 Materials and methods

Experimental plan

For the experiments, adult specimens of *Mytilus galloprovincialis* were used. A total of 36 individuals of mussels were obtained from a local supplier in Palermo. The mussels were transferred to a holding tank (1m x 1m, depth: 1m, water height: 80 cm, volume: 800 L at 13 ± 3 °C) with re-circulated, filtered seawater and a flow-through of dissolved oxygen at 8 mg l^{-1}. All animals were acclimatized for one week, during which time they were feed with commercially available invertebrate food (Azoo, Taikong Corp. Taiwan). The mussels had a mean (± standard deviation) weight of 18.30 gr ± 1.36 and length of 4.55cm ± 0.21. After acclimation, 12 individuals were divided randomly between two tanks (85 cm x 43cm, depth: 49 cm, water height: 27 cm, volume: 250 L), one as the control and one for the experimental tests (six for each tank and the latter subjected to acoustic stimulus). The animals were not fed in the 24 hours before the start of the experiment. Near the tanks, a workstation was created for the sound-generator and recording systems, a schema of the tanks is represented in Fig. 20. In the experimental group, the animals were exposed to an acoustic stimulus; after three hours, haemolymph and the digestive gland were extracted from each individual for biochemical subsequent assays. The experiments were performed in triplicate using 18 specimens for the control and 18 for the experiment. The number of the animals was chosen according to Vazzana et al., (2016) and to guarantee an equitable distance of the individuals from each other, with respect to the walls of the tank and with respect to the acoustic emission considering any reflections or refraction of the acoustic wave. All individuals handling the animals and all experiments were approved according to current regulations regarding animal experimentation in Italy.

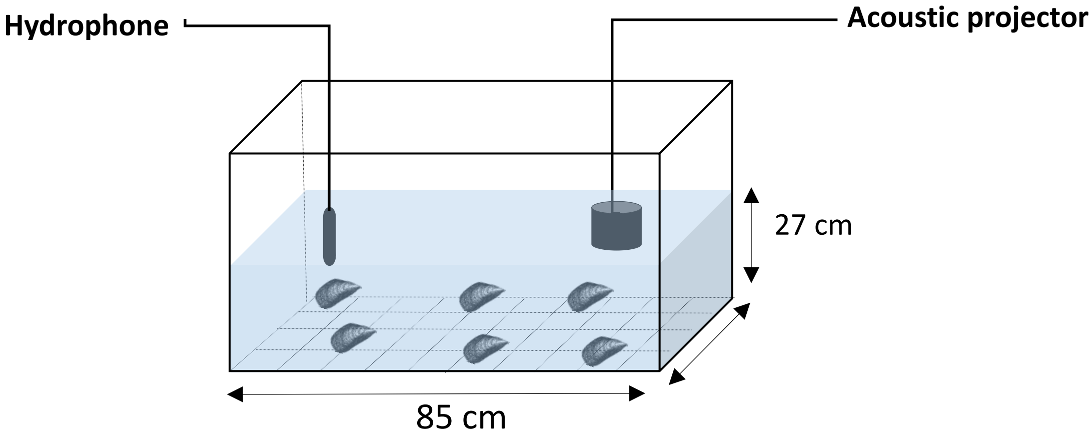


Fig. 20 Schematic view of tank. Six individuals of *M. galloprovincialis*, a sound projector and a hydrophone were positioned in each tank (control and experimental). In the control tank, the projector did not emit any acoustic stimulus.

Acoustic stimulus

The acoustic stimulus consisted of a linear sweep with lower - upper frequencies of 100 to 200 kHz and a duration of 1second. We used a projector (built in our laboratory) driven by a signal generator (Agilent 33210A, USA) (Fig. 20). The animals in the control tank were not subjected to any acoustic stimulus. To avoid any differences between the two tanks, an identical silent projector was positioned in the control tank. To characterize the background noise in both tanks, and the acoustic stimulus in the experimental tank, a hydrophone (ResonTC 4034-3, Denmark; receiving sensitivity of -218 dB re 1V/µPa +2dB and -4dB in the range 1Hz - 250 kHz) was connected to an analogical/digital card (Avisoft USGH416b, Germany; sampling frequency 500 kHz). Specific software (Avisoft recorder USGH software, Germany) was used to manage the acquisition system. During the trials, the acoustic stimulus was monitored for 30 seconds every 30 minutes.

The power spectral density of the tank background noise and of the recorded acoustic stimulus in the test tank is shown in the Fig. 21. The Sound Pressure Level in the test tank during acoustic stimulation ranged between 145 and 160 dB (re $1\mu Pa_{rms}$, time average size 30 ms), by contrast, in the control tank the level was 140±1 dB (re $1\mu Pa_{rms}$, time average size 30 ms).

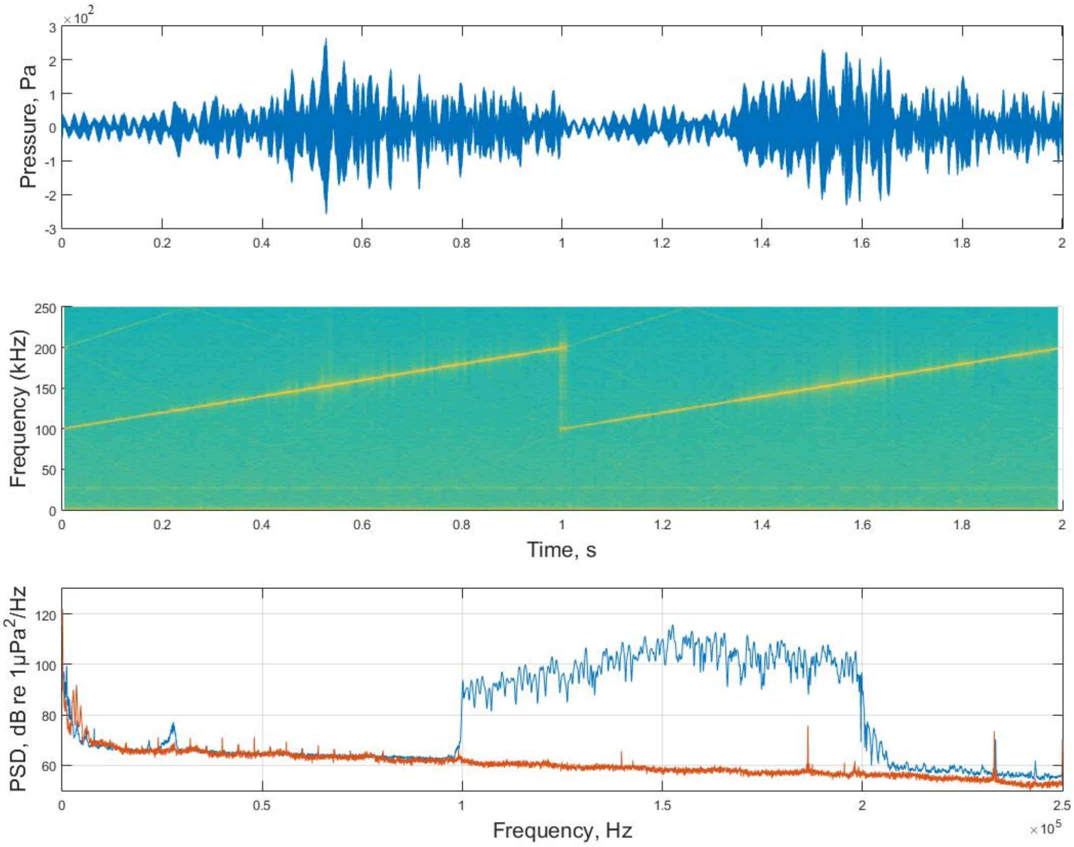


Fig. 21 Upper: Oscillogram of two sweeps. Middle: spectrogram of two sweeps (sampling frequency: 500000 sample per second, FFT size 8192 sample, time segments overlap 50%). Lower: mean power spectral density (dB re 1 $\mu Pa^2/Hz$) of two sweeps (blue) and the background noise (red).

Haemolymph collection

At the end of acoustic emission (three hours), the mussels (control and experiment) were removed from the aquaria. Haemolymph (800 µl) was collected from the adductor muscle of each mussel using a 1 ml sterile syringe in the presence of 200 µl of an anticoagulant solution (0.45 M NaCl, 30 mM sodium citrate, 26 mM citric acid and 10 mM EDTA). Total number of haemocytes per ml (THC) was determined using a Neubauer haemocytometer chamber.

Digestive gland extraction

The digestive gland was removed from each specimen using a scalpel and stored at −80 °C until time of analysis. The glands were homogenized separately in 1 ml of PBS solution (NaCl 137 mM, KH_2PO_4 1.76 mM, Na_2HPO_4, 8.1 mM, KCl 2.7 mM, $CaCl_2$ 1.19 mM, and $MgCl_2$ 1.05 mM). Tissue homogenate was centrifuged at 9000g at 4 ºC for 25 minutes and the supernatant used to evaluate protein concentration, glucose levels, cytotoxic activity and enzyme activity (alkaline phosphatase, esterase, peroxidase).

Glucose evaluation

Glucose levels in the digestive gland were determined using an Accutrend GCkit (Roche) according to the manufacturer's instructions.

Cytotoxic assay

Cytotoxic activity of the mussel plasma (v/v) was evaluated using sheep erythrocytes at 1% in TBS Ca^{2+} as target cells. After incubation at 37 °C for one hour, the samples were centrifuged at 400 g and 4 °C for ten minutes. Optical density was measured at 540 nm using a microplate reader (GloMax®-MultiDetection System; Promega Corporation, Madison, Wisconsin, USA). To calculate the degree of hemolysis we used the following formula:

$$\textbf{Degree of hemolysis} = \frac{\text{O.D. measured release} - \text{O.D.T. spontaneous release}}{\text{O.D. complete release} - \text{O.D.T. spontaneous release}} \times 100$$

Where:

O.D. measured release was the Optical Density value obtained from each sample.

O.D.T. spontaneous release was the Optical Density value of blood cell control obtained incubating erythrocyte only in in ISO–Ca^{2+}

O.D. complete release was the Optical Density of total hemolysis obtained incubating only the erythrocyte then crushed with distilled water.

Enzyme assay in M. galloprovincialis digestive gland

After estimate of total proteins in the digestive glande of each sample, according to the Bradford method (1976), the enzyme activities (alkaline phosphatase, esterase and peroxidase) were evaluated. Alkaline phosphatase, esterase and peroxidase activity were performed as in Section 3.1.1

Statistical Analysis

All the data were statistically analysed to assess possible differences between the control and experimental groups. The data were analysed using a non-parametric test (Mann-Whitney U test) as our data did not fall into a normal distribution (verified by a Shapiro-Wilk tests $p < .05$). Statistical differences between control and experiment are shown on the error bars for each graph as ***$p<.001$, **$p<.01$, *$p<.05$

3.2.2 Results

Mussel *M. galloprovincialis* exposed to three hours of noise at very high frequency showed changes in a number of biochemical parameters.

Total haemocyte count (THC) in haemolymph of *M. galloprovincialis* (Fig. 22) showed higher values in experimental individuals ($1*10^{6}\pm1.3*10^{6}$) than in the control ($1.4*10^{6}\pm8*10^{5}$), although differences were not significant ($p=.936956$; $Z= -0.07$).

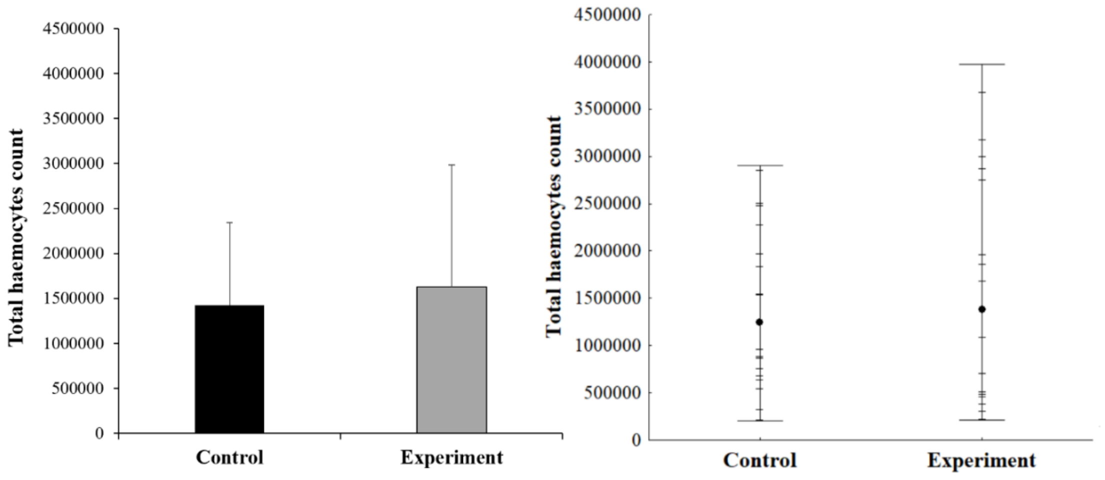


Fig. 22 Left: Bar graph of THC of of *M. galloprovincialis* haemolymph, expressed as a mean ± SD. Right: dot plot of THC represented like raw data (−), median values (•) and percentiles values (⌶).

Glucose levels in the digestive gland of animals treated with acoustic stimulus for three hours were significantly lower (p=.049811; Z=1.96) than that of the control groups, as shown in Fig. 23.

In particular, glucose levels in the control were 367±96.3 mg/dl while in the experiment they were 301±69 mg/dl.

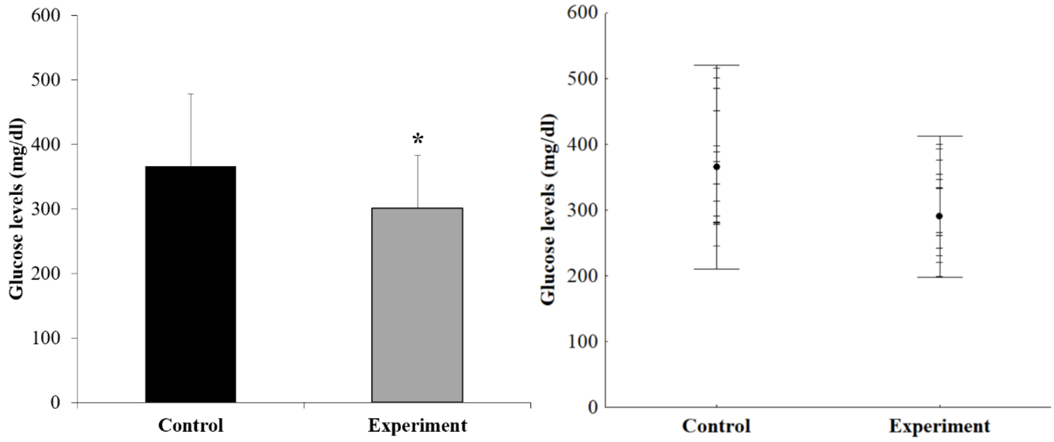


Fig. 23 Left: Bar graph of the glucose levels of mussels exposed to acoustic stimulus for three hours, expressed as a mean ± SD. Asterisks represent significant differences (*p < .05) calculated using Mann-Whitney U test to compare the means values.
Right: dot plot of glucose levels represented like raw data (−), median values (•) and percentiles values (⌶).

The mean percentage of cytotoxic activity in the digestive gland of mussels was significantly (p=.020049; Z=2.32) lower in mussels subjected to acoustic stress (75.01±10.3 %) for three hours compared to controls (85.7±18.8 %), as shown in Fig. 24.

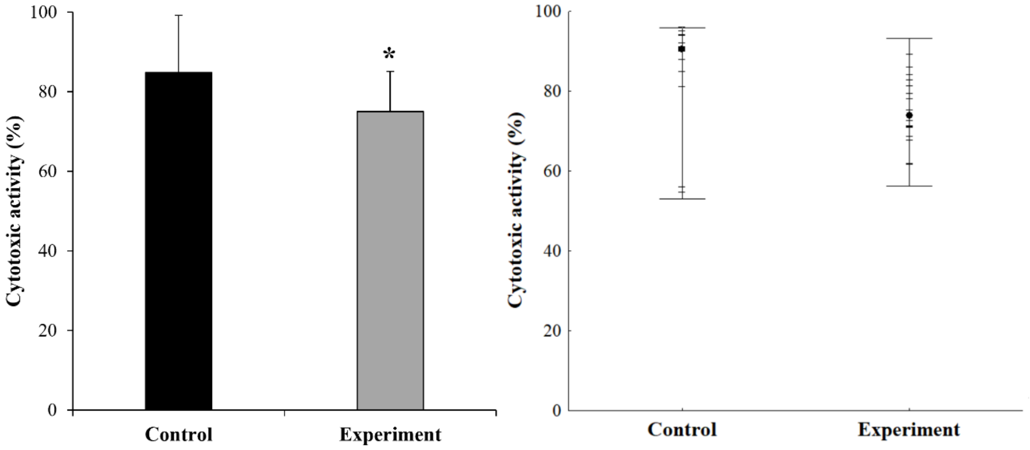


Fig. 24 Left: Bar graph cytotoxic activity of *M. galloprovincialis* digestive gland expressed as a mean ± SD. Asterisks represent significant differences (*p < .05) calculated using Mann-Whitney U test to compare the means values. Right: dot plot of cytotoxic activity of *M. galloprovincialis* digestive gland represented like raw data (−), median values (•) and percentiles values (⌶).

Enzyme activity of alkaline phosphatase, esterase and peroxidase (Fig. 25) in the digestive gland of mussels showed significantly lower values in experimental individuals respectively of 68.9 ± 15.44 U/µg than control with 99.9 ± 61.6 U/µg (p=.047996; Z=1.97), of 27.5± 9.61 U/µg than control with 37.2±15.51 U/µg (p=.046237;Z=1.99), and 8.9±4.56 U/µg than control with 13.63±5.79 U/µg (p=.010869;Z=2.54).

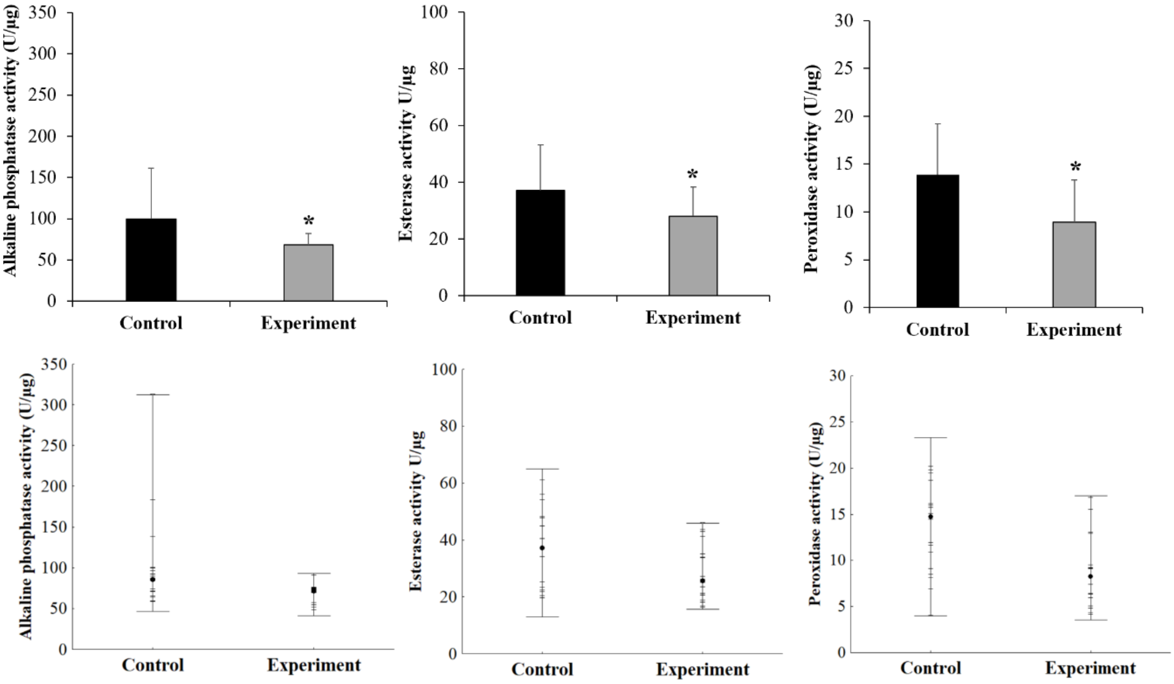


Fig. 25 Up: Bar graph Alkaline phosphatase, esterase and peroxidase activity of *M. galloprovincialis* digestive glands expressed in U/ml and presented as a mean ± SD. Asterisks represent significant differences (*p < .05) calculated using Mann-Whitney U test to compare the means values. Down: dot plot of Alkaline phosphatase, esterase and peroxidase activity of *M. galloprovincialis* digestive glands represented like raw data (−), median values (•) and percentiles values (⌶).

3.2.3. Discussion

The mussel *M. galloprovincialis*, exposed for three hours to very high frequency noise in the 100-200 kHz band, showed significant biochemical changes in the digestive gland. It is known that human activities in the sea and inland waters cause the introduction of noise at various frequencies and it has been demonstrated that low-frequency noise causes negative reactions in many aquatic and marine organisms. To date, this study presents the first results showing that high frequency over 200 kHz produces biochemical changes in mussels.

It's known that noise pollution can influence haematological parameters (Perazzolo et al., 2002; Celi et al., 2013) in marine organisms. One of the most useful parameters is THC, used to evaluate the health of aquatic organisms and the effects of stressful conditions on marine crustacean species (Le Moullac et al., 1998; Sánchez et al., 2001; Filiciotto et al., 2014). Vazzana et al. (2016) showed that THC values increased significantly in mussels exposed to acoustic treatment. Similarly, under acoustic stimuli, the THC of our *M. galloprovincialis* samples increased (although not significantly), and could suggest that exposure to acoustic noise increased circulating cells. Other experiments and the use of a greater number of individuals would be necessary to verify the result and this increase of cells in the hemolymph. These data are also in agreement with Malagoli et al. (2007), who showed that the number of circulating immunocytes in *M. galloprovincialis* increases following exposure to air and to mechanical and salinity stress. In bivalve molluscs, the digestive gland plays a central role in metabolism (Canesi et al., 2007). Vazzana et al. (2016) showed that haemolymphatic glucose levels increased in *M. galloprovincialis* after exposure to an acoustic stimulus; this is probably due to a decrease in glycogen content in the digestive gland as a result of high-glycolytic activity (Pekkarinen and Suoranta, 1995; Ngo et al., 2011). In fact, Sonawane and Sonawane (2018) reported that during acute and chronic stress exposure, there was a significant reduction in the glycogen content in the digestive gland, which suggests an increase in the glycolytic activity of the gland. In our study, we showed that animals treated with acoustic stimulation had high concentrations of glucose in the digestive gland ready to be released into the blood circle. This suggests higher energy requirements and, therefore, a greater breakdown of glycogen for stressed animals. It is known that molluscs, similar to all invertebrates, possess only an innate or natural immunity, recognizing and eliminating the non-self through cellular and humoral components. The digestive gland plays an important role also in innate immunity (Smith, 2001); in fact, it is involved in the clearance of pathogens, in the treatment of antigens and in metabolic changes induced by infection (Luchtel et al., 1997; Alday-Sanz et al., 2002). Cytotoxicity is one of the most important functions in immune activities (Franceschi et al., 1991; it has been well preserved throughout evolution and described in both invertebrates and in vertebrates (Ratcliffe et al., 1985; Savary and Lotzová, 1986). Wittke and Renwrantz (1984) showed that circulating cells (immunocytes) in *Mytilus edulis* are able to produce cytotoxic substances that lyse human erythrocytes. A cytotoxic protein complex was also found in *M. galloprovincialis* hemolymph (Hubert et al., 1997). Malagoli et al. (2008) showed that a reduction in cytotoxic activity could compromise the health of mussels. Our results show that the cytotoxic activity of the gland was significantly influenced by acoustic stimulus: a decrease in the percentage of cytotoxic activity in subjects exposed to acoustic stress compared to controls were observed. Overall, cytotoxicity is a dynamic parameter that can be used as an indicator of immune efficiency and, therefore, of health in mussels. Enzymes produced in the digestive gland, in addition to being involved in nutrient transport and digestion, modulate the immunological processes of haemocytes, such as phagocytosis (Chen et al., 2007). In this study, all enzyme evaluated after the stress condition were found to decrease. The digestive gland is the major site of xenobiotic uptake and oxyradical-generating biotransformation enzymes (Livingstone et al., 1992).

Santovito et al. (2005) showed that in both the digestive gland and gills of *M. galloprovincialis* (with some tissue-specific differences), antioxidant enzymes, such as SOD, GPX and CAT, are expressed. Parisi et al. (2017) showed that in mussels exposed to experimental conditions, phosphatase and esterase activity in the digestive gland was lower than in the control group. Environmentally stressful situations compromise this enzyme activity. Here, we also evaluated peroxidase activity in the digestive gland, highlighting a significant reduction in these enzymes in stressed animals.

It has long been known that humoral and cellular immune-related parameters in bivalves are sensitive to stress factors, such as salinity, nutrient availability, water temperature, dissolved oxygen, and parasites (Auffret et al., 2004; Giron-Perez, 2010). In this study, for the first time, we showed a decrease in various immunological and biochemical parameters in animals exposed to very high frequency acoustic treatment, highlighting that noise pollution in the marine environment emanating from different sound sources at very high frequencies (i.e. sonars) can be considered a pollutant capable to compromise the immune system. Therefore, this can make the animals more susceptible to disease causing alterations also to the ecosystem level. More attention must therefore be paid to all human activity that causes high frequency noise pollution.

3.3 *In-situ* experiment: the biochemical effects of watergun acoustic emission on vertebrates (*Chromis chromis*) and invertebrates (*Holothuria tubulosa* and *Arbacia lixula*)

The airgun is a technique that produces sound in order to provide a better understanding of the deep structure of the sea floor by constructing images (McCauley et al., 2000; Gausland, 2003); however, the frequencies emitted by this type of analysis (impulsive noise with low frequencies lower than 1 kHz) fall within the frequency range of sounds detected by many marine species (Popper et al., 2003b; Popper & Fay, 2011; Ladich & Fay, 2013). Several countries apply a principle of precaution in seismic investigations, limiting the length and duration of exploration (Lewandowski, 2015). Neverless, to date, the effect of this technique on marine life is poorly understood. Greater knowledge of its impact is essential given that the airgun and watergun could precede DSM to study the ocean floor. Despite the fact that various scientific studies have studied the effects of noise pollution on biodiversity, the effects of airgun and watergun acoustic emission on sea urchin, sea cucumber and juvenile fish have not been analysed. During 2nd and 3rd year of study, were performed the CNR research project: IMPLEMENTATION OF THE SOS CONVENTION - *Offshore Platforms & Impacts between* MATTM and CNR-DTA, (Capo Granitola, Campobello di Mazara) Work Package E: *In-depth technical and scientific evaluation of the effects on marine ecosystems of airgun technologies and the effects of a watergun*. The measurement campaign involved several research groups (for a total of approximately 15 people including researchers, scholarship holders and technicians) and multiple skills including ethology, biochemistry, bioacoustics and marine acoustics. Therefore, the biochemical responses of certain target invertebrate species – the sea cucumber (*Holothuria tubulosa*) and sea urchin (*Arbacia lixula*) – and juvenile specimens of the vertebrate (*Chromis chromis*) were evaluated in this project. Given the effects of the acoustic impact on other invertebrate species (see Section.1.6.1), and given the effects observed on echinoderms in Section 3.1, it was hypothesised that impulsive emissions with low frequency could have significant biochemical effects on echinoderms. Furthermore, given the importance of cortisol to study the stress responses it was hypothesised that this parameter could give important information in juvenile individuals extracting it from their whole body by modifying the experimental protocols of other authors, since blood cannot be taken given the small size of individuals. The purpose of this biological section was to analyse acoustic impacts on test species, in particular using the coelomic fluid of echinoderms, the peristomial membrane of the sea urchin and the total body of juvenile fish. Results led to a greater understanding of the effects of the watergun technique on marine ecosystems.

3.3.1 Materials and methods

Experimental Area.

The experiment was conducted at Capo Granitola (Campobello di Mazara, Italy), at the National Research Council (CNR). In order to obtain realistic results as a reference for the impacts of these activities on marine ecosystems, experimental tests carried out at sea were chosen. The main problems encountered with the tank tests were thus eliminated, i.e. the non-reproducibility of real acoustic marine stress in the tanks. Furthermore, keeping specimens in the sea during experiments enabled stress levels related to captivity and the handling of individuals to be reduced. In order to transport samples quickly to the laboratories for biochemical analysis and to avoid the noise of a motor boat (which would have caused further acoustic stress) the animals were placed near the institute and the port of Capo Granitola, where the seabed is sandy with a depth of approximately 8 m (watergun position 37°34.205'N - 12°39.337'E; position of the cage with animals 37° 34.225 'N - 12° 39.360' E). Fig. 26 shows the experimental area and Fig. 27 show the boat used for the experimental plan, the CNR Institute, the site in front of CNR and the laboratory.

Fig. 26 Representation of the experimental area showing the site where the cages with the animals were placed, the watergun site and CNR, where laboratory activities and samplings were carried out.

Fig. 27 Top left: the boat used for the experimental plan; Top right: the site in front of the CNR Institute; Bottom left: the CNR building; Bottom right: the sampling laboratory.

Experimental plan

The experimental plan involved the use of 20 adult individuals of *Holothuria tubulosa* (weight ranging between 70±7 g and 12±2 cm in length), 20 adults of *Arbacia lixula* (weight ranging between 50 ± 8 g and 9 cm in diameter) and 20 juveniles of *Chromis chromis* (0.042±0.018 g and 1,6 ± 0,17 cm in length). All specimens were collected in a natural environment and transported directly to the test area for the four-day acclimatization period. Individuals of sea urchin and sea cucumber were placed in the same cage (1.60m x 80 cm x 80 cm) while juvenile individuals of fish in separate smaller cages (Fig. 28). The animals were stressed with watergun acoustic noise and were taken for biological sampling at four different experimental times (taking 5 specimens at a time): before beginning acoustic stress (group t0), at the end of the acoustic stress (lasted 20 minutes, group t1), three hours after the end of acoustic emission (group t2) and 24 hours after the end of acoustic emission (group t3).

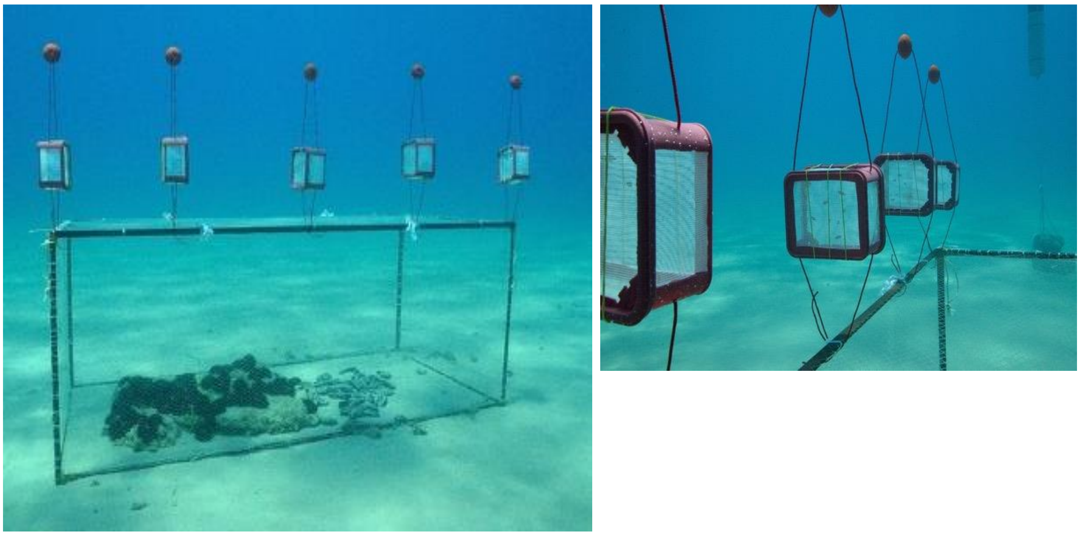
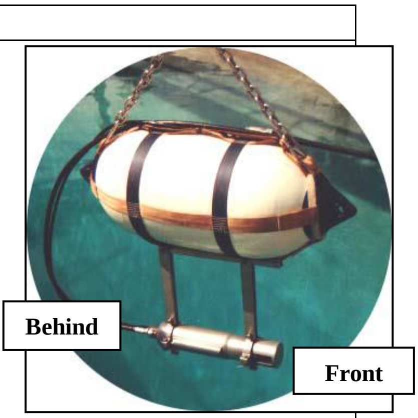


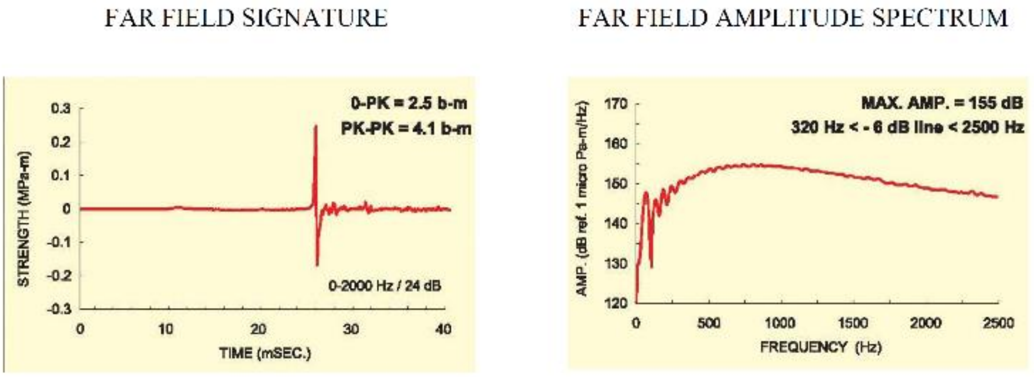
Fig. 28 Cages arranged on the bottom of the sea. On the left, the large cage containing sea urchins and sea cucumbers, and the small cages containing juvenile fish individuals. On the right, a particular of the small cages.

Acoustic signals

The acoustic source used for the experimental tests was the S15 watergun (Sodera, France), a pneumatic seismic compressed air source. Tab.1 shows the main characteristics of the acoustic source used. The watergun has a theoretical signature similar to the classic air-gun, but with a higher peak frequency (Fig. 29).

Tab. 1 Main characteristics of the acoustic source used (S15 watergun)

MATERIAL: Stainless Steel	 
LENGTH: 516 mm	
WIDTH: 152 mm	
WEIGHT: 17.3 Kg	
AIR PRESSURE: from 10 bars to 207 bars	
AIR REQUIREMENT: 0.16 liter per shot.	
FIRING CYCLE: 0.25 SEC.	
COMPRESSOR REQUIREMENT: 87 Nm^3/h	
MINIMUM FIRING DEPTHS: • Horizontal: 0.23 m • Vertical: 0.30 m	
MAXIMUM FIRING DEPTH: 550 m	



FAR FIELD SIGNATURE

FAR FIELD AMPLITUDE SPECTRUM

Fig. 29 Signature and theoretical spectrum of the Watergun at a pressure of 138 bar (as manual instruction).

Characterization of watergun sources at 50 m (far field) - acoustic pressure and particle velocity

In order to characterize the acoustic signal produced by the watergun at a 50 m distance, the acoustic signal was recorded both using an autonomous pressure sensitive recorder (SM2, Wildlife Acoustics, US) and using an autonomous system (M20, Geospectrum ltd, Canada) equipped with a sensor for measuring the movement of particles in three dimensions and acoustic pressure. The positions of the source and the recording points are listed in Tab.2 and represented in Fig.30.

Tab. 2 Source positions and recording points

Positions of the emission and registration points at 50 m		
Position name	Latitude	Longitude
Watergun	37°34.205’ N	12° 39.337’ E
SM2	37°34.225’ N	12° 39.360’ E
M20	37°34.225’ N	12° 39.360’ E

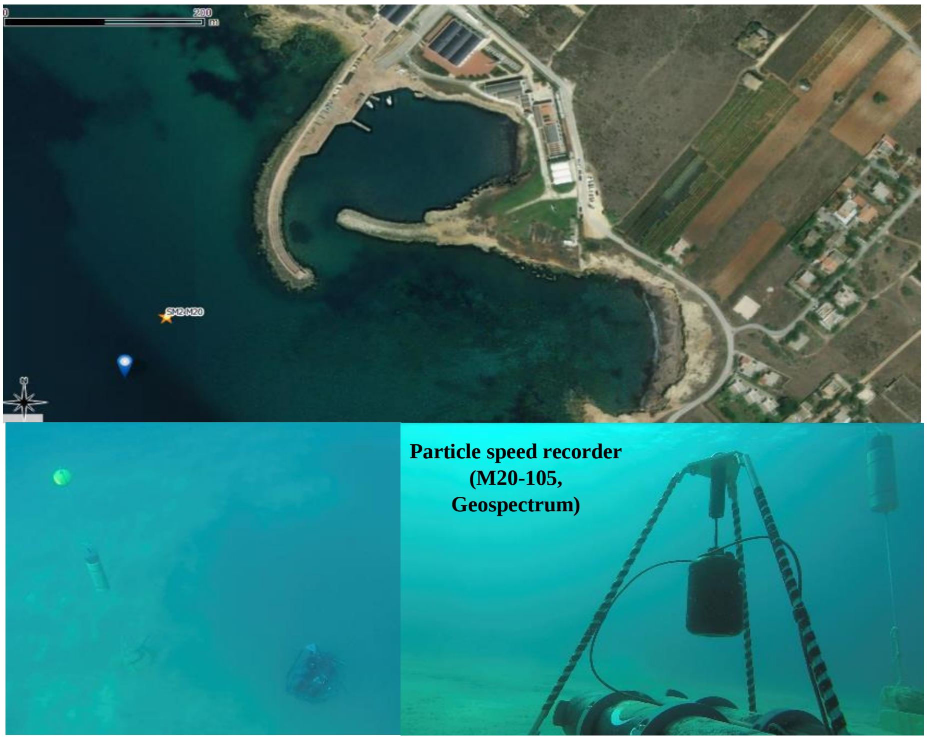


Fig. 30 Representation of the emission and recording point at 50 m and under, the two recording systems SM2 and M20 *in-situ* during the measurements.

Far field acoustic pressure

The CNR bioacusticlab characterized the acoustic signal produced by the watergun at a 50 m distance and a depth of six m from the surface. The acoustic signal was recorded, at the sampling frequency of 192 kHz, using an autonomous recorder (SM2, Wildlife Acoustics, US) with input sensitivity of -170 ± 5 dB re 1 V/µPa in the 8 Hz frequency band 150000-Hz, and - 166 ± 1 dB re 1 V/ Pa in the 100–100000 Hz band.

The position of the watergun from the hydrophone was kept always the same and in front during all the emissions and in total 102 pulses were recorded. For each pulse detected, an interval of 100 ms of amplitude was taken into consideration: 20 ms before the impulse and 80 ms after the impulse. For each pulse, the following quantities were estimated: Cumulative Energy (µPa^{2}s), Spectrogram (dB re

$1\mu Pa^2/Hz$), PSD spectral power density (dB re $1\mu Pa^2/Hz$) and acoustic pressure (μPa) (see Fig. 31)

For the experimental plan, signals were emitted for a total of 20 minutes, with an interval of eight seconds between one shot and another. This type of emission reflects a normal geophysical investigation campaign.

The average value and the standard deviation of the estimated quantities of all the pulses are summarized in Tab. 3.

Tab. 3 Mean value and the standard deviation of the estimated quantities via SM2

Impulse	**(Mean±σ)**
1) T90% (s)	0.021 ± 0.003
2) Lpp (dB re 1μPa)	172.0±0.1
3) SPLrms90% (dB re 1μPa)	162.3±0.6
4) SEL (dB re $1\mu Pa^2 S$)	145.6±0.3
5) Fpeak (Hz)	850±77
6) BW_{-3dB} (Hz)	688±115

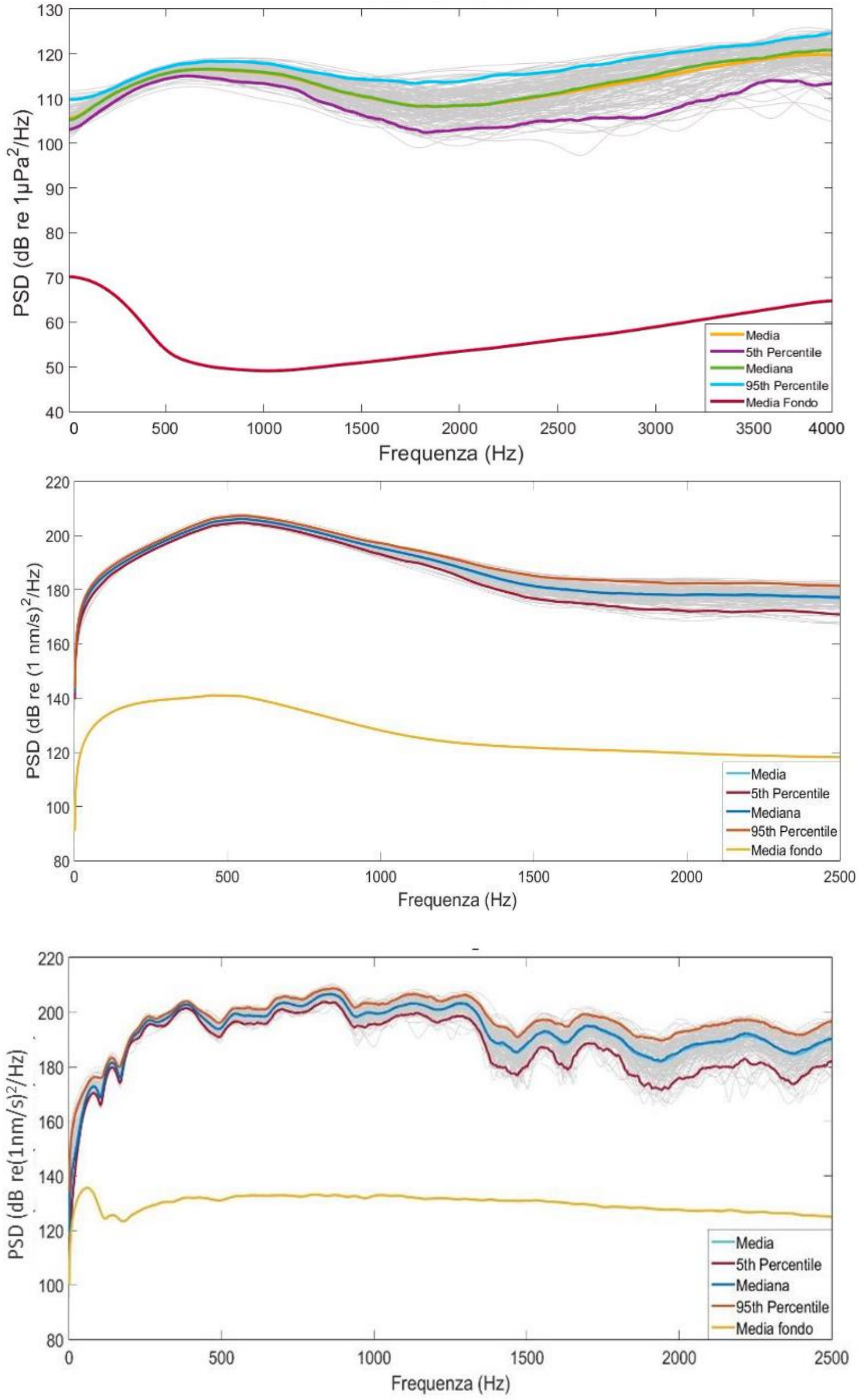


Fig. 31 Upper figure: Power Spectral Density of pressure (dB re 1μPa^2/Hz). Middle figure: Power Spectral Density of particle acceleration for horizontal component. Lower figure: Power Spectral Density of particle acceleration for vertical component.

Biological samples

At different experimental times (before watergun emission (t0), at the end of watergun emission lasted 20 minutes (t1), three hours (t2) and 24 hours (t3) after the end of watergun emissions individuals of *Holothuria tubulosa*, *Arbacia lixula* and *Chromis chromis* were taken from the cages and transported to the laboratory. The sea cucumbers and juveniles fishes were weighed and their length was measured with an ichthometer (Fig. 32 and Fig. 33), while the sea urchins were weighed and their diameter measured with a calliper (Fig. 34). Coelomic fluid was subsequently taken from the sea cucumbers and sea urchins using ISO-EDTA anticoagulant (NaCl 0.5M, Tris-HCl 20mM, EDTA 30 mM, pH 7.4) as shown in Fig.32D and 34C and the volume was measured. The coelomic fluid of each individual was filtered with a fine mesh net and the amount of fluid taken from each animal was recorded. A cell count was performed on each sample with a Neubauer chamber. The samples were centrifuged for ten minutes at 400g and the cell-free coelomic fluid (CfCF) was separated from the cellular pellet. The supernatants were stored in eppendorf stock of 100 µl for subsequent biochemical assays. The peristomial membrane around Aristotle's lantern (Fig. 34D) was sampled in each sea urchin using sterilized scissors and tweezers. All samples were stored at -20 °C for subsequent molecular and biochemical assays. *Chromis chromis* specimens were anesthetized with lethal doses of tricaine (0.05% w/v) methanesulfonate-MS222 (Sigma Aldrich), as shown in Fig. 33A. Individuals were frozen for subsequent extractions (from the whole body) and measurements of cortisol levels. Samples were weighed on thawing (before biochemical analysis) following the protocol of Barcellos et al., (2007) and Ramsay et al., (2006).

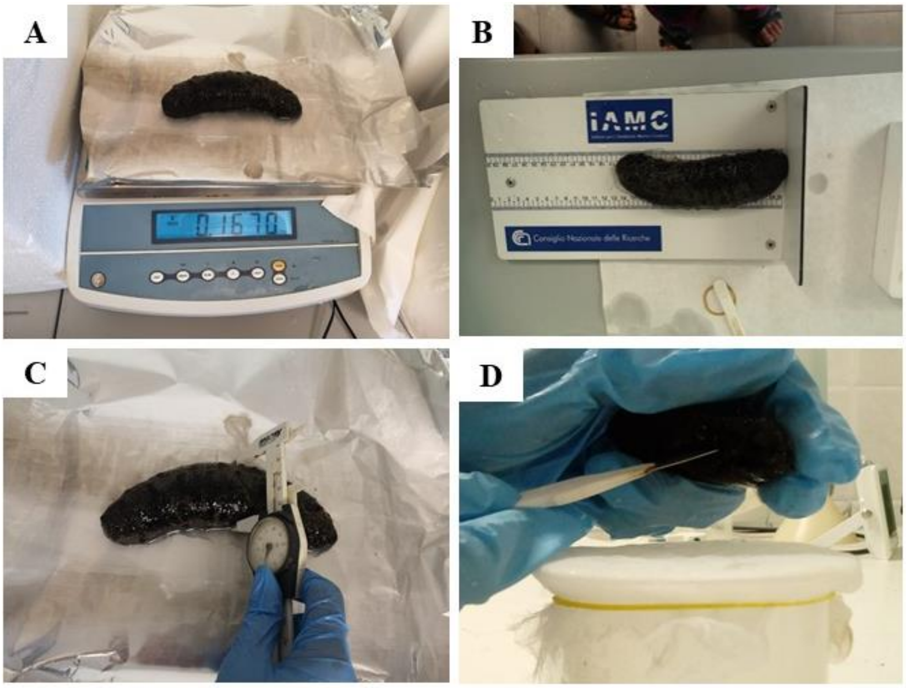


Fig. 32 Sampling phases of coelomic fluid from each individual of *H. tubulosa*. A) the animals were weighed; B) lengths measured; C) thickness measured; D) coelomic fluid sampled.

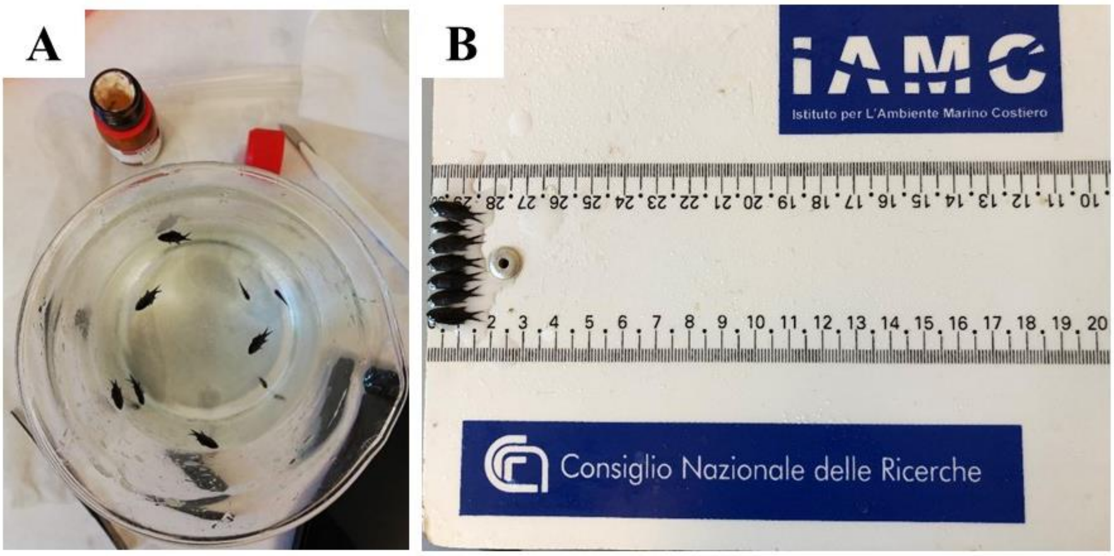


Fig. 33 Representation of sampling phases of *Chromis chromis*. A) lethal anesthesia of animals B) Length measurement of fish using an ichthyometer.

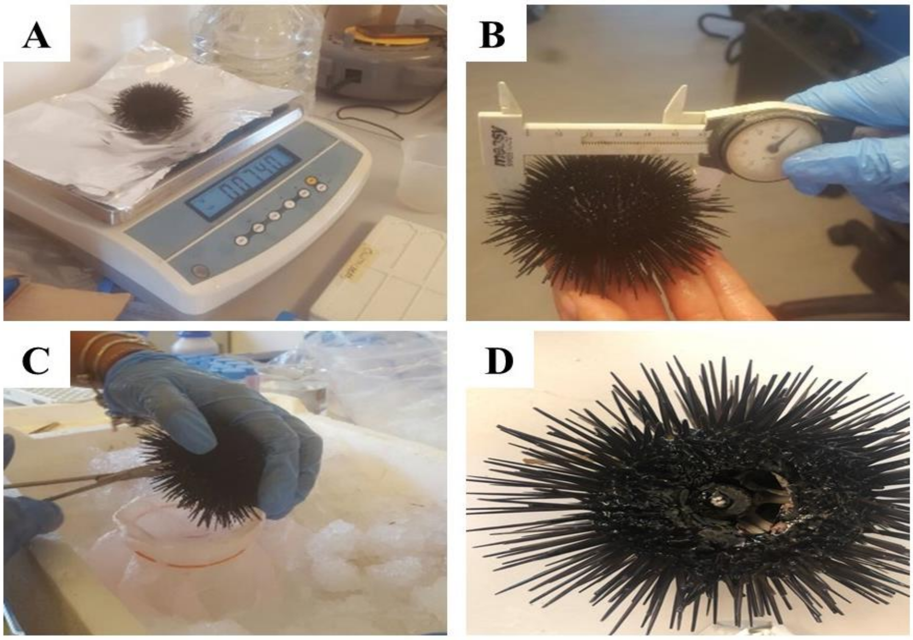


Fig. 34 Sampling phases of the coelomic fluid from each individual of *A. lixula*. A) the animals were weighed; B) diameters measured; C) coelomic fluid sampled; D) peristomial membrane sampled.

Sea Urchin peristomial membrane exctraction

The peristomial membrane (PM) were thawed and homogenised using a glass piston homogenizer on ice with 400 µl of RIPA buffer 1X pH 7.5, supplemented with a cocktail of protease inhibitors (1:200). Subsequently the samples were sonicated for one minute and centrifuged at 6800 rpm for 30 minutes at 4 °C. The supernatants were collected and stored in different aliquots (100µl) for molecular and biochemical analysis.

Protein Concentration

Protein Concentration (PC) was measured in cell-free celomic fluids of *Holothuria tubulosa* and *Arbacia lixula* with a Quibit® 2.0 fluorometer (Invitrogen, Life Technologies, Inc., Burlington, Ontario, Canada) immediately after sampling in three replicas for each sample. The data were quantified with standards. PC was measured using the Bradford method (Bradford, 1976) on extracts of sea urchin peristomial membrane in three replicas for each sample. The Bradford method was modified and PC was measured in a 96-well plate using 20 µl of sample and 180 µl of Bradford solution. Absorbance was read at 595 nm.

Enzyme assays

The protocols used to analyse esterase, alkaline phosphatase and peroxidase activity are already described in Section 3.1.1. These assays were performed on CfCF of *H. tubulosa* and *A. lixula* (50 µl sample with 50 µl of buffer) and peristomial membrane of *A. lixula* (25 µl sample with 25 µl of marine solution and 50 µl of buffer). Enzyme activities were measured in three replicas for each sample. The assays were carried out at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) using samples previously stored in different stock of 100µl to avoid repeated thawing.

Superoxide dismutase (SOD) activity was determined (in three replicas for each samples) only in peristomial membranes of *A. lixula* using an assay kit for SOD determination (Sigma-Aldrich. Product number 19160). In particular, the samples were incubated using a 96-well plate following manual instruction. Absorbance was measured at 450 nm using a microplate reader (GloMax; Promega Corporation, USA).

The enzyme activity was elaborated using the formula:

SOD activity (inhibition rate%) =
{[(Ablank1-Ablank3) -(Asample-Ablank2)]/(Ablank1-Ablank3)} *100

Where:

Ablank1, *Ablank3* and *Ablank2* are the Absorbance value obtained incubating the solutions provided in the SOD kit following the manual instruction.

Asample was the Absorbance value obtained by the sample incubated following manual instruction of the SOD kit.

Using an Inibition curve (prepared by WSZ-1 assay with different incubation time) the Concentraion of SOD (U/ml) was obtained.

SDS-PAGE and western blot

Using western blot analyses (Towbin et al., 1979), HSP70 protein expression was performed using the same protocol as in Section 3.1.1. For each sample of *A. lixula* peristomial membrane, 10 μg/ml migrated in 7.5 % SDS-PAGE gels in three replicas. Westerblot and densitometric analysis was then carried out. Densitometric analysis of the immunoblotted bands was performed using ImageJ software. Densitometry data were expressed as the mean values of different experiments and reported as a percentage of the integrated density value. The integrated density value (IDV) of the relevant bands was normalized to the IDV of the beta-actin bands (data not shown).

Cortisol extraction in Chromis chromis juvenile

Cortisol levels could not be measured using conventional methods applied to plasma due to the small size of the *Chromis chromis* individuals. Methods for the extraction of cortisol from the entire body of the individual were developed by modifying methods given by Ramsay et al., (2006), Sink et al., (2007), Barcellos et al., (2007), Peterson & Booth, (2010), and Guest et al., (2016). The animals were thawed, weighed and cut into small pieces. The minimum weight necessary to obtain detectable cortisol levels was between 0.06g and 0.1g. If the fish had less weight, they were added together. They were then homogenised with 5 ml of PBS (NaCl 137 mM, KH_2PO_4 2 mM, Na_2HPO_4 10 mM, KCl 2.7 mM) and sonicated for two minutes. Then, the samples were transferred in a 15 ml tube (since the diethyl ether ruins the plastic or polycarbonate tubes) and the previous one was cleaned with 1 ml of PBS. Subsequently, 5 ml of diethyl ether were added and vortexed for one minute. The sample was centrifuged for 10 min at 3000 rpm and frozen at -20 °C for two hours. After the two hours, the diethyl alcohol was recovered, transferred to a new 15 ml tube and left to evaporate overnight under a hood. The contents were resolubilized with 1 ml of PBS. Cortisol was measured using the ELISA kit (Cusabio Biotech Co., Ltd.).

Statistical analysis

A non-parametric Kruskal-Wallis test was used on the biochemical parameters (protein concentration, peroxidase activity, superoxide dismutase activity, esterase activity, alkaline phosphatase activity and HSP70 expression protein). For each biochemical parameter analysed, a statistical test was performed between the control samples and the various experimental times. The purpose of this was to test the differences in the medians of the analysed groups because the variables did not meet the conditions of normality (Shapiro-Wilk's test). All the statistical analyses were carried out using the statistical software R (R3.2.2.).

3.3.2 Results

Fig.35 shows the results for total coelomic fluid volume sampled from the animals. In *Holothuria tubulosa*, total coelomic fluid volume decreased three hours after the end of acoustic emission (t2; 15.2±4.73 ml) compared to the control group (t0; 27.25±15.62 ml). In *Arbacia lixula* total volume did not change during the experimental time compared to group t0. However, the differences in the results were not significant for either species.

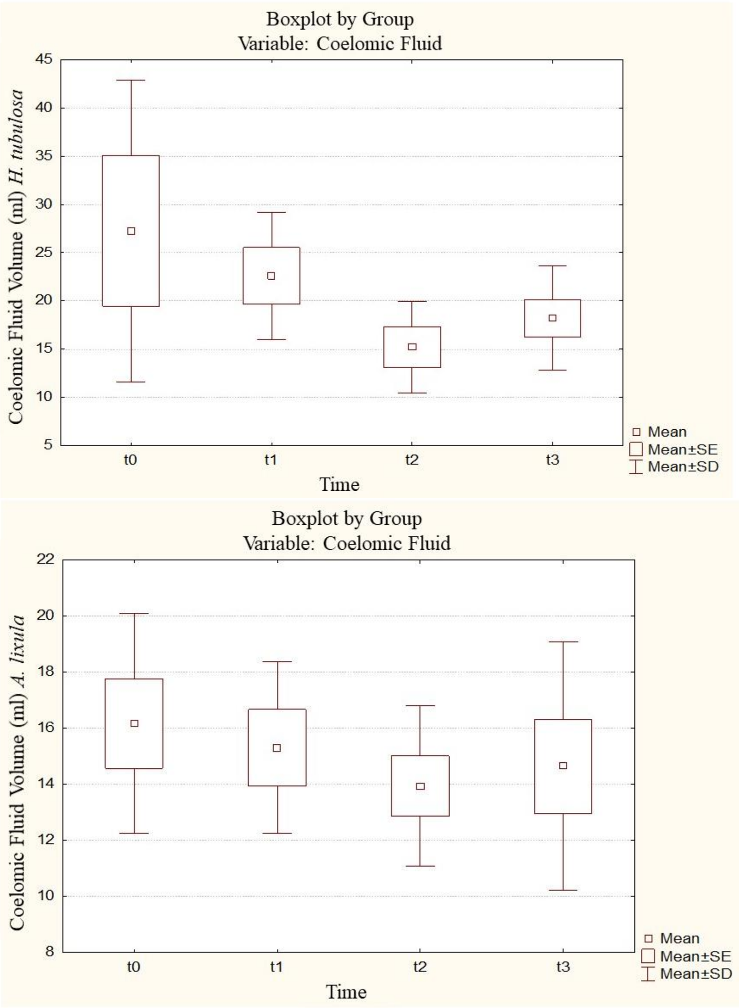


Fig. 35 Total volume of coelomic fluid sampled from *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission(t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in millilitres (ml) as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Fig. 36 shows the results for Total Haemocyte Count (THC). Changes in THC in both species were not significant at the different experimental times compared to control (t0), although an increase in cell number was found in both species at the end of watergun emission lasted 20 minutes (t1).

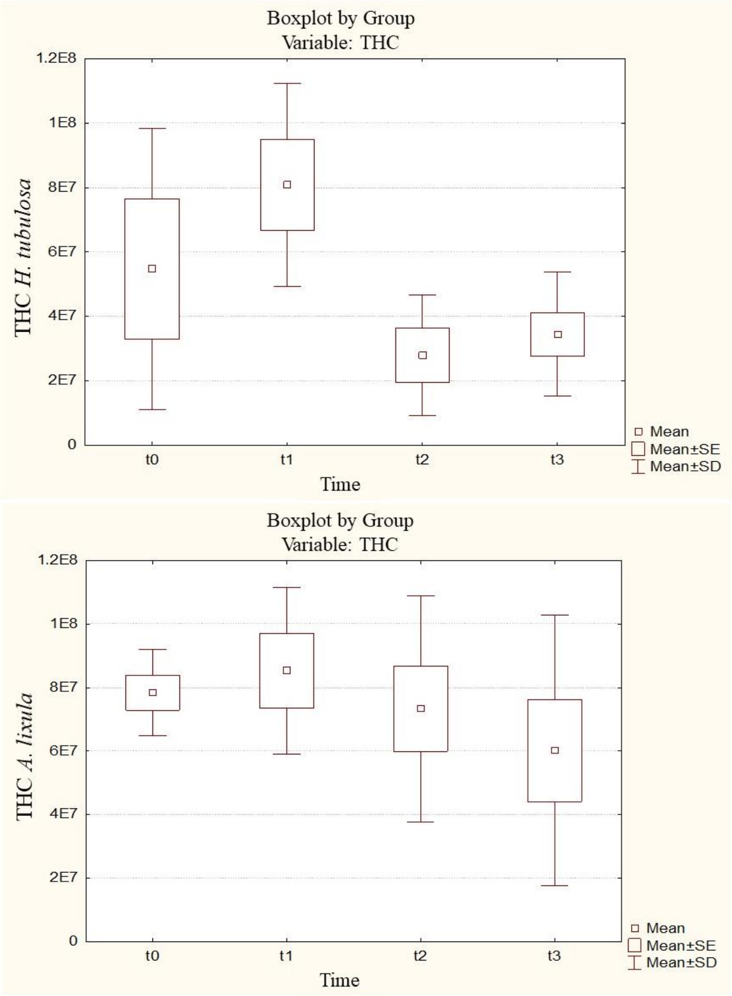


Fig. 36 Total Haemocyte Count (THC) of *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

A decrease in Protein Concentration (PC) (Fig. 37) three hours after the end of watergun emission (t2, 279±30.40 µg/ml in *H. tubulosa* and 301±18.99 µg/ml in *A. lixula*) was recorded in both species compared to control (t0, 360±8.14 µg/ml in *H. tubulosa* and 328±21.77 µg/ml in *A. lixula*), however, the decrement was not significant.

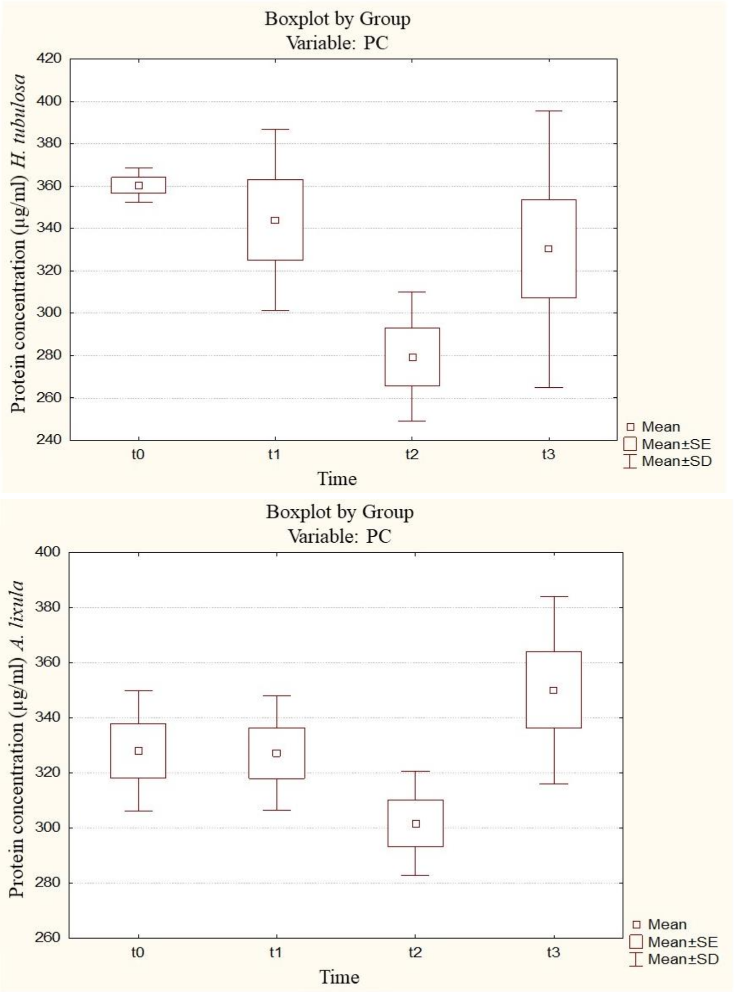


Fig. 37 Protein Concentration in *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in µg/ml, as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Regarding the alkaline phosphatase activity analysed in the CfCF of both species also in this case the data showed the same trend with a first decreament at t1 (2.04±1.22 U.L/µg in *H. tubulosa* and 2.85±2.40 U.L/µg in *A. lixula*) and a subsequent increment at t2 experimental times (5.17±2.88 U.L/µg in *H. tubulosa* and 6.22±2.72 U.L/µg in *A. lixula*) compared to control group t0 (2.57±1.40 U.L/µg in *H. tubulosa* and 4.27±1.20 U.L/µg in *A. lixula*) as showed in Fig. 38. However, the differences were not significant.

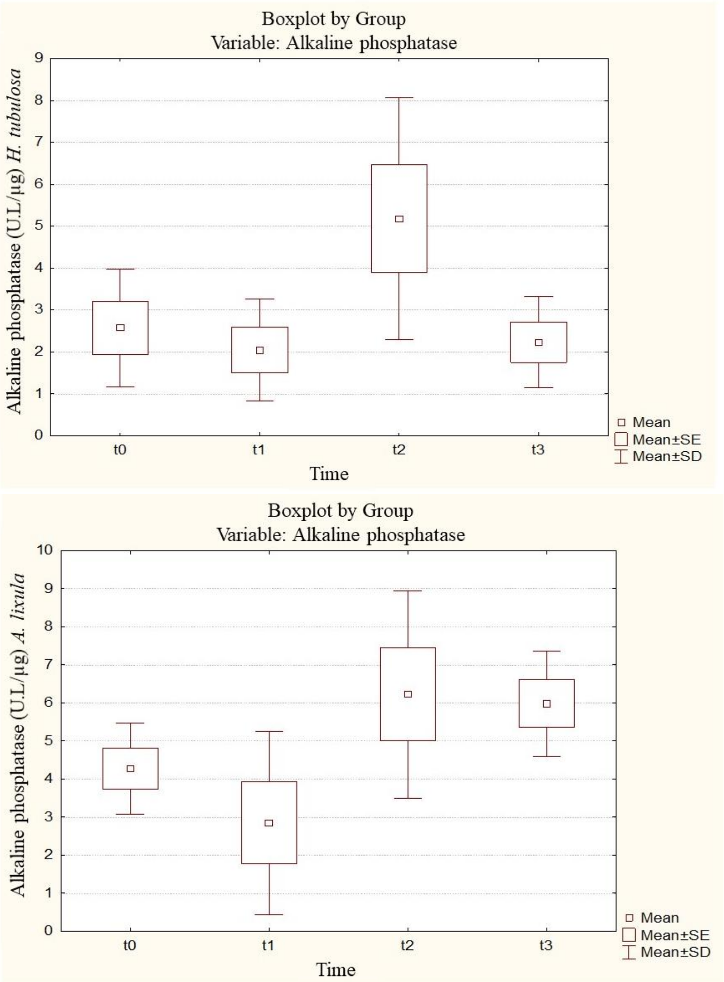


Fig. 38 Alkaline Phosphatase activity of cell-free coelomic fluid of *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in U.L/µg, as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

At the end of emission that lasted 20 minutes (t1) the esterase activity in CfCF of both invertebrate species treated with watergun acoustic emission increases compared to the control specimens (t0) respectively 3.40 ±2.01 U.L/µg in *H. tubulosa* and 5.23±1.46 U.L/µg in *A. lixula* (Fig. 39). Three hours after the end of emission (t2) in *H. tubulosa* the esterase activity decreases to return at values similar to the control 24 hours after the end of acoustic emission (t3, 1.89 ±1.46 U.L/µg). In *A. lixula* the esterase activity remains higher until 24 hours after the end of acoustic emission (t3, 9.17±4.3 U.L/µg) compared to the control (t0). However, the results were not significant.

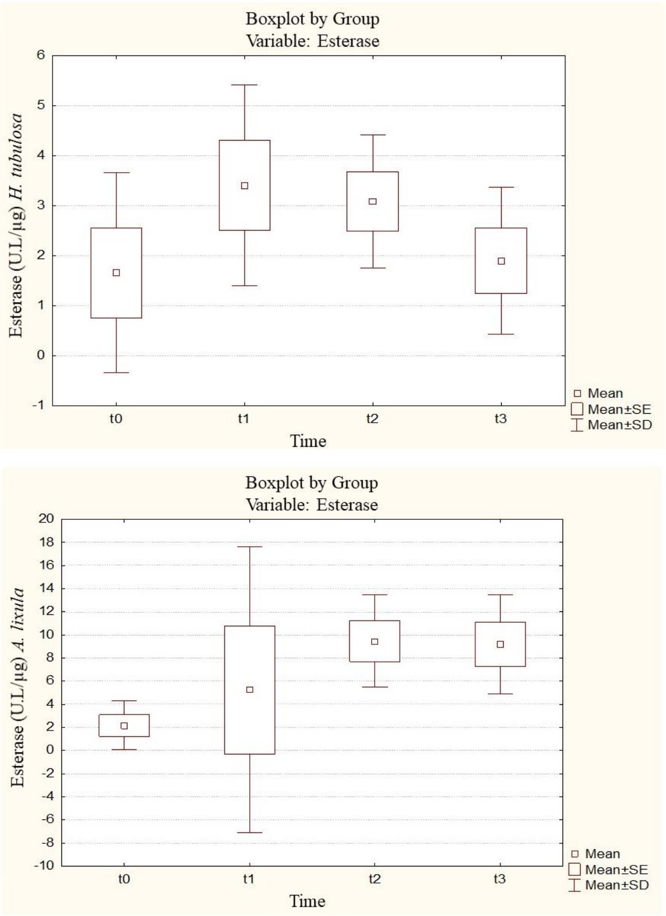


Fig. 39 Esterase activity of cell-free coelomic fluid of *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in U.L/µg, as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Although higher in the *H. tubulosa* control group compared to the *A. lixula* control group, peroxidase activity (Fig. 40) increased at t1 in both species with maximum levels at t2. CfCF peroxidase activity, in *H. tubulosa* (t2) was 0.01± 0.002U.L/mg, compared to t0 group at 0.0074±0.0014 U.L/mg. Peroxidase acitivity in *A. lixula* t2 group increased significantly (p=.0045) at 0.0072±0.0018 U.L/mg compared to t0 group at 0.002±0.0024 U.L/mg. Values remained significantly high (p=.0045) in t3 group at 0.0069 ±0.001 U.L/mg compared to t0 group. These increments were significant only in sea urchins.

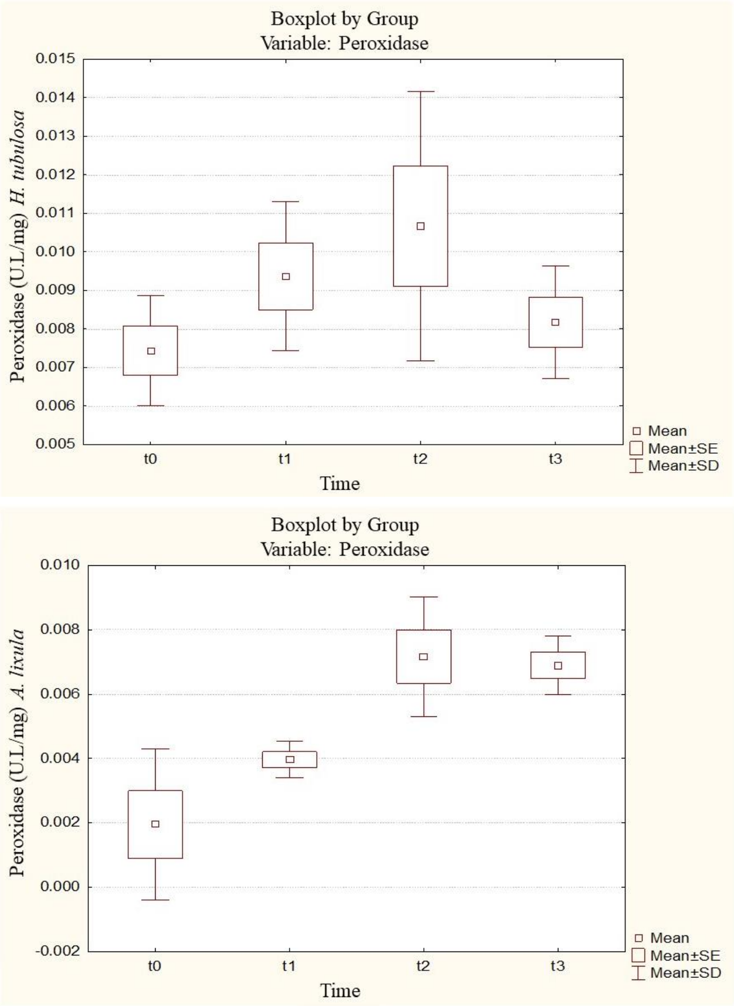


Fig. 40 Peroxidase activity of cell-free coelomic fluid of *H. tubulosa* (up) and *A. lixula* (down) at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in U.L/mg, as mean ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Arbacia lixula peristomial membrane results

Regarding peristomial membrane protein concentration (PC), a decrease was seen in results at the end of acoustic emission lasted 20 minutes (t1, 0.61±0.34 mg/ml) compared to the control group (t0, 0.99 ±0.22 mg/ml). An increase in protein concentration was seen three hours (t2, 1.08 ±0.17 mg/ml) and 24 hours after the end of watergun acoustic emission (t3, 1.42 ±0.29 mg/ml). However, these results were not significant (Fig. 41).

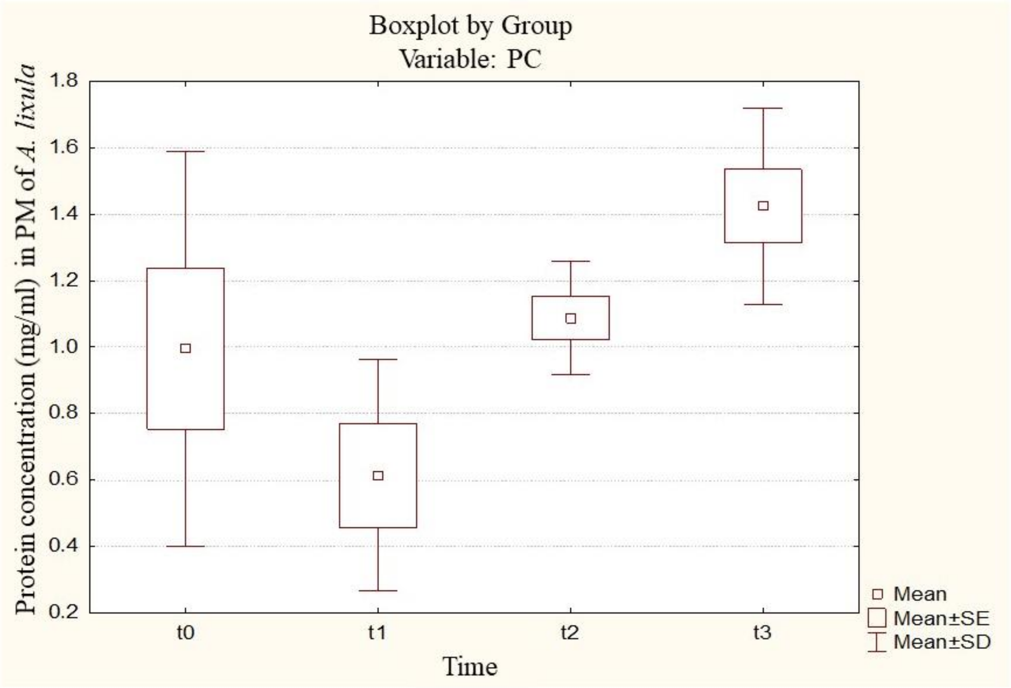


Fig. 41 Protein Concentration of peristomial membrane of *A. lixula* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values were expressed in mg/ml as a means ±SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Significant increases in peroxidase activity (p=.0234) were found in group t1 (9.85 ±2.42 U/µg) compared to group t0 (4.62±3.06 U/µg). Enzyme activity in groups t2 and t3 were similar to group t0, at 5.37±1.94 U/µg and 4.95±0.73 U/µg, respectively (Fig. 42)

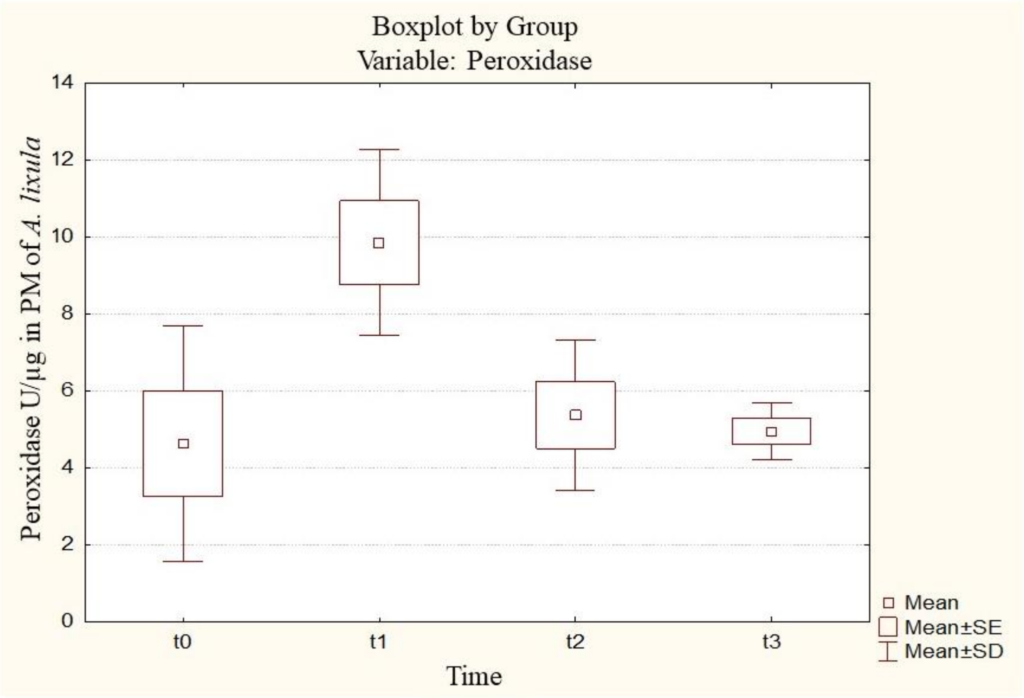


Fig. 42 Peroxidase activity evaluated in peristomial membrane of *A. lixula* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values were expressed in U/µg as a means ±SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Superoxide dismutase (SOD) activity (Fig. 43) significant increased (p=.0113) at the end of acoustic emission lasted 20 minutes (t1): enzyme activity in t0 group was 1±0.03 U/ml whilst it was found to be 5±0.06 U/ml in t1 group (Fig.43). Enzyme activity reached control values (t0) both in group t2 (1.3±0.05U/ml) and in group t3 (1.5±0.02 U/ml) following this increase.

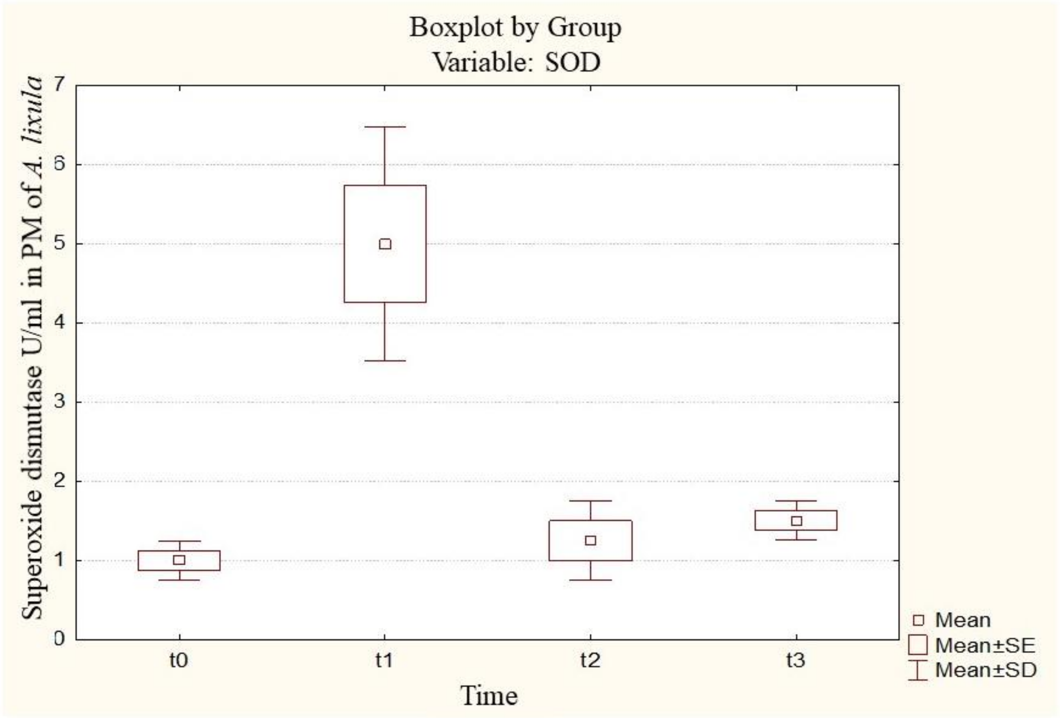


Fig. 43 Superoxide dismutase activity evaluated in peristomial membrane of *A. lixula* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values were expressed in U/ml as a means ±SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Esterase activity rose significantly (p=.0008) in group t1 (29.14±6.80 U/µg) compared to group t0 (11.41±5.9U/µg) and remained significantly higher in group t2 (19.68±0.61 U/µg) compared to control. 24 hours after the end of watergun acoustic emission (t3), esterase activity (13.82±2.10 U/µg) was similar to control (t0) (Fig. 44).

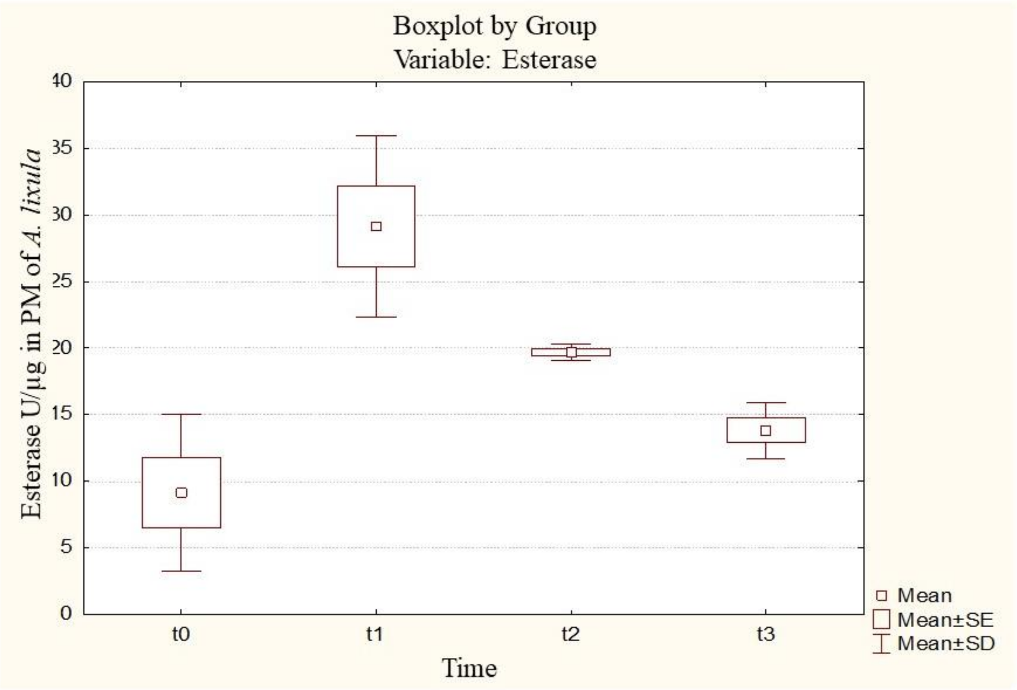


Fig. 44 Esterase activity in peristomial membrane of *A. lixula* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values were expressed in U/µg as a means ±SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Similary, alkaline phosphatase activity in peristomial membrane of *A. lixula*, showed a significant increase (p=.0581) at the end of watergun acoustic emission lasted 20 minutes (t1) compared to group t0 without acoustic emission (Fig. 45). In fact, alkaline phosphatase activity in group t1 was 18.43±6.99 U/µg whilst in group t0 was 8.46 ±6.29 U/µg. Enzyme activity reached control values three hours (t2, 11.31±1.29 U/µg) after the end of acoustic emission and remained similar until 24hours (t3, 8.90 ±1.68 U/µg) after the end acoustic emission.

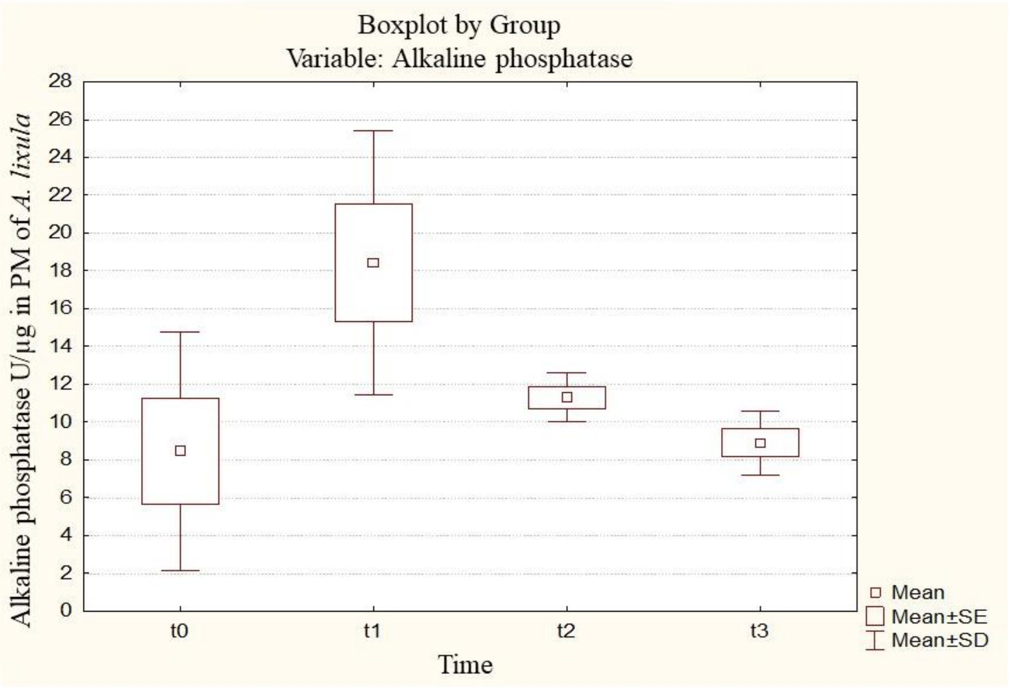


Fig. 45 Alkaline phosphatase activity evaluated in peristomial membrane of *A. lixula* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values were expressed in U/µg as a means ±SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Densitometric analysis of HSP70 immunoblotting (Fig. 46) showed an increased value of HSP70 in the experimental group at the end of acoustic emission lasted 20 minutes (t1) compared to the control group (t0). Three hours (t2) and 24h (t3) after the end of acoustic emission the value of HSP70 expression reached control values (t0). However, the results were not significant.

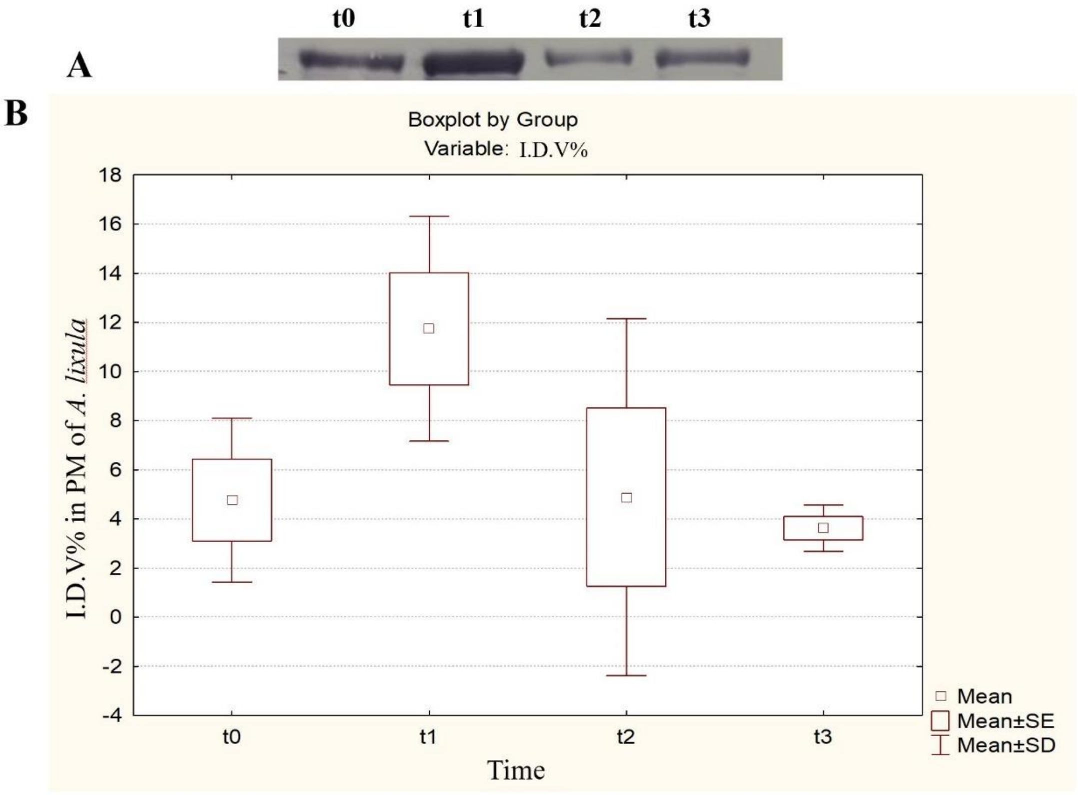


Fig. 46 A) A representative western blot of HSP70 (t0, t1, t2, t3) of peristomial membrane of *A. lixula*. B) Integrated optical density histogram (IDV) of the HSP70 protein bands. Significant differences were calculated using Kruskal Wallis multiple comparison test.

Results of cortisol levels in C. chromis juveniles.

Significant differences in cortisol levels measured on the whole body of *C. chromis* were found compared group t0, as shown in Fig. 47. Cortisol levels increased at the end of watergun emission (t1, 93.3±16.68 ng/ml) and three hours after the end of watergun emission (t2, 112.7±12.29 ng/ml) compared to control group (t0, 55.8±11.33 ng/ml). However, the results were significant only in t2 group (p=.0119).

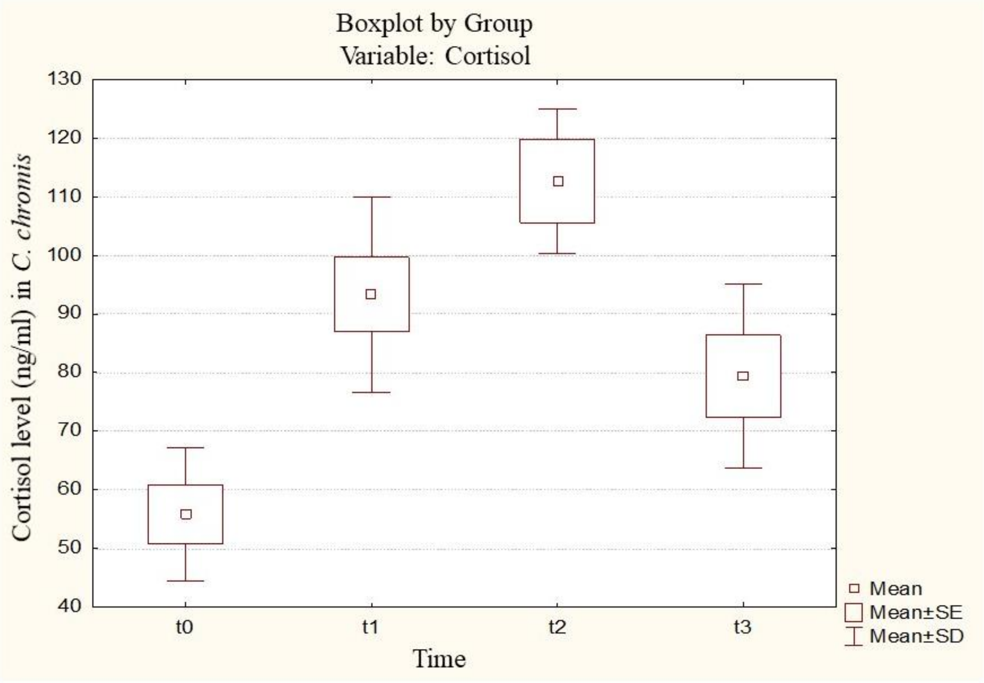


Fig. 47 Cortisol levels of *C. chromis* at different experimental times: before acoustic emission (t0), at the end of emission lasted 20 minutes (t1), 3 hours (t2) and 24 hours (t3) after the end of watergun emission. Values are expressed in ng/ml, as means ± SD. Significant differences were calculated using Kruskal Wallis multiple comparison test.

3.3.3 Discussion

H. tubulosa and A. lixula cell-free coelomic fluid

A number of studies have demonstrated the effects of airguns on fish (Santulli et al., 1999; Buscaino et al., 2010) and invertebrates (Celi et al., 2013; Vazzana et al., 2016; Filiciotto et al., 2014; 2016; 2018). However, to date, no data have been reported on the effects of watergun noise pollution on echinoderms.

Our study analysed the effects of this noise on two species of echinoderms, *H. tubulosa* and *A. lixula* for the first time, and coelomic fluid extracted from these invertebrates was measured as a possible useful biomarker. Some previous studies showed changes in coelomic fluid volume in echinoderms under stress conditions (Virkar, 1966; Buchanan, 1969; Pierce, 1971; Binyon, 1972; Ellington et al., 1974), by observing, for example, variations in dependence of intracellular isosmotic regulation (Ellington et al., 1974). Binyon, (1972) also showed that there may be an optimal ratio between coelomic and body volume which the animal tends to maintain. In our study, a decrease in coelomic fluid extracted from *H. tubulosa* was found, compared to unexposed individuals, particularly three hours after the end of watergun emission. However, no significant differences were observed.

In *A. lixula*, coelomic fluid volume remained constant throughout the different experimental times and, also in this case, no significant differences were apparent. Based on this experimental model, watergun emissions did not cause changes in coelomic fluid volume in either echinoderm species.

Other parameters relate to immune responses. Innate immunity in echinoderms is divided into cellular and humoral immunity. Celomocytes mediate cellular responses through cytotoxicity (Arizza et al., 2007), the production of antimicrobial agents (Schillaci et al., 2010), phagocytosis and encapsulation (Chia & Xing, 1996) while different humoral factors of the coelomatic fluid (Canicatti, 1991) play an important role in the defense against pathogens and other foreign substances (Coffaro & Hinegardner, 1977; Smith et al., 1996; Gross et al., 1999; Pancer et al., 1999; Gross et al., 2000; Pancer, 2000; Kudriavtsev & Polevshchikov, 2004). In our study, changes in THC, PC and enzyme activity in cell-free coelomic fluid of *A. lixula* and *H. tubulosa* were determined for the first time. THC is an important parameter in the study of immune responses in marine organisms. It is useful in the evaluation of crustacean health, the effects of stress conditions (Smith et al., 1995; Jussila et al., 1997; Hennig et al., 1998; Le Moullac et al., 1998; Sánchez et al., 2001; Celi et al., 2013; Filiciotto et al., 2014) and it is affected in invertebrates by different types of stress (Lorenzon et al., 1999; Malagoli et al., 2007; Yao & Somero, 2013). Recent studies report the effects of acoustic stress on crustaceans and bivalve molluscs; in both cases effects have been demonstrated on total circulating hemocyte count (THC) and on total proteins (Celi et al., 2013; Vazzana et al., 2016; Filiciotto 2014; 2016; 2018). In the case of sea cucumber, scientific literature shows that THC can be modulated by several factors. However, most research analysed variations in THC in sea cucumber species based on different types of feeding (Zhang et al., 2010; Zhao et al., 2011; Chi et al., 2014; Wei et al., 2015) and not due to acoustic impact. In our study, THC levels increased in both species of echinoderm at the end of watergun emission lasted 20 minutes. However, these increments were not significant. Despite acoustic stimuli can modulate the THC in invertebrates (Celi et al., 2013, 2015; Filiciotto et al., 2014; Vazzana et al., 2016; Day et al., 2016b), this does not seem to be the case for echinoderms. Given the variability found in results in literature and the difference compared to our research, the results obtained confirmed that responses to stress, in our case, acoustic, change in a species-specific way. The acoustic stimulus of this experimental plan does not seem to significantly affect the total number of circulating haemocytes in echinoderms. The immune system of many invertebrates is based also on proteins, involved in the recognition of foreign particles (Vargas-Albores & Yepiz-Plascencia, 2000), in the entrapment of invading organisms and in the reduction of blood loss following wounds (Hall et al., 1999; Montaño-Pèrez et al., 1999). Total protein concentration is an important parameter to describe the stress response in marine organisms and its variation is a typical response of invertebrates in the presence of different types of stress (Le Moullac et al., 1998; Durand et al. 2000; Speed et al., 2001; Auffret et al., 2006). Blood protein levels change with changes in environmental and physiological conditions and play key roles in invertebrate physiology (Chang, 2005; Lorenzon et al., 2011), providing valuable information which allow us to define their condition (Ozbay & Riley, 2002; Chen and Cheng, 1993) and, acting as a stress indicator, to monitor their health status (Lorenzon et al., 2011). In several invertebrate species, acoustic stress influences and modulates total protein levels (Filiciotto et al., 2016; 2018) but nothing is known about the acoustic impact on total proteins in echinoderms. Our results showed the same effects on the two species of echinoderms without significant differences. In both species, total protein levels decreased three hours after the end of the acoustic emission, returning to values similar to organisms not exposed to acoustic stress 24 hours after the end of emission. The return of protein levels to values similar to controls indicates a possible recovery of the animals, since total protein levels can be expression of adaptive characteristics or acclimatization strategies (Da Silva-Castiglioni et al., 2007). In literature, results concerning total protein levels of organisms exposed to acoustic stress are contrasting. In fact, in the presence of acoustic stress, total protein levels increase in mussels (Vazzana et al., 2016) and in crustaceans (Filiciotto et al., 2016) or remained unchanged in other invertebrate species (Christian et al., 2003; Andriguetto-Filho et al., 2005; Celi et al., 2013) and fish species (Celi et al., 2016). Our results confirm that total protein levels depend on the species (Shafir et al., 1992; Palacios et al., 2000; Da Silva-Castiglioni et al., 2007; Buckup et al., 2008), the type of stress, the fact that acoustic stimuli are perceived differently in different environmental conditions and acoustic properties (e.g. source level, frequency, duration). The biochemical analysis of the cell-free coelomic fluid also included activity of immunity-related enzymes, such as alkaline phosphatase and esterase. Alkaline phosphatase and esterases play an important role in the immunity of marine organisms. They are present, for example, in fish mucus and their activity can be modified by the presence of bacteria and parasitic infections (Ross et al., 2000). Alkaline phosphatase activity can be used to assess immune status in invertebrates (Mou et al., 1999; Liu et al., 2000; Sarlin & Philip, 2011) and, in literature, it has been shown that circulating coelomocytes of *Holothuria polii* contain this enzyme (Canicattí, 1990). Alkaline phosphatase is commonly used as an indicator to assess immunity and health status in sea cucumber (Liu et al., 2008; Wang et al., 2008a) and studies show that activity of this enzyme is improved in coelomocytes and coelomic fluid by diet (Sarlin & Philip, 2011; Liu et al., 2012; Ma et al., 2013; Wang et al., 2015; Yang et al., 2015; Wen et al., 2016; Li et al., 2018; Dang et al., 2019). In sea urchin, on the other hand, alkaline phosphatase activity has been studied with more detail in larvae and eggs (eg Stumpp et al., 2013) but not in coelomic fluid. However, no studies have been reported in literature that analyse enzyme activity in echinoderms exposed to noise pollution. In our study, no significant changes were found in alkaline phosphatase activity in both echinoderms exposed to noise pollution, and, more specifically, no significant increase was found three hours after the end of acustic stimulus. Results in literature are different; some authors report an increase in enzyme activity in stressed animals (Seitkalieva et al., 2016) and others a decrease (Mazorra et al., 2002; Jing et al., 2006 a,b). Suresh et al. (1993), for example, studied alkaline and acid phosphatase levels in hemolymph of a number of molluscs when exposed to different concentrations of copper. They showed that, depending on the species, metal concentrations and length of exposure, hypersynthesis of acid and alkaline phosphatases can occur. In addition, Chandy & Patel, (1985) found that no significant changes were observed in enzyme activity at certain concentrations of metal ions. In our study, decreases in enzyme activity at the end of acoustic emission lasted 20 minutes are in agreement with results obtained for other invertebrates under different stress conditions (Mazorra et al., 2002; Jing et al., 2006a, b; Chandurvelan et al., 2013; Parisi et al., 2017). These changes in enzymatic activities could be caused by changes in hormone levels that have a knock-on effect on lymphatic cells and alkaline phosphatase activity, as observed in *Apostichopus japonicus* (Zhao et al., 2010). In fact, improvements in alkaline phosphatase activity may indicate an improvement in the immune function of echinoderms (Zhao et al., 2012). Moreover, enzyme activity of alkaline phosphatase also seems to be influenced by gender, in particular in males than females. It has been shown that these enzymes are more abundant in cell lysates than in cell-free coelomic fluids and, therefore, analysis of enzyme activity in coelomocytes is suggested in order to further evaluate their impact (Jiang et al., 2017).

Furthermore, in first experimental plan (see Section 3.1), in cell free coelomic fluid of sea urchin (stressed with acoustic frequency ranging between 100-200 kHz) significant increases in alkaline phosphatase activity were found, highlighting the fact that acoustic emissions of different intensity and duration increase this enzyme activity.

As regards the esterases, they are one of the most common biomarkers of environmental stress used in aquatic organisms (Forget et al., 2003). In literature, changes in esterase activity are correlated with the performance and survival of some invertebrates (Galloway et al., 2002; Rickwood & Galloway, 2004; Barata et al., 2004; Hannam et al., 2008; Ren et al., 2015), and esterase inhibition has been proposed as a biomarker capable of predicting the ecological effects of pollutants (Douhri & Sayah, 2009). Various studies have examined this activity in invertebrates stressed with different types of stress, both in hemolymph and in tissues (Hyne et al., 2003; Sun et al., 2006; Parisi et al., 2017). Other studies have characterized esterases in sea cucumbers (Wu et al., 2013) and AChE activity has been determined in *Holothuria leucospilota* and *Holothuria atra* (Kolasinski et al., 2010). To date, no scientific research has studied this enzyme activity under acoustic stress conditions in echinoderms, and, more specifically, in cell-free celomic fluid. The results of our study show that for both species, esterase levels increase at the end of watergun acoustic emission lasted 20 minutes. However, in both cases no significant changes were found. However, in *A. lixula* the levels of enzyme activity are higher than in *H. tubulosa* and remain high until 24hours after the end of watergun emission compared to control. Although our results are not significant, the trends of esterase activity observed in the sea urchin are in agreement with the results of the experimental plan in Section 3.1, in which individuals of *A. lixula* were exposed to acoustic frequencies of 100-200 kHz and demonstrated significant increases in this enzyme activity in cell-free celomic fluid. This data is in agreement with data reported by Xue & Renault, (2000), who observed an increase in enzyme activity in cell-free coelomic fluid of oysters infected with Bonamia compared to control individuals.

In contrast, Wang et al. (2012) showed a reduction in esterase activity in *Perna viridis* haemocytes exposed to different types of environmental stress, such as pH, dissolved oxygen quantity and salinity. The results in literature are conflicting and may depend on the matrix, the species and the type of stress analysed (Jing et al., 2006a, b).

Many environmental pollutants or different types of stress can also induce oxidative stress in marine organisms. Organisms adapt to increased ROS production by regulating the activity of antioxidant enzymes (Livingstone, 2003). ROS are formed by the univalent reduction of molecular O_2 that produces superoxide radicals (O^{2-}) and by a reduction of superoxide hydrogen peroxide (H_2O_2) and hydroxyl radicals ($HO\cdot$) through the iron-catalyzed Haber-Weiss reaction (Fridovich, 1986). These reactions occur in many metabolic processes in chloroplasts, cytosol and mitochondria (Asada & Takahashi, 1987; Halliwell & Gutteridge, 1999) and concentrations of these radicals increase under stress (Fridovich, 1986; Asada & Takahashi, 1987). The organisms defend themselves against ROS with antioxidants and non-enzymatic deoxidizers (i.e. ascorbate, tocopherols, carotenoids) or through the production of enzymes, such as superoxide dismutase which removes O_2 and produces H_2O_2, catalase which removes H_2O_2, and glutathione peroxidase which removes H_2O_2 and other peroxides (Asada & Takahashi, 1987; Halliwell & Gutteridge, 1999). These enzymatic radical scavengers play a compensatory role in maintaining radical detoxification (Choi et al., 1999) and are considered important biomarkers of stress, especially in aquatic organisms (Winston et al., 1991). The inability to detoxify excess ROS production can cause significant oxidative damage including lipid peroxidation (Halliwell & Gutteridge, 1999), which leads to altered cellular functions and changes in the physicochemical properties of cell membranes (Rikans & Hornbrook, 1997). Peroxidase is one of the antioxidant enzymes involved in the elimination of reactive oxygen species (Beyer et al., 1991; Ahmad, 1995); it acts as an important microbicide agent which forms a highly toxic complex and was evaluated for the first time by Guardiola et al. (2014). As regards acoustic impact on peroxidase activity in echinoderms, information is not found. However, the presence of antioxidant activity in coelomic fluid of different species of sea cucumbers was demonstrated by Hawa et al. (1999). In our study, this enzymatic activity was analysed in cell-free coelomic fluid of *H. tubulosa* and *A. lixula* and showed the same increasing trend in both species up to three hours after the end of watergun emission. Enzyme activity levels subsequently decreased, only in sea cucumber, returning to levels similar to those of untreated organisms. However, results were not significant in *H. tubulosa* and are in agreement with Fangyu et al.

(2011). The authors evaluated the effect of antioxidant defense in celomic fluid of *Apostichopus japonicus* in the aestivation phase and, although variations were found to depend on environmental conditions in individual months, significant differences were not observed. Regarding *A. lixula*, we showed significant differencences in peroxidase activity. Our results are in agreement with results for peroxidase activity in cell-free celomic fluid in Section 3.1, in which *A. lixula* individuals were subjected to high-frequency acoustic stress and in which this enzymatic activity increased significantly. Moreover, these results are in accordance with increases in enzymatic activity found in juvenile shellfish individuals exposed to endosulfan (Halliwell & Gutteridge, 1999) or other pollutants (Doyotte et al., 1997; Brouwner & Brouwner, 1998; Geracitano et al., 2002; Geret et al., 2002; Barata et al., 2005). Moreover, this increase in peroxidase activity (also accompanied by increases in esterase activity) seems to be in agreement with results reported by Franco et al. (2016), which showed a significant correlation between esterase activity and antioxidant activity in mussels. This suggests that esterase activity increases in response to oxidants as a compensatory mechanism. It is possible that the increasing trend up 24 hours after the end of acoustic emission observed in our data is related to continuous peroxidase enzyme production and release in cell-free coelomic fluid in response to oxidative stress, as demonstrated in other cases (Monari et al., 2007). Without doubt, the activation of antioxidant defenses in coelomic fluid in some species of echinoderms is a defensive mechanism against ROS invasion (Fangyu et al., 2011); however, even in this case, our results show that all biochemical responses depend on the type of stress and the species analysed.

In summary, the watergun impulsive acoustic emissions caused significant effects only in *A. lixula* coelomic fluid and in particular only in peroxidase activity. Despite this, through this study it was possible to understand the enzymatic responses over time in two echinoderms species. This was a preliminary study and further experiments were done for which the samples will be analysed later and analyzing a greater quantity of individuals. Moreover, probably at coelomic fluid level this type of stress influences only the antioxidant enzymes. To understand this, since the coelomic fluid did not give significant differences, and since significant differences were observed only in *A. lixula* it was decided to analyse the peristomial membrane which is subject to vibrations when struck by an acoustic wave. Further studies are needed to understand if these results depend on the matrix analysed or on the species studied.

The results of this research were presented at the 2019 Effects Of Noise On Aquatic Life conference which took place in Den Haag (The Netherlands) July 7-12 (see Chapter 7).

Arbacia lixula peristomial membrane

The body structure of sea urchin is characterized by a rigid case inside which all the organs are contained. The only soft part, and undoubtedly subject to vibrations, is the peristomial membrane located around Aristotle's lantern. The endoskeletal shell of the sea urchin may have a "soundbox" effect when struck by an acoustic wave, causing a subsequent vibration of the peristomial membrane. It is precisely for this reason that the peristomal membrane was chosen to be analysed; PC and various enzyme activities (esterase, alkaline phosphatase, peroxidase and superoxide dismutase) were evaluated in this specific *A. lixula* tissue for the first time.

PC decreased at the end of watergun acoustic emission lasted 20 minutes, whilst at three hours and 24 hours after the end of emission, PC returned to values similar to those of unexposed individuals, suggesting that the animals implement an adaptive or acclimatization strategy (Da Silva-Castiglioni et al., 2007). PC does not undergo significant changes, according to Celi et al. (2016); however, it is clear from literature that this is a highly variable parameter in invertebrates, that depend on the type and duration of stress, on the type of species and genus, environmental conditions and the age of individuals (Romeo & McEwen, 2006; McLaughlin et al., 2009; Coeurdacier et al., 2011; Weinstock, 2011; Adamec et al., 2012; McEwen, 2012; Miller et al., 2012; Celi et al., 2015; Filiciotto et al., 2016; Vazzana et al., 2017).

Furthermore, it has been demonstrated in rats that decreases in PC levels can be caused by increases in ROS activity following exposure to noise (Manikandan et al., 2005). This then causes harmful effects on proteins, nucleic acids and lipid membranes, thus interrupting cellular functions and damaging their integrity (Endo et al., 2005; Manikandan et al., 2005). In this study, in accordance with Manikandan et al. (2005), decreases in PC observed at the end of watergun emission lasted 20 minutes could be due to increases in ROS reactivity levels.

ROS production following stress conditions has been demonstrated in echinoderm immunocytes (Coteur et al., 2001;2002) and peroxidase activity biochemically measured in cellular lysates (Canicattì, 1990). Superoxide dismutase, catalase and glutathione peroxidase activities have been demonstrated in several phyla invertebrates (Livingstone, 1991). For example, peroxidase has been used to study immune levels in sea urchins *Lytechinus variegatus*, *Strongylocentrotus* *franciscanus* and *Strongylocentrotus purpuratus* (Du et al., 2013), and increases in levels of this enzyme have been found in gametes and embryos exposed to pollutants (Lister et al., 2012; 2016). This type of enzymatic activity has never been studied in the peristomial membrane of sea urchins. In our research, significant increases in peroxidase activity were shown at the end of watergun acoustic emission lasted 20 minutes, returning to control values three hours and 24hours after the end of noise emission. These results are in agreement with a number of scientific studies showing that peroxidase activity increases in tissues of different invertebrate species exposed to stress conditions (Lima et al., 2007; Pan et al., 2009; Parrilla-Taylor et al., 2011; Vidal-Liñán et al., 2014, 2016; Haque et al., 2019; Cao et al., 2019). Since reactive oxygen species are sub-products of aerobic respiration and substrate oxidation (Dalton et al., 1998), and because they play a crucial role in the regulation of various physiological activities, such as cellular apoptosis and tissue dysfunction (Dalton et al., 1999; Bogdan et al., 2000), it is reasonable to suppose that increases in enzymatic activity observed in our study are an attempt by the sea urchin's immune system to compensate for oxidative stress caused by an increase in ROS production in peristomal membrane cells (Aruoma, 1998; Gutteridge et al., 2000). In addition to peroxidase, the antioxidant enzyme superoxide dismutase was also analysed in the peristomal membrane of the sea urchin. Significant increases in superoxide dismutase were also observed at the end of watergun acoustic emission lasted 20 minutes with a subsequent recovery three hours and 24 hours after the end of noise emission. Cellular oxidative damage, antioxidant capacity and enzymatic activities were measured in the tissues of three species of sea urchins (Du et al., 2013) and these studies showed that antioxidant capacity and enzymatic activities can be important mechanisms to mitigate tissue damage. This has also been demonstrated in gametes and embryos of *Sterechinus neumayeri*, in which significant increases in SOD were found when exposed to polycyclic aromatic hydrocarbons (Lister et al., 2015; 2016). However, information on SOD in echinoderms is conflicting. Wang et al. (2008a) observed significant decreases in superoxide dismutase in *Apostichopus japonicus* exposed to different temperatures and salinity. Furthermore, SOD levels changed significantly in the same species during different periods of estivation, in some cases increasing and in others decreasing (Wang et al., 2008b; 2010). SOD activity in *A. japonicus* muscle changed after evisceration, with no significant changes during tissue regeneration. Furthermore, the genus of echinoderm influences SOD expression (Jiang et al., 2017) and at high temperatures, levels of this enzyme in sea cucumber increases significantly, suggesting an increase in oxidative stress (Yunwei et al., 2007). The same occurs in the tissue of other invertebrates with changes in environmental conditions, such as pH, salinity, temperature and pCO_2 (Verlecar et al., 2007; Tomanek et al., 2011; Matozzo et al., 2013). Results from our study confirm the above for peroxidase. Increased SOD levels suggest an increase in oxidative stress with the removal of O_2 and the production of H_2O_2, subsequently removed by the peroxidase enzyme. This is confirmed by increases in both of the antioxidant enzymes at the end of acoustic emission lasted 20 minutes.

In addition, in the case of hydrolases, significant increases in levels of esterase activity measured in the peristomal membrane were apparent immediately at the end of acoustic emission and three hours after the end of acoustic emission. Values decreased, however, 24 hours after the end of emission, returning to values measured in unexposed individuals. Several studies in literature show variations in levels of esterase activity in the tissues of invertebrates subjected to stress conditions (Galloway et al., 2002; Vioque-Fernández et al., 2007; Kopecka-Pilarczyk, 2009; Parisi et al., 2017) however, the data are conflicting and no study has analysed this type of activity in the peristome of the sea urchin to date. Parisi et al. (2017), for example, showed that in conditions of high temperature and anoxia, levels of enzyme activity in the mussel gland decrease. However, this enzymatic activity increased in dependence of the diet administered indicating a subsequent activation of enzymatic activity. Goswami et al. (2014) demonstrated a decrease in esterase activity in the tissues of molluscs exposed to Cu and Cd. However, combined exposure to the two metals caused an induction and, therefore, an increase in enzyme level. Biomarker expression can also vary in different species, as observed by Brown et al. (2004), who analysed the effects of copper exposure on three marine invertebrates. Enzyme levels increased in *Patella vulgata*, in contrast to *Mytilus edulis* and *Carcinus maenas*. Biomarkers in different sentinel species can provide a different "stress diagnosis" and can be used to understand the physiological level of organism impairment. Furthermore, immune responses may be highly specific to tissue. Franco-Martinez et al. (2016) demonstrated numerous changes in the levels of some esterase enzymes compared to control in mussel tissues exposed to different combinations of metals. Furthermore, they showed that esterase enzyme levels in some tissues are higher than others in the same individual, confirming that enzymatic response may change depending on the species and the tissue analysed. Conflicting results in literature on esterase activity could also be due to genetic and molecular polymorphism of the different types of esterases.

Furthermore, distributions and physiological roles of this enzyme differ between species (Massoullie et al., 1993; Scaps et al., 1996; Forget and Bocquene, 1999), and their degree of inhibition (Lundebye et al., 1997) and levels are highly variable, both within and between populations (Rattner and Fairbrother, 1991). Additionally, the increases in esterase activity found in our study could indicate an inflammatory state of the peristomal membrane, which shows that levels of esterase activity can be used as a biomarker in echinoderms exposed to acoustic stress. As for the esterase, alkaline phosphatase also showed significant increases at the end of watergun emission lasted 20 minutes and recovery of enzyme levels already three hours after the end of acoustic emission. This activity has already been studied in sea urchins (Hsiao & Fujii, 1963; Anderson, 1968; Pfolh, 1965,1975; Durkina & Evtushenko, 1991; Menzorova et al., 2014); however, in this study we evaluated alkaline phosphatase activity in the peristomal tissue for the first time. Under stress conditions, numerous studies show a decrease in enzyme activity (Mazorra et al., 2002; Jing et al., 2006b; Chandurvelan et al., 2013; Parisi et al., 2017) while others show an increase (Matozzo et al., 2013; Seitkalieva et al., 2016) or the absence of significant changes (Zhao et al., 2010). High temperatures and pH, for example, cause significant increases in alkaline phosphatase levels (Liu et al., 2004; Hu et al., 2015), demonstrating that these changes significantly affect the immunity of some invertebrates. Zang et al. (2012) showed that during viscera regeneration in sea cucumber, alkaline phosphatase in the muscle tissue reaches a peak on the 10th day after evisceration, then decreases gradually until finally returning to normal. In regenerated tissues, on the other hand, the authors found a tendency towards a increase in alkaline phosphatase starting from the 20th day after evisceration. Even variations in salinity can change the levels of this enzyme in the muscle tissue of other invertebrates (Pinoni et al., 2004). It is also possible that, under stress conditions, the levels of this enzyme change based on the tissue being analysed (Li et al., 2012). In fact, Jing et al. (2006b) demonstrated a different response in alkaline phosphatase levels in different tissues of the same individual exposed to various different copper concentrations for different time intervals. Enzyme activity increased or decreased compared to the control based on the sampling time, metal concentrations and tissue type. Literature shows, therefore, that levels of this enzyme depend on the type of stress, the species and the tissue analysed. In numerous studies on invertebrates exposed to pollutants (metals, for example), protein concentration decreases together with enzyme activity. This is the case with Hg, for example, in the digestive gland and in the gills of *Ruditapes philippinarum*, probably due to the affinity of the metal with the sulfhydryl group (Blasco et al., 1993). In our study, the pollutant in question is an acoustic stress that does not create any kind of affinity with chemical groups of proteins and enzymes. The result is not inhibition of enzyme activity but an increase in production, in response to stress to restore homeostasis. As in the case of esterase activity, we can assume that increases in enzyme levels at the end of acoustic emission lasted 20 minutes is due to inflammation of the membrane resulting from a "soundbox" effect in the skeleton of sea urchin. In response to stress conditions, living organisms express specific sets of proteins, such as HSPs. There is no scientific literature on the expression of HSPs in the peristomal membrane of sea urchins exposed to acoustic stress; however, other authors have analysed the expression of these proteins in other matrices of invertebrates exposed to noise. Celi et al. (2015) observed that the

HSP27 proteins increased in lysates of crustacean haemocytes exposed to acoustic stress. The same was found for HSP70 in the hemolymph of other species of crustaceans (Celi et al., 2013; Filiciotto et al., 2014). Exposure to noise causes increases in HSP70 levels even in humans (Wu et al., 2001), in birds (Hoekstra et al., 1998) and in fish (Vazzana et al., 2017). In accordance with data reported in literature, our results show an increment in expression levels of HSP70 at the end of watergun emission lasted 20 minutes, which subsequently return to levels similar to individuals not exposed to stress. However, this result was not significant. The HSPs could be involved in cytoprotective and antiapoptotic activities following exposure to environmental noise, thus inducing a stress response. Individuals respond to acoustic stress after a short period of time and re-establish homeostasis three hours after the end of noise emission. Although not significant, the trend of our results could confirm that this type of acoustic emission can induces a response to stress and that heat shock proteins are satisfactory biomarkers to establish the state of health of echinoderms subjected to environmental noise as showed in Section 3.1 in cells of *A. lixula* exposed to acoustic stress. The enzyme activity analysed in peristomial membrane of *A. lixula* showed significant changes after noise emission including sea urchin, peristomial membrane and the enzyme activity on the list of possible bioindicators and biomarkers of acoustic impact on invertebrates.

Cortisol level in Chromis chromis

It is known that changes in biochemical parameters (Heath, 1990) are found in fish stressed with acoustic noise; cortisol is the main biomarker which indicates the animal's state of well-being. Cortisol is a glucocorticoid and is released in the blood stream, through activation of the hypothalamic-pituitary-interrenal axis (HPI), to restore homeostasis after stress conditions (Mommsen et al., 1999; Pankhurst, 2011). Its secretion causes a series of behavioural and physiological changes (Mommsen et al., 1999; Moberg & Mench, 2000) which guarantee the survival and well-being of individuals. Measurement of this parameter in fish blood was also used to evaluate activity of the HPI axis (Baker et al., 2013). Many research papers in scientific literature analysed cortisol levels in the plasma of adult fish subjected to different types of stress (Barton, 2000; Grutter & Pankhurst, 2000; Vazzana et al., 2002; Hosoya et al., 2007; Ramsay et al., 2009; Archard et al., 2012; Triki et al., 2016) and, in recent years, this field of study has also concerned the acoustic impact of anthropogenic activities. A number of authors testing various types of noise in different species of fish, observed significant changes in blood cortisol levels (Smith et al., 2004a; Wysocki et al., 2006; Will et al. 2007; Picciulin et al., 2010; Filiciotto et al., 2013; Bruintjes & Radford 2014; Celi et al., 2016). Vazzana et al. (2017), for example, observed significant changes in the plasma parameters of adult *Chromis chromis* individuals subjected to acoustic stress; however, cortisol levels were not analysed. In this study, we sought to evaluate cortisol levels in juvenile individuals of *Chromis chromis* for the first time. The small size of the individuals (which prevented blood sampling) led to the development of a new experimental protocol for corticosteroid extraction from the entire body.

Furthermore, the ability to analyse cortisol levels on a new matrix other than plasma seemed to improve the quality of the result (Carbajal et al., 2019); other authors have previously performed cortisol extraction on the entire body for other species of fish (de Jesus & Hirano, 1992; Feist & Schreck, 2002; Ramsay et al., 2006, 2009). The results of our work showed significant increases in hormone levels up to three hours after the end of watergun emission. 24 hours after the end of acoustic emission, hormone levels returned to values similar to those of individuals not exposed to acoustic stress. Our results are in agreement with data in literature, in particular with the work of Santulli et al. (1999), who observed significant increases in plasma and tissue cortisol levels in fish exposed to airgun emission, with recoveries 72 hours after emission. However, stress levels also depend on the type of noise emitted; Filiciotto et al. (2017), for example, did not observe significant changes in cortisol levels in juvenile sea bream individuals when stressed with offshore aquaculture noise. This difference compared to our work may depend on the period of exposure to noise (Davidson et al., 2009) and on the fact that physiological responses to stress vary between species (Barton, 2002). Our results confirm that cortisol in the tissue of juvenile individuals could be considered a reliable parameter in the study of stress in fish, as observed for plasma parameters (Pickering 1981; Wells & Pankhurst, 1999; Martinez- Porchas et al., 2009; Gronquist & Berges 2013). Further analyses will be carried out on other juvenile fish species subjected to the same experimental plan in order to compare expression of hormone levels in individuals subjected to the same type of acoustic stress.

3.4 The effect of low frequency noise on the behaviour of juvenile *Sparus aurata*

Different types of human activities are causing an increase in underwater noise in marine ecosystems (Slabbekoorn et al., 2010; Hawkins et al., 2015) and for this reason it is considered a real pollutant by the World Health Organization (Kunc et al., 2016), the International Maritime Organization and the Marine Strategy Framework Directive (MSFD) of the European Union (2008/56/EC). The MSFD promotes the achievement of a good quality environmental status for European waters by 2020 and in particular, the descriptor 11.2 on "continuous low frequency sound" aims to monitor trends in the ambient noise level within the 1/3 octave bands of 63 and 125 Hz (centre frequencies). Although several scientific works highlight the importance of understanding better the effects of this new pollutant on individuals, on populations and therefore on whole ecosystems, anthropogenic noise is the least studied source of pollution (Borsani et al., 2015; Hawkins et al., 2015). It is known that anthropogenic sounds overlap the biologically important sounds of animals (Hastings & Popper, 2005; Slabbekoorn et al., 2010), induces different types of responses in fish (Bart et al., 2001; Smith et al., 2004a;b; Popper et al., 2005; Sandström et al., 2005) and has a significant impact on deep sea habitats and marine organisms (Myrberg, 1980; McIntyre, 1995; Popper et al., 2004). As described in the Section 1.6.1 and 1.6.2, several scientific studies have shown that noise pollution has effects at a physical, physiological (Santulli et al., 1999; Wysocki et al., 2006; Buscaino et al 2010; Filiciotto et al., 2017) and anatomical level (Popper et al., 2005; Halvorsen et al., 2012c; Casper et al., 2013a, b). Moreover, further effects have also been observed at a behavioural level (Sarà et al., 2007; Popper & Hastings, 2009a, b; Buscaino et al., 2010; Kunc et al., 2016; Hawkins & Popper, 2017; Spiga et al., 2017). The behavioural responses of marine organisms to anthropogenic sound may affect feeding (Purser & Radford, 2011; Wale et al., 2013; Voellmy et al., 2014; Magnhagen et al., 2017); reproduction and larval development (Aguilar de Soto, 2013; Nedelec et al., 2014, 2015, 2017; Montie et al., 2017); predator-prey interactions (Wale et al., 2013; Simpson et al., 2015, 2016; La Manna et al., 2016); communication (Picciulin et al., 2012; Alves et al., 2017; Shannon et al., 2016); orientation and habitat selection (Holles et al., 2013; Lecchini et al., 2018); and, growth rates and mortality (Filiciotto et al., 2013; Debusschere et al., 2014; McCauley et al., 2017; Kuşku et al., 2018). Acoustic stress can also change the group cohesion, motility, and swimming height of fish (Hawkins et al., 2014; Neo et al., 2014, 2015; 2016; Nedelec et al., 2016a) with negative effects inferred on their capture rate, abundance and distribution (Skalski et al., 1992; Engås et al., 1996; Løkkeborg et al., 2012). There are several studies on behavioural responses of marine organisms subjected to acoustic stress, however the variables that influence behavioural changes are poorly understood. For example, the emission of impulsive or continuous sounds (Neo et al., 2014) or the day/night alternation (Lankford et al., 2003; Vera et al., 2014; Neo et al., 2018) can modify the effect of noise on marine organisms. Although there are many studies on adult fish, impacts of noise on the behaviour of marine juveniles' organisms have not yet been fully investigated (Spiga et al., 2017; Holmes et al., 2017; Ferrari et al., 2018; Zhou et al., 2018). According to some authors, adult fish are more sensitive and vulnerable to acoustic stress than juveniles due to the larger size of the swim bladder and its "resonance" effect (Casper et al., 2013a). However, for juvenile individuals stress can be particularly harmful as it can negatively affect growth (McCormick et al., 1988; Woodley et al., 2003) and eventually, result into an increase of risk of predation (Sogard, 1997). Changes in this period of life can cause dramatic changes in the structure, physiology and behaviour of species and any stress, which changes their sensory information, can negatively affect their ability to assess risk and select appropriate reactions. This could make them more vulnerable to predators by influencing their survival (Mesa, 1994; McCormick et al., 2002; Nilsson et al., 2007; Munday et al., 2010; McCormick & Lönnstedt, 2013) and the future generations. Numerous studies have analysed the effects of specific acoustic emissions (natural or synthetic) on marine organisms, but few have examined the effects of different frequencies on groups of individuals of the same species. Since there are few studies on juvenile fish and since, to our knowledge, there are none comparing the effects of different acoustic frequencies on their behaviour, we studied the stress effects of four different low frequency bands in the acoustic spectrum (1/3 octave centred at 63 Hz, 125 Hz, 500 Hz and 1000 Hz) on juvenile, gilthead seabreams (*Sparus aurata*, Linnaeus, 1758). This is an important commercial fish species which lives in small groups in the demersal zone at a usually depth range of 1 to 30 m (Lloris, 2005). The effects of noise on dispersion, motility and swimming height of the group were analysed. The aims of work are to contribute a better understanding of the effects of the acoustic stress of maritime activities on juvenile individuals of a commercially important fish species, identifying which frequencies have the greatest impact on them and whether they habituate to the sound.

3.4.1 Materials and methods

Experimental animals

The experiments were carried out at the Universitat Politècnica de València (UPV, Gandia Campus, Spain). We used 90 juveniles of *Sparus aurata* coming from a marine farm in Sagunto (Spain) with a weight 14.1 ± 0.9 gr and length 10.5 ± 0.2 cm (mean ± Standard Deviation). Fish were maintained in a circular tank (radius 2 m, water depth 0.75 m). The water of the tank was filtered and re-circulated, with a constant temperature of 12 ± 2 °C, a dissolved oxygen concentration of 8 mg l^{-1}. The fish were maintained in a natural photoperiod and were fed with commercial dry pellets (0.5% of body weight). The animals were not fed for 24 hours before the experiment. Experiments were conducted under authorisation of the, Dirección General de Producción Agraria y Ganadería, Generalitat Valenciana, Spain (authorisation number 2018/VSC/PEA/0156) (Fig.48).

Fig. 48 On the left the fish transport system from the aquaculture center to the UPV laboratory. On the right the acclimatization tank with the oxygenation system.

Acoustics: emission and recording systems

The juvenile individuals of *Sparus aurata* were continuously stressed (seven hours) with white noise filtered at 1/3 octave frequencies of 63 Hz, 125 Hz, 500 Hz, and 1000 Hz at 140-150 dB re 1 µPa at 1m sound pressure level (SPL, dB re 1 µPa) (see Fig. 49). The 63 and the 125 Hz frequency bands are indicating in the MSFD as the descriptors to monitor the level of noise in the sea (Dekeling et al., 2014). Moreover, hearing of fish have the best auditory sensitivity at the frequencies up to 1 kHz (Popper et al., 2003a,b). The estimated sound exposure level for seven hours ranged from 184 to 194 dB, re 1µPa^2 s. The signal was generated using MATLAB® code specifically developed for this work and emitted via a Red Pitaya emission-acquisition system through an amplifier (TA-F161 Sony Integrated Stereo Amplifier) to an underwater loudspeaker (Beyma-UA-UPV prototype) (Fig. 50). The emitted acoustic signal was recorded in the tank using two hydrophones (Reson TC4034) at the beginning and end of the acoustic stress.

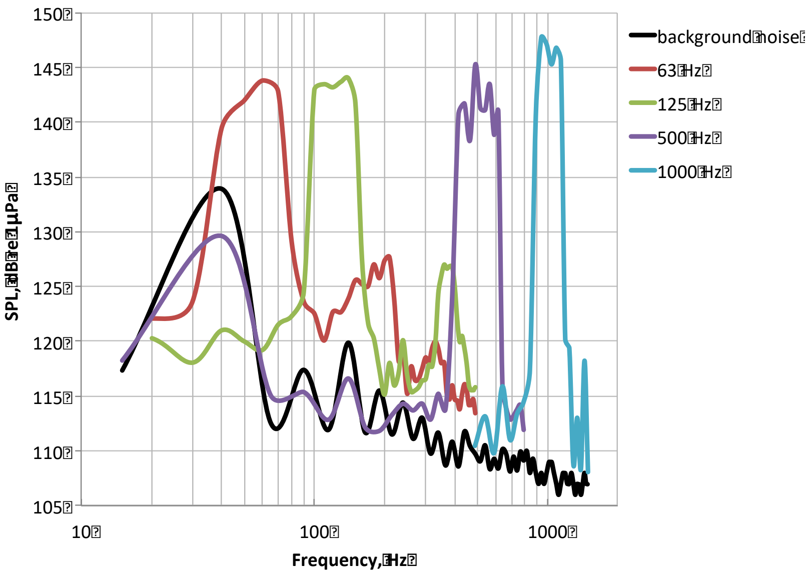


Fig. 49 Power Spectrum (dB re 1 µPa) of tank background noise and different acoustic stimulus.

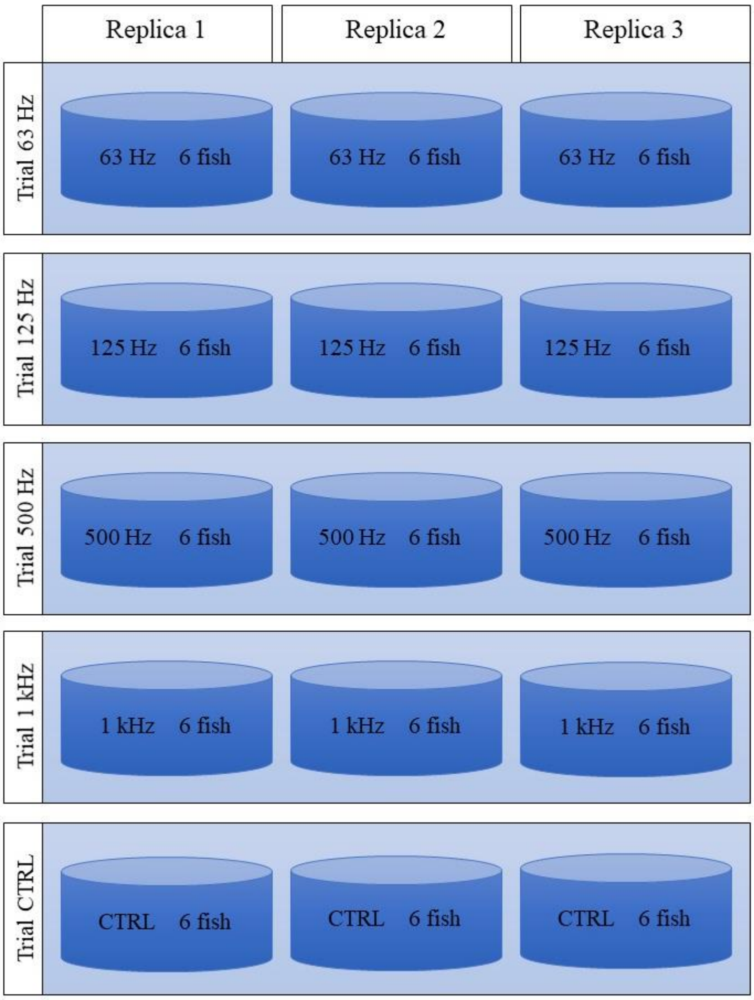
Fig. 50 Acoustic emission system. At the top left are the systems for acoustic emission and video recording. Lower left the emitter inside the tank and the baskets where the fish were placed. On the right the acoustic emitter.

Experimental plan

In total 15 trials were performed, consisting of (Fig.51) three test replicas for each experimental frequency (63, 125, 500, and 1000 Hz) and a further three replicates for the control trial, without any sound emission. For each replicate six fish were used thus totalling 90 fish across all the trials. The experimental trials lasted about one month and the order of the replicates was chosen randomly. The animals to be tested were randomly chosen from the maintenance tank 24 hours before the beginning of each trial, and transferred to the experimental tank, which was identical to the maintenance tank, for acclimatisation (Fig. 52). The hydrophone and the underwater speaker were present in the tank during all the trials, with the speaker turned off during the control trials. Each trial lasted 435 minutes: 15 minutes before the emission and 420 minutes (seven hours) of acoustic exposure.

In order to understand the starting response and the presence of a possible habituation effect of the acoustic stress, considering the autonomy of the two cameras, we chose a specific sampling design for video collection. Each trial, the sampling was divided temporally, as follows:

- 15 minutes before the acoustic emission (Before);
- the first 60 minutes of the acoustic stress emission (D1);
- the final 15 minutes of every hour for the following six hours during which the acoustic stress was emitted, (D2 to D7).



	Replica 1	Replica 2	Replica 3
Trial 63 Hz	63 Hz 6 fish	63 Hz 6 fish	63 Hz 6 fish
Trial 125 Hz	125 Hz 6 fish	125 Hz 6 fish	125 Hz 6 fish
Trial 500 Hz	500 Hz 6 fish	500 Hz 6 fish	500 Hz 6 fish
Trial 1 kHz	1 kHz 6 fish	1 kHz 6 fish	1 kHz 6 fish
Trial CTRL	CTRL 6 fish	CTRL 6 fish	CTRL 6 fish

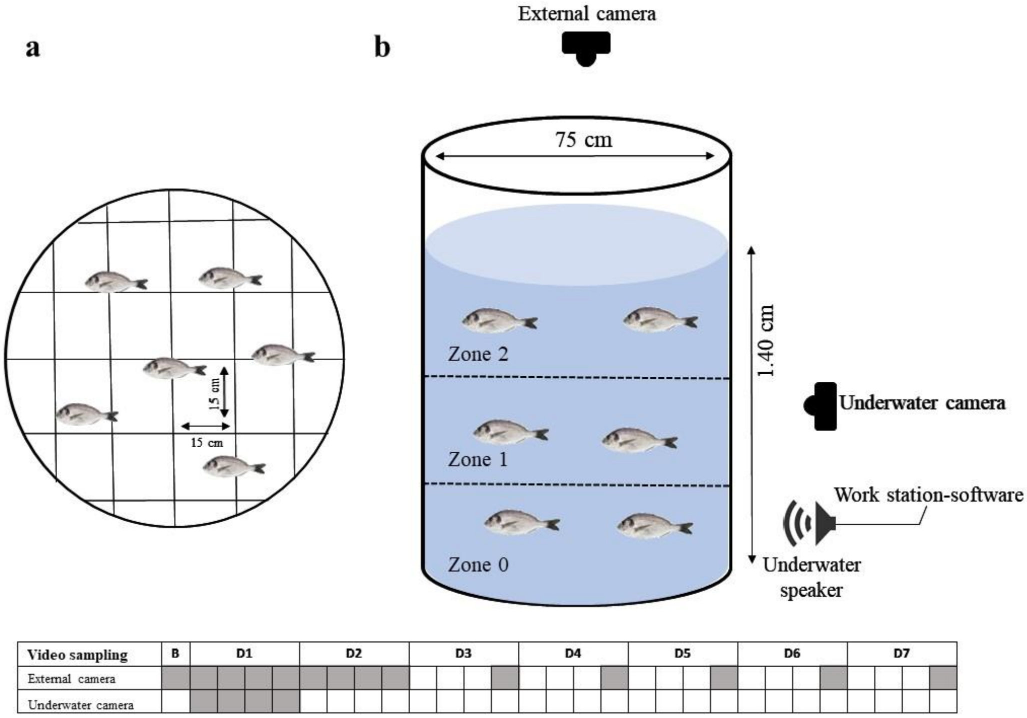
Fig. 51 Experimental plan with replicas for each of four frequency band and control.

Fig. 52 Random arrangement of fishes in experimental tank.

Behavioural analysis

To facilitate the monitoring of the fish behaviour with the video cameras, the fish were confined in a smaller cylindrical net cage (height 1.40 m and diameter 75 cm). The behaviour of the animals was recorded using an underwater camera (GoPro HERO4) (1 m distance from the cage and 35 cm depth) and an external camera (Axis camera 1346) placed at 1m from the top of the cage (Fig. 53B). The arrangement of the cameras was chosen to make the whole cage net visible in the cameras field of view.

For each trial, the camera positioned on the top of the cage (see Fig. 53B) recorded for a total of 165 minutes: 15 minutes before sound emission (Before), then 60 minutes during the acoustic emission (D1), and then during the final 15 minutes of each hour for the next six hours of acoustic emission (D2, D3, D4, D5, D5, and D7). The underwater camera, used for recording the swimming height, recorded only 60 minutes in the D1 period due to battery limitations (see Fig.53B). Analysis of recorded videos was performed to evaluating the fish behaviour in terms of their dispersion, motility, and swimming height (Tab.4) in the water column. These parameters were chosen because other authors demonstrated are effective to highlight behavioural responses following acoustic stress (Hawkins et al., 2014; Neo et al., 2014, 2015, 2016; Nedelec et al., 2016a). The dispersion and the motility behaviours were assessed using recordings from the top camera while swimming height was assessed using recording from the underwater camera. To extract the dispersion and motility data, the bottom of the cage was virtually divided into a grid of 15 cm squares (the total surface of the bottom was 4416 cm^2) (see Fig.53A). For the swimming height evaluation, the water column was virtually divided in three zones (see Fig. 53B).



Video sampling	B	D1				D2				D3				D4				D5				D6				D7			
External camera	▨	▨	▨	▨	▨	▨	▨	▨	▨				▨				▨				▨				▨				▨
Underwater camera		▨	▨	▨	▨																								

Fig. 53 A) Schematic representation of the grid created at the bottom of the basket for the study of dispersion and motility of fishes. B) Schematic representation of the cylindrical cage net, underwater speaker, external and underwater camera; the water column was ideally divided into three parts to study the swimming height of fishes: zero zone (lower), one zone (intermediate), two zone (higher). Below: Timing of each trial and video monitoring. Each square represents 15 minutes.

Dispersion

To analyse the dispersion the recordings from the video camera positioned above the tank was used which framed the bottom of the cage net in the field of view. Dispersion was calculated by counting the number of squares occupied by the fishes (by virtually drawing a closed polygon around the fish group) in one still image every 30 seconds (see Tab. 4). The area occupied was expressed as cm^2 per still image. The first measure was collected at time zero.

Motility

For the motility analyses, the same recordings were used as for the dispersion. A 10 second snippet of video was analysed every five minutes of the recording (starting at time zero). For each snippet the number of grid squares crossed by each fish in that time period were counted (see Tab. 4). The number of squares was then converted into an approximate swimming speed (cm/sec) by considering the size of each square (15 cm^2) and the time period of analysis (10 seconds). In this way, the motility was evaluated for each fish and then averaged.

Swimming height

The swimming height was obtained using the underwater camera (GoPro) video recordings (see Fig. 53 B) which viewed the side of the cage net. The cage was virtually divided into three zones: zone zero (the deepest), zone one (intermediate) and zone two (the highest). Every five minutes, starting at time zero, the zone occupied by each fish was noted (see Tab. 4). These six values were averaged. This parameter was measured only during the first hour of acoustic emission (D1).

Tab. 4 Behavioural categories description.

	Description	Sampling design	Sampling effort	Unit
Dispersion	Area occupied by fishes measured as number of squares occupied by fish per single square area (225 cm^2).	One data every 30 seconds along all video recording both in Before and During sound exposure.	338 frames per trial and 5070 frames in total.	Cm^2
Motility	The number of squares crossed by each fish in 10 second. These 6 values were averaged.	One data every 5 minutes.	41 values for each trials and 615 values in total.	Cm/s
Swimming height	The zone occupied by each fish. These 6 values were averaged.	One data every 5 minutes (only During period).	13 values for each trial and 195 values in total.	Dimensionless Index

Statistical analysis

The experiments were performed with three replicas of six individuals of juveniles of *S. aurata* for each frequency and with three replicas of six individuals for the control trial (Fig. 51). Dispersion, motility and swimming height data were tested for normal distribution using Shapiro-Wilk tests. Non-parametric U-Mann Whitney tests were carried out to evaluate differences in dispersion and motility between the control and acoustic trials for each period of sampling. In order to investigate the non-linear effect of the experimental time (before and during the noise exposure) on behavioural parameters, Generalized Additive Models (GAMs) were carried out for each trial using mgcv package (Wood et al. 2016) in R (v.3.4.0). We used dispersion and motility parameters as dependent variables and the experimental time as smooth term. The family distribution applied in the models was changed in according to the results of normal distribution tests (Gaussian family for normally distributed data and Gamma family for not-normal distributed data). The model diagnostic was checked for each model. Concerning the swimming height, the Kruskal-Wallis test and multiple comparisons post-hoc test were applied comparing different trials only for the first hour of acoustic exposition (D1).

3.4.2 Results

The analysis of the "Dispersion" and "Motility" behaviours between Test and Control groups in the sampling period in the 15 minutes before acoustic emission did not show any differences (see Tab. 5).

Tab. 5 Results of U-Mann Whitney test to explore significant differences in the behaviours Dispersion and Motility between Control groups and each Test groups at different frequencies band (1/3 octave band centred at 63 Hz, 125 Hz, 500 Hz, and 1 kHz) inside each period (Before, D1, D2, D3, D4, D5, D6, D7). Values in bold indicate significant differences between Control groups and Test groups.

		Dispersion			**Motility**		
		Z	**p-level**	**Valid N**	**Z**	**p-level**	**Valid N**
63 Hz	**Before**	-0.64	0.5221	93	0.84	0.4025	12
	D1	12.64	**0.0000**	363	5.64	**0.0000**	39
	D2	3.54	**0.0005**	93	3.97	**0.0001**	12
	D3	0.94	0.3488	93	2.25	**0.0243**	12
	D4	-0.85	0.3969	93	2.92	**0.0036**	12
	D5	-1.32	0.1865	93	3.03	**0.0024**	12
	D6	-0.85	0.3931	93	2.25	**0.0243**	12
	D7	0.72	0.4721	93	3.03	**0.0024**	12
125 Hz	**Before**	-1.33	0.1838	93	-0.03	0.9770	12
	D1	15.94	**0.0000**	363	5.61	**0.0000**	39
	D2	2.62	**0.0094**	93	3.80	**0.0002**	12
	D3	0.20	0.8445	93	0.61	0.5444	12
	D4	0.68	0.4942	93	-0.78	0.4357	12
	D5	1.97	**0.0484**	93	-1.48	0.1379	10
	D6	1.57	0.1167	93	0.32	0.7508	12
	D7	1.42	0.1543	93	1.04	0.2987	12
500 Hz	**Before**	-0.25	0.7990	93	0.12	0.9081	12
	D1	4.17	**0.0000**	363	0.24	0.8105	39
	D2	-0.13	0.9003	93	1.94	0.0531	12
	D3	-1.27	0.2053	93	0.75	0.4529	12
	D4	-1.10	0.2700	93	1.50	0.1333	12
	D5	-0.16	0.8755	93	-0.20	0.8399	12
	D6	-2.59	**0.0095**	93	0.06	0.95402	12
	D7	-4.01	**0.0001**	93	0.00	10.000	12
1 kHz	**Before**	0.88	0.3797	93	0.09	0.9310	12
	D1	0.43	0.6692	363	1.31	0.1888	39
	D2	-3.18	**0.0017**	93	0.70	0.4884	12
	D3	-3.79	**0.0001**	93	1.85	0.0647	12
	D4	-1.57	0.1163	93	3.12	**0.0018**	12
	D5	-3.25	**0.0012**	93	1.53	0.1260	12
	D6	-3.16	**0.0016**	93	3.26	**0.0011**	12
	D7	-3.93	**0.0001**	93	1.33	0.1842	12

Dispersion behaviour

The analysis of dispersion showed significant differences during the first hour of acoustic emission (D1) for all frequencies except for 1 kHz (Tab. 5 and Fig. 54). In D1 a large decrease in dispersion was observed (equivalent to an increase in cohesion) compared to the control groups. For example, for 125 Hz, before emission dispersion was 2922±631 cm^2 (written as mean ± SD) while in D1 the values decrease to 1939± 603 cm^2. At the lower sound frequencies (63 and 125 Hz) the differences between the control and acoustic groups decreased with time of exposure (Fig. 54) with a gradual return to approximately control values. Conversely, at the higher frequencies (500 and 1 kHz) the differences increased over time, with higher values for the acoustic test groups compared to the control groups. The GAMs analysis confirmed the effect of time of exposure at all frequencies (see Tab. 6) with higher explained deviance in 63 Hz, 125 Hz and 500 Hz. The smooth term for control trial significant modelled the data but with a very low explained deviance (4.09%) (see Tab. 6).

Tab. 6 Results of Generilized Additive Models (GAMs) considering, as dependent variable, Dispersion and Motility parameters and, as smooth term, experimental time.

Dispersion ~ s (Experimental time)

Trials	CTRL (n=1011)		Estimate	Std.Error	t value	p value
		Intercept	7,93	0,005	1541	.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	8,359	1,45	<0.001	4,09%
	63 Hz (n=1014)		Estimate	Std.Error	t value	p value
		Intercept	7,811	0,0069	1123	.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	23,62	26	<0.001	40,20%
	125 Hz (n=997)		Estimate	Std.Error	t value	p value
		Intercept	7,789	0,00693	1124	.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	21,41	23,28	<0.001	33,10%
	500 Hz (n=1004)		Estimate	Std.Error	t value	p value
		Intercept	7,905	0,00628	1259	.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	26,24	25,45	<0.001	34,60%
	1 kHz (n=1013)		Estimate	Std.Error	t value	p value
		Intercept	7,9511	0,005921	1343	.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	24,6	14,26	<0.001	25,50%

Motility ~ s (Experimental Time)

Trials	CTRL (n=123)		Estimate	Std.Error	t value	p value
		Intercept	3,185	0,068	46,7	<.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	1	0,22	0,622	0,20%
	63 Hz (n=119)		Estimate	Std.Error	t value	p value
		Intercept	2,179	0,079	27,5	<.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	6,881	4,009	<0.001	25,70%
	125 Hz (n=997)		Estimate	Std.Error	t value	p value
		Intercept	2447,24	16,56	147,8	<.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	12,55	37,41	p<0.001	33,30%
	500 Hz (n=118)		Estimate	Std.Error	t value	p value
		Intercept	1,119	0,029	38,2	<.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	7,78	2,56	<0.05	16,50%
	1 kHz (n=121)		Estimate	Std.Error	t value	p value
		Intercept	2,68	0,0947	28,29	p<.001
			edf	F	p-value	Dev. Explained
		Smooth term (Experimental time)	4,309	1,418	0,212	7,90%

Motility behaviour

A general and significant decrease of motility was observed for the fishes exposed to acoustic noise. As for the dispersion, this reduction was largest in the first hour of exposition (D1). The analysis of motility showed significant differences during the first hour of acoustic emission (D1) for 63 and 125 Hz (Tab. 5 and Fig.54) with values that changed from 3.3 ± 1.3 cm/s to 1.5 ± 1.1 cm/s for the 63 Hz trial and from 3.2 ± 0.3 cm/s to 1.7 ± 0.9 cm/s for the 125 Hz trial. At 63 Hz the animals did not return to the control values for the rest of the trial, butdisappeared in the 125 Hz trial after three hours of exposure (D3). At 500 Hz no significant differences were observed between the control and acoustic test groups, even though on average lower values of motility were recorded during the first four hours of acoustic expositions. The 1 kHz groups showed significant differences compared to control groups after in four and six hours (D4 and D6) of exposition to the noise (see Fig.54). In summary, the time of exposure was found to significantly affect the motility behaviour of the fish at most of the tested acoustic frequencies (63, 125 and 500Hz), with higher explained deviance for 63 Hz and 125 Hz trial. No significant effect was found for control and the highest test frequency of 1 kHz (see Tab. 6).

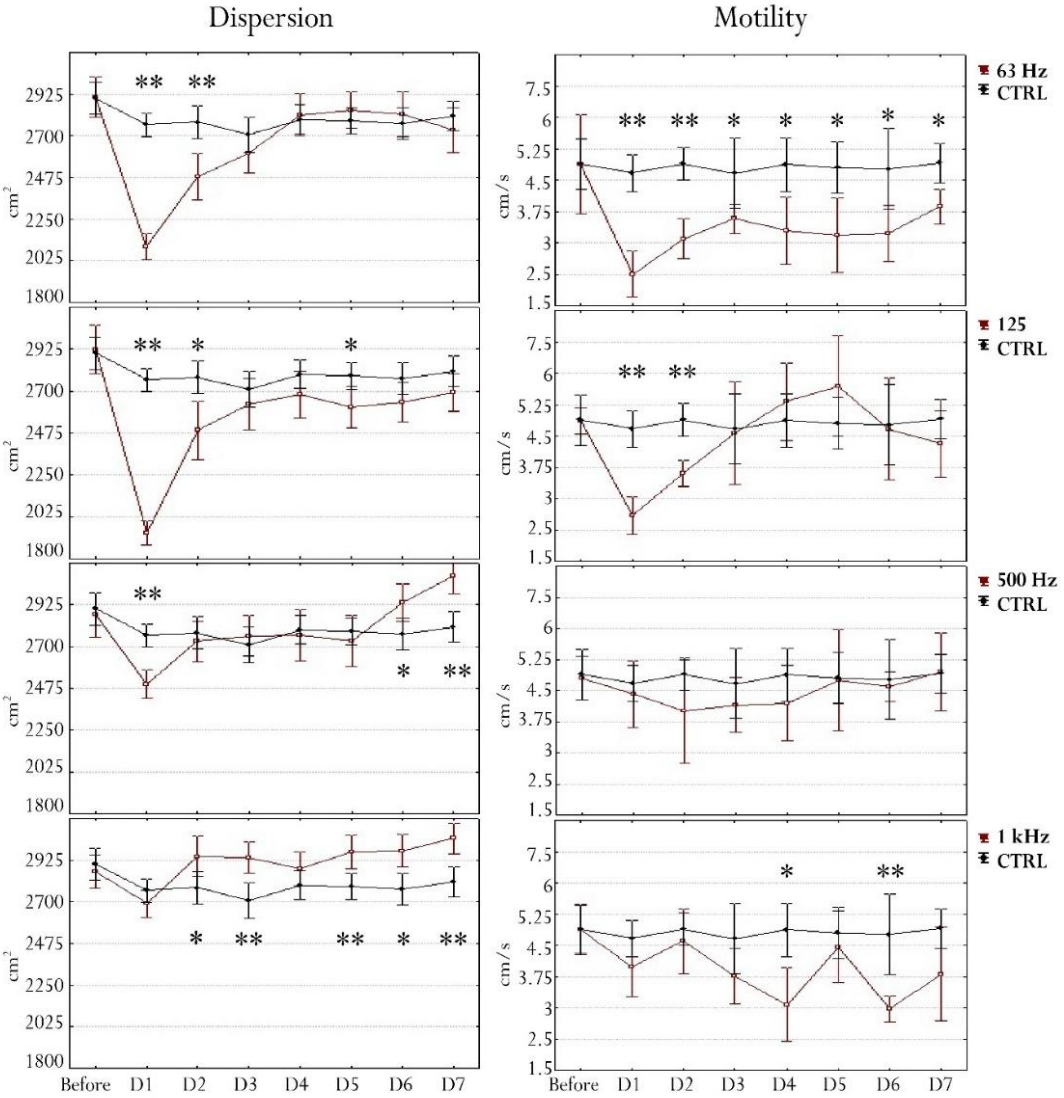


Fig. 54 Left: Average ± 95% confidence interval of the dispersion obtained for each replicate by counting the number of squares occupied by the group of fishes every 30 seconds. Right: Average ± 95% confidence interval of motility obtained for each replicate by counting the number of squares crossed by each fish in 10 second and then averaged. Statistically differences are showed within the same period between control and acoustic groups (*p <.05, **p<.0001).

Swimming height behaviour

Fish exposed to noise were generally observed to swim towards the bottom (Fig. 55). The swimming height of the fish, assessed during the first hour of acoustic exposure, showed significant differences of between the acoustic groups versus control groups (Tab. 7). Only the 125 Hz tests did not show any differences compared to control (Tab. 7).

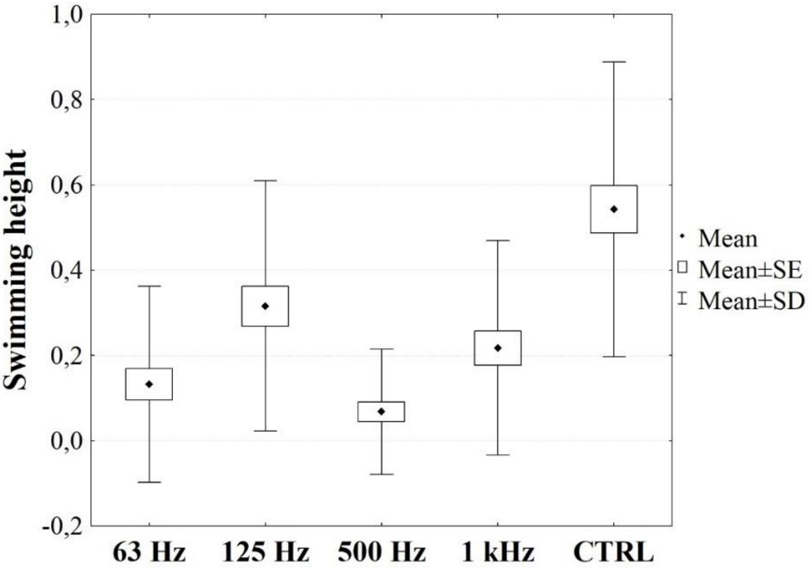


Fig. 55 The swimming height obtained dividing the water column in different zone: 0 for the lowest, 1 for the middle and 2 for the higher. The multiple comparison post hoc test showed significant differences between CTRL trials vs. all sound type trials (*p<.05) excluded the 125 Hz trials (p=.13).

Tab. 7 Results of Kruskal Wallis and Multiple comparisons post-hoc test for Swimming height (z' values). Values in bold are significant at p <.05 while values in bold with* are significant at p<.0001.

		63 Hz	**125 Hz**	**500 Hz**	**1 kHz**	**CTRL**
63 Hz	K-W test H (4, N=195) = 54.5 **p<0.0001**		**2,82**	0,92	1,51	**5.29***
125 Hz		**2,82**		**3,74**	1,31	2,47
500 Hz		0,92	**3,74**		2,43	**6.21***
1 kHz		1,51	1,31	2,43		**3,78**
CTRL		**5.29***	2,47	**6.21***	**3,78**	

3.4.3 Discussion

The results of this study have highlighted significant variations in the behavioural responses of juvenile fish depending on the exposure to different acoustic frequencies. The greatest impact was observed at lower acoustic frequencies (1/3 octave band centred at 63 Hz and 125 Hz). However, also the higher frequencies elicit significant behaviour changes especially in the last hours of the monitoring (see for example the dispersion at 1 kHz).

Regarding the dispersion, it was observed that the control specimens (i.e. groups that did not receive any sound exposure) occupied a larger area compared to the test groups. However, control fishes tended to be closer to each other and occupied on average half of the available area. Considering that our study was carried out during daylight hours, this result is in agreement with Hawkins et al. (2012), who observed a greater cohesion during daylight hours in fish *Sprattus sprattus* exposed to acoustic stress. Our results demonstrate that all frequencies tested had a significant effect on the dispersion. The initial effects were noticeably different depending on frequency but generally occurred immediately after exposure to the noise. The fish also showed evidence of returning to control behaviour over time with the length of time for this to happen also depending on the particular acoustic frequency.

The 63 Hz and 125 Hz sound exposures had an immediate behavioural effect visible in the first two hours of emission that was greatest at 125 Hz. After that, a gradual habituation effect was observed. At 63 Hz, the specimens showed values comparable to the control at the third hour which remained for the last five hours of exposure, whereas at 125 Hz the average dispersion stayed slightly lower with a significant decrease occurring on the fifth hour of exposure (D5). For the 500 Hz tests, a significant reduction of dispersion was observed during the first hour of acoustic emission followed by a significant increase in the last two hours. For the 1 kHz tests, the animal's dispersion increased significantly after the second hours of emission.

The differences in dispersion behaviours during low and high frequency exposition could possibly be due to differing sound sensitivities of the fish. Nowadays, the audiogram of many fish species, in this case of juvenile *S. aurata*, is not known, but the frequency ranges from 50 and 1122 Hz (with an SPL of 140-150 re 1μPa) is generally heard by most fish species (Popper et al., 2003a, b). Even if more specific studies need to be performed, we suggest that the fish responded differently to the frequencies used in experimental plan due to their different acoustic sensitivity to the acoustic stimulus. The recovery time observed only for the 63 Hz and 125 Hz treatments could be due to either habituation or sensory adaptation (Domjan, 2010). The possibility of the species to get used to acoustic stress has been observed in previous studies (Neo et al., 2014, 2015, 2016, 2018; Nedelec et al., 2016a) and according with Neo et al., (2014), our continuous noise may have favoured a partial habituation effect compared to an intermittent noise. When an organism is exposed to a continuous noise it is subjected to a continuous stimulation that involves a rapid habituation to stress and therefore a more rapid recovery (Rankin & Broster, 1992; Rankin et al., 2009). In fact, in the presence of intermittent sounds the recovery to pre-exposure levels in sea bass was slower (Neo et al., 2014; Koolhaas et al., 2011; Rankin et al., 2009).

Our results confirmed the effect of anthropogenic noise on group dispersion and they are in agreement with the formation of closer groups as described in Fewtrell and McCauley, (2012). We confirm that dispersion can be a good behavioural impact indicator even using a small experimental arena, where the ability of the individuals to see each other is elevated.

Regarding motility, significant effects on behaviour were found during the first hour of emission at lower frequencies (63 Hz and 125 Hz). The reduction of motility observed at these frequencies is not in agreement with the results obtained by Buscaino et al. (2010) and Neo et al. (2016) who tested animals in the frequency range between 0.1 kHz and 1 kHz. However, adult individuals of *S. aurata* and different acoustic frequencies were used in those studies, and for this reason a different response in juveniles might be expected (Holmes et al., 2017; Ferrari et al., 2018).

The recovery of motility over time to control values could be due to habituation or sensory adaptation and/or muscle fatigue (Domjan, 2010; Neo et al, 2014). Between the two lowest tested frequencies, 63 Hz seems to have the most impact and the animals never showed a recovery behaviour. Conversely, at 125 Hz the animals returned to the control values on the third hour of exposure, subsequently exceeding them.

These increases in speed, even if not significant, may also depend on sudden reactions and accelerations (Blaxter et al., 2009; Fewtrell & McCauley, 2012; Kastelein et al., 2008; Pearson et al., 1992; Purser & Radford, 2011; Wardle et al., 2001), which are caused by Mauthner cells. These play important roles in anti-predation and anxiety behaviours (Cachat et al., 2010; Eaton et al., 1977) which could indicate negative impact of low frequencies on fish motility. Reactions of this type have also been observed in this study at 1 kHz where the data showed a higher variability. This could be influenced by a considerable amount of attacks and startle reactions. Probably, the animals become more irritated and scared.

The swimming depth significantly decreased at all frequencies except at 125 Hz. Changes in swimming depth due to noise are in agreement with the results of Sarà et al. (2007) on tuna subjected to motorboat noises. Several studies have shown that fish dive deeper after noise exposure inducing movements in the vertical axis along the water column and not in the horizontal axis (Doksæter et al., 2012; Fewtrell & McCauley, 2012; Gerlotto & Fréon, 1992; Handegard et al., 2003; Slotte et al., 2004). Moreover, our results are in agreement with Neo et al. (2016), who observed an increase in swimming depth in *S. aurata* adults as a response to acoustic stress. Moreover, Neo et al. (2016) observed that the fishes when exposed to an acoustic stimulus tended to get close to the emitter increasing their swimming depth. This was explained as a possible phonotactic response due to the curiosity towards the sound emitted (Nelson & Johnson, 1972; Weilgart, 2007). However, in our study, the greater depth of swimming due to curiosity can be discounted because the fish tended to be in the part of the basket more distant from the acoustic emitter. The observed behaviour of swimming towards the bed could be due to anxiety situations (Cachat et al., 2010; Israeli-Weinstein & Kimmel, 1998; Kuwada et al., 2000; Luca & Gerlai, 2012; Skilbrei & Holst, 2009; Wilson & Dill, 2002) also observed in outdoor studies (Gerlotto & Fréon, 1992; Handegard et al., 2003; Slotte et al., 2004). Our study confirms that swimming depth can be a good behavioural indicator of response to stress, demonstrating an attempt to escape and perhaps a reduction in risk of predation.

In our work, we did not consider the sensory adaptation at the base of the recovery of the variables since it is a type of recovery that requires longer times, even weeks (Smith et al., 2004a, b.; Wysocki & Ladich, 2005). Furthermore, the possibility that fish get used to acoustic stress does not exclude the presence of any negative impacts (Bejder et al., 2009). In fact, although fish get used to stress, it is possible to find effects on essential functions such as the distribution and organization of the group with consequences at a physiological (Anderson et al., 2011; Filiciotto et al., 2013) and auditory level (Vasconcelos et al., 2007). The behavioural changes observed, although it has not been demonstrated, could have consequences on the survival, reproduction, foraging, and growth of the species and on the time of surveillance of the offspring which could also be adversely impacted (Picciulin et al., 2010; Blom et al., 2019). The laboratory data certainly are different from the data that can be obtained in natural environment; however, they are useful since fish in the wild showed major behavioural reactions to acoustic stress compared to the captive fishes (Benhaïm et al., 2012; Lepage et al., 2000). Although in our work some variables in the behaviour analysis (500 Hz on motility and 125 Hz on swimming height) have not shown significant differences, this does not indicate the absence of an effect since individuals can respond to stress with different strategies (Koolhaas et al., 2011; Silva et al., 2010). Even unknown environmental conditions can influence behaviour (Brewer, 2000). Our data have not been correlated to physiological parameters, although probably a hormonal variation would have been found following acoustic stress (Anderson et al., 2011; Santulli et al., 1999; Smith et al., 2004a; Wysocki et al., 2006). However, the correlation between the behavioural and physiological response in stress conditions is not clear (Silva et al., 2010). Although further studies are needed, even on other juvenile fish species, this result could be important to begin to understand which specific frequencies influence them.

The results of this research were presented (see Chapter 7) at the 2019 Effects Of Noise On Aquatic Life conference wich take place in Den Haag (The Netherlands) from July 7-12 and at the XI Congreso Iberoamericano de Acústica; X Congreso Ibérico de Acústica; 49º Congreso Español de Acústica -TECNIACUSTICA' 18 (FIA 2018). Cádiz 24-26 October, 2018.

4. Technical-technological module

During the third year of study, have been spent six months at the HR Wallingford company (Howbery Park Wallingford, Oxfordshire OX108BA United Kingdom). HR Wallingford is an independent, non-profit-distributing, commercial research organization operating globally in the fields of civil engineering, environmental hydraulics and water management. During this period, the results obtained from behavioural analyses on juvenile individuals of *Sparus aurata* from an experimental plan carried out at Gandia Campus in Spain were used (Section 3.4). Data from *in vivo* analyses were used to create a numerical model that would allow us to predict acoustic impact of human activities on marine organisms. I was given initial training to learn about the different activities carried out at the company and then how to create the numerical model. In the final stage, when the numerical model was ready, one model for each experimental frequency and for the control of the experimental plan effectuated in Spain was elaborated. The models were created using the results of dispersion, motility and swimming height from *in vivo* experiments on *S. aurata* juveniles. The models were considered reliable once behavioural analysis, carried out in the same way as the *in vivo* experiments, reported the same results. Agent Based Models (ABMs), such as Boids, allow simulation of anthropic or environmental impact on fish populations. This type of model is important in a context of environmental impact assessment (EIA). The ability to use ABM models for EIA was studied by HR Wallingford and led to the creation of the HydroBoids model (Benson et al., 2016). The algorithms underlying the model's operation were described by Benson et al. (2016). HydroBoids is an ABM that has existed for several years and has recently undergone significant development. It was used in a collaboration with the Zoological Society of London to identify the breeding grounds for Smelt in the Thames estuary (https://www.zsl.org/conservation/smelt-osmerus-eperlanus) and with the University of Southampton to study the interaction of eels with hydroelectric turbines (http://nexus.soton.ac.uk/vaccinating-the-nexus). The model is currently encoded in Matlab and new algorithms will be incorporated into the model by studying the behavioural variations of different species of fish. The model is developed in such a way that changes to the software can be made and the results can be viewed immediately for rapid development and good quality control. It offers useful information on population dynamics and is easily adaptable to a wide range of scenarios. The results obtained from the behaviour of the *S. aurata* juveniles in the *in vivo* experiment carried out in Spain have enabled further development of the model with the creation of new algorithms.

4.1 Acoustic effects on the behaviour of *Sparus aurata* juveniles: *in vivo* measurements and agent-based modelling

In the Section 1.6.2 it has been highlighted that the acoustic emissions of anthropic activities have negative effects on the behaviour of marine organisms, from invertebrates, to vertebrates and mammals. In particular, anthropic noise can also influence schooling (Halfwerk et al., 2015), motility (Mendl, 1999; Buscaino et al., 2010) and swimming depth (Neo et al., 2014, 2015, 2016, 2018) and several studies confirm negative impacts on juvenile fish at a physiological (Filiciotto et al., 2013, 2017) and behavioural level (Holmes et al., 2017; Simpson et al., 2015).

Despite the growing evidence in the literature on the potential impacts of noise on fish, the ability to predict the impacts is less developed, largely due to the wide range of behaviours exhibited by different fish species. Other researchers have previously used agent-based models to predict fish response to anthropogenic activity (Willis, 2011; Herbert et al., 2012; West et al., 2011; Peck & Hufnagl, 2012; Testa et al., 2012; Willis, 2007) and despite uncertainty in the predictions provided by such models (Willis, 2011) these techniques can still contribute to the EIA process in the analysis of acoustic impacts on marine biodiversity (Chan & Blumstein, 2012; Kight & Swaddle, 2011). Further research into ways of improving agent-based modelling techniques is therefore justified.

Like all models, agent-based models rely on good data for calibration and validation. *In-situ* data on fish behaviour in response to noise is generally difficult to obtain in a controlled manner, is expensive to collect and measurements can often be sparse and therefore hard to interpret. Laboratory experiments are therefore commonly used for observing and quantifying fish behaviours in response to stresses such as underwater sound. Laboratory methods on their own are useful for understanding fish behaviour, but scaling up to real world behaviour is problematic due to differences in the fish status (e.g. stress levels) or the absence of important behavioural cues (e.g. flows, deep water, food or predators). Numerical models offer a way of bridging the gap between laboratory and field scale. Results obtained *in vivo* can be used to parameterise numerical agent-based models of fish behaviour (Benson et al., in prep.). The parameterised models may then be applied to real world situations to evaluate the potential impacts of acoustic emissions in order to help mitigate against them. Validation of such field scale models is still a difficult challenge, but understanding gained from the modelling process itself can, for example, be used to target the collection of more relevant *in-situ* validation data (such as suitable measurement locations or depths) thus minimising costs.

In this study, the results obtained from experimental plan in Section 3.4 of an *in vivo* experiment were applied to a numerical agent-based model of fish behaviour. From these data, a numerical agent-based model was developed to simulate the basic behaviours of the fish. The overarching aim of the work was to assess the behavioural changes in *Sparus aurata* when subjected to underwater sound at a range of frequencies and apply the observed behaviours to a numerical agent-based model. It is envisaged that the model presented here might lead to larger scale ecological modelling of potential impacts of anthropogenic noise for this species.

4.1.1 Materials and methods: Numerical modelling methodology

Fish behaviour model

To obtain fish behavioural model the behavioural data obtained by *in vivo* experiment were used (see Section 3.4 for experimental plan and fishes manteinance). The model framework of the ABM, called HydroBoids, consists of two parts: a general fish tracking model and a species-specific behaviour model.

For fish tracking, a coupled Lagrangian-Eulerian model was used which tracked the movement of Lagrangian particles (or fish) due to advection and dispersion by the flow field provided by a Eulerian framework hydrodynamic model. No flows were required in the present study since the fish were in a still water tank and therefore the flow velocities were set to zero in the model.

To simulate behaviour, the fish were assigned physiological traits (e.g. size and swim speed) and were programmed to perform particular tasks (e.g. schooling and vertical migration). The fish were able to make decisions based on a combination of their local environment (e.g. underwater sound level), their own physiology (e.g.

hearing threshold and frequency sensitivity) and reactional behaviour (e.g. changing depth and swim speed).

Modelled behaviours

In an initial phase prior to model development, in order to be able to transform the behavioural data obtained in the *in vivo* experiment into a numerical model, it was necessary to form a conceptual model of how the fish behaved when they were exposed to acoustic stress. This was fundamental for choosing the correct basic animal behaviours on which to base the numerical model. To do this, the fishes behavioural responses were analysed as described in Section 3.4.1 to obtain the numerical data relating each of the three behavioural parameters (swimming height, cohesion, motility). Based on these quantitative data and many hours of visual observations of the fish behaviour, an understanding was obtained at a conceptual level as to what basic behaviours the fish performed which led to the changes in the three measured parameters. During this process an assessment was also made as to whether different parameters could influence each other (e.g. if cohesion could be influenced by swimming speed or swimming height affected cohesion, etc.). Efforts were made during this process to try not to overcomplicate the model, choosing the fewest behaviours possible to achieve a suitable comparison with the measurements. The physiological characteristics and behaviours of *Sparus aurata* that were determined to be important for the current modelling work were as follows:

1. Swim speed and directional error
2. Vertical migration and holding stationary when at the bed

3. Schooling behaviour
4. Swim velocity change

Swim speed and directional error

The main physiological characteristic parameterised in the model was the fish swim speed, which was assigned to each individual in the population from a normal distribution of speeds. The simulated fish then moved under their own propulsion according to a correlated random walk (CRW) algorithm (Codling et al., 2008; Willis, 2011) whereby the direction of the fish at each model time step was dependent on the direction at the previous time step (Fig. 56). A directional error term was added at each time interval chosen randomly from a normal distribution with a predefined standard deviation. Standard deviations were prescribed separately for the horizontal (azimuth) and the vertical (elevation) angles, with maximum limitations of 180º and 90º respectively.

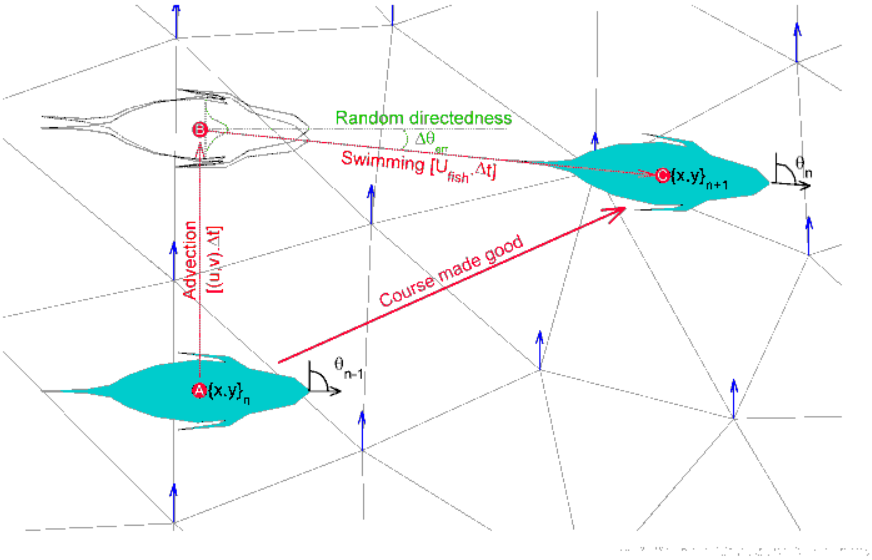


Fig. 56 Schematic of 2D fish movement using a correlated random walk and advection by hydrodynamic flows (indicated as blue vectors on at triangular mesh nodes)

Vertical migration and holding at the bed

Fish within the model were assigned a preferred height above the bed, or depth below the surface, which they actively tried to maintain. Vertical migration occurred in the model simply by modifying each fish's elevation angle (positive or negative swim angle from horizontal) at every model time step. Reasons for such a behaviour include fright response or predator avoidance (Cachat et al., 2010; Skilbrei & Holst, 2009) and selective tidal stream transport (Harrison et al., 2014). In addition, to simulate the observed fishes' response to noise, when the fish reached the bed they were commanded to stop swimming horizontally so they remained stationary.

Schooling behaviour

Schooling behaviour was already included in the HydroBoids model and is based on the Boids method of Reynolds, (1987). In this method, three simple rules are prescribed to all the individuals to control schooling. These rules are: school centring (or cohesion); collision avoidance (or separation); and, velocity matching (or alignment). The three behaviours mean that the fish interact with their local neighbours by steering towards the centre of the group, but avoid contact, whilst also matching the groups average speed and direction. A key parameter for schooling was maximum school speed. This is used to limit the combined swim speed vectors for school cohesion, separation and alignment to a specified maximum magnitude, thus limiting the overall school speed.

Swim velocity change

During the laboratory testing for this study, the fish were observed to change their swim speed and direction at seemingly random intervals. Reasons for this observation included behaviours not explicitly included in the model such as scavenging for food or aggressive behavioural interactions with nearby fish. To capture this behaviour, a heuristically based model was included in the ABM as part of this study whereby randomly selected fish (chosen according to the probability of velocity) changed their speed (S) and direction (θ) at time interval i according to the following formulae:

(1) $$S_i = \max\{S_{ini}[1 + N(0,1).U(0,1)], 0\}$$

(2) $$\theta_i = \theta_{i-1} + N(0,1)\frac{\pi}{2}\partial t$$

Where:

S_i , θ_i = speed and direction (in radians) at the present time interval (i)

S_{ini} = normal swim speed of the fish (initialised at the start of the simulation)

$N(0,1)$ = a random decimal number chosen from a normal distribution with zero mean and standard deviation of one.

$U(0,1)$ = a random decimal number between 0 and 1 chosen from a flat distribution.

∂t = time step interval (s)

The term $\frac{\pi}{2}\partial t$ at the end of Equation 2 resulted in a heading change with a standard deviation of 90 degrees in one second.

Decision making

An important component of any agent-based model is the mathematical implementation of how individuals make decisions. In HydroBoids, at each model time step interval each fish decides which behaviour out of the set of defined behaviours to undertake. This occurs mathematically using a probability of each behaviour occurring (across the whole population) which is set by the user for each of the chosen behaviours to be modelled. The method is shown schematically in Fig. 56. The probability for all the behaviours is specified in such a way so that they sum to less than or equal to one. If less than one, the remaining fraction accounts for the fish not making a decision at every time interval (i.e. they continue as on the previous time interval). During the simulation, at each time step a random number is generated (represented as the spinning the arrow in Fig.57) for each fish to determine which activity to perform during that time step. This process is overridden if the fish individual finds itself in a situation involving high risk such as a dangerously high stimulus above a specified threshold (in this case a loud underwater noise level) or in the presence of a predator that is within a specified range. In such instances the fish is assumed to be in *fright mode* and the probability is ignored and the individual responds regardless.

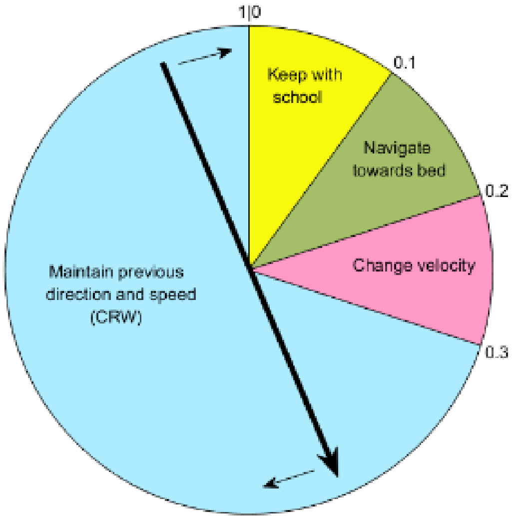


Fig. 57 Schematic diagram of decision making by probability assuming three behaviours, each with a probability of 0.1 in this example. The black arrow represents a random number generator which is spun at each time step for each fish and points to a number between 0 and 1 to choose a behaviour.

Model setup

A HydroBoids model was set up to mimic the experimental setup described in Section 3.4.1. The model results were output to computer generated video animations which were then manually processed in the same way as the laboratory camera recordings (Fig. 58). Only the first hour of sound emission was simulated since the recorded behavioural changes during the laboratory experiments were visually most pronounced during this time period.

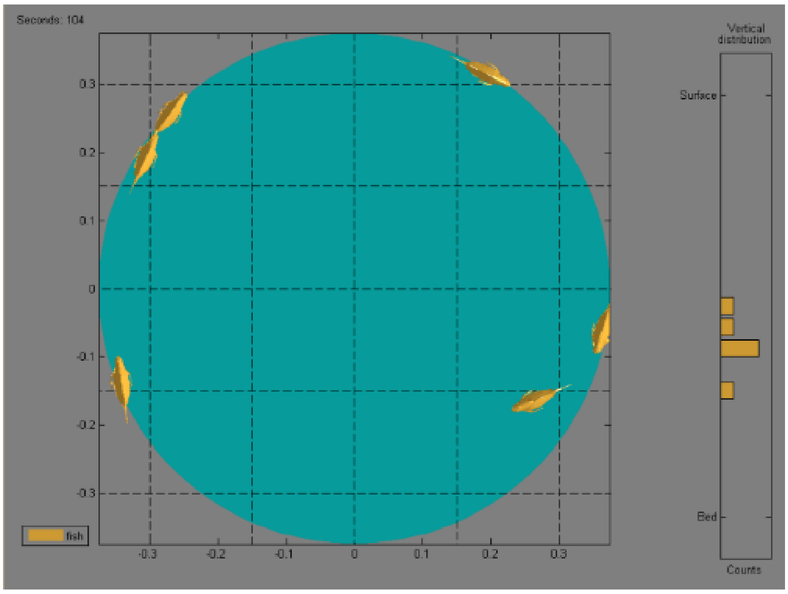


Fig. 58 A single frame of the HydroBoids model output. On the right is a histogram indicating the height of the fish in the water column. The time in seconds is displayed in the top-left corner.

Parameter selection

To reproduce the behavioural patterns of the fish over the full hour of the observations for each treatment, the modelling was divided into four separate modelled periods: from zero to five minutes; from five to 15 minutes; from 15 to 30 minutes; and from 30 minutes to one hour. These time intervals were chosen based on the observed changes in fish behaviour, which tended to occur more rapidly towards the start of the experiment and gradually reduce over time.

Before modelling, the *in vivo* video recording results were first analysed separately for the four different time intervals to provide data on the group cohesion, motility, and swim height as described in Section 3.4.1. Then, starting with the control (no sound) condition, an iterative process was carried out whereby numerous models were run, modifying the model parameters each time, with the objective to obtain results similar to the different metrics analysed in the real videos.

During the *in vivo* experiments, it was observed that, at the start of the acoustic emission, the fish swam very quickly to the bottom and stopped. Therefore, a short initial sound reaction model, lasting just 17 seconds, was first simulated using the final positions of the fish from the control model at the start, to obtain the initial position of the fish from which to start the models of the four treatment frequencies. The final position of the fish from the reaction model was then taken to be used in the sound treatment at 63 Hz model for the period from zero to five minutes. The modelling was then progressed to the next time interval (five to 15 minutes) and so forth, each time using the final position of the fish as the start position in the next model. After a full set of parameterised models for the 63 Hz treatment were completed (i.e. covering the full period from control to 1 hour exposure), the sound exposure experiments were then modelled in the same manner for each of the other sound exposure frequencies (125 Hz, 500 Hz and 1 kHz).

The results obtained from the elaboration of the numerical model on cohesion, motility and depth again were then compared to the results obtained from *in vivo* experiments. If the result was not similar to within a predefined error, the model was corrected and rerun. Behavioural data were again processed manually and compared with data obtained from the *in vivo* experiment. These steps were repeated until the results of the numerical model were comparable to those of the *in vivo* experimental plan.

Statistical analysis

The processed video and model animation data were analysed using Tukey's multiple comparison tests. Statistical analysis was used to evaluate the significant differences between the control and each experimental treatment. For the *in vivo* experiments, the control and each individual acoustic treatment were repeated and analysed three times. For each individual model, only a single model was analysed for each treatment since the model was representative of the average behaviour *in vivo*. The results are expressed as a mean ± S.E.

4.1.2 Results

In vivo experiment results

During the *in vivo* experiments, significant differences in group cohesion, motility and swimming height were observed in fish behaviours between each frequency treatment and the control during the first hour of emission. Even if results are relative to the Section 3.4, we subsequently report a table that resumes the data obtained *in vivo* only regard the first hour of acoustic emission, being that the numerical model was realized only for this part of experiment. The behavioural results obtained for the control and each treatment are shown in Tab. 8.

Tab. 8 The table shows the behavioural results obtained elaborating the *in vivo* video recording. The data are expressed as mean values.

Laboratory measurements	**Motility (cm/sec)**	**Cohesion (cm^2)**	**Swimming height (%)**		
	During	During	zero	one	two
Control	4.67	2760	55	36	9
63Hz	2.25	2101	89	9	2
125Hz	2.6	1938	77	15	8
500Hz	4.41	2498	93	6	1
1kHz	4.04	2689	80	18	2

Group cohesion was significantly reduced with acoustic emissions of 63 Hz, 125 Hz and 500 Hz ($p<.001$) with respect to the control. The cohesion value for the control was 2760± 76.90 cm^2 and decreased to 2101± 172.37 cm^2 at 63 Hz, 1938-± 202.2 cm^2 at 125 Hz and 2498-± 195.04 cm^2 at 500 Hz (Tab.8). At the frequency of 63 Hz the fish reacted quickly to the acoustic emission and clustered close together at one point. Instead, at the frequency of 125 Hz, even as the levels of cohesion increased, the fish did not cluster at one point but close to each other at the edge of the basket. At high frequencies the fish reacted quickly but were more distant from each other

Motility was significantly reduced during both the 63 Hz and 125 Hz acoustic emissions ($p<.001$) compared to the control. In the control experiment, the average fish motility was 4.67 ± 0.14 cm/s while at 63 Hz and 125 Hz it was 2.25 ± 0.11 cm/s and 2.60 ± 0.08 cm/s respectively (Tab.8). At all frequencies emitted the fish remained immobile after the emission began. Among the frequencies different recovery times were in fact the fish began to move more quickly at high emission frequencies. Moreover, startle reactions have been observed mainly at the frequencies of 125 Hz.

For swimming height (Tab.8) a significant increase ($p<.001$) in the percentage of fish within zone zero (near-bed) was observed during all emitted frequencies with respect to control conditions. In the control experiment, the percentage of fishes in zone zero was 55.12%. For the sound treatments, the percentage of fishes in zone zero was 89.3%, 76.5%, 93.6% and 80.3% for the 63 Hz, 125 Hz, 500 Hz and 1 kHz frequencies respectively. Conversely, the percentage of fish in zone one (mid-depth) decreased significantly ($p<.001$) for all emitted frequencies with respect to the control. In the control experiment, the percentage of fish in zone one was 35.5%. In the experimental condition, the percentage of fish in zone one was 8.5%, 15.4%, 6.0% and 17.2% for each frequency respectively. A significant reduction in the percentage of fish in zone two (near-surface) was observed only at frequencies of 63 Hz ($p<.01$), 500 Hz ($p<.001$) and 1 kHz ($p<.01$). In the control experiment, the percentage of fishes in zone two was 9.4%. In the experimental condition, the percentage of fishes in zone two was 2.2%, 8.1%, 0.4% and 2.1% at each frequency respectively. The most noticeable effects observed on swimming height were observed at the beginning of the acoustic emission. If the fish were in the upper area of the water column, they reacted quickly by shedding downward at all emission frequencies. These effects were more evident at low emission frequencies with longer recovery times compared to high emission frequencies.

Model results

During the tests, it was found that some of the model parameters needed to be modified over time to obtain similar results to the laboratory measurements. These parameters were:

- Probability of schooling (PbS)
- Probability of velocity change (PbVC)
- Probability of navigation to the bed (PbN)
- Swim speed distribution (mean and standard deviation) (SSD)
- Maximum schooling speed (MSS)

Based on the *in vivo* results (Tab. 8), the criteria for deciding if a good model calibration had been achieved was chosen as the absolute difference between the average model result and the data (averaged over each time interval), with values of: 0.4 cm/s for average swim speed; 200 cm^2 for the cohesion; and 1.5% error for the percent of fish in each height zone.

Select calibration parameter values

To obtain suitably calibrated values of cohesion, motility and swimming height comparable to the values obtained from *in vivo* experiments, the values of the parameters for the control model used were:

PbS	0.03
PbVC	0.04
PbN	0.0014
SSD	0.04±0.007 m/s
MSS	0.1 m/s

The same values were used for all four control models since the behaviour of the fish over time did not change.

For the numerical models of with the different applied sound frequencies, since the fish react differently at the beginning of the acoustic emission, three reaction models were carried out for the frequency of 63 Hz, 125Hz and 1kHz. For the 500 Hz model the same reaction pattern as the 125 Hz frequencies was used because the fishes reacted in a very similar way. For each subsequent time interval model, the final positions of the fish were taken from the previous model.

The parameters used in the four models with applied sound frequencies are shown in Tab. 9 to 12 for 63, 125, 500 and 1000 Hz respectively. The parameters change between each time interval in all models and return to values similar to the control at different times as observed in the *in vivo* results. For the each of the four frequencies, the time varying fish behaviours meant that it was necessary to modify the parameter settings between each of the four-time intervals.

Tab. 9 The table shows the parameters used for each of the modelled time intervals for the 63 Hz treatment.

Parameters	Reaction model	0-5 min	5-15 min	15-30 min	30-60 min
PbS	0.19	0.07	0.05	0.04	0.03
PbVC	0.001	0.02	0.17	0.1	0.04
PbN	0.8	0.003	0.003	0.002	0.0015
SSD	0.2±0.007	0.008±0.0004	0.015±0.004	0.015±0.004	0.04±0.007
MSS	0.2	0.01	0.01	0.01	0.1

Tab. 10 The table shows the parameters used for each of the modelled time intervals for the 125 Hz treatment.

Parameters	Reaction model	0-5 min	5-15 min	15-30 min	30-60 min
PbS	0.13	0.09	0.06	0.04	0.04
PbVC	0.01	0.1	0.09	0.08	0.06
PbN	0.8	0.003	0.002	0.0015	0.0015
SSD	0.2±0.007	0.004±0.0002	0.025±0.007	0.03±0.007	0.03±0.007
MSS	0.2	0.01	0.1	0.1	0.1

Tab. 11 The table shows the parameters used for each of the modelled time intervals for the 500 Hz treatment.

Parameters	Reaction model	0-5 min	5-15 min	15-30 min	30-60 min
PbS	0.19	0.07	0.05	0.03	0.03
PbVC	0.001	0.1	0.09	0.09	0.05
PbN	0.8	0.004	0.004	0.004	0.035
SSD	0.2±0.007	0.004±0.0002	0.04±0.007	0.04±0.007	0.04±0.007
MSS	0.2	0.01	0.1	0.1	0.1

Tab. 12 The table shows the parameters used for each of the modelled time intervals for the 1 kHz treatment.

Parameters	Reaction model	0-5 min	5-15 min	15-30 min	30-60 min
PbS	0.13	0.04	0.04	0.04	0.03
PbVC	0.07	0.4	0.08	0.06	0.04
PbN	0.8	0.003	0.0025	0.0025	0.0015
SSD	0.2±0.007	0.008±0.0005	0.03±0.007	0.04±0.007	0.04±0.007
MSS	0.2	0.01	0.1	0.1	0.1

Comparison of the in vivo data with model results

Using the calibration parameters, the model results were analysed in the same way as the *in vivo* video recordings to obtain the values of cohesion, motility and swimming height. As shown in Tab.13, the modelled values for each metric compared well with to the equivalent values obtained from *in vivo* camera recordings.

Tab. 13 The table shows the results obtained elaborating different numerical model for the whole experimental hour. The data are expressed as mean values. The equivalent values obtained from *in vivo* behaviour analysis are shown in brackets.

Models Data	**Motility (cm/sec)**	**Cohesion (cm^2)**	**Swimming height (%)**		
			zero	**one**	**two**
Control	4.3 (4.7)	2806 (2760)	54.7 (55)	35.6 (36)	9.5 (9)
63Hz	2.6 (2.3)	2163 (2101)	86 (89)	11.5 (9)	2.5 (2)
125Hz	2.6 (2.6)	1999 (1939)	75.6 (76.4)	16.6 (15)	7.7 (8)
500Hz	4.2 (4.4)	2414 (2499)	93.6 (93.6)	5.1 (6)	1.3 (0.4)
1kHz	4.0 (4.0)	2620 (2689)	81.0 (80)	16.5 (18)	2.5 (2)

The cohesion values determined using the numerical model, as found for the results obtained from the *in vivo* experiment, were significantly reduced during the tests with acoustic emissions at frequencies of 63, 125 and 500 Hz. The cohesion value in the control was 2806 ± 566 cm^2 and decreased to 2163 ± 694 cm^2 at 63 Hz, 1999 ± 488 cm^2 at 125 Hz, 2414 ± 759 cm^2 at 500 Hz and 2620 ± 720 cm^2 at 1 kHz (Tab.13). In the numerical model, as for the *in vivo* results, the most evident impacts were observed at 63 Hz and 125 Hz.

Motility values obtained from the numerical model, similarly the values obtained from *in vivo* experiment, significantly reduce at 63 Hz, 125 Hz compared to the control. For the control fish, the motility value was 4.30 ± 0.48 cm/sec while in 63

Hz and 125 Hz was respectively 2.57 ± 1.53 cm/sec and 2.63 ± 0.88 cm/sec (Tab.13).

The values for swimming height show a significant increase in the percentage of fish in zone zero (near bed) at all emitted frequencies with respect to the control. In the control, the percentage of fishes in zone zero was 55.1%. In the experimental condition, the percentage of fish in zone zero was 89.3% for 63 Hz, 75.6% for 125 Hz, 93.6% for 500 Hz and 81.0% for 1000 Hz. Conversely, the percentage of fish in zone one (mid depth) decreases significantly at all emitted frequencies with respect to the control. In the experimental control, the percentage of fishes in zone one was 35.5%. In the experimental condition, the percentage of fishes in zone zero was 8.5% for 63 Hz, 16.6 for 125 Hz, 5.1% for 500 Hz and 16.5% for 1000 Hz. No significant difference was observed for the numbers of fish swimming near the surface (zone two). The same statistical analysis was performed for the results obtained from the model and the results obtained were similar as show in Tab. 14.

Tab. 14 The table shows the results about statistical analysis performed using t-test on behavioural data and model data.

Cohesion	**63 Hz**	**125 Hz**	**500Hz**	**1 kHz**
Control *in vivo*	*** (p<.001)	*** (p<.001)	*** (p<.001)	N.S
Control model	***(p<.001)	***(p<.001)	***(p<.001)	N.S
Motility				
Control *in vivo*	*** (p<.001)	*** p<.001)	N.S	N.S
Control model	***(p<.001)	***(p<.001)	N.S	N.S
Swimming height zero zone				
Control *in vivo*	***(p<.001)	*** (p<.001)	*** (p<.001)	*** (p<.001)
Control model	**(p<.01)	*(p<.05)	***(p<.001)	***(p<.001)

4.1.3 Discussion and conclusion

Towards developing a time-varying behaviour model

Up to this point, the modelling has focused on assessing the changes in the fish behaviour at discrete time intervals through the duration of the experiments. The main reason for this was because the manual calibration of the model was time consuming. In future work, it is envisaged that the empirical relationship formula from the fitted curves described above could be applied within the HydroBoids agent-based model code. In doing so, the model would be able to simulate the whole behaviour cycle of the fish from control conditions, to the initial reaction to the noise followed by the gradual habituation that was observed in the laboratory measurements.

This could be achieved by deriving empirical relationships of how the parameters changed over time. Fig. 59 to 61 show power law curves fitted to the model parameters against time for the probability of navigation, schooling and velocity change respectively. In particular, the different trend of the curves for the different experimental frequencies represented in Fig.59, indicates that at the frequency of 500 Hz the fish spent most of the time in zone zero. For other frequencies the curves are very similar to each other, in fact the percentage of time spent in zone zero are similar (see Tab.13). Furthermore, in all frequencies the curves show that after the first five minutes the swimming depth begins to decrease to a greater extent at 1 kHz and 125 Hz. Overall, the fitted curves indicate that the percentage of time spent near the bed (in the zone zero) is higher in all cases than in the control, confirming an effect of the acoustic stimulus on the swimming depth of the fish. This reaction of swimming towards the bed could be due to anxiety situations (Israeli-Weinstein and Kimmel, 1998; Cachat et al., 2010; Kuwada et al., 2000; Wilson and Dill, 2002;

Skilbrei and Holst, 2009; Luca and Gerlai, 2012;) also observed in field studies (Gerlotto and Fréon, 1992; Handegard et al., 2003; Slotte et al., 2004).

Regarding the Probability of Schooling represented in Fig.60, it is possible to observe that the levels of cohesion in the first five minutes are greater for the frequencies of 125Hz. The curves of 63 Hz and 500 Hz show a very similar trend with high levels of cohesion in the first five minutes but lower than the 125 Hz curve. These three fitted curves decrease after only the first five minutes indicating a recovery in the levels of cohesion faster at the frequency of 500 Hz and slower at 125 Hz. The curve for cohesion at a frequency of 1 kHz is not very different from the control and maintains a constant trend over time. The differences in Probability of Schooling could be due to different sound sensitivities of the fish being that the frequency range received by most fish ranges from 50 to 1122 Hz (with an SPL of 140-150 re 1μPa) (Popper et al., 2003). Moreover, the recovery may be due to sensory adaptation or habituation (Domjan, 2010) to continuous noise (Neo et al., 2014).

The numerical model has also confirmed variations in the Probability of Velocity Change (Fig. 61). This parameter is different for the four acoustic frequencies at the beginning of the emission. In fact, the probability value is higher at a frequency of 1 kHz. At 500 Hz and 125 Hz the initial value is the same. At all frequencies the value decreases after the first five minutes of acoustic emission. The highest three frequencies return to a similar Probability of Velocity Change after the first 5 minutes, whereas at 63 Hz the value remains higher. This suggests that recovery is slower at 63 Hz. After an hour of acoustic emission, at all frequencies, this parameter returns to values similar to the control. Also in this case, the difference in the effects of different acoustic frequencies may be due to states of anxiety and fear of fish and to the greater quantity of C-start responses (Blaxter et al., 2009; Fewtrell & McCauley, 2012; Kastelein et al ., 2008; Pearson et al., 1992; Purser & Radford, 2011; Wardle et al., 2001). The different responsens at different experimental frequencies showed different sensibility of fish probably due to their hearing capability. Moreover, these responses can affect anti-predation and anxiety behaviours (Cachat et al., 2010; Eaton et al., 1977).

In Fig. 62, another relationship is derived for the swimming speed using a logarithmic fit to the model parameters. The curves show that swimming speed levels remain high for control fish around 0.04 cm/s. The curves for all four frequencies are similar, in fact the swimming speed is zero at the beginning of the acoustic emission in all cases indicating a reaction to all emission frequencies perhaps caused also in this case by fear and anxiety (Cachat et al., 2010). Between the four frequencies the recovery time changes with respect to the control values, and they recover more quickly at 500 Hz and 1 kHz. In all cases the recovery starts after five minutes from the acoustic emission. At 125 Hz the swimming speed never returns to the control levels. Also, in this case, such as the probability of schooling the recovery may be due to sensory habituation to continuous noise (Neo et al., 2014).

For the maximum school speed (MSS) the relationship differed from the other parameters because it acted more like a switch, with the school either being stationary or moving (Fig.63). Hence, it was not possible to fit a curve to the data in this case. Instead, this could be modelled using a timer relative to the reaction time. Interestingly, the switch occurred after five minutes for all of the frequencies other than 63 Hz, in which the switch occurred after 30 minutes. This highlights a significant difference in the fish behaviour at 63 Hz compared to the other frequencies tested, possibly indicating a more pronounced reaction to the noise at this frequency.

The results obtained from the numerical model are consistent with the results obtained from the processing of *in vivo* video recordings. Furthermore, it would be possible to use the results obtained from the model to construct a model that changes over time using the different parameters shown in Fig. 59 to Fig. 63 and combining them together. In fact, the levels of Probability of navigation to bed and Probability of schooling are correlated and influenced by the levels of Probability of velocity change, Swimming speed and Maximum school speed.

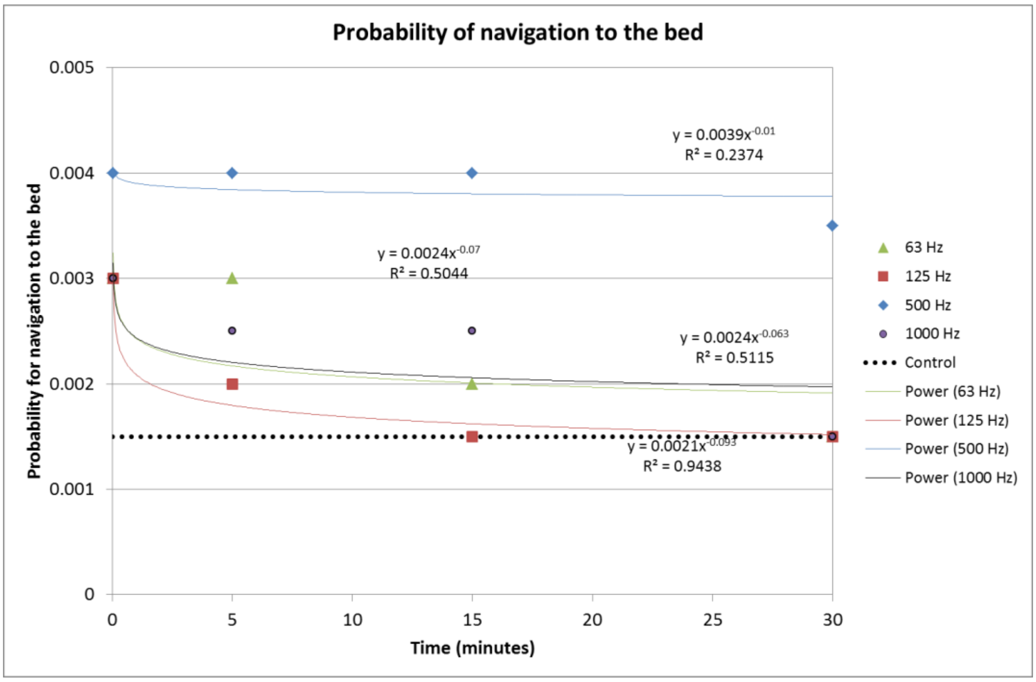


Fig. 59 Power law curves fitted to the model parameters against time for the probability of navigation, to the bed of fish

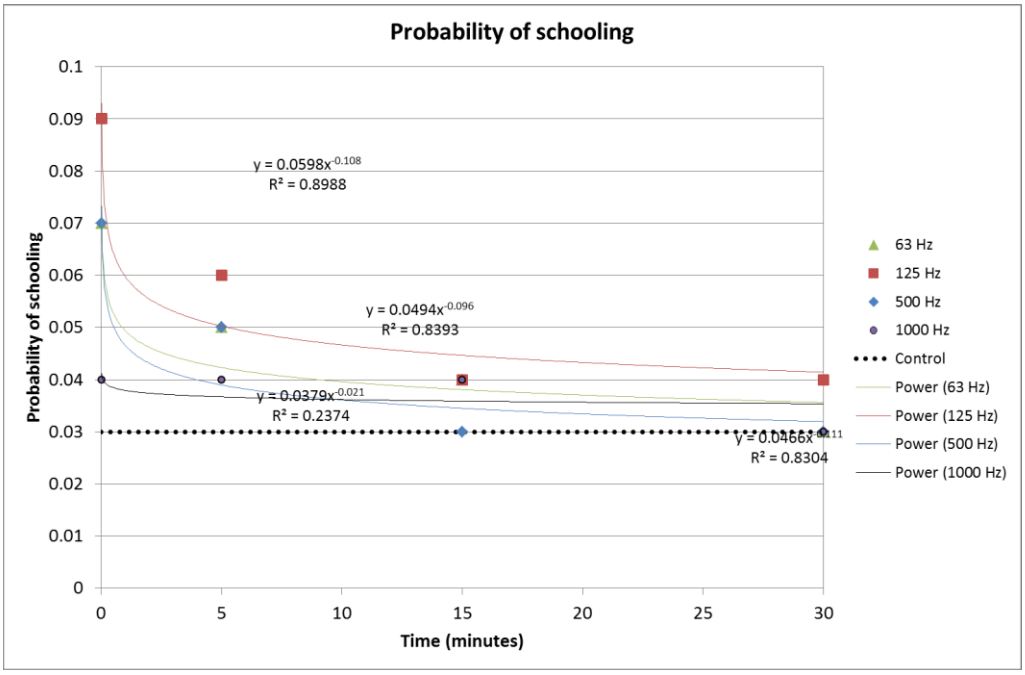


Fig. 60 Power law curves fitted to the model parameters against time for the probability of schooling of fish.

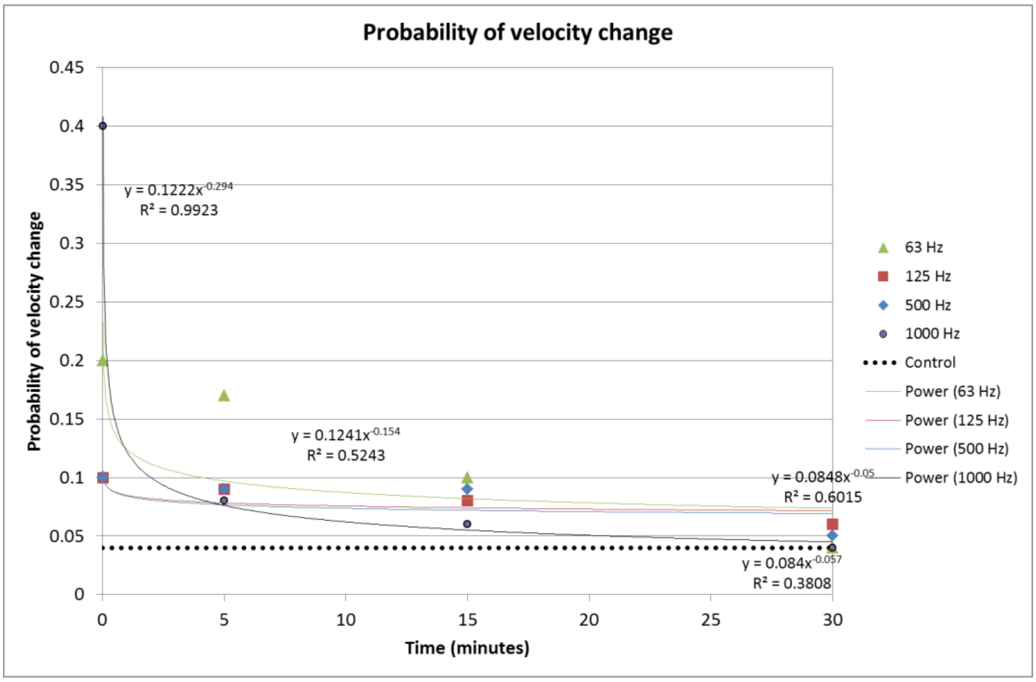


Fig. 61 Power law curves fitted to the model parameters against time for the probability of velocity change of fish.

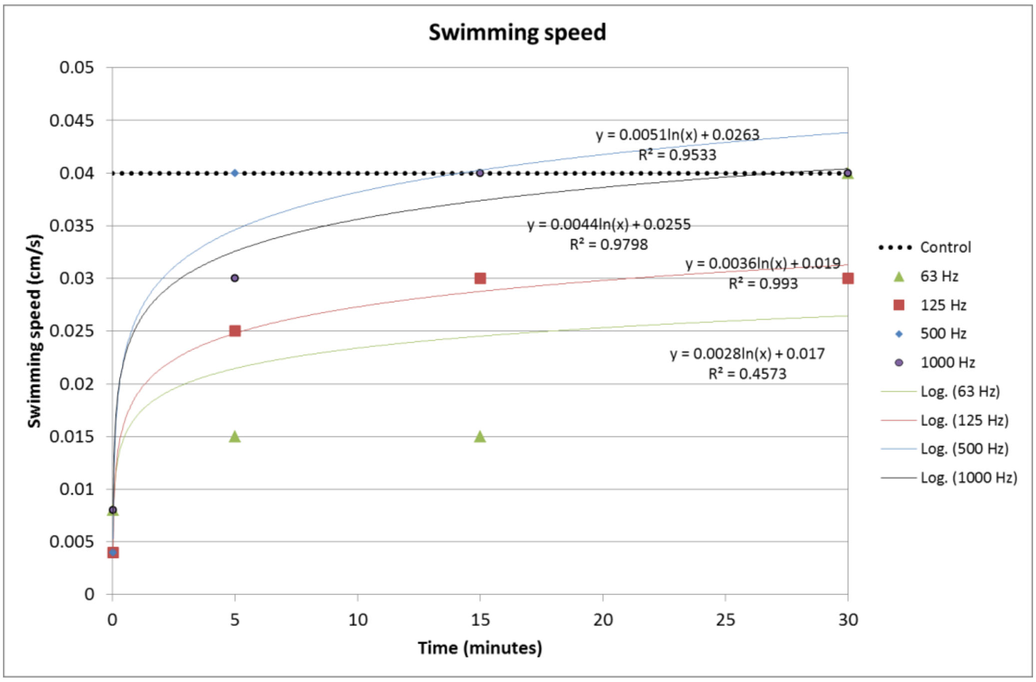


Fig. 62 Representation of relationship derived for the swimming speed using a logarithmic fit to the model parameters against time.

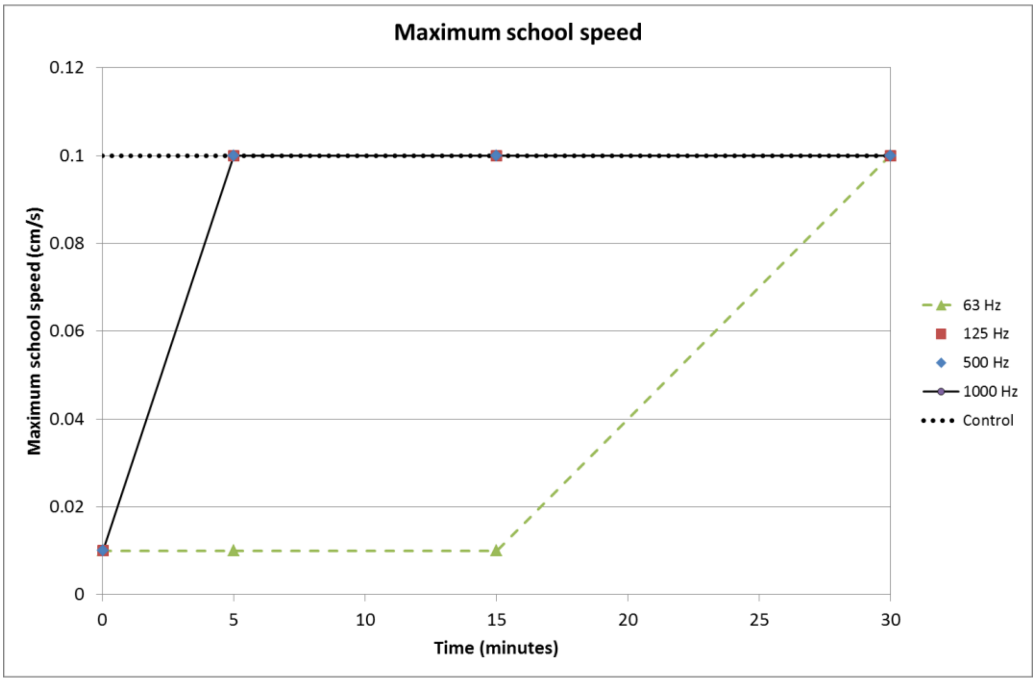


Fig. 63 Representation of the maximum school speed (MSS) for each experimental frequencies and control.

How does the model help in our understanding of the fish behaviour?

In the literature, many scientific studies have assessed acoustic impacts of several human activities on marine organisms at a physical, physiological and behavioural level. These data are useful to understand the impacts on marine biodiversity being that human activities in the sea have increased over time and as noise levels in the sea will increase in the future (Andrew et al., 2002; Hildebrand, 2009). However, today it is essential to have a tool that can predictions to be made of the behavioural responses of marine organisms to an acoustic stress, and the associated impacts, before they are subjected to it. An organization or an industry that plans to carry out activities at sea can take advantage from this numerical model, to predict the impacts that the acoustic emissions of their activities will cause on biodiversity. Knowing the frequencies and the acoustic intensities that will be emitted, and inserting them in the numerical model, it will be possible to understand if those acoustic emissions will have effect on behavioural parameters such as swimming speed, swimming depth or group cohesion.

An important feature of the numerical model developed in this study is that it was calibrated using the behavioural responses of juvenile fish. Changes in the early stages of fish life can cause dramatic effects in the structure, physiology and behaviour of species. Moreover, the acoustic stress, which changes their sensory information, can negatively affect their ability to assess risk and select appropriate reactions. The consequences could therefore be significant, making them more vulnerable to predators by influencing their survival (McCormick et al., 2002; Nilsson et al., 2007; Munday et al., 2010, McCormick & Lönnstedt, 2013) and the future generations. Consequently, behavioural responses to underwater noise as investigated here can have important and negative effects on entire ecosystems

(Borsani et al., 2015) and at commercial level as it could influence the capture rates of the species causing their dispersal or removal from a site (Løkkeborg et al., 2012).

A key advantage of the numerical model approach is the possibility to predict the behavioural responses of a fish species over time and include any recovery or habituation. It should be possible to estimate if specific acoustic frequencies will have effects and for how long they will have negative effects. The model obtained showed that different frequencies can have different effects at the time of acoustic emission and that subsequent recoveries over time can change based on acoustic emission. The *in vivo* recordings analysis and the numerical model results highlight the importance that the low acoustic emission frequencies that the Marine Strategy Framework Directive requires to be monitored (Dekeling et al., 2014) are the most impacting on all behavioural parameters studied in *S. aurata* juvenile fishes.

Looking forward, by including similar data as recorded here relating to other species that have different hearing thresholds and behavioural responses and implementing the numerical model, it might be possible to select the frequencies and acoustic emission intensities that have less impact on the species present at a particular the site. Such a tool would be of considerable importance for EIAs helping to predict noise impacts. Experiments carried out in the laboratory generally provide incomplete information and *in-situ* experiments on the other hand are difficult to manage (i.e. control) for testing the acoustic effects of human activities. In this respect the numerical model will be able to make use of existing results from numerous scientific studies present in the literature, making them no longer simply information or dissemination of a result, to provide a real tool that can be used for forecasting and mitigation of the noise impact on marine biodiversity.

In conclusion, there are few examples in the literature of numerical models of the behaviour of fish, in particular juveniles, that are subjected to acoustic stimuli. Developing such models requires observation data for calibration and validation. During this study, through the development of a numerical model, it has been shown that it is possible to use data obtained from *in vivo* experiments for the creation of a model that allows to predict the effects of the acoustic stress at a range of frequencies associated with different human activities on the behaviour of fish. These results are important as changes in fish behaviour can have important consequences for their survival. Furthermore, it was important to test some frequencies that the Monitoring Guidance for Underwater Noise in European Seas - Part II recommends to observe during the emissions into the sea.

HydroBoids is an ABM that through various types of research is undergoing interesting developments (https://www.zsl.org/conservation/smelt-osmeruseperlanus). Through this experimental plan, it was possible to investigate the interaction of marine organisms with the acoustic impact of human activities. It was possible to associate new algorithms to the model.

This model could potentially be useful in the EIA process for future mining in the ocean depths or for predicting the impacts of other acoustic activities. In fact, this model allows a deepening the knowledge and the analysis on population dynamics. Understanding of the potential impacts of anthropogenic noise on marine species requires an understanding of how a species feels, what frequencies and levels of sound it can hear and its probable responses. To date, in the bibliography studies have increased and some recent studies informing about behavioural variations are available (Neo et al., 2018).

The model confirmed that behaviours change depending on the frequency of emission and in general at all frequencies of the experimental plan the fish prefer to remain in deeper waters starting to return to the surface only after the first hour of emission. The fish respond with a vertical and not a horizontal behaviour (Slotte et al., 2004; Sabet et al., 2016). This reaction was observed at all emission frequencies with effects more evident at 63 Hz and 500 Hz.

The values used influenced the results of the model (Fonoberova et al., 2013; Ginot et al., 2006; Simons et al., 2013). In particular, the final result depends on the interaction of the parameters between them.

One of the most important results of this numerical model was the need to create reaction models to reproduce the reaction that fish have when the acoustic emission begins. The fish reacted differently to the acoustic emission frequencies: or quickly collected in a single point or distributed to the edge of the basket at different distances according to the frequency of emission. Thanks to the model, it was also possible to confirm that depending on the emission frequency the fish can recover their behaviour after an hour. In fact, at the frequency of 63 Hz and 1 kHz all the parameters of the model return to being the same as those of the model for control. This confirms the possibility of fish recovery after a time interval of exposure to the acoustic stimulus (Holmes et al., 2018), in this case after one hour. The same does not happen for the frequency of 125 Hz in which the only parameters that return to the control values are MSS and PbN. This confirms the results obtained *in vivo*, the acoustic emission has impacts on cohesion (PbS), on the speed of change of swimming direction (PbVC) and on the speed of the individual sign (SSD). The change in the model of the swimming direction parameter is in agreement with Mueller-Blenkle et al. (2010). These results confirm that acoustic impacts have consequences on fish startle responses (Bruintjes et al., 2016). As for the frequency of 500 Hz instead the only values to recover are PbS, SSD and MSS. This indicates and confirms that this emission has an impact on the depth of swimming and on the change of direction of the fish. Also, in this case the results confirm startle responses but which differ according to the emission frequency (Kastelein et al., 2008).

Empirical relationships were derived from curves fitted to the time-varying model parameters (PbS, PbN, PbVC, SSD and MSS). This opens up the possibility to carry out further studies in which the HydroBoids code could be modified to simulate the time-varying fish behaviour observed throughout the *in vivo* experiments in a single model run. This would then form the basis for using the model in real world scenarios where impacts of anthropogenic noise on *Sparus aurata* could be assessed.

The results of this research were presented (see the Chapter 7) at the 80° National Congress Of Italian Zoological Union (UZI). Roma, 23 - 26 September 2019

5. Standard and standardization module

For this part of study were performed six months at The Italian Institution for research and promotion of standardization (ENR) based in Palermo. This period was carried out during the second and third year of study and was divided in four phases:

1) General study of the structure, writing and function of national and international technical rules and/or standards;

2) Bibliographic research on technical rules and standards regarding the acoustic impact of marine-maritime activities;

3) Bibliographic research on technical rules and standards regarding the acoustic and environmental impact of Deep Sea Mining;

4) Final elaboration of the first technical standard concerning the acoustic impact of the Deep Sea Mining for the preservation of marine biodiversity.

The time spent in this institution also allowed to follow a professional training course by participating in different activities and events, such as the Blue Sea Land Expo (Mazara del Vallo, Italy, October 2018) during which our study has been present in several seminars.

Results of the studies carried out during the initial phases (1, 2 and 3) allowed to confirm that, today, there are no technical regulations and standards regarding the acoustic impact of mining activities in the ocean depths.

Thanks to the final phase (study of scientific bibliography concerning the acoustic impact of different marine-maritime activity) limits and indications of use in elaboration of the subsequent technical standard were extrapolated.

A review of the scientific papers in literature, regarding noise pollution linked to various marine-maritime activities was used to write the subsequent technical standard. Relevant scientific literature has been cited in the introduction to this thesis.

This Technical Standard provides an initial document from which to derive minimum requirements and/or recommendations of use when attempting to limit possible acoustic impacts of DSM activity. Whilst the acoustic frequencies emitted during DSM activities remain unknown, this document contributes to the identification of a baseline that could be followed by different stakeholders performing these activities.

It is believed that following these guidelines while carrying out deep sea mining activities, is accordance with the precautionary principle.

All the scientific studies that was consulted during the drafting of this standard highlighted the acoustic impact which occurs on biodiversity (from invertebrates to mammals) on a physical, physiological and behavioural level. Based on the effect of a sound with a known acoustic intensity and frequency, typical, for example, of other anthropic activities, it was possible to hypothesise the possible effects of DSM activities, where the noise emitted coincided with some of these frequencies and/or intensities. Most of the published papers concerned laboratory experiments and not *in-situ* experiments. This fact may constitute a possible limit in the establishment of noise emission thresholds. In this respect, we are fully aware that the two experimental situations do not overlap. However, it was deemed appropriate to provide an initial set of indications regarding the acoustic limits to be respected, taking into consideration the type of emission of various marine/maritime activities and the possibility that DSM activities create the same frequency emissions and thus the same impacts.

Although acoustic frequencies produced by mining are not known (a great deal of information is still lacking or not available) using knowledge on the effects of various types of anthropogenic noise is, undoubtedly, a satisfactory starting point to mitigate impacts. We remain aware of the fact that there are many scientific limitations; however, we are even more aware of the fact that marine/maritime sector activities and future mining activities in the ocean depths cannot continue without indications or basic limits to contain noise impact. It was this consideration which inspired us to propose possible noise emission limits and operational advice to be used during DSM activities in order to provide (as far as possible) impact mitigation measures.

There is still time to provide a set of indications before DSM activities begin. "Prevention is better than a cure"; 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. In this contest, a multidisciplinary approach was recognised as providing a more useful and meaningful approach, given the fact that the large number of factors involved in marine noise pollution gives rise to a number of difficulties, such as establishing fixed and unambiguous parameters, and distances for safe noise monitoring.

Scientific papers were studied and grouped in terms of emission frequencies and acoustic emission intensity. Subsequently, these papers were grouped in terms of physical, physiological and behavioural damage to marine species (from invertebrates to vertebrates and mammals). This allowed to obtain three acoustic emission intensity ranges: serious impact, average impact and low impact. In most cases, bibliographic research reported emissions expressed in dBrms re 1μPa and this allowed us to extrapolate limit values. For example, levels causing severe animal injury appear to be much higher than 180 dB rms re 1μPa (OGP-IAGC). However, most of the scientific studies we studied with impacts on physiology, physics and animal behaviour concerned emissions ≥ 130 dBrms re 1μPa. This has allowed to establish that emissions above 130 dBrms re 1μPa cause "serious impacts" on biodiversity, in many cases irreversible. Given the lower percentage of studies 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, from 90 dBrms re 1μPa to 130 dBrms re 1μPa, cover a type of impact defined as "average impact". These levels 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 contribute to the reduction of noise impact. Furthermore, the acoustic limits selected through the study of scientific literature were confirmed by the results of the indoor and outdoor experiments discussed above. A range of scientific information was taken into consideration when indicating emission frequencies and, for most species, sound sensitivity begins below 100 Hz and continues up to several hundred hertz, or several thousand hertz in a few species (Mann et al., 1997, 2001). Moreover, as stated in the introduction, the "Monitoring Guidance for Underwater Noise in European Seas - Part II" suggests monitoring trends linked to environmental noise levels (annual average values measured in RMS re 1μPa) emitted within 1/3 octave bands with central frequencies of 63 and 125 Hz. Monitoring these frequencies was advised for this reason. "Guidelines for the management of anthropogenic noise impact on cetaceans in the ACCOBAMS area" were used to indicate possible time intervals for the monitoring of acoustic and visual activities, which should be carried out throughout the duration of the noise emissions. This document also provided information on the professional figure of a Marine Mammals Observer (MMO). The Guidelines for Germany, BfN 2013:1, provided useful suggestions on minimum distances for monitoring and measuring noise emissions.

There are still many gaps concerning some aspects of acoustic impacts; this information has been provided in the technical standard using specific bibliographical references. For example, data for determining distances are insufficient; however, in general, the closer the animal is to the source, the more likely it is that the high energy will have an effect (Popper et al., 2019). In this context, regulators need to consider noise levels at the origin and noise reception by animals. Regarding particle movement, the International Organization for Standards in ISO/DIS 1683 (2013) recommends the following values: 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.

Noise levels from impulsive emissions have also been indicated in the technical standard. At present there are no national or international standards relating to the exposure of fish to impulsive sounds. However, information relating to the latter has been collected by the National Marine Fisheries Service (NMFS). Starting from the mortality of species that have been exposed to explosive noise (Popper & Hastings 2009a), NMFS developed intermediate criteria for pile driving (FHWG 2008, Woodbury & Stadler 2008; Stadler & Woodbury 2009; Caltrans, 2009), specifying a maximum SPL peak of 206 dB re 1μPa, a maximum cumulative SEL (Sound Exposure Level) of 187 dB re 1μPa^2 s^{-1} for fish ≥2 gr and 183 dB re 1 μPa^2 s^{-1} for fish <2 gr (Carlson et al., 2007). Moreover, Halvorsen et al. (2011, 2012a, c) and Casper et al. (2012, 2013a, b) describe the effects of impulsive sound on different species by formulating the Response Severity Index (RSI). In particular, they determined maximum sound pressure levels associated with different RSI levels and found that tissue damage increases with increasing cumulative SEL and single shot SEL. For this reason, use of these indices was also recommended. In addition, the scientific research of Popper et al. (2014; 2019) was used to indicate emission levels. Popper et al. (2019) organize the scientific data according to species, type of damage detected and type of source or sound exposure.

However, in literature, there are also other metrics for pulsed sounds: Sound Exposure Level (SEL) for single and cumulative sounds; Peak sound pressure level; Peak-to-peak sound pressure level. SEL, for example, can be considered a measure of the energy content of the impulse (Good Practice Guide No.133-Underwater Noise Measurement), although available data on this latter did not allow us to identify reliable sound levels. It was possible, however, to extract some useful basic information from other scientific documents to include in the subsequent technical standards. Other recommendations in the subsequent technical standard were made using the following bibliographic sources: in the case of SEL, significant negative effects already occur at levels above 120 dB re μPa^{2}s (183 dB re: μPa^{2}s) (Southall et al., 2007; Borsani & Franchi, 2011) and 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 μPa^{2}s (Malme et al., 1983; Southall et al., 2007; Borsani & Franchi, 2011; Ljungblad et al., 1988; Todd et al., 1996; McCauley et al., 1998). 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., 2018; McCormick et al., 2018b) and drilling seems to be recommended over piling (Broudic et al., 2014). It is also useful to calculate peak compressional sound pressure levels and peak rarefactional sound pressure levels, pulse duration and pulse repetition frequency. The impact of these activities could be contained by using the "soft-start" technique (gradual start to activities), which can guarantee the escape of marine organisms from the areas involved in DSM activities. To minimise additional noise, “soft starting” should not last longer than 40 minutes (Joint Nature Conservation Committee). Furthermore, power increases during a soft-start technique should not exceed 6 dB every five minutes (Guidelines for the management of anthropogenic noise impact on cetaceans in the ACCOBAMS area). In the case of drilling for DSM activities, a reduction in drilling times could contribute to a decrease in acoustic impacts, taking advantage of any resting sessions. It is important, however, to bear in mind that the acoustic conditions and pressure levels may change, depending on the type of environment (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. Another way to minimise sound impacts would be to minimise 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 mixed understanding of the effects of noise on marine animals (Drazen et al., 2019). From bibliographic research, it emerged that documents such as 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) are useful for good monitoring. All these mentioned documents enabled us to extract some of the information contained in this standard. Despite the considerable number of variables at stake, providing information on possible limits regarding exposure to noise is of great importance in order to direct all marine and maritime activities towards greater environmental friendliness and eco-sustainability, and to raise awareness among all stakeholders to ensure greater protection of the marine environment, also from the point of view of noise impact. Below is proposed initial technical rules on noise pollution for Deep Sea Mining activities.

5.1 A proposal of technical rules for noise pollution of Deep Sea Mining activity

TECHNICAL STANDARD

ENR
14001
SEPTEMBER, 2019

The acoustic impact of maritime activities related to Deep Sea Mining (DSM): Minimum standards of admissibility

Requirements

The Italian Institution for research and promotion of standardization

ENR

Index

PREFACE

This document (Technical standard ENR 14001:2019) has been elaborated by a special Technical Committee for the realization of a voluntary technical standard to define the potential minimum criteria of acceptability of the acoustic impact of the sea-shore activities related to Deep Sea Mining (DSM)

1. Introduction

Anthropic noise is now considered to be a serious cause of pollution. EU Member States shall develop strategies to maintain good environmental status in their seas (Marine Strategy Framework Directive, MSFD; Monitoring Guidance for Underwater Noise in European Seas - Part II).

In 2010 the European Commission published standards and criteria for achieving these objectives (Commission Decision 2010/477/EU), but to date data on anthropogenic noise are still incomplete. Noise has become part of the descriptors of good environmental status such as descriptor 11 (Noise/Energy) and in particular Indicator 11.1.1 for low and medium frequency impulsive sounds and Indicator 11.2.1 for low frequency continuous sounds (environmental noise) (Monitoring Guidance for Underwater Noise in European Seas - Part II).

Given the recent increase in anthropogenic noise, it is essential to consider the acoustic impact of maritime-sea activities related to mining in the depths of the ocean.

Noise monitoring is essential to prevent impacts on the marine ecosystem as much as possible and is an indispensable factor to be included in all phases of these activities. This is consistent with the possible balance between reducing noise impacts on ecosystems and socio-economic development.

The drafting of the following standard takes into account the different conclusions that exist today between the scientific results obtained in the field and in the laboratory. It is taken into account that possible biological responses, obtained from scientific research in the field of "noise pollution", may be difficult to assess due to ecosystem variability and incomplete scientific information on anthropogenic noise at sea.

In the standard under consideration, the difficulty of assessing acoustic impacts has been taken into account together with the high variability and interaction with different environmental aspects.

It is therefore difficult to develop plans to contain anthropogenic noise given the lack of scientific knowledge of the sounds emitted, the exposure thresholds and any responses triggered at the ecosystem level by these activities.

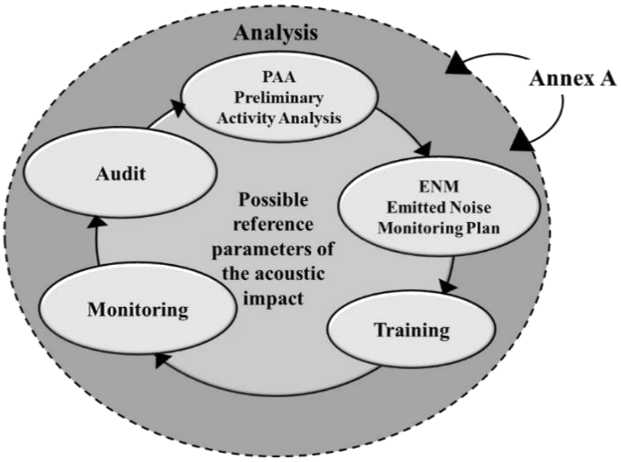
Despite this, efforts are being made to develop mitigation strategies that take into account biological information or possible changes to sound sources in order to minimise the resulting noise impacts as far as possible.

The multidisciplinary approach is the basis for the drafting of this standard, the result of an Innovative PhD course with industrial characterization PON 2014-2020 carried out at the University of Palermo focused on the study of acoustic impacts of the activities of Deep Sea Mining.

The standard provides guidance on the behaviors to be implemented in all operational phases for the management of acoustic impact.

Introduction of the Thesis contains a summary of the scientific results (bibliographic data) that have been taken as a reference in the drafting of this standard.

The following diagram represents the description of a careful and precise analysis in order to carry out an efficient and effective acoustic monitoring.



In this Standard, the following verbal forms are used:

— “shall” indicates a requirement;
— “should” indicates a recommendation;
— “may” indicates a permission;
— “can” indicates a possibility or a capability.

2. Aim and Scope of Application

The purpose of this standard is to suggest possible ways of managing the acoustic impact of Deep Sea Mining (DSM) marine activities operating in the range between 10 Hz and 10 kHz.

The standard aims to provide stakeholders with references and/or guidance to reduce the noise impact by ensuring business continuity.

Interested parties may improve their environmental performance by following the minimum noise acceptability criteria set out in the following standard.

The problem of noise pollution is now relevant in order to improve environmental performance; an organization that will follow the indications of the following standard can take it into account in the course of its activities and contain it.

This standard is aimed at organizations that need a reference for noise pollution control.

The ultimate aim is to carry out these activities by controlling and reducing as much as possible the noise emissions and therefore the environmental impacts that could result from them. It will therefore be possible to improve the environmental performance and achieve the objectives set before the start of the activities.

3. Preliminary analysis

3.1 PRELIMINARY ACTIVITY ANALYSIS (PAA)

Before the start of the activities, the organization shall carry out a Preliminary Analysis of the Activities to be carried out (PAA).

In the PAA, the organization shall demonstrate that it is fully aware of all activities and has taken into account the resulting environmental impacts.

This will involve the analysis of some points such as:

a) Type of activity;
b) Technical characteristics of the equipment involved;
c) Actions likely to have a significant environmental impact;
d) Timing of activities;
e) Responsibilities and risks of the different levels of activity;
f) Type and characteristics of the environment of interest;

g) Structure of the ecosystem and of the biodiversity of the area, its mapping and monitoring;
h) Methods for the assessment and analysis of environmental indicators for environmental monitoring;
i) Noise emissions from machinery used before and during operation;
j) Possible emergency situations, in particular those with an environmental impact;
k) Environmental goals and the processes by which they are achieved;
l) Acoustic impacts and how to mitigate them;
m) Environmental aspects that the activities will be able to control and/or influence and those that they will not be able to control;
n) Operational measures to prevent or reduce undesirable effects which may be caused by external environmental conditions;
o) Expected environmental conditions throughout the period of activity and any climate and/or sea current changes that could affect safety conditions;
p) Human resources involved and their training.

The PAA aims to improve environmental performance by reducing noise impact.

The results of the PAA shall be recorded; the organization involved shall therefore document and leave tangible traces of the preliminary analysis of the activities carried out and their final results.

The related documents shall therefore:

a) Be dated and signed by all the people who have attended and/or participated in the preliminary analysis;
b) Provide details of all decisions taken regarding the conduct of activities and their management;
c) Identify the environmental criteria used for the decisions taken;
d) Identify the potential expected environmental impacts;
e) Identify the environmental aspects considered;
f) Identify which measures will be taken to reduce the impacts referred to in point (d)
g) Identify the environmental aspects not covered and justify the choice;

On the basis of the complexity of the site and the activity itself, which will be evaluated through the PAA, it is essential the presence of professionals with specific skills necessary for the analysis of the site where the activity will be carried out. Marine/biological, environmental (e.g. ecological, taxonomic, biological, ethological-naturalistic, geological and geochemical) and technical-acoustic (e.g. engineering-physical) skills are required for the monitoring of the noise emitted as per points 2.2 and 3. Expertise in laboratory techniques and technologies is recommended

The professionals involved shall have skills in environmental, acoustic and conservation / environmental protection.

It is also possible to include professionals from other sectors, not included in the list above, where necessary. This list can be modified according to the characteristics of the site, the area involved and the type of activity carried out.

It is therefore essential to form a team of professionals competent in the environmental and engineering sectors related to the activities.

The ultimate aim of the preliminary analysis is to have a picture as clear as possible of the current state of the underwater environment and the consequences of the activity that will be carried out on it. The PAA provides a snapshot of the previous status at the start of the activity and is useful for continuous monitoring.

3.2 EMITTED NOISE MONITORING PLAN (ENM)

Taking into account all the elements mentioned in point 3.1, it will be possible to program the noise emitted monitoring plan (ENM). This will be essential for analyzing and monitoring the environmental performance of the activity carried out.

The monitoring plan allows the organization not to deviate from the plans and objectives of this standard.

The ENM shall be documented and the controls carried out shall be recorded (e.g. methods, periodicity, personnel involved).

In the management of the ENM, it is necessary to take into account the skills required under point 3.1.

During the preparation of the ENM, the organization shall demonstrate that it has taken into account all environmental aspects and possible impacts by establishing analysis and monitoring timescales and the instruments to be used.

Below you can find a reference list for the preparation of the ENM:

a) The environmental objectives set shall be measurable, monitored, updated and reported;
b) Significant environmental aspects shall be monitored during the activities;
c) The frequency and responsibility of the planned activities shall be clearly defined;
d) The competences necessary for the activities to be carried out shall be defined in detail, ensuring the competence of the professionals chosen;
e) The type of ecosystem and the presence of any reproductive areas shall be determined;
f) It should be assessed whether the site involved is a sanctuary of reproduction of certain species;
g) The timing and type of analyses to be carried out shall be defined;
h) Methods of acoustic measurement and analysis shall be defined;
i) Noise emission measurements during the activities shall be carried out at a reference distance of 500 m. The choice of any different reference distances (not exceeding 500 m) shall be justified and signed by the technical staff involved.
j) The time interval in which measurements are to be made shall be managed as above;
k) The instruments used for the measurements shall be correctly calibrated and maintained by appropriate standards;
l) The quality of water and sediment before, during and at the end of the activities shall be taken into account;
m) Consideration shall be given to any contamination that may affect the noise impact;
n) The possible presence and maintenance of endemic and bacterial species typical of the site in question and their maintenance shall be assessed;
o) A census shall be made of the biodiversity, the species present and their breeding periods;
p) Any behavioural changes in the species present shall be considered;
q) The protection of a part of the area characterized by the same biodiversity shall be ensured to allow future re-colonization and gene flow;
r) The oxygen content at different depths shall be considered;
s) Salinity at different depths shall be assessed;

The organization shall provide evidence that all measurements performed will be carried out in accordance with recognised international technical standards.

Particular attention shall be paid to trends of environmental noise levels (annual average values measured in RMS re 1µPa) emitted within the 1/3 octave bands with center frequencies at 63 and 125 Hz.

Aoustic and visual monitoring activities should be carried out throughout the duration of the emission.

Software may be required to compile a log line at regular time intervals (e.g. every 30 minutes). Sampling should be ensured with adequate timing. A sampling of at least six hours equally distributed during the 24 hours is strongly recommended.

Due to the variability of the species present in the sea and the variability of their acoustic ranges, their anatomy and physiology, it is difficult to establish fixed and unique distances for a safe monitoring. However, it is recommended to monitor and measure the emission at a minimum (and no more than) 500 m distance from the emission source. In this context, regulators should consider the levels of noise source and reception by animals.

The depth at which the noise measurements will need to be made:

a) It will depend on the type of site and the depth of extraction;
b) It should be assessed with the person in charge (e.g. a physicist);
c) It shall be documented and justified;
d) Provide information on the acoustic impact on the whole water column involved in the activities.

It is necessary to evaluate the pressure levels and the movement of the particles of the sounds produced before, during and after the activities.
The physics of sound propagation shall be taken into account in any acoustic impact assessment.

It is essential to consider that any changes in pH and/or temperature may affect the propagation of acoustic waves (e.g. changes in the thermocline).

It is necessary to measure the vibrations created in the seabed during the activities.

The analysis of biological samples and the results should not exceed a time interval of ten hours. This will allow you to control any emergency situations that are not immediately visible. It may be desirable to monitor behavior and perform statistically significant random biological sampling (e.g. blood and/or tissue, analysis of the state of internal organs) at different time intervals for the evaluation of cellular and molecular stress indicators. For this type of analysis it will be necessary to carry out control sampling prior to the start of activities in the absence of noise emissions themselves. The possibility of carrying out these analyses will be evaluated by the biologist and the body and/or organization involved.

If the above analyses can be carried out, it will be necessary to evaluate also the juvenile individuals of the species present in order to know their impact on fitness, therefore the future impact on the ecosystem as a whole.

It will be the type of analysis carried out and indicators studied that will determine the environmental value considered during the activities carried out with a view to mitigating anthropogenic noise.

It may be necessary to continuously monitor activities to implement any mitigation actions of impacts caused by sudden and unexpected events.

Competent personnel shall select the tools used to carry out the analyses. These may vary according to the environmental conditions of the site and the economic conditions of the project. The same applies to the monitoring times recommended in the above points.

The personnel involved shall document and leave traceability of the planned monitoring plan, of the instruments used, of the time intervals in which the analyses have been carried out.

All information shall be documented, stored and collected over time. The persons responsible for the different monitoring steps identified in the same documents.
The monitoring plan may be modified for a reasonable cause on the basis of needs that may arise and are not foreseeable. However, it shall comply with all the controls and criteria set out in points 3.1 and 3.2.

3.3 TRAINING OF THE STAFF INVOLVED

A proper monitoring plan shall assess the competence and training of the operators involved.

Operators or professionals shall be selected on the basis of the skills required by the PAA referred to in point 3.1.

The figures involved shall give evidence of adequate competence with respect to the requirements and the organization shall ensure that the competences in question are in place.

Operators should be competent and aware. They should also be involved in and undergo training courses and their knowledge and/or preparation on the issue of environmental/acoustic impact shall be assessed.

All operators involved should be informed about the practices and good behavior to be maintained to minimise acoustic impacts, promoting and encouraging continuous improvement.

Staff involved in activities should be fully aware of the consequences of their actions on the environment or of any negligence on their part; they should also be aware of the importance of their contribution to minimizing the acoustic impacts of the activities carried out.

The organization shall monitor the constant updating of the personnel involved, verify their knowledge of the environmental aspects, the environmental policy, the possible impacts and the importance of their contribution.

4. Monitoring of the noise emitted

4.1 MANAGEMENT OF THE EMITTED NOISE MONITORING PLAN (ENM)

Once the Preliminary Activity Analysis (PAA) and the Noise Emitted Monitoring Plan (ENM) referred to in points 3.1 and 3.2 have been carried out, it will be essential to manage the monitoring of the noise impact.

It is a matter of implementing all the controls foreseen in the ENM, in the methodology and in the timing foreseen.

Constant, continuous and detailed documentation of the measurements carried out according to the indications provided for in the ENM shall be guaranteed.

It will be necessary to provide specific action plans for any unforeseeable conditions and assess any emergency situations.

The organization shall monitor all types of non-voluntary changes through strategic mitigation actions.

The biological and environmental parameters referred to in point 3.2 shall be monitored at statistically significant time intervals chosen on the basis of the machinery used, the type of maritime activity carried out, the type of ecosystem involved, the type of extraction and the type of deposit, according to the requirements of the ENM.

If some parameters do not respect the biological sustainability ranges and/or in emergency situations, the activities shall be stopped immediately.

The results obtained from the different monitoring phases shall be evaluated and compared to detect any changes at the end of each sampling and analysis.

The results of the analyses should be: reliable, reproducible, traceable, documented and transparent.

In the case of significant changes in parameters, the actions to be taken to bring them back to the safe limit values should be planned.

The cause of the change in parameters should be determined and, if possible, reduced or eliminated in future phases of the activities concerned.

The organization shall plan actions to mitigate possible impacts on the environment or biodiversity, be able to respond to different types of emergency situations, ensure a periodic review of the processes carried out and the actions implemented.
Everything shall be documented and the communication and/or information to the parties involved ensured.

4.2AUDIT

Audits shall be planned and carried out at predetermined time intervals.

The purpose of the audits shall be to assess the conformity of the process with this technical standard.

The personnel involved in the audits should have the necessary technical and scientific skills.

All audit findings shall be taken into account by the competent technical staff.

5. Possible reference parameters of the acoustic impact

On the basis of the elaboration of the scientific literature, considering the frequency bands between 10 Hz and 10 kHz below some parameters that can be taken as reference in the management of the emitted noise:

Severe impact

- Deep sea mining and marine-sea activities carried out above 130 dB_{rms} re 1 µPa can cause a serious impact on biodiversity.

Medium impact

- Deep sea mining and marine-sea activities with noise levels between 90 dB_{rms} re 1 µPa and 130 dB_{rms} re 1 µPa may cause an average impact on biodiversity. However, it is possible to limit and/or reduce the impacts as described below.

Low impact

- Deep sea mining and marine-sea activities with noise levels below 90 dB_{rms} re 1 µPa can cause low impact on biodiversity.

In the case of impulsive emissions, where the emissions are greater than:

1. SPL_{peak} 207 dB re 1 µPa
2. SEL_{ss} 174 dB re 1 $\mu Pa^2 s^{-1}$
3. SEL_{cum} 204 dB re 1 $\mu Pa^2 s^{-1}$

The noise impact must be considered serious.
The above values shall be respected within 24 hours of observation.

Impacts can be reduced by considering the following **Impact Mitigation Parameters (IMP)**:

a) Reduce as much as possible the operating times, in the case of the drilling DSM, by taking advantage of any resting sessions. All this bearing in mind that the acoustic conditions and pressure levels depending on the type of environment may change;

The assessment of these timescales should be considered in the monitoring plan.

b) Minimise the time required for 24hour operation, including by using alternative technologies. All of this by ensuring a proper balance between the company's business and any impact on biodiversity;

Break times can be reduced to a minimum only for activities with noise levels lower than those mentioned above.

c) Use possible sound inhibitors (bubble curtains, blasting mats, etc.) to contain noise emissions in a restricted area;

d) Evaluate the possibility of carrying out activities in the non-reproductive months;

e) Reduce activities to a minimum number of possible months within a year so as to allow for a possible recovery of species and ecosystems;

f) Assess the possibility of creating any Areas of Particular Environmental Interest (APEI);

g) Ensure the presence of a Marine Mammals Observer (MMO) that supports the figure of the biologist before, during and at the end of the activities for the possible sighting of mammals.

The MMO shall guarantee the absence of mammals at least 30 minutes before the start of activities within a 500 m radius.

h) Establish safety areas around the source where the noise emitted can be deactivated or reduced in case of animal sightings;
i) Reduce noise levels to the lowest possible level, especially at night;
j) Ensure the presence of additional professionals who work alongside the biologist and who can contribute to the development of work as appropriate for the protection of biodiversity (eg. geochemical for water analysis, ecologist for the evaluation of ecosystems);
k) Evaluate the Response Severity Index (RSI), determining the maximum sound pressure levels associated with different levels of RSI. Tissue damage increases with increasing SEL_{cum} and SEL_{ss};
l) Use of soft start techniques (gradual increases in energy levels);
m) Evaluate the use of drilling compared to piling, with the latter having a greater impact.

6. Conclusion

The study of scientific literature and experiments carried out during this have enabled us to confirm that the various anthropogenic activities that produce noise can negatively influence marine organisms and that an interdisciplinary approach could provide more solid and satisfactory interpretation of acoustic impacts. The main objective of this study was to investigate the acoustic pollution contributions by deep sea mining in the marine environment. The lack of knowledge on the frequencies and intensity of sounds emitted by mining activities has made it difficult until now to develop technical standards or guidelines aimed at reducing impacts on marine biodiversity and with this also the implementation of indoor or outdoor experiments that simulate them. Despite this, a first attempt was made during this PhD to provide a basis to prevent damage to ecosystems and, therefore, to contain the acoustic impact. Given the fact that, to date, frequencies and acoustic intensities emitted by mining activities have not yet been made known, we have shown that scientific data on acoustic impacts caused by other marine/maritime anthropogenic activities are an important basis in understanding possible damage due to DSM and the possibility and/or methods available to limit that damage. In the future, when frequencies and acoustic intensities are released, it will be possible to update and/or improve written technical standards. For this reason, this PhD project was inspired by scientific knowledge on the acoustic impacts of other human activities on biodiversity on physical, physiological and behavioural levels. On the basis of this, experiments were carried out to test the effects of different types of anthropic acoustic emissions, both indoors and outdoors, on vertebrate and invertebrate species. In fact, since the main criteria for acoustic emissions resulting from anthropic activities have been developed to date for marine mammals (Southall et al., 2007; Erbe et al., 2016; NMFS, 2016) (mortality levels, lesions of tissues, hearing, behaviour and physiology), the PhD project was intended to contribute to knowledge on the effects on fish and invertebrates, which are important as they are the basis of the human food web and as the environmental bioindicators. The experiments carried out confirmed the need to increase knowledge on these effects (fish and invertebrates) with appropriate metrics by performing indoor and outdoor experiments capable of analyzing the effects of different frequencies on different species. Moreover, this would allow selection of frequencies and emission intensities which have a lower impact on marine life. For the first time it was possible to test acoustic impacts on echinoderms, confirming that responses of marine organisms vary in a species-specific way. In particular, sea urchins were found to be sensitive to different types of acoustic emission while sea cucumbers were more resistant. New matrices and new biomarkers have been analysed on these organisms, demonstrating their possible validity in the study of acoustic impact. It has been shown that cell-free coelomic fluid in echinoderms, the peristomal membrane in sea urchins and the digestive gland in mussels constitute useful matrices for the study of invertebrate response to this type of stress. The usefulness of different biomarkers in the study of acoustic impact on invertebrates (THC, protein concentration, HSP, glycogen quantity) has been confirmed while enzyme activities (esterase, alkaline phosphatase, peroxidase, superoxide dismutase) and cytotoxicity were used for the first time to study the acoustic impact. This PhD project also provided a contribution to understanding acoustic impact on the behaviour of juvenile fish individuals following exposure to four different acoustic frequencies, based on which it emerged that effects depend on the acoustic frequency emitted and the recovery of the individuals from the time of acoustic emission. The results obtained are important because they allowed us to select frequencies with a lower impact on the behaviour of juveniles, which could affect the survival of the species and subsequent generations. Furthermore, the two frequencies that the EU directive (MSFD) asks to be taken into consideration (63Hz and 125Hz) were the most impactful. We believe that, given the variability in hearing sensitivities, further studies of this type should also be carried out on other juvenile fish species to understand the effects of the same frequencies on different species. Taking into consideration the fact that laboratory conditions and the results obtained do not reflect the reality of deep sea environments and the excessive variables involved, we analysed the effects of acoustic emissions from activities that could precede mining activities (watergun) during *in-situ* experiments. We showed that, this type of acoustic emission has significant effects in cortisol levels of fish and in enzyme activity of the peristomial membrane of sea urchins. However, the effects in the cell-free coelomic fluid of echinoderms were significant only in sea urchins and only for peroxidase activity. We have shown that, also in this case, effects depend on the species analysed and on the type of acoustic emission. Furthermore, even if not significant, changes in biochemical parameters were also observed long after the end of acoustic emission. These preliminary results allowed us to understand the trend response of different biochemical parameters to repeat the analyses repeating the experimental plan. Moreover, this type of acoustic emission probably were more impactful for sea urchins and affects more the antioxidant responses. Many abyssal processes are influenced by food availability (Smith et al., 2008) and its distribution (Jumars, 1981), by temperatures (McClain et al., 2012) and by the abundance of fauna (Glover et al., 2002) and pressure (Childress, 1995); for this reason long-term experiments are important in order to learn about the recovery potential of underwater life. Despite this, the discovery of effects from short-term experiments on a physical, physiological and behavioural level is already a good baseline for future experiments and for understanding the impacts on biodiversity.

There is no doubt that if future mining activities emit the frequencies and acoustic intensities studied in this PhD project from the start, impacts on a physical-physiological level for echinoderms and mussels, and on a behavioural level for fish juveniles will be significant. Using data from scientific literature and the results of our experiments, we were able to determine frequencies with greater and lesser impact and contribute to the development of an initial technical standard for future mining activities in the ocean depths. The experiments carried out were useful in order to obtain the two most important products of the PhD course: the technical standard and the numerical model for the prediction of acoustic impacts on vertebrate behaviour. We were also able to confirm that for the development of a set of environmental guidelines, variability in environmental and biological parameters must be considered. Furthermore, a standardized method is needed for these new activities in order to monitor sound, calibrate the instruments and ensure validity of the data collected (Przeslawski et al., 2018). The written technical standard, therefore, proposes operational modalities that could contribute to containing acoustic impact. To date there is little information and many uncertainties about the effects of noise to be able to formulate absolute thresholds for ecologically sustainable sound levels (Merchant et al., 2016); in spite of this, initial indications to contain impacts are, undoubtedly, of notable use. Knowing something is certainly more useful than knowing nothing. The results of this PhD course are essential to provide a baseline for knowledge on the possible acoustic impact of DSM given the fact that, by 2020, the mining of minerals will begin in the ocean depths and it is essential to understand the contribution of the sound source to the soundscape (Hatch et al., 2008). The ultimate goal of our work is not to prevent mining activities from being carried out, but to prevent them from starting without appropriate environmental protection measures for biodiversity. In fact, the results of this PhD course aim to contribute to the creation of evaluation procedures and a mining code to protect biodiversity and marine ecosystems, of which we are an integral part. We believe that our results provide a scientific and normative basis to further understanding of the effects of noise pollution from DSM activities by finding a balance between resource exploitation and maintaining the deep marine environment. Before new human activities with potential acoustic impact begin, greater knowledge and better collaboration is needed. We are still in time to make intelligent, reasoned decisions by evaluating the advantages of these activities with respect to the economic and environmental costs for ecosystems and/or services that probably could not be restored. It is extremely important to inform policy makers and develop effective management strategies and appropriate mitigation methods. Despite the results of this PhD course, the gaps are still considerable and we believe the lack of information could be due to high variability between species and within species, to interaction with other environmental variables and the lack of knowledge on the technologies that will be used. It is essential to know acceptable or less harmful thresholds for biodiversity which will probably be produced by these activities and for this reason further experiments of this type should be carried out on other vertebrate and invertebrate species to compare their effects. It is highly likely that the consequences of the new activities will be irreversible if we do not try to change the direction of events and our actions. To do this, better communication between the interested political parties, scientists and industries involved is imperative. The scientific community, the mining industries and its regulators have the power to improve the environmental performance of this new sector and they must act before it is too late.

7. Funding Acknowledgments

The scientific studies carried out during this PhD have had different types of funding.

The studies of the Section 3.1, 3.2 and Chapter 4 and 5 were supported by grants from MIUR (Ministry of Education, University and Research), Fondo Finalizzato alla Ricerca di Ateneo (FFR, 2018-2021) to M.V.

The study of the Section 3.3 was supported by grants from MIUR (Ministry of Education, University and Research), Fondo Finalizzato alla Ricerca di Ateneo (FFR, 2018-2021) to M.V. and by the CNR research project: IMPLEMENTATION OF THE SOS CONVENTION - Offshore Platforms & Impacts between MATTM and CNR-DTA, (Capo Granitola, Campobello di Mazara) Work Package E: In-depth technical and scientific evaluation of the effects on marine ecosystems of airgun technologies and the effects of a watergun

The study of the Section 3.4 study was conducted in the framework of the PhD program in Mediterranean Biodiversity XXXII cycle (International) of the University of Palermo. I. Perez-Arjona, M. Bou-Cabo and V. Espinosa acknowledge the financial support of the European Comission -project 11.0661/2018794607/SUB/ENV.C2. Risk-based Approaches to Good Environmental Status (RAGES). The other founding supports come from PhD Innovative with Industrial Characterization PON 2014-2020 and the projects CAIMAR Joint Laboratory Italy-Argentina (Laboratori Congiunti Bilaterali Internazionali of the Italian National Reseach Council, 2017-2019) and BOSS – Study of bioacoustics and applications for the sustainable exploitation of marine resources (Projects of major importance in the Scientific and Technological Collaboration Executive Programmes, funded by the Italian Ministry of Foreign Affairs and International Cooperation).

Each experimental part of this thesis was carried out thanks to the collaboration of different professional figures and research bodies which I thank.

I thank my supervisor of the University of Palermo, Prof.ssa Mirella Vazzana (supervisor). Thank you for helping me during these three years of research and for helping me to grow professionally and personally.

I thank the ENR and Cetena members. Prof. Francesco Beltrame, Dr. Virginia Dagostino, Dr. Salvatore Vacante, Dr. Andrea Andò, Dr. Antonio Ferraro and Dr. Emilio De Angelis. Their contribution was essential to write the technical standard. In them I found friends and thanks to them I learned a lot.

I thank the members of the CNR: Salvatore Mazzola (co-supervisor), Giuseppa Buscaino and Maria Ceraulo. Thank you for their valuable collaboration in the realization of the experimental plans of the thesis. They too contributed to my professional and personal growth.

I thank the members of HR Wallingford, Dr. Giovanni Cuomo and Dr. Tom Benson for their help in the realization of the numerical model. I also thank all the guys of the research team who have been supportive when I was in England.

I would like to thank UPV professor, Prof. Eduardo Belda Pérez (supervisor), Prof.ssa Isabel Pérez Arjona (co-supervisor) and their research team. With them, it was possible to carry out the experiments on the juveniles of *S. aurata*. I thank them for their support and collaboration despite the difficulties and distance.

I thank Valentina Lazzara for the physical and psychological support. In her I found not only a precious help in the laboratory but a precious friend.

I thank my colleague and to date friend, Luca Vecchioni for putting up with me and supporting me. It was one of the most important parts of my PhD at psychological and moral level.

I thank Prof. Arizza for his availability, his support, his advice and his guide. Thank you for helping me grow.

In addition to the figures who helped me in carrying out my scientific research, an important thanks goes to my family. They have contributed to the realization of this thesis and for this reason, I thank them without spending a lot of words. They really know what I think and what I feel and will go beyond the words they will read.

The most important thanks go to the most important person. I thank my mother for making me the person I am today. Thank you for believing in me and I know she would have been proud of me today. I dedicate this PhD to her; I dedicate this thesis to her and I dedicate to her my single day and my single goal. It has not been easy without her and it will not be easy in the future, but with her in my heart, I will find the right way. Her dream is reality today. Thank you, mom.

In the end, but not for importance, I thank the most important people in my life my father, my sister, Calogero and Giorgio for their support in a particular moment of our life. With them I thank all my family, I could not ask for a better family than the one I have.

8. Glossary

Abiotic sound: sounds generated by abiotic/natural components such as water currents, geophysical processes, waves, rain, ice.

Acclimatization period: experimental period in which animals are allowed to acclimatize and then get used to the new environment.

Acetylcholinesterase (AChE): is an enzyme belonging to the class of hydrolases and in particular of esterases. It recognizes specific substrates, in this case choline esters, and is important for example in the transmission of nerve impulses. It is an important biomarker used in environmental monitoring.

Acoustic impact: typical scientific way of identifying the possible effects or “impact” of anthropogenic noise emissions.

Acoustic source: where the acoustic field and therefore the acoustic emission is generated.

Acoustic stress: shift from the equilibrium and homeostasis conditions of the organism due to an acoustic emission.

Acoustic trauma: is an injury to the inner ear or to the other type of tissue that’s usually caused by exposure to a high-decibel noise.

Acoustic waves: a type of propagation of energy through a medium. An example are mechanical and longitudinal waves resulting from an oscillation of pressure traveling through a solid, liquid or gas in a wave model. Acoustic waves travel with a characteristic acoustic speed that depends on the medium they pass through. Some examples of acoustic waves are audible sound coming from a loudspeaker traveling in the air, soil movement from an earthquake traveling through the earth or ultrasound used for medical imaging traveling through the body.

Adaptation: ability of living organisms to change metabolic, physiological and behavioral processes, in line with the new environmental conditions in which they live.

Adenosine Di-Phosphate (ADP): nucleotide deriving from adenosine triphosphate (or ATP) due to the loss of a group of phosphate with the consequent release of energy. It is produced with an exoergonic reaction and is then reused by the cell to recreate ATP with an endergonic reaction when energy is needed.

Adenosine Mono-Phosphate (AMP): also known as 5'-adenyl acid, it is a purine nucleotide involved in many biochemical reactions, it is also present in DNA and RNA. It is composed of a phosphate, a pentose sugar (a ribose) and a nitrogenous adenine base. Allosteric effector in the regulation of carbohydrate metabolism and constituent of important coenzymes. It also performs an important function in the incorporation of amino acids into proteins and is the nucleotide present in RNA.

Adenosine Tri-Phosphate (ATP): a triphosphate ribonucleotide consisting of a nitrogenous base, adenine, ribose (pentose sugar) and three phosphate groups. It is one of the reagents necessary for the synthesis of RNA, but above all it is the chemical link between catabolism and anabolism and constitutes its energetic current. It is hydrolyzed to

ADP which is converted back to ATP through various processes. It is the high energy compound required by almost all the endoergonic metabolic reactions.

Adrenaline: a typical chemical mediator of the vertebrate class, a hormone and a neurotransmitter belonging to a class of substances called catecholamines. Plays the role of neurotransmitter.

Agent Based Models (ABMs): numerical model that allow the simulation of anthropic or environmental impact on marine organisms.

Agonistic behaviour: any social behaviour related to fighting. However, the term has broader meaning respect to "aggressive behaviour" because it includes threats, displays, retreats, placation, and conciliation.

Airgun: technique that produce sound and that provide a better understanding of the deep structure of the sea floor by constructing images. Its operation is based on the use of guns that shoot air. Normally the frequencies emitted was <1 kHz.

Alarm reactions: sudden reaction linked to the perception of a potentially dangerous situation. Initial phase of the body's response to stressful stimuli.

Albumin/globulin ratio: amount of albumin in the serum divided by the globulins. This value is used to identify causes of change in total serum protein.

Aldosterone: steroid hormone that regulate the levels of sodium, potassium and the volume of extracellular fluids.

Alkaline Phosphatase (AKP): a metalloenzyme which catalyses the nonspecific hydrolysis of phosphate monoesters. Are involved in the degradation of foreign proteins, lipids and carbohydrates. Always used as biomarker to evaluate the state of health of organisms.

Ambient noise level: is the background sound pressure level in a given position, specified as the reference level for studying a new intrusive sound source. It is any sound other than the sound being monitored and is also called background noise level, reference sound level or ambient noise level.

Anthropic noise: unwanted sound produced by several type of anthropogenic activities.

Antiprotease activity: ability to avoid the activity of the protease enzyme capable of catalyzing the breakdown of the peptide bond between the amino group and the carboxylic group of proteins.

Antioxidant enzyme: enzymes that counteract the harmful action of free radicals that contain oxygen, exerting a protective action on cellular integrity.

Area: areas located outside the national jurisdiction. Areas that belong to everyone and nobody. Located after the Exclusive Economic Zone.

Apoptosis: form of programmed cell death and is a distinct process compared to cell necrosis. Under normal conditions it contributes to maintaining the number of cells in a system.

Areas of Particular Environmental Interest (APEI): o protected areas, are sites that are created to maintain biodiversity and allow the recolonization of the area exploited by mining.

Auditory Brainstem Response (ABR): an auditory evoked potential extracted from ongoing electrical activity in the brain and recorded via electrodes placed on the scalp. The measured recording is a series of six to seven vertex positive waves labeled with Roman numerals in Jewett and Williston convention. Occur in the first 10 milliseconds after onset of an auditory stimulus. Is considered an exogenous response because it is dependent upon external factors.

Auditory frequencies: indicates the range of audible frequencies. It varies according to the species and organism considered.

Auditory sensitivity: represents the threshold of audibility. For example, in the case of man with the passing of the years the threshold of audible sounds decreases.

Auditory threshold: the minimum sound level of a pure tone that an auditory system with normal hearing can hear without any other sound present. The absolute threshold refers to the sound that can only be heard by the body.

Backscattering: reflection of matter, radiant energy, waves, particles or signals that go back in the same direction from which they come, but in the opposite direction.

Barotrauma: tissue injury caused by the lack of balance between the air pressure of a body cavity and the pressure of the surrounding environment. They occur when the body moves suddenly from or to a condition where the pressure is higher.

Benthic communities: composed of macroinvertebrates, such as annelids, mollusks, and crustaceans. These organisms inhabit the bottom substrates and play a vital role in maintaining sediment and water quality.

Biochemical responses: changes in biochemical biomarkers in the presence of different types of stress that cause a shift from homeostasis conditions.

Biodiversity: variability among living organisms from all sources, including terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part. Includes diversity within species, between species, and of ecosystems.

Ecologically or Biologically Significant Marine Areas (EBSA): special areas in the ocean that have important purposes to support the proper functioning of the oceans and its many services.

Biological rest areas: areas similar to the disturbed sites, which could then recover in the future their structure and biodiversity.

Biomarker: or biological marker, measurable indicator of some biological state or condition. Measured and evaluated to examine biological processes, pathogenic processes, or pharmacologic responses.

Biotic sound: sounds emitted voluntarily or involuntarily by organisms and by human activities.

Blood count: test that determines the amount of blood cells, the levels of the hematocrit, and hemoglobin.

Blue Mining (2014-2018): technological project for deep-sea mineral resource. Is a part of the European Union's Seventh Framework Programme and consists of 11 EU countries and 19 research institute and companies.

Blue Nodules (2016-2020): a research and innovation project to develop a deep-sea mining system for the harvesting of polymetallic nodules with minimum environmental impact

Buffer: solution prepared in laboratory in which another reagent was dissolved to perform biochemical assay.

Catecholamines: secreted hormones that circulate in the blood and released by the glands in stressful situations. The most important catecholamines are adrenaline, norepinephrine, dopamine and dobutamine. They derive from the amino acid tyrosine.

C-start reaction: a bending of the body that takes the form of the letter C and can change schooling patterns, the positions in water columns and swimming speeds.

Cyclic Adenosine Mono-Phosphate (cAMP): cell metabolite produced by the adenylate cyclase enzyme from ATP. It is involved in signal transduction mechanisms within living cells in response to various stimuli, such as those that are unable to cross the cell membrane.

Cardiac output: volume of blood being pumped by the heart, in particular by the left or right ventricle, per unit time. Is the product of the heart rate (HR), or the number of heart beats per minute (bpm), and the stroke volume (SV), which is the volume of blood pumped from the ventricle per beat

Cardiorespiratory level: refers to a measure of an organism's heart and respiratory levels. It can be used to measure the individual's physiological state.

Cell-free coelomic fluid: coelomic fluid without cells. It is obtained after centrigugation and is also called supernatant.

Cellular lysate supernatant: obtained after destroying the cells (with the appropriate buffer different according to the cells and the assay to be made) and centrifuged everle.

Chemosynthetic bacteria: bacteria that live without having to depend on the organic molecules of other living organisms. High energy inorganic substances are transformed into low energy substances by oxidation.

CHIRP: sonar that sends a sweep of frequencies ranging from low to high offering a wider range of information and high-resolution images.

Chronic acoustic stress: long-term loss of homeostasis due to noise emissions.

Circular economy: an economic system aimed at eliminating waste and a continuous use of resources. It employs reuse, sharing, repair, renovation, regeneration and recycling to create a closed loop system, minimizing the use of resource inputs and the creation of waste or pollution.

Clarion-Clipperton Zone (CCZ): also known Clipperton fracture zone is a geological underwater fracture zone of the Pacific Ocean rich in mineral deposits.

Clearance rates: volume of water cleared of food per unit time useful to estimated feeding rates.

Cobalt-rich ferromanganic crusts: mineral deposits formed by the precipitation of minerals at depths ranging from 400 to 7000m and contain cobalt, nickel, traces of rare earth elements and mineral debris

Coelomic fluid: fluid present inside the cavity of coelomated organisms, comparable to human blood and which contains coelomocytes.

Coelomocyte: cellular elements found within the celomatic fluid.

Commission on marine mammals: independent government agency mandated by the Marine Mammal Protection Act (MMPA) to promote the conservation of marine mammals and their environment.

Common heritage of humanity (CHM): a principle of international law which maintains that certain territorial areas and elements of the common heritage of humanity, cultural and natural, are equally equal to all and should be maintained and protected for future generations.

Community: also called biocoenosis, indicates the set of species of an ecosystem that lives in a given environment, or, better, in a given biotope, the area in which the physical-chemical and environmental conditions are constant.

Conceptual model: representation of a system, consisting of the composition of concepts that are used to help know, understand or simulate a subject or object represented by the model. It is therefore a set of concepts. It is formed after a process of conceptualization or generalization.

Connectivity: the degree to which the populations present in different areas of the distribution of the species are linked by the exchange of gametes, larvae, recruits, juvenile forms, or adults.

Continuous noise: further way to characterize the sound in relation to the emission modes of the source. A continuous sound lasts over time without significant duration pauses.

Cortisol: steroid hormone, that is, deriving from cholesterol, belonging to the category of glucocorticoids. It is synthesized on stimulation of the adrenocorticotropic hormone (ACTH), sometimes associated with stress and inflammatory states. Is released in the blood stream, through activation of the hypothalamic-pituitary-interrenal axis (HPI), to restore homeostasis. Is the main biomarker which indicates the animal's state of well-being.

Cytoprotective role: ability to protect against various environmental or pharmacological agents

Cytotoxicity: effect of a chemical, physical or biological agent capable of inducing damage to a cell. Can be an efficient natural defence system in vertebrates and invertebrates; lysins for example can be secreted in the body fluids or act at the membrane level of the effector cell.

Cumulative Sound Exposure Level (SELcum): way to express the duration of the sound that should be specified because there is no accepted standard duration over which the summation of energy is measured. In fact, SEL can be calculated for a single pulse or signal, often called a single shot SEL (SELss). It can also be calculated for multiple pulses or signals to generate a value equivalent to a single exposure for cumulative sound energy (SELcum).

Demersal zone: part of the sea or ocean close to the seabed and benthos. The demersal area is just above the benthic area.

Differential Haemocytes Counts (DHC): relative percentage of each type of cells and also helps to reveal abnormal cell populations.

Digestive gland: organ that is part of the digestive system of mussels is the main production center of enzymes involved in the defense mechanisms of digestion and absorption of food. Equipped with specialized intestinal epithelial cells involved in the production of immune system molecules.

DISCOL (German project): a DIS-turbance and re-COL-onization experiment in a Manganese Nodule Area in the South East Pacific Ocean off Peru. First large-scale experiment to investigate the possible impacts of manganese nodule mining from the deep sea. It was funded from 1988 to 1997 by the former Ministry of Science and Technology of the Federal Republic of Germany. During this time, a total of four cruises with the German research vessel SONNE were carried out to monitor the recolonization process and thus the recovery of the ecosystem of the previously disturbed environment.

Deep Sea Mining (DSM): extraction of minerals from deposits found in ocean depths.

Developmental delays: failure to achieve the goals of embryonic development in the expected time.

Dispersion: indicated the level of fish group cohesion.

Dopamine: is an organic chemical of the catecholamine and phenethylamine families. It functions both as a hormone and a neurotransmitter, and plays several important roles in body. of several type of organisms.

Drilling: type of mining activities that contribute to low frequency underwater noise. Broadband noise generated from drilling is continuous sounds.

Echolocation: also called biosonar, is a biological sonar used by some mammals. Basically, animals emit sounds that bounce when meeting objects and, estimating the time elapsed for the return of the sound, they understand the distance.

Echo sounders: ultracoustic ecometer instrument used to measure the depth of the sea, transmitting sound impulses.

Echinoderms: invertebrates of the phylum of marine deuterostomes. Called in this way because they are often covered with limestone plates. Echinoderms are coelomated deuterostomes closely related to chordate and hemicordate.

Ecological service: benefits arising from the ecological functions of ecosystems that accrue to all living organisms, including animals and plants, rather than to humans alone. Examples of ecological services include purification of air and water (e.g.performed by the filtration of marine invertebrats), maintenance of biodiversity, decomposition of wastes, soil and vegetation generation and renewal, pollination and natural vegetation, groundwater recharge through wetlands, seed dispersal, greenhouse gas mitigation, and aesthetically pleasing landscapes.

Ecosystems: the set of living organisms called biotic factors and non-living matter called abiotic factors that interact in a given environment constituting a self-sufficient and dynamic balance system.

Ecosystem services: benefits that humans freely gain from the natural environment and from properly-functioning ecosystems. Such as ecosystems that provide such things like agricultural produce, timber, and aquatic organisms such as fishes and crabs.

Electrolyte content: measurement of the level of electrolytes, minerals with an electric charge when they are dissolved in a liquid, such as blood. The blood electrolytes for example are sodium, potassium, chloride and bicarbonate which regulate the nervous and muscular functions and maintain, among the various functions, the water balance.

Environmental Impact Assessment (EIA): process of assessing the likely environmental impacts of a project or development that also takes into account the interconnected socio-economic, cultural and human health impacts, both positive and negative.

Environmental impact: any change to the environment, whether adverse or beneficial, wholly or partially resulting from an organization's activities, products, or services.

EU Habitats Directive (92/43/CEE): directive approved on 21 May 1992 by the European Commission which aims to promote the maintenance of biodiversity through the conservation of natural habitats in the European territory.

Exclusive Economic Zone (EEZ): Up to a distance of 200-350 nautical miles from the coast, each country enjoys decision-making power over that area of water, the possibility of using it or exploiting it, and exercises jurisdiction over the area. This part is called the Exclusive Economic Zone (EEZ) for which the extension may vary for different countries.

Excretion rates: measure of the levels of elimination of something from the organism. It is closely related to the filtration rate, the reabsorption rate and the secretion rate. An example is the role played by the kidneys.

Exploitation: phase in which the site that has been explored is used to find what it contains.

Exploration: phase in which the site to be exploited is studied and analyzed to know its content and properties. It precedes exploitation.

Exploitation authorization: written permissions, then documents, which allow you to exploite the mining site. Issued by ISA or state based on the part of the sea where the mining site is located.

Exploration licenses: written permissions, then documents, which allow you to analyze and study the site. Issued by ISA or state based on the part of the sea where the mining site is located.

Empirical relationships: correlation supported by experiment and observation but not necessarily supported by theory.

Endemic species: species of living organisms that live exclusively in an area and characterize it. They are not found elsewhere.

Energy balance: balance of energy levels entering and leaving an ecosystem. The evolution of the time of an ecosystem is characterized by flows of energy and matter. The main source of energy for any ecosystem is solar radiation which creates the conditions of temperature and light necessary for vital processes. This is then transmitted to subsequent ecosystem levels (e.g. plants and photosynthesis).

Environmental Impact Statement (EIS): a document that describe the effects for proposed activities on the environment that is defined, in this case, as the natural and physical environment and the relationship of people with that environment.

Esterase: enzymes of the hydrolase class that catalyze the hydrolysis of the ester bond. They are involved in digestive, inflammatory and detoxification processes of organisms. Esterase is one of the most common biomarkers of environmental exposure used in aquatic organisms and changes in esterase activity are correlated for example with the performance and survival of some invertebrates.

FAO: United Nations Food and Agriculture Organization is a specialized United Nations institution with the aim of helping to increase nutrition levels, increase productivity, improve the lives of populations and contribute to world economic growth.

Global Sea Mineral Resources: mining company's, a world leader in the highly specialised fields of dredging, marine engineering and environmental remediation.

Glucocorticoids: a class of corticosteroids that bind to the glucocorticoid receptor that is present in almost every vertebrate animal cell. Glucocorticoids are part of the feedback mechanism in the immune system which reduces certain aspects of immune function, such as inflammation. An example is the cortisol, the most important human glucocorticoid that regulates or supports a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions.

Glucose level: parameter indicating the levels of sugar in the blood or in other biological matrices, in particular glucose, also known as glycose or dextrose which is an aldehyde monosaccharide.

Glycolysis: is the process of breaking down glucose and that can take place with or without oxygen. It produces two molecules of pyruvate, two molecules of ATP, two molecules of NADH, and two molecules of water.

Good Environmental Status (GES): According the Marine Directive, is important to achieve Good Environmental Status of EU marine waters by 2020. The Directive defines Good Environmental Status (GES) as: “The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive”

Guidelines: is a statement by which to determine a course of action and aims to streamline particular processes according to a set routine or sound practice.

Habitat fragmentation: division of a habitat into different parts that are still difficult to communicate with each other. Often it is caused by impacts of anthropogenic activities.

Haemocytes: cellular elements that characterize hemolymph, a fluid typical of some invertebrate species and comparable to blood.

Hearing ability: ability to perceive sounds through an organ such as the ear, by detecting vibrations, changes in the pressure of the surrounding medium through time.

Heart rate: speed of the heartbeat measured by the number of beats of the heart per minute (bpm). The heart rate can vary according to the body's physical needs.

Heat shock proteins: protein synthesized when cells are exposed to high temperatures or high stress conditions. It protects cells from possible damage and often facilitates the proper folding of proteins.

Hemolymph: is a fluid analogous to the blood in vertebrates that circulates in the interior of some invertebrate body remaining in direct contact with the animal's tissues. It is composed of a fluid plasma in which hemolymph cells called hemocytes are suspended.

High frequency sound: the acoustic frequency is the number of complete cycles that a sound wave performs in one second and is measured in Hertz (Hz). The high frequencies are between 1,600 and 20,000 Hz.

HMS Challenger expedition: expedition carried out between 1872 and 1876. It was a scientific program that brought many discoveries that laid the foundation for oceanography. These include the discovery of mineral deposits. The name derives from the name of the ship that was used.

Homeostasis: natural tendency to achieve stability of both internal and behavioral chemical-physical properties. It unites all living organisms, for which this regime must be maintained over time, even with changes in external conditions, through precise self-regulating mechanisms.

Humoral factor: they constitute the different elements transported in the circulatory system (different from cells) and which play important roles in the immune defenses (e.g. production of antibodies).

Hydrocortisone levels: measure of the active form levels of cortisone also called cortisol (see cortisol). It is part of the glucocorticoid class.

Hydrophone: a microphone used to record or listening to underwater sound.

Hypothalamic-Pituitary-Interrenal axis (HPI): is a complex set of direct influences and feedback interactions among three components: the hypothalamus, the pituitary gland , and the adrenal.Normally is activated in response to most forms of stress in fishImmune characteristics.

Immunocytes: cells responsible for the functioning of the immune system and the performance of defense functions.

Impact mitigation: reduction of damage or effects that can be produced on an environment.

Impulsive sounds: characterized, unlike continuous sounds, by sudden variations in sound level. Impulsive noise can be characterized by short pulses (e.g. hammering operations) or long pulses (e.g. grinding operations).

Innate immunity: or nonspecific immunity, is a subsystem of the immune system. Is a non-specific immunity present since birth, and is not able to give specific and selective responses to pathogens. With limited specificity and no cellular memory. It includes soluble cells and molecules that defend the host (called self) from infection and colonization of other organisms (called non-self).

Intermittent noise: characterized by interruptions that repeat at more or less regular intervals.

International Seabed Authority (ISA): an autonomous intergovernmental body with 168 members and was founded in 1982 by UNCLOS. is responsible for mineral resources and the marine environment in the Area. Moreover, has the obligation to make the CHM principle operational and is a judicial officer for the law of the sea. It can issue exploration and exploitation licenses, monitoring mining activities.

International Marine Mining Society (IMMS): founded in 1987, is a professional society (non profit) whose members share a common interest in marine minerals as a resource for study and sound application to meet world demands for strategic minerals.

International Organization for Standards (ISO): funded in 1947, isan international standard-setting body composed of representatives from various national standards organizations. The organization promotes worldwide proprietary, industrial, and commercial standards.

Interspecific relationship: interaction that takes place in a community between individuals of different species, within an ecosystem.

Intraspecific relationship: interaction that takes place between individuals of the same species.

Invertebrate: animals that neither possess nor develop a vertebral column derived from the notochord.

Lactate: is a byproduct of the anaerobic lactacid metabolism. It is a toxic compound for cells, the accumulation of which correlates, for example, with the appearance of so-called muscle fatigue.

Lagrangian-Eulerian model: used to denote a family of modeling and simulation techniques wherein droplets or particles are represented in a Lagrangian reference frame while the carrier–phase flow field is represented in an Eulerian frame.

Lateral line: sensorial system characteristic of fish, consisting of a series of receptor organs arranged along the sides of the animal. It constitutes a line visible to the naked eye that starts from the spiracles and reaches the tail and is equipped with receptors called neuromasts. Used for example for locating or avoiding obstacles.

Legal and Technical Commission (LTC): members elected by the Council of the International Seabed Authority on the basis of their qualifications in the fields of mineral resources, oceanography, protection of the marine environment, or economic or legal matters relating to ocean mining.The main functions are to review applications for plans of work for exploration in the International Seabed Area and to advise the Council on such applications, as well as to make recommendations regarding protection of the marine environment and monitor compliance with the rules, regulations and procedures for exploration and exploitation of the seabed.

Low frequency sounds: frequency is the number of complete cycles that a sound wave performs in one second and is measured in Hertz (Hz). The low frequencies have a range that goes between 20 and 400 Hz.

Lysozyme: also known as muramidase or N-acetylmuramide glycancanololase, is an antimicrobial enzyme produced by animals that are part of the innate immune system. Lysozyme is a glycoside hydrolase that catalyzes the hydrolysis of the 1,4-beta bonds between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan, which is the main component of the gram-positive bacterial cell wall. This hydrolysis in turn compromises the integrity of the bacterial cell walls causing the lysis of the bacteria.

Mauthner cells: pair of large and easily identifiable neurons located in the rhombus 4 of the navel in fish and amphibians which are responsible for a very fast escape reflex.

Manganese nodules: mineral deposite formed by sedimentation processes of metals dissolved in sea water from the erosion of the continents or given by hydrothermal sources. It was at depth of approximately 3000-6000 m and are composed of manganese, iron oxides, rare earth metals, platinum, tellurium, and other elements

Marine Strategy Framework Directive (MSFD Directive 2008/56/EC): also called Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 that establishing a framework for community action in the field of marine environmental policy. Is a European Directive aimed at achieving or maintaining Good Environmental Status in European seas

Marine Mammals Observer (MMO): a professional in environmental consulting who specializes in whales and dolphins.

Metamorphoses: functional or structural modification of an animal during development, in the passage from the larval to the adult phase.

MIDAS: Managing Impacts of Deep-seA reSource exploitation - was a multidisciplinary research programme investigating the environmental impacts of extracting mineral and energy resources from the deep-sea environment. This included the exploitation of materials such as polymetallic sulphides, manganese nodules, cobalt-rich ferromanganese crusts, methane hydrates and the potential mining of rare earth elements. MIDAS was funded under the European Commission's Framework 7 initiative from November 2013 for a period of 3 years, and has recently completed its programme of research.

Mechanical propagation: propagation of the perturbation, acoustic waves for example, in a gaseous, liquid or solid medium with the transport of energy.

Metabolic rates: the amount of energy used by an animal per unit of time.

Mining code: set of guidelines or rules to manage the activities of extraction of minerals from the deposits of the ocean depths. It is still being written by the ISA.

Mining deposits: mineral deposits present in the ocean depths and created by various geological and chemical phenomena.

National Marine Fisheries Service (NMFS): U.S. federal agency and a division of the National Oceanic and Atmospheric Administration and the U.S. Department of Commerce. It is responsible for managing living marine resources and their habitat around the U.S. exclusive economic zone that extends 200 nautical miles from the coast.

Nautilus Minerals: underwater mining exploration company based in Toronto, Ontario, Canada. It is the first company to commercially explore the seabed for massive sulphide systems.

Neptune Minerals: Underwater mining exploration company founded in January 2011 to explore and develop Seafloor Massive Sulfide (SMS) deposits.

Neubauer chamber: is a thick crystal slide with the size of a glass slide (30 x 70 mm and 4 mm thickness) with central area divided in several squares in which the cell counts are performed. It is used with microscope observation.

Noise: unwanted sound that can have negative effects on the organisms that perceive it.

Non Net Loss (NNL) of biodiversity: expression that refers to the possibility of guaranteeing the absence of biodiversity loss

Noise pollution: expression that identifies the noise among the category of pollutants.

Noradrenaline: hormone that is part of the catecholamine class. Among its functions it plays the role of neurotransmitter and is a stress hormone. Together with epinephrine, it provokes the attack or flight response, activating the sympathetic nervous system to increase the heart beat, release energy in the form of glucose from the glycogen and increase muscle tone. Norepinephrine is released when a series of physiological changes are triggered by an event.

Numerical model: mathematical models that use some sort of numerical time-stepping procedure to obtain the models behavior over time. The mathematical solution is represented by a generated table and/or graph.

Optital Density (OD): is a measurement of a refractive medium or optical component's ability to slow or delay the transmission of light. It measures the speed of light through a substance, affected primarily by the wavelength of a given light wave.

OMCO: mining project that has conducted or sponsored research on processing, waste disposal, and plant location. Process engineering research has been conducted since 1978.

O:N ratio: atomic ratio of oxygen to nitrogen (O:N) obtained by dividing the oxygen consumption rate by the ammonia excretion rate correlated for example with protein catabolism and to lipid and carbohydrate catabolism.

Oxygen consumption: the amount of oxygen taken in and used by the body per minute; thus, it is the rate of oxygen use.

Particle movement: a sound wave propagates because particles next to a vibrating source are moved backwards and forwards in an oscillatory motion; these particles then move the particles next to them and so on, resulting in the propagation of vibratory energy.

Pelagic stages: stage of pelagic larvae that can disperse over great distances, colonize new territories and move away from habitats that have become overcrowded or otherwise unsuitable.

Pellet: in this case referred to the cellular pellet obtained after centrifugation of a sample (e.g coelomatic fluid) and separated from the supernatant.

Peristomial membrane: membrane of sea urchin located around Aristotle’s lantern.

Permanent Threshold Shift (PTS): expression used when hearing loss is lost permanently due to strong acoustic stress. Normally caused by high intensity sound.

Peroxidase: enzyme involved in disease resistance and stress response. Used by the organisms to defend themselves against ROS production. Peroxidase removed H_2O_2.

Physical and physiological responses: changes in immune parameters or the state of tissues and organs as a result of stress or environmental changes.

Physoclistosis: fish for which the swim bladder does not communicate with the digestive system.

Piling: mining technique characterised by impulsive ad high intensity of acoustic emission.

Plumes: clouds of toxic material produced by the phases of mineral extraction which can also be propagated for considerable distances.

Precautionary approach: is a strategy for approaching issues of potential harm when extensive scientific knowledge on the matter is lacking. It emphasizes caution, pausing and review before leaping into new innovations that may prove disastrous.

Prevention: prevent the occurrence or spread of unwanted or harmful environmental damages.

Prime Crust Zone (PCZ): mineral deposit of Central equatorial Pacific Ocean rich in cobalt-rich ferromanganese crusts.

Primary response: one of the reactions of organisms to restore homeostasis. oncerns the sympathetic nervous system, the hypothalamic-pituitary-interrenal axis, catecholamines and the release of glucocorticoids.

Primer: a short single-stranded nucleic acid utilized in the initiation of DNA synthesis.

Prospecting: non-destructive investigation technique of the subsoil, which consists in measuring some physical properties of the soil that can reveal its structure, as well as the presence of buried objects.

Projector: instrument used for playing recorded or programmed sounds.

Reflected: a wave, which propagates along the interface between different media, changes direction due to an impact with a reflective material

Refracted: deviation suffered by a wave that takes place when it passes from one medium to another optically different in which its propagation speed changes.

Resonance: physical condition that occurs when a forced oscillating system is subjected to periodic stresses with a frequency equal to the oscillation of the system itself.

Respiratory rates: is a number of breaths per minute.

Response Severity Index (RSI): index related to the appearance of tissue damage with respect to noise emission levels

RNA: or ribonucleic acid, is a polymeric molecule implicated in various biological roles of coding, decoding, regulation and expression of genes.

ROS: or reactive oxygen species, are chemically reactive chemical species containing oxygen. The most important are the superoxide anion O^{2-}, hydrogen peroxide H_2O_2 and the hydroxyl radical HO·.

Scanning Electron Microscopy (SEM): it is an electron microscope that does not use light as a radiation source but an electroelectric source that is typically a tunsten filament that emits a flow of primary electrons concentrated by a series of electromagnetic lenses and deflected by an objective lens.

Schooling patterns: fish interaction behavior during which group cohesion levels can change.

SEA: or Strategic environmental assessment. Is a process aimed at integrating environmental considerations into development plans and programs, to improve overall decision-making quality.

Seabed mining: other way, besides Deep Sea Mining, to identify the mineral extraction processes from the ocean depths.

Seafloor Massive Sulfide (SMS): mineral deposits formed by hydrothermal fluids (contact between the hot magma of active volcanic centers and the cold water of the oceanic depths) and mainly concentrated in the Pacific, Atlantic, Arctic and Indian Oceans. The composition of these deposits depends on the depth, which is between 2-3 km and 1500 m. Are rich in different minerals, in particular, copper and zinc, but also gold and silver.

Secondary response: response of organisms to restore the homeostasis. It comes into play after the primary responses. It concerns physiological metabolism such as hematological and immune characteristics and changes in respiratory rate.

Sensory hair cells: sensory receptors of both the auditory system and the vestibular system in the ears of all vertebrates, and in the lateral line organ of fishes. Through mechanotransduction, hair cells detect movement in their environment.

Sydney Sustainable Futures Institute: Australian-based institute that deals with research in different sectors to promote sustainable development, for example in proposing other methods to recover minerals avoiding the exploitation of deep deposits.

SOD: antioxidant enzyme that act against ROS production, in particular removing O_2 and produces H_2O_2 subsequently removed by catalase or peroxidase enzymes.

Soft-start technique: It is a technique that has been proposed with regard to noise emissions at sea which requires a particular procedure for gradually increasing noise levels and not suddenly. In particular power increases during a soft-start technique should not exceed 6 dB every five minutes.

Solwara site: Seafloor Massive Sulfide (SMS) deposits of Papua New Guinea that in which Nautilus Minerals won the first license in the wordl and is the owner of the “Solwara Project 1”.

Sonar: acronym of the English expression sound navigation and ranging. It is a technique that uses underwater sound propagation for navigation, communication, to detect the presence and position of ships or submarines, or to analyze the seabed.

Sound: mechanical propagation of waves through a medium carrying energy.

Sound Exposure Level (SEL): is a measure of energy that takes into account both received level and duration of exposure. SEL is a common metric since it allows sound exposures of different durations to be related to one another in terms of total acoustic energy.

Sound Pressure Level (SPL): a logarithmic scale to represent the sound pressure of a sound relative to a reference pressure, and it's measured in units of decibels (dB). It is a means of characterizing the amplitude of a sound. There are several ways sound pressure can be measured. The most common of these are the root-mean-square (rms) pressure, the peak pressure, and the peak-to-peak pressure.

Soundscape: term used to indicate the natural acoustic environment.

Sound frequencies: number that defines how many times a sound wave oscillates per second, it is measured in cycles per second, more commonly called Hertz (Hz).

Sound pressure: is the local pressure deviation from the ambient (average or equilibrium) atmospheric pressure, caused by a sound wave. In air, sound pressure can be measured using a microphone, and in water with a hydrophone. The SI unit of sound pressure is the pascal (Pa).

Spectral characteristics: spectrum gives all information about when the signal occurred in time.

Spectrogram: graphical representation of the intensity of a sound as a function of time and frequency.

Stakeholders: it is generally any person influential towards an economic initiative, a company or any other project.

Statocyst: sensory balance receptor present in some aquatic invertebrates, including molluscs, bivalves, cnidarians, ctenophores, echinoderms, cephalopods and crustaceans. It is also involved in the perception of sounds or better of the movement of particles.

Startle reaction: also called Startle Pattern is an extremely rapid psychophysiological response of an organism to a sudden and unexpected stimulus such as a loud sound or a blinding flash of light.

Stress: disturbance of the equilibrium conditions of an organism and therefore of its homeostasis.

Sublethal effects: capable of giving rise to pathological events or biological damage, but without causing death.

Swim bladder: internal organ of fish which contributes to the ability to control buoyancy. It is used to adapt the specific weight of the fish to the environment by filling with gas, exploiting the Archimedes principle, so that you can swim with less energy and can make vertical movements without swimming.

Power Spectral Density (PSD): measure of signal's power content versus frequency. A PSD is typically used to characterize broadband random signals. The amplitude of the PSD is normalized by the spectral resolution employed to digitize the signal.

Technical standard: is a standard or requirement established for a repeatable technical activity. It is usually a formal document that establishes uniform engineering or technical criteria, methods, processes and practices.

Temporary Threshold Shift (TTS): hearing loss for short periods of time following severe acoustic stress. The recovery time varies between species and depending on the noise emission.

Tertiary response: response that plays its role after the primary and secondary response to reestablished homeostasis. Are relates for example to growth, behaviour, reproduction and survival o fthe organisms.

Total Haemocytes Count (THC): count of the total number of hemocytes present in a hemolyph sample. Is one of the most useful parameters used to evaluate the health of aquatic organisms and the effects of stressful conditions.

Total protein concentrations: total levels of proteins contained in a sample, generally measured by the Bradford method. This too is one of the most useful parameters used to evaluate the health of aquatic organisms.

Trial: set of the number of replicas made for each experimental test.

Underwater noise: unwanted or potentially dangerous sound that propagates in deep water different from the soundscape.

Underwater sound: set of the typical and natural sounds of the deep waters, comparable to the soundscape.

United Nations General Assembly: is one of the six principal organs of the United Nations (UN), the only one in which all member nations have equal representation. It is the main deliberative, policy-making, and representative organ of the UN.

United Nations Conference on the Law of the Sea (UNCLOS): also called the Law of the Sea Convention or the Law of the Sea treaty, is the international agreement that resulted from the third United Nations Conference on the Law of the Sea (UNCLOS III), which took place between 1973 and 1982. It defines the rights and responsibilities of States in the use of the seas and oceans, defining guidelines governing negotiations, the environment and the management of mineral resources.

Ventilation rate: is usually defined as the rate at which external air (fresh air) flows into the body.

Vertebrate: a subphylum of corded animals characterized by a skeletal bone and / or cartilage structure (e.g fish).

Vulnerable Marine Ecosystems (VME): areas that may be vulnerable to the impacts of human activities. This concept emerged from discussions in the United Nations General Assembly (UNGA) and gained momentum after UNGA resolution 61/105.

Watergun: technique that, such as airgun, produce sound and that provide a better understanding of the deep structure of the sea floor by constructing images. Its operation is based on the use of guns that shoot water.

Wavelengths: distance between corresponding points of two consecutive waves usually indicated with λ.

World Health Organization: ONU special health agency, founded on 22 July 1946 and entered into force on 7 April 1948 with headquarters in Geneva. Its role is the achievement by all populations of the highest possible level of health, defined as a condition of complete physical, mental and social well-being, and not only as the absence of disease or infirmity.

Zooplankton: it is one of the three types of plankton, with phytoplankton and bacteriumplankton. It is composed of animal organisms that are not autonomous in movement, but which allow themselves to be carried away by the current.

9. Congresses

1. Lo Presti, D., Mauro, M., Ferrantelli, V., Vazzana, M. Diclofenac effects in sea urchin gametes and fertilization. XIII Meeting Of Doctors In Ecology And Science Of Aquatic Systems (SiTE). Palermo, 3-5 May 2017. Pag.72

2. Mauro, M., Mazzola, S., Beltrame, F., Vazzana, M. "Environmental Impact Assessment Relating to Industrial Activities Concerning Mining of the Seabed (Deep Sea Mining, DSM)". MIUR annual event. National Research Program "Research & Innovation" 2014-2020. Innovation in action: projects, skills, resources. Rome, 7 December, 2017, Communication Room of the MIUR.

3. Mauro, M., Mazzola, S., Buscaino, G., Camilleri, G., Beltrame, F., Cuomo, G., Ceraulo, M., Vazzana, M. Deep Sea Mining and the possible increase of metals in the water column: how will echinoderms and mussels respond? XIV Meeting Of Doctors In Ecology And Science Of Aquatic Systems (SiTE). Genova, 9-11 May, 2018 pag.7 and pag.70

4. Mauro, M., Buscaino, G., Ceraulo, M., Inguglia, L., Beltrame, F., Ducato, A., Papale, E., Vazzana, M., Mazzola, S. Aquatic acoustic noise: behavioural and molecular responses in echinoderms, the case of *A. lixula* (Linnaeus,1758) sea urchin. 79° National Congress Of Italian Zoological Union (UZI). Lecce, 25 - 28 September 2018.pag.117

5. Mauro, M., Buscino, G., Beltrame, F., Mazzola, S., Vazzana, M. Blue Sea Land. Expo of the Mediterranean, African and Middle Eastern Clusters. Mazara del Vallo, 4-7 October, 2018. Deep Sea Mining: new resource or new problem? pag.13

6. Mauro, M., Belda, E., Bou, M., Espinosa, V., Pérez-Arjona, I., Beltrame, F., Buscaino, G., Ceraulo, M., Mazzola, S., Vazzana, M. Effect of submarine acoustic noise in juvenile sea bream (*Sparus aurata*) and mussels (*Mytilus galloprovincialis*). FIA 2018. XI Congreso Iberoamericano de Acústica; X Congreso Ibérico de Acústica; 49º Congreso Español de Acústica - TECNIACUSTICA'18. Cádiz, 24 - 26 October, 2018. pg. 1.181 ISBN: 978-84-87985-30-4. ISSN: 2340-7441 (Digital version)

7. Mauro, M., Pérez-Arjona, I., Belda, E., Ceraulo, M., Bou-Cabo, M., Benson, T., Espinosa, V., Cuomo, G., Beltrame, F., Mazzola, S., Vazzana, M., Buscaino, G. Low frequencies noise effects on behaviour of *Sparus aurata* juveniles. The Effects of Noise on Aquatic Life. 2019 Effects Of Noise On Aquatic Life conference will take place in Den Haag (The Netherlands) from July 7-12. pag. 123

8. Buscaino, G., Ceraulo, M., Giacalone, V.M., Papale, E., Gregorietti, M., Mazzola, S., Grammauta, R., Moyano, M.P.S., Di Fiore, V., Dioguardi, M.,

Mauro, M., Celi, M., Vazzana, M. Effects of Water gun on echinoderms: biochemical changes on sea urchin (*Arbacia lixula*) and Sea cucumber (*Holothuria tubulosa*). The Effects of Noise on Aquatic Life. 2019 Effects Of Noise On Aquatic Life conference will take place in Den Haag (The Netherlands) from July 7-12. Pag.18

9. Mauro, M., Pérez-Arjona, I., Belda, E., Ceraulo, M., Bou-Cabo, M., Benson, T., Espinosa, V., Cuomo, G., Beltrame, F., Mazzola, S., Vazzana, M., Buscaino, G. Anthropogenic noise: the behavioural responses of *Sparus aurata* juveniles as the basis for a numerical model. 80° National Congress Of Italian Zoological Union (UZI). Roma, 23 - 26 September 2019.pag.119. ISBN – 9788883442445

10. Mauro, M., Andò, A., Beltrame, F., Mazzola, S., Buscaino, G., Vazzana, M. Deep Sea Mining study and development of legislation about human activities at sea. Blue Sea Land. Mazara del Vallo, 18 October 2019

Organizing Committee:

11. Cosmic Project " COnfezione Smart Per Prodotti Ittici Progetto COSMIC”. FEAMP 2014-2020. Misura 1.26. Department Stebicef, Via Archirafi,18 15 p.m, 12 September, 2019

    Organization of the Project Final Meetin with presentation of the results obtained. Molecules extracted from marine invertebrates with antimicrobial activity on pathogenic microorganisms (potential cotaminants of food) could be used as natural compounds to replace common chemical compounds and preserve food. Antimicrobial peptides from the coelomic fluid of *H. tubulosa* and *A. lixula* were used. *H. tubulosa* has resisted Gram-positive and Gram-negative bacteria in sea bream.

12. Screening Project: “Scarti ittici: Valorizzazione e sfruttamento biotecnologico”. PO FEAMP 2014-2020. Misura 1.26 Department Stebicef, Via Archirafi,18. 9 a.m, 12 September, 2019. Organization of the Project Final Meetin with presentation of the results obtained. The use of waste from production processes such as fishing or the transformation of fish products is consistent with the Blue Economy. The molecules extracted from waste are substances that find a market in pharmaceutical, medical, nutraceutical and cosmetic applications. The goal was to transform the waste from the fish supply chain from waste to resource.

10. Scientific Paper

Published

1. Parisi, M.G., Mauro, M., Sarà, G., Cammarata, M. Temperature increases, hypoxia, and changes in food availability affect immunological biomarkers in the marine mussel *Mytilus galloprovincialis*. (2017). J Comp Physiol B. DOI 10.1007/s00360-017-1089-2

2. Lazzara, V., Arizza, V., Luparello, C., Mauro, M., Vazzana, M. Bright spots in the darkness of cancer: A review of starfishes-derived compounds and their anti-tumor action. (2019) Mar. Drugs 2019, 17, 617; doi:10.3390/md17110617

3. Vazzana, M., Mauro, M., Ceraulo, M., Dioguardi, M., Papale, E., Mazzola, S., Arizza, V., Beltrame, F., Inguglia, L., Buscaino, G. Underwater high frequency noise: biological responses in sea urchin *Arbacia lixula* (Linnaeus, 1758). 2020. Comparative Biochemistry and Physiology, Part A. 242, 110650. https://doi.org/10.1016/j.cbpa.2020.110650

Submitted

4. Inguglia, L., Chiaramonte, M., La Rosa, S., Vazzana, M., Mauro, M., Queiroz, V., Arizza, V. Allograft Inflammatory factor AIF-1: early immune response in the Mediterranean sea urchin *Paracentrotus lividus.* Zoology

5. Inguglia, L., Chiaramonte, M., Di Stefano, V., Schillaci, D., Cammilleri, G., Pantano, L., Mauro, M., Vazzana, M., Ferrantelli, V., Nicolosi, R., Arizza, V. *Salmo salar* fish waste oil: Fatty acids composition and antibacterial activity. Peer J.

6. Mauro, M., Pérez-Arjona, I., Belda Perez, E.J., Ceraulo, M., Bou-Cabo, M., Benson, T., Espinosa, V., Cuomo, G., Beltrame, F., Mazzola, S., Vazzana, M., Buscaino, G. The effect of low frequency noise on the behaviour of juvenile *Sparus aurata.* JASA

7. Mauro, M., Andò, A., Ferraro, A., Dagostino, V., Vacante, S., De Angelis, E., Buscaino, G., Ceraulo, M., Beltrame, F., Mazzola, S., Vazzana, M. A proposal of technical rules for noise pollution of Deep Sea Mining activity. Marine Policy

8. Vazzana, M., Ceraulo, M., Mauro, M., Papale, E., Dioguardi, M., Mazzola, S., Arizza, V., Chiaramonte, M., Buscaino, G. Effects of acoustic stimulus on biochemical parameters in digestive gland of *M. galloprovincialis* (Lamark, 1819). JASA

9. Mauro, M., Buscaino, G., Mazzola, S., Vazzana, M. Deep Sea Mining activity: how we can understand their possible acoustic impacts? Review Marine Pollution Bulletin

In preparation

10. Acoustic effects on the behaviour of *Sparus aurata* juveniles: *in vivo* measurements and agent-based modelling

Other:

11. Vazzana, M., Arizza, V., Nicolosi, R., Celi, M., Lazzara, V., Affranchi, F., Rubino, M.L., Mauro, M., Settanni, L., Cirlincione, I.F., Ferrantelli, V., Cammilleri, G. (2019). Cosmic Project Final Book. COnfezione Smart Per Prodotti Ittici Progetto COSMIC. FEAMP 2014-2020. Misura 1.26.

12. Arizza, V., Vazzana, M., Schillaci, D., Nicolosi, R., Cusimano, M.G., Inguglia, L., Chiaramonte, M., Mauro, M., Ferrantelli, V., Cammilleri, G., Badalamenti, G. (2019). Screening Project Final Book. Scarti ittici: Valorizzazione e sfruttamento biotecnologico. PO FEAMP 2014-2020. Misura 1.26

11. References.

ACCOBAMS. (2010). Guidelines to address the issue of the impact of anthropogenic noise on marine mammals in the ACCOBAMS area. Document prepared by Gianni Pavan for the ACCOBAMS Secretariat, SC4/2006.

Adamec, R., Hebert, M., Blundell, J., Mervis, R.F. (2012). Dendritic morphology of amygdala and hippocampal neurons in more and less predator stress responsive rats and more and less spontaneously anxious handled controls. Behav. Brain Res. 226, 133–146.

Aguilar de Soto, N., Delorme, N., Atkins, J., Howard, S., Williams, J., and Johnson, M. (2013). Anthropogenic noise causes body malformations and delays development in marine larvae. Sci. Rep. 3, 2831

Aguilar de Soto, N. (2016). Peer-reviewed studies on the effects of anthropogenic noise on marine invertebrates: from scallop larvae to giant squid. In: Popper, A.N., and Hawkins, T. (Eds.). The Effects of Noise on Aquatic Life II. Adv. Exp. Med. Biol. 875: 17-26, New York: Springer Press

Ahmad, S. (1995). Oxidative Stress and Antioxidant Defenses in Biology. Chapman & Hall, New York, NY, USA.

Ahnert, A., Borowski, C. (2000). Environmental risk assessment of anthropogenic activity in the deep-sea". J. Aquatic Ecosyst. Stress Recovery 7 (4), 299–315.

Akamatsu, T., Nanami, A., Yan, H.Y. (2003). Spotlined sardine *Sardinops melanostictus* listens to 1-kHz sound by using its gas bladder. Fisheries Science 69, 348–354

Akinrotimi, O.A., Abu, O.M.G., Ansa, E.J., Edu, O.M., & George, O.S. (2009) Haematological responses of *Tilapia guineensis* to acute stress. International J. Nat. App.Sci. 5, 338–343.

Alday-Sanz, V., Roqueand, A., Turnbull, J.F. (2002). Clearing mechanisms of *Vibrio vulnificus* biotype I in black tiger shrimp *Penaeus monodon*. Dis. Aquatic Organisms 48, 91–99.

Aleynik, D., M. E. Inall, A. Dale, and A. Vink. (2017). Impact of remotely generated eddies on plume ispersion at abyssal mining sites in the Pacific. Sci. Rep. 7: 16959.

Alves, D., Amorim, M.C.P., Fonseca, P.J. (2016). Assessing acoustic communication active space in the Lusitanian toadfish. J. Exp. Biol. 219, 1122e1129.

Alves, D., Amorim, M.C.P., and Fonseca, P.J. (2017). Boat noise reduces acoustic active space in the Lusitanian toadfish *Halobatrachus didactylus*. Proc. Mtgs. Acoust. 27(1), 010033

Amann, H. (1985). Development of ocean mining in the Red Sea. Mar. Min. 5, 103e116

Amon, D.J., Ziegler, A.F., Dahlgren, T.G., Glover, A.G., Goineau., A., Gooday., A.J., Wiklund., H. and Smith., C.R. (2016). Insights into the abundance and diversity of abyssal megafauna in a polymetallic-nodule region in the eastern Clarion-Clipperton Zone. Sci Rep 6: 1–12. July. Nature Publishing Group.

Amon, D., Ziegler, A., Drazen, J., Grischenko, A., Leitner, A., Lindsay, D., Voight, J., Wicksten, M., Young, C., Smith, C. (2017). Megafauna of the UKSRL exploration contract area and eastern Clarion-Clipperton Zone in the Pacific Ocean: Annelida, Arthropoda, Bryozoa, Chordata, Ctenophora, Mollusca. Biodiversity Data J. 5: e14598.

Amorim, M.C.P. (2006). "Diversity of sound production in fish," In: Ladich, F., Collin, S.P., Moller, P., and Kapoor, B.G. (Eds.). Communication in Fishes. Enfield: Science Publishers 1, 71–104.

Amorim, M.C.P., Conti, C., Sousa-Santos, C., Novais, B., Gouveia, M.D., Vicente, J.R., Modesto, T., Goncalves, A., Fonseca, P.J. (2016). Reproductive success in the Lusitanian toadfish: influence of calling activity, male quality and experimental design. Physiology Behav. 155, 17e24.

Amos, A.F., Roels, O.A. (1977). Environment aspects of manganese nodule mining. Mar. Policy 1, 156–163.

Amoser, S., and Ladich, F. (2003). Diversity in noise-induced temporary hearing loss in otophysine fishes. J. Acoust. Soc. Am. 113, 2170–2179.

Anderson, W. A. (1968). Cytochemistry of sea urchin gametes: III. Acid and alkaline phosphatase activity of spermatozoa and fertilization. J. Ultrastruc. Res. 25(1–2), 1-14

Anderson, P.A., Berzins, I.K., Fogarty, F., Hamlin, H.J., and Guillette, L.J. (2011). Sound, stress, and seahorses: the consequences of a noisy environment to animal health. Aquaculture 311(1), 129-138

Andersen, C., Rupke, L., Hasenclever, J., Grevemeyer, I., Petersen, S. (2015). Fault geo-metry and permeability contrast control vent temperatures at the Logatchev 1 hydrothermal field, Mid-Atlantic Ridge, Geology 43, 51–54

Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., Boissery, P. (2004). Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Mar.Pollut. Bull. 49, 704–712

André, M., Morell, M., Mas, A., Solé, M., van der Schaar, M., Houégnigan, L., Zaugg, S., Castell, J.V., Baquerizo, C., Rodríguez, R. L. (2010). Best practices in management, assessment and control of underwater noise pollut1ion. CONAT150113NS2008029, Laboratory of Applied Bioacoustics, Technical University of Catalonia, Barcelona, Spain. Available via http://www.lab.upc.es.

André, M., Van der Schaar, M., Zaugg, S., Houégnigan, L., Sánchez, A.M. (2011a). Sea observatories and acoustic events: towards a global monitoring of ocean noise. 978-1-4577-0164-1/11/$26.00 ©2011 IEEE

André, M., Solé, M., Lenoir, M., Durfort, M., Quero, C., Mas, A., Lombarte, A., Van der Schaar, M., López-Bejar, M., Morell, M., Zaugg, S., and Houégnigan, L. (2011b). Low-frequency sounds induce acoustic trauma in cephalopods. Front. Ecol. Environ. 9(9): 489–493,

Andrew, R.K., Howe, B.M., Mercer, J.A., Dzieciuch, M.A. (2002). Ocean ambient sound: comparing the 1960s with the 1990s for a receiver off the California coast. Acoust. Res. Lett. Online 3, 65–70.

Andrews, C.D., Payne, J.F., and Rise, M. L. (2014). Identification of a gene set to evaluate the potential effects of loud sounds from seismic surveys on the ears of fishes: a study with *Salmo salar*. J. Fish Biol. 84, 1793–1819

Andriguetto-Filho, J.M., Ostrensky, A., Pie, M.R., Silva, U.A., and Boeger, W.A. (2005). Evaluating the impact of seismic prospecting on artisanal shrimp fisheries. Cont. Shelf Res. 25, 1720–1727.

Anestis, A., Lazou, A., Pörtner, H.O., Michaelidis, B. (2007). Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long termacclimation at increasing ambient temperature. Am. J. Physiol. Regul. Integr. Comp. Physiol. 293, 911–921.

Antoni, M., Muench, F., Kunz, U., Brötz, J., Donner, W., and Ensinge, W. (2017). Electrocatalytic applications of platinum-decorated TiO_2 nanotubes prepared by a fully wet-chemical synthesis. J. Mat. Sci. 57, 7754–7767.

Archard, A.G., Earley, R.L., Hanninen, A.F., Braithwaite, V.A. (2012). Correlated behaviour and stress physiology in fish exposed to different levels of predation pressure. Funct.Ecol.2012,26, 637–645.

Ardron, J.A., Ruhlb, H.A., Jones, D.O.B. (2018). Incorporating transparency into the governance of deep-seabed mining in the Area beyond national jurisdiction. Mar.Policy 89 58–66

Arizza, V., Giaramita, F.T., Parrinello, D., Cammarata, M., Parrinello, N. (2007). Cell cooperation in coelomocyte cytotoxic activity of *Paracentrotus lividus* coelomocytes. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 147, 389–394

Aruoma, O.I. (1998). Free radicals, oxidative tress, and antioxidants in human health and disease, J. Am. Oil Chem. Soc. 75, 199–212.

Armstrong, C., Foley, N., Tinch, R., and van den Hove, S. (2012). Services from the deep: steps towards valuation of deep-sea goods and services. EcosystemEcosyst. Services Serv. 2, 2–13.

Asada, K., Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis. In: Kyle, D.J., Osmod, C.B., Arntzen (Eds.), Photoinhibition, Elsevier, Amsterdam (1987) pp.228-287.

Ascension Holdings. (2016). Seafloor Metal Sulphide Deposits [ONLINE] Available at: http://www.ascensionholdings.co.uk/index.php/sms (Accessed February 01 2017).

Au, W.W. L. (1993). The sonar of dolphins. Springer-Verlag, New York, NY.

Auffret, M., Mujdzic, N., Corporeau, C., Moraga, D., (2004). Xenobiotic induced immunomodulation in the European flat oyster, *Ostrea edulis*. Mar. Environ. Res.54,585–589

Auffret, M., Rousseau, S., Boutet, I., Tanguy, A., Baron, J., Moraga, D., Duchemin, M. (2006). A multiparametric approach for monitoring immunotoxic responses in mussels from contaminated sites in Western Mediterranea. Ecotoxicol. Environ. Saf. 63, 393–405.

Bailey, H., Senior, B., Simmons, D., Rusin, J., Picken, G., Thompson, P.M. (2010). Assessing underwater noise levels during pile-driving at an offshore windfarm and its potential effects on marine A. Mar. Pollut. Bull. 60, 888–897

Baker, E.T., German, C.R. (2004). On the global distribution of hydrothermal vent fields. In: German, C.R., Lin, J., Parson, L.M. (Eds.), Mid-ocean Ridges: Hydrothermal Interactions between the Lithosphere and Oceans. Amer Geophysical Union, Washington, pp. 245e266.

Baker, C., German, R. (2009). Going for gold! Who will win in the race to exploit ores from the deep sea? Ocean. Chall. 16, 10–17

Baker, E., Beaudoin, Y. (2013). Deep Sea Minerals: Seafloor Massive Sulphides, a Physical, Biological, Environmental, and Technical Review, vol. 1A. SPC.

Baker, M.R., Gobush, K.S., Vynne, C.H. (2013). Review of factors influencing stress hormones in fish and wildlife. J. Nat.Conserv., 21, pp.309-318,

Banner, A., and Hyatt, M. (1973). Effects of noise on eggs and larvae of two estuarine fishes. Trans. Am. Fish Soc. 102: 134–136.

Barata, C., Solayan, A., Porte, C. (2004). Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to *Daphnia magna*. Aquat. Toxicol. 66, 125e139

Barata, C., Varo, I., Navarro, J.C., Arunc, S., Portec, C. (2005). Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. Comp. Biochem. Phys. C 140,175 – 186

Barber J.R., Crooks K.R. & Fristrup K.M. (2009). The costs of chronic noise exposure for terrestrial organisms. Trends in Ecol. Evol. 25, 180–189.

Barcellos, L.J.G., Ritter, F., Kreutz, L.C., Quevedo, R.M., da Silva, L.B., Bedin, A.C., Finco, J., Cericato, L. (2007). Whole body cortisol increases after direct and visual contact with a predator in zebrafish, *Danio rerio*. Aquaculture. 272, 774–778.

Bart, A.N., Clark, J., Young, J., Zohar, Y. (2001). Underwater ambient noise measurements in aquaculture systems: a survey. Aquacultural Engin. 25, 99–110.

Barton, B.A. (2000). Salmonid Fishes Differ in Their Cortisol and Glucose Responses to Handling and Transport Stress. North American J. Aquaculture.62:12–18, 2000

Barton, B.A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42, 517e525.

Beaulieu, S.E. (2010). InterRidge Global Database of Active Submarine Hydrothermal Vent Fields: prepared for InterRidge, Version 2.0. World Wide Web Electronic Publication

Beaulieu, S.E., Graedel, T.E., Hannington, M.D. (2017). Should we mine the deep seafloor? Earths Future 5, 655–658.

Beckmann, N., Morse, M.P., and Moore, C.M. (1992). Comparative study of phagocytosis in normal and diseased hemocytes of bivalve mollusc *Mya arenaria*. J. Invertebr. Pathol. 59, 124–132.

Bejder, L., Samuels, A., Whitehead, H., Finn, H., Allen, S. (2009). Impact assessment research: use and misuse of habituation, sensitisation and tolerance in describing wildlife responses to anthropogenic stimuli. Mar. Ecol. Prog. Ser. 395, 177– 185.

Bell, J. B., C. H. S. Alt, and D. O. B. Jones. (2016). Benthic megafauna on steep slopes at the northern mid-Atlantic ridge. Mar. Ecol. 37: 1290–1302.

Benhaïm, D., Péan, S., Lucas, G., Blanc, N., Chatain, B., Bégout, M.-L. (2012). Early life behavioural differences in wild caught and domesticated seabass (*Dicentrarchus labrax*). Appl. Anim. Behav. Sci. 141, 79–90.

Benson, T., Rossington, K., Bruintjes, R. (2016). Putting fish in the tank: An Agent Based Modelwith flow interaction. XXIIIrd TELEMAC-MASCARET User Conference, 11 - 13 October 2016, Paris, France.

Bertheussen, K. (1979). The cytotoxic reaction in allogeneic mixtures of echinoid phagocytes. Exp. Cell Res. 120, 373–381

Beyer W, Imlay J, Fridovich I. (1991). Superoxide dismutase. Prog Nucleic Acid Res Mol. Biol. 40, 221–253.

Binyon, J. (1972). Physiology of Echinoderms. Pergamon Press, Oxford, 264 pp.

Bayne, B.L., Thompson, R.J., Widdows, J., (1976). Physiology: I. In: Bayne BL, editor, Marine mussels: their ecology and physiology. Cambridge: Cambridge University Press; p. 121-206.

Blasco, J., Puppo, J., Sarasquete, M.C. (1993). Acid and alkaline phosphatase activities in the clam *Ruditapes philippinarum*. Mar. Biol. 115, (1) 113-118.

Blaxter, J.H.S., Gray, J.A.B., Denton, E.J. (2009). Sound and startle responses in herring shoals. J. Mar. Biol. Assoc. United Kingdom 61, 851–869.

Blom, E. L., Kvarnemo, C., Dekhla, I., Schöld, S., Andersson, M. H., Svensson, O., Amorim, M. C. P. (2019). Continuous but not intermittent noise has a negative impact on mating success in a marine fish with paternal care. Sc. Rep. 9 (1), 5494.

Blöthe, M., Wegorzewski, A., Müller, C., Simon, F., Kuhn, T., and Schippers, A. (2015). Manganese-cycling microbial communities inside deep-sea manganese nodules. Environ. Sci. Technol. 49, 7692–7770.

Blue Nodules. (2016). Press Release February 5 2016 European Consortium Launches Blue Nodules Project. Available online at: http://www.blue-nodules. eu/ (Accessed August 15, 2016).

Boeger, W.A., Pie, M.R., Ostrensky, A., Cardoso, M.F. (2006). The effect of exposure to seismic prospecting on coral reef fishes. Braz. J. Oceanogr. 54, 235–239.

Bogdan, C., Rollinghoff, M., Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity, Curr. Opin. Immunol. 12, 64–76

Bolle, L.J., de Jong, C.A.F., Bierman, S.M., van Beek, P.J.G., van Keeken, O.A. (2012). *Common sole* larvae survive high levels of pile-driving sound in controlled exposure experiments. PLoS One 7, e33052.

Bolton-Warberg, M., Coen, L.D., Weinstein, J.E. (2007). Acute Toxicity and Acetylcholinesterase Inhibition in Grass Shrimp (*Palaemonetes pugio*) and Oysters (*Crassostrea virginica*) Exposed to the Organophosphate Dichlorvos: Laboratory and Field Studies Arch. Environ. Contam. Toxicol. 52, 207–216.

Bonanno, A., Goncharov, S., Mazzola, S., Popov, S., Cuttitta, A., Patti, B., Basilone, G., Di Nieri, A., Patti, C., Aronica, S., Buscaino, G. (2006).Acoustic evaluation of anchovy larvae distribution in relation to oceanography in the Cape Passero area (Strait of Sicily).Chem. Ecol. 22, 265-273.

Bonga, S.E.W. The stress response in fish. Physiol. Rev. (1997) 77, 591–625.

Booman, C., Dalen, J., Leivestad, H., Levsen, A., van der Meeren, T., Toklum, K. (1996). Effekter av luftkanonskyting på egg, larver og yngel. Undersøkelser ved Havforskningsinstituttet og Zoologisk laboratorium, UIB.

Boyd, I.L., Frisk, G., Urban, E., Tyack, P., Ausubel, J., Seeyave, S., Cato, D., Southal, B., Weise, M., Andrew, R., Akamatsu, T., Ling, R.D., Erbe, C., Farmer, D., Gentry, R., Gross, T., Hawkins, A., Li, F., Metcalf, K., Miller, J.H., Moretti, D., Rodrigo, C., Shinke, T. (2011). An international quiet ocean experiment. Oceanography 24, 174–181.

Borsani, J.F., Farchi, C. (2011). Guidelines for the study and regulation of anthropogenic noise introduced into the sea and inland waters (Part Two). ISPRA.

Borsani, J., Faulkner, R., Merchant, N. (2015). Impacts of Noise and Use of Propagation Models to Predict the Recipient Side of Noise. Report prepared under contract ENV.D.2/FRA/2012/0025 for the European Commission. Centre for Environment, Fish. Aquac. Sc., UK.

Boschen, R.E., Rowden, A.A., Clark, M.R., Gardner, J.P.A. (2013). Mining of deep-sea seafloor massive sulfides: a review of the deposits, their benthic communities, impacts from mining, regulatory frameworks and management strategies. Ocean. Coast Manage 84, 54-67

Boschen, R.E., Rowden, A.A., Clark, M.R., Barton, S.J., Pallentin, A., Gardner, J.P.A. (2015). Megabenthic assemblage structure on three New Zealand seamounts: Implications for seafloor massive sulfide mining. Mar. Ecol. Prog. Ser. 523, 1-14.

Boschen, R.E., Collins, P.C., Tunnicliffe, V., Carlsson, J., Gardner, J.P.A., Lowe, J., McCrone, A., Metaxas, A., Sinniger, F., Swaddling, A. (2016). A primer for use of genetic tools in selecting and testing the suitability of set-aside sites protected from deep-sea seafloor massive sulfide mining activities. Ocean & Coastal Management 122 37-48.

Bossart, G. D. (2011). Marine Mammals as Sentinel Species for Oceans and Human Health. Veterinary Pathology, 48(3), 676–690.

Bracciali, C., Campobello, D., Giacoma, C., Sará, G. (2012). Effects of nautical traffic and noise on foraging patterns of Mediterranean damselfish (*Chromis chromis*). PLoS One 7 (7), e40582.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principles of protein-dye binding. Anal. Biochem. 72, 248–254.

Branscomb, E.S., Rittschof, D. (1984). An investigation of low frequency sound waves as a means of inhibiting barnacle settlement. J. Exp. Mar. Biol. Ecol. 79, 149–154

Brandt, M.J., Diederichs, A., Betke, K., Nehls, G. (2011). Responses of harbour porpoises to pile driving at the Horns Rev II offshore wind farm in the Danish North Sea. Mar. Ecol. Prog. Ser. 421, 205–216

Bregman, A.S. (1990). Auditory Scene Analysis: the Perceptual Organization of Sound MITPress: Cambridge.

Brehmer, P., Sarré, A., Guennégan Y., Guillard, J. (2019). Vessel Avoidance Response: A Complex Tradeoff Between Fish Multisensory Integration and Environmental Variables, Reviews in Fisheries Science & Aquaculture.

Brewer, M. (2000). Research design and issues of validity. In: Reis, H., Judd, C. (Eds.), Handbook of Research Methods in Social and Personality Psych. Cambridge University Press, Cambridge, pp. 3e16.

Broudic, M., Voellmy, I., Dobbins, P., Robinson, S., Berggren, P., Laing, S., Blake, L., Radford, A., Lepper, P., Pace, F. (2014). Underwater noise emission from the NOAH's drilling operation at the NAREC site, Blyth, UK. In: Proceedings of the 2nd International Conference on Environmental Interactions of Marine Renewable Energy Technologies (EIMR2014). Blyth, UK, April 2014

Brouwner, M., Brouwner, T.H. (1998). Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, *Callinectes sapidus*. Arch. Biochem. Biophys. 351, 257 – 264

Brown, R.J., Galloway, T.S., Lowe, D., Browne, M.A., Dissanayake, A., Jones, M.B., Depledge, M.H. (2004). Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat.Toxicology 66 (2004) 267–278

Bruintjes, R., Radford, A.N. (2013). Context-dependent impacts of anthropogenic noise on individual and and social behaviour in a cooperatively breeding fish. Anim. Behav. 85, 1343–1349.

Bruintjes, R., and Radford, A.N. (2014). Chronic playback of boat noise does not impact hatching success or post-hatching larval growth and survival in a cichlid fish. PeerJ 2: e594.

Bruintjes, R., Renshaw, E., Rossington, K., Armstrong-Smith, E., Tozer, B., Jones, D., Botterell, Z., Benson, T., Simpson, S.D. (2014). A tool to predict the impact of anthropogenic noise on fish. In: 2nd International Conference on Environmental Interactions of Marine Renewable Energy Technologies (EIMR2014). 28 April – 02 May 2014, Stornoway, Isle of Lewis, Outer Hebrides, Scotland.

Bruintjes, R., Purser, J., Everley, K.A., Mangan, S., Simpson, S.D., and Radford, A.N. (2016). Rapid recovery following short-term acoustic disturbance in two fish species. Open Science 3(1): 150686.

Bruintjes, R., Simpson, S.D., Harding, H., Bunce, T., Benson, T., Rossington, K., and Jones, D. (2017). The impact of experimental impact pile driving on oxygen uptake in black seabream and plaice. Proc. Mtgs. Acoust. 27: 010042.

Bruland, K.W., Middag, R., Lohan, M.C. (2014). Controls of trace metals in seawater, Treatise Geochem. 2 (8) 19–51.

Brungart, D.S. (2001). Informational and energetic masking effects in the perception of multiple simultaneous talkers. J. Acoust. Soc. Am. 110, 2527–2538

Buchanan, J.B. (1969). Feeding and the control of volume within the tests of regular sea-urchins. J. Zool.159 (1) pp.51-64

Buckup, L., Dutra, B.K., Ribarcki, F.P., Fernandes, F.A., Noro, C.K., Oliveira, G.T., Vinagre, A.S. (2008). Seasonal variations in the biochemical composition of the crayfish *Parastacus defossus* (Crustacea, Decapoda) in its natural environment. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 149, 59–67.

Buhl-Mortensen, L., Vanreusel, A., Gooday, A.J., Levin, L.A., Priede, I.G., Buhl-Mortensen, P., Gheerardyn, H., King, N.J., Raes, M. (2010). Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. Mar. Ecol. 31: 21–50.

Buscaino, G., Buffa, G., Sarà, G., Bellante, A., Tonello, A.J., Sliva Hardt, F.A., Jussara Cremer, M., Bonanno, A., Cuttitta, A., Mazzola, S. (2009). Pinger affects fish catch efficiency and damage to bottom gill nets related to bottlenose dolphins. Fish Sci. 75, 537–544.

Buscaino, G., Filiciotto, F., Buffa, G., Bellante, A., Stefano, V.D., Assenza, A., Fazio, F., Caola, G., Mazzola, S. (2010). Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.). Mar. Environ. Res. 69, 136–142.

Buscaino, G., Gavio, A., Galvan, D., Filiciotto, F., Maccarrone, V., de Vincenzi, G., Mazzola, S., Orensanz, J.M. (2015). Acoustic signals and behaviour of *Ovalipes trimaculatus* in the context of reproduction. Aquatic Biol. 24 (1), pp. 61-73.

Buscaino, G., Ceraulo, M., Pieretti, N., Corrias, V., Farina, A., Filiciotto, F., Maccarrone, V., Grammauta, R., Caruso, F., Giuseppe, A., Mazzola, S. (2016). Temporal patterns in the soundscape of the shallow waters of a Mediterranean marine protected area. Sci.Rep.6, 34230.

Buscaino, G., Mattiazzo, G., Sannino, G., Papale, E., Bracco, G., Grammauta, R., Carillo, A., Kenny, J.M., De Cristofaro, N., Ceraulo, M., Mazzola, S. (2019). Acoustic impact of a wave energy converter in Mediterranean shallow waters. Sci. Rep. 9 (1), 9586.

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K.M., Wu, N., Wong, K., Roy, S., Suciu, C., Goodspeed, J., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Tan, J., Denmark, A., Gilder, T., Kyzar, E., Dileo, J., Frank, K., Chang, K., Utterback, E., Hart, P., Kalueff, A.V. (2010). Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat. Protoc. 5, 1786–1799.

Caiger, P.E., Montgomery, J.C., Radford, C.A. (2012). Chronic low-intensity noise exposure affects the hearing thresholds of juvenile snapper. Mar. Ecol. Prog. Ser 466: 225–232.

Caltrans (California Department of Transportation). (2009). Technical guidance for assessment and mitigation of the hydroacoustic effects of pile driving on fish (http://ter.ps/3xk). Appendix I, revised 2012, Compendium of pile driving sounds

Calvo, G., Mudd, A. Valero, and A. Valero. (2016). Decreasing ore grades in global metallic mining: A theoretical issue or a global reality? Resources, 5(4), 36.

Cammarata, M., Vazzana, M., Cervello, M., Arizza, V., Parrinello, N. (2000). Spontaneous cytotoxic activity ofeosinophilic granule cells separated from the normal peritoneal cavity of *Dicentrarchus labrax*. Fish Shellfish Immunol. 10 (2), 143–154.

Canesi, L., Borghi, C., Fabbri, R., Ciacci, C., Lorusso, L.C., Gallo, G., Vergani, L. (2007). Effects of 17beta-estradiol on mussel digestive gland. General Comp. Endoc. 153(1-3), 40-6.

Canicattí, C. (1990). Lysosomal enzyme pattern in Holothuria polii coelomocytes. J. Invert. Pathol. 56, 70–4

Canicatti, C. (1991). Binding properties of *Paracentrotus lividus* (Echinoidea) hemolysin. Comp. Biochem. Physiol. A Comp. Physiol. 98, 463–468.

Cao, R., Zhang, T., Li, X., Zhao, Y., Wang, Q., Yang, D., Qu, Y., Liu, H., Dong, Z.,Zhao, J. (2019). Seawater acidification increases copper toxicity: A multi-biomarker approach with a key marine invertebrate, the Pacific Oyster *Crassostrea gigas.* Aquatic Toxic. 210, 167–178

Carballal, M., Lopez, C., Azevedo, C., and Villalba, A. (1997). Enzymes involved in defence functions of hemocytes of mussel *Mytilus galloprovincialis*. J. Invertebr. Pathol. 70, 96–105

Carbajal, A.,Reyes-López, F.E., Tallo-Parra, O., Lopez-Bejar, M., Tort,L. (2019). Comparative assessment of cortisol in plasma, skin mucus and scales as a measure of the hypothalamic-pituitary-interrenal axis activity in fish. Aquaculture 506, 410-416.

Carlson, T.J., Hastings, M.C., Popper, A.N. (2007). Update on recommendations for revised sound exposure guidelines for fish during pile driving activities. CALTRANS-Arlington Memo Update 12-21-07

Carroll. A.G., Przeslawski. R., Duncan. A., Gunning. M., Bruce.B. (2017). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Poll. Bull.114,9–24

Casaretto, L., Picciulin, M., Olsen, K., Hawkins, A.D. (2014). Locating spawning haddock (*Melanogrammus aeglefinus,* Linnaeus, 1758) at sea by means of sound. Fish. Res. 154, 127–134.

Casper, B.M., Popper, A.N., Matthews, F., Carlson, T.J., Halvorsen, M.B. (2012). Recovery of Barotrauma Injuries in Chinook Salmon, *Oncorhynchus tshawytscha* from Exposure to Pile Driving Sound. PLoS One 7, p. e39593

Casper, B.M., Halvorsen, M.B., Matthews, F., Carlson, T.J., Popper, A.N. (2013a). Recovery of barotrauma injuries resulting from exposure to Pile driving sound in two sizes of hybrid striped bass. PLoS One 7, p. e73844

Casper, B.M., Smith, M.E., Halvorsen, M.B., Sun, H., Carlson, T.J., Popper, A.N. (2013b). Effects of exposure to pile driving sounds on fish inner ear tissues. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 166, 352–360

Celi, M., Filiciotto, F., Parrinello, D., Buscaino, G., Damiano, M.A., Cuttitta, A., D'Angelo, S., Mazzola, S., Vazzana, M. (2013). Physiological and agonistic behavioural response of *Procambarus clarkii* to an acoustic stimulus. J. Exp. Biol. 216, 709–718

Celi, M., Filiciotto, F., Vazzana, M., Arizza, V., Maccarrone, V., Ceraulo, M., Mazzola, S., Buscaino, G. (2015). Shipping noise affecting immune responses of European spiny lobster *Palinurus elephas* (Fabricius, 1787). Can. J. Zool. 93, 113–121.

Celi, M., Filiciotto, F., Maricchiolo, G., Genovese, L., Quinci, E.M., Maccarrone, V., Mazzola, S., Vazzana, M., and Buscaino, G. (2016). Vessel noise pollution as a human threat to fish: assessment of the stress response in gilthead sea bream (*Sparus aurata*, Linnaeus 1758). Fish Physiol. Biochem. 42(2), 631–641.

Ceraulo, M., Bruintjes, R., Benson, T., and Rossington, K., Farina, A., Buscaino, G. (2016). Relationships of sound pressure and particle velocity during pile driving in a flooded dock. Acoustical Society of America [DOI: 10.1121/2.0000295] Proc. Meet. Acoust. 27, 040007

Ceraulo, M., Papale, E., Caruso, F., Filiciotto, F., Grammauta, R., Parisi, I., Mazzola, S., Farina, A., Buscaino, G. (2018). Acoustic comparison of a patchy Mediterranean shallow water seascape: *Posidonia oceanica* meadow and sandy bottom habitats. Ecol. Indic. 85, 1030–1043.

Chainy, G.B., Paital, B., Dandapat, J. (2016). An overview of seasonal changes in oxidative stress and antioxidant defence parameters in some invertebrate and vertebrate species. J. Sci. 2016 (2016) 8

Chandy, J.P., Patel, B. (1985). Do selenium and glutathione (GSH) detoxify mercury in marine invertebrates? Effects on lysosomal response in the tropical blood clam *Anadara granosa*. Dis. Aquat. Org., 1, 39–47

Chandurvelan, R.,Marsden, I.D., Gaw, S., .Glover, C.N. (2013). Biochemical biomarker responses of green-lipped mussel, *Perna canaliculus*, to acute and subchronic waterborne cadmium toxicity Aquatic Toxic. 15, 303-313

Chang, E.S. (2005). Stressed-out lobsters: crustacean hyperglycemic hormone and stress proteins. Integr. Comp. Biol. 45, 43–50.

Chan, A.A.Y.-H., Giraldo-Perez, P., Smith, S., Blumstein, D.T. (2010). Anthropogenic noise affects risk assessment and attention: the distracted prey hypothesis. Biol. Lett. 6, 458–461.

Chan, A.Y., Blumstein, D.T. (2012). Anthropogenic noise's first reverberation into community ecology. Proc. Royal Soc. B: Biol. Sci. 279, 2725–2726

Chapman, C., Hawkins, A. (1969). The importance of sound in fish behaviour in relation to capture by trawls. FAO Fisheries and Aquaculture Report (FAO) 62 (3), 717–729.

Charifi, M., Miserazzi, A., Sow, M., Perrigault, M., Gonzalez, P., Ciret, P., Benomar, S., Massabuau, J. (2018). Noise pollution limits metal bioaccumulation and growth rate in a filter feeder, the Pacific oyster *Magallana gigas*. PLoS ONE 13(4): e0194174.

Chen, M.Y., Yang, H.S., Delaporte, M., Zhao, S. (2007). Immune condition of *Chlamys farreri* in response to acute temperature challenge. Aquaculture 271, 479–487.

Cherkashev, G.A., Ivanov, V.N., Bel'tenev, V., Lazareva, L.I., Rozhdestvenskaya, I.I., Samovarov, M.L. (2013). Massive sulfide ores of the northern equatorial Mid-Atlantic Ridge, Oceanology 53,607–619.

Chia, F.S., Xing, J. (1996). Echinoderm coelomocytes. Zool. Stud. 35, 231–254

Chia, C.,Liu, J., Fei, S., Zhang, C., Chang, Y., Liu, X., Wang, G. (2014). Effect of intestinal autochthonous probiotics isolated from the gut of sea cucumber (*Apostichopus japonicus*) on immune response and growth of *A. japonicus*. Fish Shell. Immun. 38,(2)367–373

Childress, J.J. (1995). Are there physiological and biochemical adaptations of metabolism in deep-sea animals. Trends Ecol. Evol. 10,1–36

Choi, J., Roche, H., Caquet, T. (2000). Effects of physical (hypoxia, hyperoxia) and chemical (potassium dichromate, fenitrothion) stress on antioxidant enzyme activities in chironomus riparius mg. (diptera, chironomidae) larvae: potential biomarkers. Environ. Toxic. Chem. 19, (2), 495–500

Chong, Z. R., Yang, S. H. B., Babu, P., Linga, P, and Li, X.-S. (2016). Review of natural gas hydrates as an energy resource: prospects and challenges. Appl. Energy 162, 1633–1652.

Christian, J.R., Mathieu, A., Thompson, D.H., White, D., Buchanan, R.A. (2003). Effect of Seismic Energy on Snow Crab (*Chionoecetes opilio*). Environmental Funds Project No. 144. Fisheries and Oceans Canada. Calgary (106p)

Christian, J.R., Mathieu, A., Buchanan, R.A. (2004). Chronic Effects of Seismic Energy on Snow Crab (*Chionoecetes opilio*). Environmental Funds Project No. 158. Fisheries and Oceans Canada. Calgary (25pp).

Christiansen, B., Denda, A., Christiansan, S. (2019). Potential effects of deep seabed mining on pelagic and benthopelagic biota. Mar. Policy

Clark, C.W., Ellison, W.T., Southall, B.L., Hatch, L., Van Parijs, S.M., Frankel, A., Ponirakis, D. (2009). Acoustic masking in marine ecosystems: intuitions, analysis and implications.Mar. Ecol.-Prog. Ser. 395, 201–222

Clark, M.R., Durden, J.M., Christianse, S. (2019). Environmental impact assessments for deep-sea mining: Can we improve their future effectiveness? Mar. Policy

Clifford, N.J., Soar, P.J., Harmar, O.P., Gurnell, A.M., Petts, G.E., Emery, J.C. (2005). Assessment of hydrodynamic simulation results for eco-hydraulic and ecohydrological applications: a spatial semivariance approach. Hydrological Proc. 19, 3631–3648.

Codarin, A., Wysocki, L.E., Ladich, F., Picciulin, M. (2009). Effects of ambient and boat noise on hearing and communication in three fish species living in a marine protected area (Miramare, Italy). Mar. Pollut. Bull. 58, 1880–1887.

Coeurdacier, J.L., Dutto, G., Gasset, E., Blancheton, J.P. (2011). Is total serum protein a good indicator for welfare in reared sea bass (*Dicentrarchus labrax*)? Aquat. Living Resour. 24, 121–127.

Coffaro, K.A., Hinegardner, R.T. (1977). Immune response in the sea urchin *Lytechinus pictus*. Science 197, 1389–1390.

Coffey Natural Systems. (2008). Environmental Impact Statement, Solwara 1 Project. Nautilus Minerals Niugini Limited, main report Coffey Natural Systems, Brisbane, p. 226.

Collins, P.C., Kennedy, R., Van Dover, C.L. (2012). A biological survey method applied to seafloor massive sulphides (SMS) with contagiously distributed hydrothermal-vent fauna. Mar. Ecol. Prog. Ser. 452, 89–107

Collins, P.C., Croot, P., Carlsson, J., Colaço, A., Grehan, A., Hyeong, K., Kennedy, R., Mohn, C., Smith, S., Yamamoto, H., Rowden, A. (2013). A primer for the Environmental Impact Assessment of mining at seafloor massive sulfide deposits. Mar. Policy. 42,198–209.

Cooper, K., Boyd, S., Aldridge, J., Rees, H., (2007a). Cumulative impacts of aggregate extraction on seabed macro-invertebrate communities in an area off the east coast of the United Kingdom. J. Sea Res. 57, 288–302.

Cooper, K., Boyd, S., Eggleton, J., Limpenny, D., Rees, H., Vanstaen, K., (2007b). Recovery of the seabed following marine aggregate dredging on the Hastings Shingle Bank off the southeast coast of England. Estuar. Coast. Shelf Sci. 75, 547–558.

Coeurdacier, J.L., Dutto, G., Gasset, E., Blancheton, J.P. (2011). Is total serum protein a good indicator for welfare in reared sea bass (*Dicentrarchus labrax*)? Aquat. Living Resour. 24,121–127

Corliss, J.B., Dymond, J., Gordon, L.I., Edmond, J.M., von Herzen, R.P., Ballard, R.D., Green, K., Williams, D., Bainbridge, A., Crane, K., van Andel, T.H. (1979). Sub- marine thermal springs on the Galápagos Rift. Science 203, 1073–1083.

Corliss, J.B., Baross, J.A., Hoffmann, S.E. (1981). An hypothesis concerning the relationship between submarine hot springs and the origin of life on Earth. Oceanol. Acta 4 (Suppl.), 59–69.

Correa, J.M.G., Sempere, J.B., Juanes, F., Rountree, R., Ruiz, J.F., Ramisa, J. (2018). Recreational boat traffic effects on fish assemblages: First evidence of detrimental consequences at regulated mooring zones in sensitive marine areas detected by passive acoustics. Online 2018. Ocean Coast. Manag. 168, 22–34

Coteur, G., Danis, B., Fowler, S.W., Teyssie, J.L., Dubois, P., Warnau, M. (2001). Effects of PCBs on Reactive Oxygen Species (ROS) Production by the Immune Cells of *Paracentrotus lividus* (Echinodermata). Mar.Poll. Bull. 42 (8) 667–672

Coteur, G., Warnau, M., Jangoux, M., Dubois, P. (2002). Reactive oxygen species (ROS) production by amoebocytes of *Asterias rubens* (Echinodermata). Fish Shell. Immun. 12 (3), 187–200

Courtenay, S.C., Boudreau, M., Lee, K. (2009). Potential Impacts of Seismic Energy on Snow Crab: An Update on the September 2004 Peer Review. Fisheries Oceans Canada, Moncton.

Cox, K., Brennan, L.P., Gerwing, T.G., Dudas, S.E., Juanes, F. (2018). Sound the alarm: a meta-analysis on the effect of aquatic noise on fish behavior and physiology. Glob Chang Biol. 24(7), 3105– 3116

Craik, N. (2016). ISA Discussion Paper No. 4. Enforcement and Liability Challenges for Environmental Regulation of Deep Seabed Mining, Kingston, Jamaica.

Crawford, A.M., Hollingshead, S.C., Scott, S.D. (1984). Geotechnical engineering properties of deep-ocean polymetallic sulfides from 21N, East Pacific Rise. Mar. Min. 4, 337–354.

Cronan, D.S. (1980). Underwater Minerals. Academic Press.

Das, A. (2019). Underwater radiated noise: A new perspective in the Indian Ocean region. J. Nat. Mar. Found. India.15,65-77

Da Silva-Castiglioni, D., Dutra, B.K., Oliveira, G.T., Buckup, G.B. (2007). Seasonal variations in the intermediate metabolism of *Parastacus varicosus* (Crustacea, Decapoda, Parastacidae). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 148, 204–213.

Dalen, J., Knutsen, G. (1987). Scaring effects in fish and harmful effects on eggs, larvae and fry by offshore seismic explorations. In: Merklinger, H. (Ed.), Progress in Underwater Acoustics. Springer US, pp. 93–102.

Dalen, J., Dragsund, E., Naess, A., Sand, O. (2007). Effects of Seismic Surveys on Fish. Fish Catches and Sea Mammals (Norway)

Dalton, T., Shertzer, H. (1998). Regulation of gene expression by reactive oxygen, Annu. Rev. Pharmacol. Toxicol. 39, 67–101.

Dalton, T.P., Shertzer, H.G., Puga, A. (1999). Regulation of gene expression by reactive oxygen, Annu. Rev. Pharmacol. Toxicol. 39, 67–101.

Dang, H., Zhanga, T., Yi, F., Ye, S., Liu, J., Li, Q., Li, H., Li, R. (2019). Enhancing the immune response in the sea cucumber *Apostichopus japonicus* by addition of Chinese herbs *Houttuynia cordata* Thunb as a food supplement. Aquac. Fish. 4,114-121.

Danovaro, R., Snelgrove, P. V., and Tyler, P. (2014). Challenging the paradigms of deep-sea ecology. Trends Ecol. Evol. 29, 465–475.

Davidson, J., Bebak, J., Mazik, P. (2009). The effects of aquaculture production noise on the growth, condition factor, feed conversion, and survival of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 288(3-4), 337-343.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Semmens, J.M. (2016a). Seismic air gun exposure during early-stage embryonic development does not negatively affect spiny lobster *Jasus edwardsii* larvae (Decapoda: Palinuridae). Sci Rep 6, 22723.

Day, R.D., McCauley, R.M., Fitzgibbon, Q.P., Hartmann, K., and Semmens, J.M. (2016b). Assessing the impact of marine seismic surveys on southeast Australian scallop and lobster fisheries. University of Tasmania, Hobart, October. CC BY 3.0.

Debusschere, E., De Coensel, B., Bajek, A., Botteldooren, D., Hostens, K., Vanaverbeke, J., Vandendriessche, S., Van Ginderdeuren, K., Vincx, M., Degraer, S. (2014). Situ mortality experiments with juvenile sea bass (*Dicentrarchus labrax*) in relation to impulsive sound levels caused by pile driving of windmill foundations. Plos One 9.

Debusschere, E., Hostens, K., Adriaens, D., Ampe, B., Botteldooren, D., De Boeck, G., De Muynck, A., Sinha, A.K., Vandendriessche, S., Van Hoorebeke, L. (2016). Acoustic stress responses in juvenile sea bass *Dicentrarchus labrax* induced by offshore pile driving. Environ. Pollut. 208, 747–757.

De Groot, S.J. (1979). An assessment of the potential environmental impact of large-scale sand-dredging for the building of artificial islands in the North Sea. Ocean Manag. 5,211–232.

Dekeling, R.P.A., Tasker, M.L., Van der Graaf, A.J., Ainslie, M.A, Andersson, M.H., André, M., Borsani, J.F., Brensing, K., Castellote, M., Cronin, D., Dalen, J., Folegot, T., Leaper, R., Pajala, J., Redman, P., Robinson, S.P., Sigray, P., Sutton, G., Thomsen, F., Werner, S., Wittekind, D., Young, J.V. (2014). Monitoring

Guidance for Underwater Noise in European Seas, Part II: Monitoring Guidance Specifications, JRC Scientific and Policy Report EUR 26555 EN, Publications Office of the European Union, Luxembourg.

De Jesus, E.G.T., Hirano, T. (1992). Changes in whole body concentrations of cortisol, thyroid hormones and sex steroids during early development of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 85, 55–61

De Jong, C.A.F., Ainslie, M.A., Blacquiere, G. (2011). Standard for measurement and monitoring of underwater noise, Part II: procedures for measuring underwater noise in connection with offshore wind farm licensing. In: D.-G.f.W. A (Ed.), Ministry of Infrastructure and the Environment, p. 56. TNO report, TNO-DV 2011 C251, Den Haag.

De Jong, K., Amorim, M.C.P., Fonseca, P.J., Klein, A., and Heubel, K.U. (2016). Noise affects acoustic courtship behavior similarly in two species of gobies. Proc. Mtgs. Acoust. 27 (1), 010018.

De Jong, K., Amorim, M.C.P., Fonseca. P.J., Fox C.J., Heubel. K.U. (2017). Noise can affect acoustic communication and subsequent spawning success in fish. 0269-7491/© 2017 Elsevier Ltd. All rights reserved.

De Jong, K., Amorim, M.C.P., Fonseca, P.J., Heubel, K.U. (2018) Noise Affects Multimodal Communication During Courtship in a Marine Fish. Front. Ecol. Evol. 6,113.

Deep-sea mining summit, (2016). The 5th Annual Deep Sea Mining Summit 2016. The Cumberland Hotel, London.

Deng, Z.D., Southall, B.L., Carlson, T.J., Xu, J., Martinez, J.J., Weiland, M.A., Ingraham, J.M. (2014). 200 kHz commercial sonar systems generate lower frequency side lobes audible to some marine mammals. PloS one, 9(4), e95315.

Department for Environment, Food and Rural Affairs (2012). Resource Security Action Plan: Making the Most of Valuable Materials. Available online at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/69511/pb13719-resource-security-action-plan.pdf (Accessed August 23, 2017).

De Quirós, J.B., Fernandez,A., Baird, R.W., Brownell, R.L., Aguilar de Soto, N., Allen, D., Arbelo, M., Arregui, M., Costidis, A., Fahlman, A., Frantzis, A., Gulland, M. Iñíguez, F. M. D., Johnson, M., Komnenou, A., Koopman, H., Pabst, D. A., Roe, W. D., Sierra, E., Tejedor M., and Schorr, G. (2019). Advances in research on the impacts of anti-submarine sonar on beaked whales. Proc. R.Soc.B. 286, 20182533

De Soto, N.A., Delome, N., Atkins, J., Howard, S., Williams, J., Johnson, M. (2013). Anthropogenic noise causes body malformation and delays development in marine larvae. Sci. Rep. 3,2831.

Desprez, M. (2000). Physical and biological impact of marine aggregate extraction along the French coast of the Eastern English Channel: short-and long-term post-dredging restoration. ICES J. Mar. Sci. 57, 1428–1438.

Devey, C.W., Fisher, C.R., Scott, S. (2007). Responsible science at hydrothermal vents. Oceanography 20, 162–171

Department of Fisheries and Oceans. Statement of Canadian Practice: Mitigation of Seismic Noise in the Marine Environment (2005).

Diamond Fields International (2016). News Release. July 18, (2016). Diamond Fields Announces Update on Projects. Available online at: http://www. diamondfields.com/s/NewsReleases.asp?ReportID=756418&_Type=News-Releases&_Title=Diamond-Fields-Announces-Update-on-Projects (Accessed August 10, 2016).

Doksæter, L., Handegard, N.O., Godø, O.R., Kvadsheim, P.H., and Nordlund, N. (2012). Behavior of captive herring exposed to naval sonar transmissions (1.0–1.6 kHz) throughout a yearly cycle. J. Acoust. Soc. Am. 131(2), 1632-1642.

Dolman, S.J., Weir, C.R., Jasny, M. (2009). Comparative review of marine mammal guidance implemented during naval exercises. Mar. Pollut. Bull. 58, 465–477

Domjan, M. (2010). The Principles of Learning and Behaviour, sixth ed. Wadsworth, Cengage Learning, Belmont, CA.

Douhri, H., Sayah, F. (2009). The use of enzymatic biomarkers in two marine invertebrates *Nereis diversicolor* and *Patella vulgata* for the biomonitoring of Tangier's bay (Morocco). Ecotoxicol. Environ. Saf. 72, 394–399

Doyotte, A., Cossu, C., Jacquin, M.C., Babut, M., Vasseur, P. (1997). Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve Unio tumidus. Aquat. Toxicol. 39, 93 – 110.

Drazen, J.C., Smith, C.R., Gjerde,K., Au,W., Black, J., Carter, G., Clark, M., Durden,J.m., Dutrieux, P., Goetze, E., Haddock, S., Hatta, M., Hauton, C., Hill, P., Koslow, J., Leitner, A.B., Measures, C., Pacini,A., Parrish,F., Peacock, T., Perelman, J., Sutton, T., Taymans,C., Tunnicliffe, V., Watling, L., Yamamoto, H., Young, E., Ziegler, A.F. (2019). Report of the workshop Evaluating the nature of midwater mining plumes and their potential effects on midwater ecosystems. Research Ideas and Outcomes 5: e33527

Du, C., Anderson, A., Lortie, M., Parsons, R., Bodnar, A. (2013). Oxidative damage and cellular defense mechanisms in sea urchin models of aging. Free Radic. Bio.Med., 63, 254–263.

Dukas, R. Causes and consequences of limited attention. Brain Behav. Evol. (2004) 63, 197–210.

Dunn, D.C., Van Dover, C.L., Etter, R.J., Smith, C.R., Levin, L.A., Morato, T., Colaço, A., Dale, A.C., Gebruk, A.V., Gjerde, K.M., Halpin, P.N., Howell, K.L., Johnson, D., Perez, J.A.A., Ribeiro, M.C., Stuckas, H., Weaver, P. (2018). SEMPIA Workshop Participants. A strategy for the conservation of biodiversity on mid-ocean ridges from deep-sea mining. Sci. Adv. 4: eaar4313

Dunning, D.J., Ross, Q.E., Geoghegan, P., Reichle, J.J., Menezes, J.K., Watson, J.K. (1992). Alewives in a cage avoid high-frequency sound. North Am. J. Fish Manag. 12, 407–416.

Durand, F., Devillers, N., Lallier, F.H., Regnault, M. (2000). Nitrogen excretion and change in blood components during emersion of the subtidal spider crab Maia squinado (L.). Comp. Biochem. Phys. A 127, 259–271

Durkina, V.B., Evtushenko, Z.S. (1991). Changes in activity of certain enzymes in sea urchin embryos and larvae after exposure of adult organisms to heavy metals. Marin. Ecol.Progress Series. 72, 111-115

Durden, J.M., Murphy, K., Jaeckel, A., Van Dover, C.L., Christensen, S., Ortega, A., Jones, D.O.B. (2017). A procedural framework for robust environmental management of deep-sea mining projects using a conceptual model, Mar. Policy 84,93–201.

Durden, J.M., Lallierc, L.E., Murphye, K., Jaeckelf A., Gjerdeg, K., Jones, D.O.B. (2018). Environmental Impact Assessment process for deep-sea mining in ‘the Area’. Mar. Policy 87 194–202

Earthworks. (2015). World's First Deep Sea Mining Proposal Ignores Consequences of its Impacts on Oceans [ONLINE] Available at: (Accessed November 21 2016).

Eaton, R.C., Bombardieri, R.A., Meyer, D.L. (1977). The Mauthner-initiated startle response in teleost fish. J. Exp. Biol. 66, 65–81.

Edmonds, N. J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., Wood, D.T. (2016). Review of crustacean sensitivity to high amplitude underwater noise: Data needs for effective risk assessment in relation to UK commercial species. Mar. Poll.Bull.108, 5–11

Eggleston, D.B., Lillis, A., Bohnenstiehl, D.R. (2016). Soundscapes and larval settlement: larval bivalve responses to habitat-associated underwater sounds. In:

popper, A.N., Hawkins, A. (Eds). The effects of noise on aquatic Life II, Advances in Experimental Medicine and Biology.

Ehlers, P. (2016). Blue growth and ocean governance – how to balance the use and the protection of the seas. WMU J. Marit. Affairs 15, 187–203.

Ekstrom, J., Bennun, L., Mitchell, R., (2015). A Cross-Sector Guide for Implementing the Mitigation Hierarchy, Cross Sector Biodiversity Initiative, Cambridge.

Ellefmo, S.L., Ludvigsen, M., Frimanslund, E.K.T. (2017). Full cycle resource evaluation of sms deposits along the arctic mid ocean ridge. Proceedings of the ASME 2017 36th International Conference on Ocean, Offshore and Arctic Engineering OMAE2017 June 25-30. Trondheim, Norway

Ellingtton, W., Lawrence, J.M. (1974). Coelomic fluid volume regulation and isosmotic intracellular regulation by *Luidia Clathrata* (Echinodermata: Asteroidea) in response to Hyposmotic stress. Biol.Bull. 146,20-31.

Ellis, D.V. (2001). A review of some environmental issues affecting marine mining. Mar. Georesour. Geotechnol. 19, 51–63.

Emery, K.O., Skinner, B.J. (1977). Mineral deposits of the deep-ocean floor. Mar. Min. 1, 1e71.

ENB (2017). Earth Negotiations Bulletin. Summary of the Twenty-Third Annual Session of the International Seabed Authority. Kingston. Available online at: http://enb.iisd.org/vol25/enb25151e.html (Accessed August 21, 2017).

Endo, T., Nakagawa, T., Iguchi, F., Kita, T., Okano, T., Sha, S.H., Schacht, J., Shiga, A., Kim, T.S., Ito J. (2005). Elevation of superoxide dismutase increases acoustic trauma from noise exposure. Free Rad. Biol Med. 38, 492–498.

Engås, A., Løkkeborg, S. (1996). Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 53, 2238–2249.

Engas, A., Lokkeborg S. (2002). Effects of seismic shooting and vessel-generated noise on fish behaviour and catch rates. Bioacoustics: 12, 313-316.

Erbe, C., MacGillivray, A.O., Williams, R. (2012). Mapping cumulative noise from shipping to inform marine spatial planning. J. Acoust. Soc. Am. 132, EL423–EL428.

Erbe, C. (2012b). Effects of underwater noise on marine mammals. In A. N. Popper & A. Hawkins (Eds.). The effects of noise on aquatic life (pp. 17–22). New York, NY: Springer New York.

Erbe, C., Reichmuth, C., Cunningham, K., Lucke, K., and Dooling, R. (2016). Communication masking in marine mammals: a review and research strategy. Mar. Poll. Bull. 103, 15–38.

European Commission (2017a). Report on the Blue Growth Strategy towards More Sustainable Growth and Jobs in the Blue Economy. Available online at: https://ec.europa.eu/maritimeaffairs/sites/maritimeaffairs/files/swd-2017-128_en.pdf

Exclusive Economic Zone and Continental Shelf (Environmental Effects) Act (2012). Available at: http://www.legislation.govt.nz/act/public/2012/0072/latest/DLM3955428.html (accessed 15.03.13).

Fangyu, W., Yang, Y.H., Xiaoyu, W., Kun, X., Fei., G. (2011). Antioxidant enzymes in sea cucumber Apostichopus japonicus (Selenka) during aestivation. J. Mar. Biol. Ass. UK. 2011, 91(1), 209–214. # Marine Biological Association of the United Kingdom, 2010

FAO. (2016). The State of World Fisheries and Aquaculture 2016 (Contributing to food security and nutrition for all. Rome).

Fakan, E.P., McCormick, M.I. (2019) Boat noise affects the early life history of two damselfishes. Mar.Poll. Bull.141, 493–500

Farina A, Pieretti N. (2014). Sonic environment and vegetation structure: a methodological approach for a soundscape analysis of a Mediterranean maqui. Ecol. Inform. 21, 120–132.

Fay, R. R. (1984). The goldfish ear codes the axis of acoustic particle motion in three dimensions. Science 225, 951–954

Fay, R.R., Popper, A. (1998). Comparative Hearing: Fish and Amphibians. Springer, New York, NY.

Fay, R. R. (2009). Soundscapes and the sense of hearing of fishes. Integ. Zool. 4, 26–32

Fay, R.R. and Popper., A.N. (2000). Evolution and hearing in vertebrates: The inner ears and processing. Hear. Res. 149, 1–10

Faulkner, R.C., Farcas, A., Merchan, N.D. (2018) Guiding principles for assessing the impact of underwater noise. J Appl Ecol. 55, 2531–2536.

Faucher, K., Parmentier, E., Becco, C., Vandewalle, N., Vandewalle, P. (2010). Fish lateral system is required for accurate control of shoaling behaviour. Anim. Behav. 79, 679 – 687.

Feist, G., Schreck, C.B. (2002). Ontogeny of the stress response in chinook salmon, *Oncorhynchus tshawytscha*. Fish Physiol. Biochem. 25, 31–40

Fernández, A., Edwards, J.F., Rodriguez, F., De Los Monteros, A.E., Herraez, P., Castro, P., Jaber JR, Martín V, Arbelo, M. (2005). Gas and fat embolic syndrome involving a mass stranding of beaked whales (Family Ziphiidae) exposed to anthropogenic sonar signals. Veterinary Path 42(4), 446-457.

Ferrari, M.C.O., McCormik, M.I., Meekan, M.G., Simpson, S.D., Nedelec, S.L., Chivers, D.P. (2018). School is out on noisy reefs: the effect of boat noise on predator learning and survival of juvenile coral reef fishes. The Royal Society.

Fewtrell, J.L., McCauley, R.D. (2012). Impact of air gun noise on the behaviour of marine fish and squid. Mar. Pollut. Bull. 64, 984–993

FHWG (Fisheries Hydroacoustic Working Group) Memorandum, agreement in principle for guidelines for injury to fi sh from pile driving activities, NOAA's Fisheries Northwest and Southwest Regions, US Fish and Wildlife Service Regions 1 and 8, California/Washington/ Oregon Departments of Transportation, California Department of Fish and Game, and US Federal Highway Administration. June 12 (2008)

Fiechter, J. (2012). Assessing marine ecosystem model properties from ensemble calculations. Ecol. Modell. 242, 164–179.

Filiciotto, F., Giacalone, V.M., Fazio, F., Buffa, G., Piccione, G., Maccarrone, V., Di Stefano, V., Mazzola, S. & Buscaino, G. (2013) Effects of acoustic environment on gilthead sea bream (*Sparus aurata*): sea and onshore aquaculture background noise. Aquaculture 414–415, 36–45.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Di Stefano, V., Mazzola, S., Buscaino, G. (2014). Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. Mar. Poll.Bull. 84,104–114

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Arizza, V., de Vincenzi, G., Grammauta, R., Mazzola, S., Buscaino, G. (2016). Underwater noise from boats: Measurement of its influence on the behaviour and biochemistry of the common prawn (*Palaemon serratus*, Pennant 1777). J. Exp. Mar. Biol. Ecol. 478,24–33

Filiciotto, F., Cecchini, S., Buscaino, G., Maccarrone, V., Piccione, G., Fazio, F. (2017). Impact of aquatic acoustic noise on oxidative status and some immune parameters in gilthead sea bream *Sparus aurata* (Linnaeus, 1758) juveniles. Aquaculture Res. 48, 1895–1903.

Filiciotto, F., Moyano, M.P.S., de Vincenzi, G., Hidalgo, F., Sciacca, V., Bazterrica, M.C., Corrias, V., Lorusso, M., Mazzola, S., Buscaino, G., Gavio, M.A. (2018). Are semi-terrestrial crabs threatened by human noise? Assessment of behavioural and biochemical responses of *Neohelice granulata* (Brachyura, Varunidae) in tank. Mar. Poll. Bull. 137, 24–34

Fitzgibbon, Q.P., Daya, R.D., McCauley, R.D., Simona, C.J., Semmensa, J.M. (2017). The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition of spiny lobster, *Jasus edwardsii*. Mar. Poll. Bull.

Folkersen, M.V., Fleming, C.M., Hasan, S. (2018a) Depths of uncertainty for deep-sea policy and legislation. Global Environ. Change 54,1–5

Folkersen, M.V., Fleming, C.M., Hasanc, S. (2018b). Deep sea mining's future effects on Fiji's tourism industry: A contingent behaviour study. Mar. Policy 96 81–89

Fonoberova, M., Fonoberov, V.A., Mezic´, I. (2013). Global sensitivity/uncertainty analysis for agent-based models. Reliability Engin. Syst.Saf.118, 8–17.

Forget, J., Bocquene, G. (1999). Partial purification and enzymatic characterisation of acetylcholinesterase from the intertidal marine copepod *Tigriopus brevicorni*. Comp. Biochem. Physiol. 123B, 345–350.

Forget, J., Beliaeff, B., Bocquene´, G. (2003). Acetylcholinesterase activity in copepods (*Tigriopus brevicornis*) from the Vilaine River estuary, France, as a biomarker of neurotoxic contaminants. Aquat. Toxicol. 62:195–204

Fossati, C., Mussib, B., Tizzib, R., Pavana, G., Pace, D.S. (2017). Italy introduces pre and post operation monitoring phases for offshore seismic exploration activities. Mar. Poll. Bull. 120, 376–378

Fouquet, Y., Cambon, P., Etoubleau, J., Charlou, J.L., Ondréas, H., Barriga, F.J.A.S., Cherkashov, G., Semkova, T., Poroshina, I., Bohn, M., Donval, J.P., Henry, K., Murphy, P., Rouxel, O. (2010).Geodiversity of hydrothermal processes along the Mid-Atlantic Ridge and ultramafic-hosted mineralization: a new type of oceanic Cu-Zn-Co-Au volcanogenic massive sulfide deposit, Am. Geophys. Union Geophys. Monogr. Series. 188,321–367,

Fouquet, Y., Lacroix, D. (2014). Deep Marine Mineral Resources, Springer, Heidelberg, 2014.

Franceschi, C., Cossarizza, A., Ortolani, C., Monti, D., Ottaviani, E. (1991). Natural cytotoxicity in a freshwater polmonate mollusc: an unotorthodox comparative approach. Advances in Neuroimmunology 1, 99–113.

Francis, C. D., & Barber, J. R. (2013). A framework for understanding noise impacts on wildlife: An urgent conservation priority. Frontiers in Ecol. Environ. 11(6), 305–313.

Franco, L., Romero, D., García-Navarro, J.A., Teles, M., Tvarijonaviciute, A. (2016). Esterase activity (EA), total oxidant status (TOS) and total antioxidant capacity (TAC) in gills of *Mytilus galloprovincialis* exposed to pollutants:

Analytical validation and effects evaluation by single and mixed heavy metal exposure. Mar. Pollut. Bull.102, 30-35.

Franco-Martinez, L., Romero, D., García-Navarro, J.A., Tecles, F., Teles, M., Tvarijonaviciute, A. (2016). Measurement of p-nitrophenyl acetate esterase activity (EA), total antioxidant capacity (TAC), total oxidant status (TOS) and acetylcholinesterase (AChE) in gills and digestive gland of *Mytilus galloprovincialis* exposed to binary mixtures of Pb, Cd and Cu. Environ. Sci. Pollut. Res. 23, 25385–25392

Fridovich. I. (1986). Biological effects of the superoxide radical. Arch. Biochem. Biophys., 247, pp. 1-11

Galkin, S.V. (1997). Megafauna associated with hydrothermal vents in the Manus back-arc basin (Bismarck Sea). Mar. Geol. 142, 197– 206.

Galloway, T.S., Millward, N., Browne, M.A., Depledge, M.H. (2002). Rapid assessment of organophosphorous/carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. Aquat. Toxicol. 61, 169– 180

Garrigue, C., Clapham, P. J., Geyer, Y., Kennedy, A. S., and Zerbini, A. N. (2015). Satellite tracking reveals novel migratory patterns and the importance of seamounts for endangered South Pacific humpback whales. R. Soc. Open Sci. 2:150489.

Gausland, I. (2003). Seismic Surveys Impact on Fish and Fisheries (Statoil 41 pp.).

Geracitano, L., Montserrat, J.M., Bianchini, A. (2002). Physiological and antioxidant enzyme responses to acute and chronic exposure of *Laeonereis acuta* (Polychaeta, Nereididae) to copper. J. Exp. Mar. Biol. Ecol. 277, 145 – 156.

Geret, F., Serafim, A., Barreira, L., Bebianno, M.J. (2002). Response of antioxidant systems to copper in the gills of the clam *Ruditapes decussatus*. Mar. Environ. Res. 54, 413 – 417.

Gerlotto, F., Fréon, P. (1992). Some elements on vertical avoidance of fish schools to a vessel during acoustic surveys. Fish. Res. 14, 251–259.

Gillard, B., Purkiani, K., Chatzievangelou, D., Vink, A., Iversen, M.H., Thomsen, L. (2019). Physical and hydrodynamic properties of deep sea mining-generated, abyssal sediment plumes in the Clarion Clipperton Fracture Zone (eastern-central Pacific). Elem. Sci. Anth. 7: 5.

Ginot, V., Gaba, S., Beaudouin, R., Aries, F., Monod, H. (2006). Combined use of local and ANOVA-based global sensitivity analyses for the investigation of a stochastic dynamic model: application to the case study of an individualbased model of a fish population. Ecol.Modell.193, 479–491

Giron-Perez, M.I. (2010). Relationships between innate immunity in bivalve molluscs and environmental pollution. Invert. Surv. J. 7, 149-156.

Gjerde, K.M. (2006). Ecosystems and Biodiversity in Deep Waters and High Seas. UNEP/Earthprint.

Glasby, G.P., Wright, I.C. (1990). Marine mineral potential in New Zealand’s exclusive economic zone. Mar. Min. 9, 403– 427.

Glasby, G.P. (2000). ECONOMIC GEOLOGY: Lessons Learned from Deep-Sea Mining". Science. 289(5479): 551–3.

Gleason, W.M. (2008). Companies turning to seafloor in advance of next great metals rush. Min. Engg. 14–16.

Glover, A.G., Smith, C.R., Paterson, G.L.J., Wilson, G.D.F., Hawkins, L., Sheader, M. (2002) Polychaete species diversity in the central Pacific abyss: local and regional patterns, and relationships with productivity. Mar.Ecol. Prog. Ser. 240:157–69

Goffredi, S. K., Johnson, S., Tunnicliffe, V., Caress, D., Clague, D., Escobar, E., Lundsten, L., Paduan, J.B., Rouse, G., Salcedo, D.L., Soto, L.A., Spelz-Madero, R., Zierenberg, R., Vrijenhoek, R. (2017). Hydrothermal ventfields discovered in the southern Gulf of California clarify role of habitat in augmenting regional diversity. Proc. R. Soc. B Biol. Sci. 284:20170817.

Gollner, S., Kaiser, S., Menzel, L., Jones, D.O.B., Brown, A., Mestre, N.C., van Oevelen, D., Menot, L., Colaço, A., Canals, M., Cuvelier, D., Durden, J.M., Gebruk, A., Egho, G.A., Haeckel, M., Marcon, Y.,Mevenkamp, L., Morato, T., Pham, C.K., Purser, A.,Sanchez-Vidal, A., Vanreusel, A., Vink, A., Arbizu, P.M. (2017). Resilience of benthic deep-sea fauna to mining activities. Mar.Envir.Res.

Goodall, C., Chapman, C., Neil, D., Tautz, J., Reichert, H. (1990). The acoustic response threshold of the Norway lobster, *Nephrops norvegicus,* in a free sound field. In: Wiese, K., W.D., K., Mulloney, B. (Eds.), Frontiers Crust.Neurob. Birkhauser, Basel, pp. 106–113

Good Practice Guide No.133. Underwater Noise Measurement. National Physical Laboratory. (2014)

Gordon, J., Gillespie, D., Potter, J., Frantzis, A., Simmonds, M.P., Swift, R., Thompson, D. (2003). A review of the effects of seismic surveys on marine mammals. Mar. Technol. Soc. J. 37, 16–34.

Gordon, Caitlin. (2018). "Anthropogenic Noise and Cetacean Interactions in the 21st Century: A Contemporary Review of the Impacts of Environmental Noise Pollution on Cetacean Ecologies". University Honors Theses. Paper 625

Goswami, P., Hariharan, G., Godhantaraman, N., Munuswamy, N. (2014). An integrated use of multiple biomarkers to investigate the individual and combined effect of copper and cadmium on the marine green mussel (Perna viridis), J. Environ. Sci. Health A.49:13, 1564-1577.

Guest, T.W., Blaylock, R.B. (2016). Andrew N. Evans. Development of a modified cortisol extraction procedure for intermediately sized fish not amenable to whole-body or plasma extraction methods. Fish Physiol Biochem, 42:1–6

Graham, A.L., and Cooke, S.J. (2008). The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*). Aquatic Conserv. Mar. Freshw. Ecosyst. 18(7): 1315-1324.

Grigoriev, A.G., Zhamoida, V.A., Gruzdov, K.A., Krymsky, R.S. (2013). Age and growth rates of ferromanganese concretions from the Gulf of Finland derived from 210Pb measurements. Oceanology 53, 345–351.

Gronquist, D., Berges, J.A. (2013). Effects of aquarium-related stressors on the zebrafish: a comparison of behavioral, physiological, and biochemical indicators. J. Aquat. Anim. Health 25:53–65.

Gross, P.S., Al-Sharif, W.Z., Clow, L.A., Smith, L.C. (1999). Echinoderm immunity and the evolution of the complement system. Dev. Comp. Immunol. 23, 429–442.

Gross, P.S., Clow, L.A., Smith, L.C. (2000). SpC3, the complement homologue from the purple sea urchin, *Strongylocentrotus purpuratus*, is expressed in two subpopulations of the phagocytic coelomocytes. Immunogenetics 51, 1034–1044

Grutter, A.S., Pankhurst, N.W. (2000). The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. J. Fish Biol. 57, Issue2.

Guardiola, F.A., Cuesta, A., Arizcun, M., Meseguer, J., Esteban, M.A. (2014). Comparative skin mucus and serum humoral defence mechanisms in the teleost gilthead seabream (*Sparus aurata*). Fish Shell. Immun. 36, (2), 545-551.

Guerra, Á., González, Á.F., and Rocha, F. (2004). A review of records of giant squid in the Northeastern Atlantic and severe injuries in Architeuthis dux stranded after acoustic exploration. ICES CM 2004/CC 29: 1–17.

Gutteridge, J.M., Halliwell, B. (2010). Free radicals and antioxidants in the year 2000. A historical look to the future, Ann. Ny. Acad. Sci. 899, 136–147.

Gwyther, D. (2008). Environmental Impact Statement, Solwara 1 Project. Nautilus Minerals Niugini Limited, Main Rep. Coffey Na. Syst. Brisbane.

Halbach, P and Fellerer, R. (1980). The metallic minerals of the Pacific Seafloor. GeoJ. 4(5): 407–421.

Halbach, P., Kriete, C., Prause, B., Puteanus, D. (1989). Mechanisms to explain the pla-tinum concentration in ferromanganese seamount crusts, Chem. Geol. 76 ,95–106

Halfwerk, W., Slabbekoorn, H. (2015). Pollution going multimodal: the complex impact of the humanaltered sensory environment on animal perception and performance. Biol. Lett. 11, 20141051.

Hall, M., Wang, R., van Antwerpen, R., Sottrup-Jensen, L., Soderhall, K. (1999). The crayfish plasma clotting protein: a vitellogenin-related protein responsible for clot formation in crustacean blood. Proc. Natl. Acad. Sci. U. S. A. 96, 1965–1970.

Halliwell, B., Gutteridge, J.M.C. (1999). Free Radicals in Biology and Medicine. Oxford University Press, Oxford

Halvorsen, M.B., Casper, B.M., Woodley, C.M., Carlson, T.J., Popper, A.N. (2011). Predicting and mitigating hydroacoustic impacts on fi sh from pile installations. NCHRP Res Results Digest 363, Project 25–28, National Cooperative Highway Research Program, Transportation Research Board, National Academy of Sciences, Washington, D.C. http://www.trb.org/Publications/ Blurbs/166159.aspx .

Halvorsen, M.B, Casper, M.C, Woodley, C.M, Carlson, T.J, Popper, A.N. (2012a). Threshold for Onset of Injury in *Chinook Salmon* from Exposure to Impulsive Pile Driving Sounds. 7(6), e38968. PloseOne.

Halvorsen, M.B., Casper, B.M., Matthews, F., Carlson, T.J., Popper, A.N. (2012b). Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker. Proc. R. Soc. B 279, 4705 – 4714.

Halvorsen, M.B., Zeddies, D.G., Ellison, W.T., Chicoine, D.R., Popper, A.N. (2012c). Effects of midfrequency active sonar on fish hearing. J. Acoust. Soc. Am. 131,599–607

Halvorsen, M.B., Zeddies, D.G., Chicoine, D., Popper, A.N. (2013). Effects of low-frequency naval sonar exposure on three species of fish. J. Acoust. Soc. Am. 134, EL205–EL210

Hamer, B., Hamer, D.P., Müller, W.E., Batel, R. (2004). Stress-70 proteins in marine mussel *Mytilus galloprovincialis* as biomarkers of environmental pollution: a field study. Environ. Int. 30, 873–882.

Hamilton, W.D. (1971) Geometry for the selfish herd. J. Theor. Biol. 31, 295– 311.

Handegard, N.O., Michalsen, K., Tjøstheim, D. (2003). Avoidance behaviour in cod (*Gadus morhua*) to a bottom-trawling vessel. Aquat. Living Resour. 16, 265–270.

Hannam, M.L., Hagger, J.A., Jones, M.B., Galloway, T.S. (2008). Characterisation of esterases as potential biomarkers of pesticide exposure in the lugworm *Arenicola marina* (Annelida: Polychaeta). Environ. Pollut. 152, 342e350

Hannington, M., Monecke, T. (2009). Global exploration models for polymetallic sul-phides in the Area: an assessment of lease block selection under the draft regulations on prospecting and exploration for polymetallic sulphides, Mar. Georesour. Geotechnol. 27, 132–159.

Hannington, M., Jamieson, J., Monecke, T., Petersen, S., Beaulieu, S. (2011). The abundance of seafloor massive sulfide deposits. Geology 39 (12), 1155–1158.

Hannington, M.D., Jamieson, J., Monecke, T., Petersen, S. (2010). Modern sea-floor massive sulfides and base metal resources: toward an estimate of global sea-floor massive sulfide potential, Econ. Geol. Spec. Publ. 15,317–338.

Hannington, M., Petersen, S., Krätschell, A. (2017). Subsea mining moves closer to shore. Nat. Geosci. 10 (3), 158

Haque, M.N., Eom, H.J., Nam, S.E., Shin, Y.K., Rhee, J.S. (2019). Chlorothalonil induces oxidative stress and reduces enzymatic activities of Na^+/K^+-ATPase and acetylcholinesterase in gill tissues of marine bivalves. PLoS ONE 14(4), e0214236.

Harrington, J.J., McAllister, J., Semmens, J.M. (2010). Assessing the Short-Term Impact of Seismic Surveys on Adult Commercial Scallops (*Pecten fumatus*) in Bass Strait. Tas. Aquac. Fish. Inst.University of Tasmania

Harrison, A. J. and Walker, A. M. and Pinder, A. C. and Briand, C. and Aprahamian, M. W. (2014). A review of glass eel migratory behaviour, sampling techniques and abundance estimates in estuaries: implications for assessing recruitment, local production and exploitation. Reviews Fish Biol. Fish. 24, 967-983

Hastings, M.C., and Popper, A.N. (1996). Effects of low-frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus*. J. Acoust. Soc. Am. 99(3): 1759-1766. PubMed: 8819864

Hastings, M.C., Popper, A.N. (2005). Effects of sound on fish. In: Stokes, S.t.J. (Ed.). California Department of Transportation California Department of Transportation Sacramento, CA, p. 82.

Hassel, A., Knutsen, T., Dalen, J., Skaar, K., Løkkeborg, S., Misund, O.A., Østensen, Ø., Fonn, M., Haugland, E.K. (2004). Influence of seismic shooting on the lesser sandeel (*Ammodytes marinus*). ICES J. Mar. Sci. 61, 1165–1173

Hawkins, A., Amorim, M. (2000). Spawning sounds of the male haddock, *Melanogrammus aeglefinus*. Environ. Biol. Fishes 59, 29e41.

Hawkins, A., Knudsen, F.R., Davenport, J., McAllen, R., Bloomfield, H.J., Schilt, C., Johnson, P. (2012). Grazing by sprat schools upon zooplankton within an enclosed marine lake. J. Exp. Mar. Biol. Ecol. 411, 59e65.

Hawkins, A.D., Roberts, L., Cheesman, S. (2014). Responses of free-living coastal pelagic fish to impulsive sounds. J. Acoust. Soc. Am. 135, 3101e3116.

Hawkins, A.D., Pembroke, A.E., Popper, A.N. (2015). Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish. 25, 39–64

Hawkins, A.D., Popper, A.N. (2017). A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. 74, 635e651.

Hatch, L., Clark, C., Merrick, R., Van Parijs, S., Ponirakis, D., Schwehr, K., Thompson, M., Wiley, D. (2008). Characterizing the relative contributions of large vessels to total ocean noise fields: a case study using the Gerry E. Studds Stellwagen Bank National Marine Sanctuary. Environ. Manag. 42, 735–752.

Hawa, I., Zulaikah, M., Jamaludin, M., Zainal Abidin, A.A., Kaswandi, M.A., Ridzwan, B.H. (1999). The potential of the coelomic fluid in sea cucumber as an antioxidant. Mal. J. Nutr. 5,55-59.

Heath, A.G. (1990). Summary and perspectives. Am Fish Soc Symp 8:183–191.

Heffernan, O. (2019). Deep-Sea Dilemma Mining the ocean floor could solve mineral shortages — and lead to epic extinctions in some of the most remoteecosystems on Earth. Nature. Vol.571.

Hein, J.R. (2008). Geologic characteristics and geographic distribution of potential cobalt-rich ferromanganese crusts deposits in the Area. In: Mining cobalt-rich ferromanganese crusts and polymetallic sulfides deposits: Technological and economic considerations, in: Proceedings of the International Seabed Authority's Workshop Kingston, Jamaica (31 July–4 August 2006) pp. 59– 90.

Hein, J.R., Mizell, K., Koschinsky, A., Conrad, T.A. (2013). Deep-ocean mineral deposits as a source of critical metals for high-and green-technology applications: comparison with land-based resources. Ore Geol. Rev. 51, 1–14.

Hein, J.R., Koschinsky, A. (2014). Deep-ocean ferromanganese crusts and nodules, Treatise Geochem. 2, 273–291.

Hein, J.R., Spinardi, F., Okamoto, N., Mizell, K., Thorburn, D., Tawake, A. (2015). Critical metals in manganese nodules from the Cook Islands EEZ, abundances and distributions, Ore Geol. Rev. 68,97–116,

Hennig, O., Itami, T., Maeda, M., Kondo, M., Natsukari, Y. and Takahashi, Y. (1998). Analyses of hemolymph immunoparameters in kuruma shrimp infected with penaeid rod-shaped DNA virus. Fish Pathol. 33, 389-393.

Herbert, R.J.H., Willis, J., Jones, E., Ross, K., Hubner, R., Humphreys, J., Jensen, A., Baugh, J. (2012). Invasion in tidal zones on complex coastlines: modelling larvae of the non-native Manila clam, *Ruditapes philippinarum*, in the UK. J. Biogeography 39, 585–599.

Herbert-Read, J.E., Kremer, L., Bruintjes, R., Radford, A.N., Ioannou, C.C. (2017) Anthropogenic noise pollution from pile-driving disrupts the structure and dynamics of fish shoals. Proc. R. Soc. B 284, 20171627. h

Hermannsen, L., Tougaard, J., Beedholm, K., Nabe-Nielsen, J., Madsen, P.T. (2015). Characteristics and Propagation of Airgun Pulses in Shallow Water with Implications for Effects on Small Marine Mammals. PLoSONE 10(7): e0133436.

Hildebrand, J.A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5e20.

Hine, P.M., Wesney, B. (1994). Interaction of phagocytosed Bonamia sp. (Haplosporidia) with haemocytes of oysters *Triostrea chilensis*. Dis. Aquat. Org. 20, 219–229.

Hirst, A.G., Rodhouse, P.G. (2000). Impacts of geophysical seismic surveying on fishing success. Rev. Fish Biol. Fish. 10, 113–118.

Hoekstra, K. A., Iwama, G. K., Nichols, C. R., Godin, D. V. and Cheng, K. M. (1998). Increased heat shock protein expression after stress in Japanese quail. Stress 2, 265-272.

Holles, S., Simpson, S.D., Radford, A.N., Berten, L., Lecchini, D. (2013) Boat noise disrupts orientation behaviour in a coral reef fish. Mar. Ecol. Prog. Ser. 485, 295– 300.

Holling, C.S. (1978). Adaptive Environmental Assessment and Management, John Wiley & Sons, Chichester, UK.

Holmes, L.J., McWilliam, J., Ferraric, M.C.O., McCormick, M.I. (2017). Juvenile damselfish are affected but desensitize to small motor boat noise. J. Exp. Mar. Biol. Eco. 494, 63–68

Holt, D.E., Johnston, C.E. (2015). Traffic noise masks acoustic signals of freshwater stream fish. Biol. Conserv. 187, 27e33.

Hoshino, T. (2015). Innovative lithium recovery technique from seawater by using world-first dialysis with a lithium ionic superconductor. Desalination 359, 59–63.

Hosoya, S., Johnson, S.C., Iwama, G.K., Gamperl. A.K.,Afonso, L.O.B.(2007). Changes in free and total plasma cortisol levels in juvenile haddock (*Melanogrammus aeglefinus*) exposed to long-term handling stress. Comp. Bioch. Phys, A: Mol. & Inte. Phys, 146 (1), 78-86.

Hsiao, S.C., Fujii, W.K. (1963). Early ontogenic changes in the concentration of alkaline phosphatase in Hawaiian sea urchins. Exp. Cell Res.. 32(2),217-231

Hu, M.,Li, L.,Sui, Y.,Li, J.,Wang, Y., Lu, W.,Dupont, S. (2015). Effect of pH and temperature on antioxidant responses of the thick shell mussel *Mytilus coruscus.* Fish Shell. Immun. 46(2),573-583

Hubert, F., Cooper, E.L., Roch, P.(1997). Structure and differential target sensitivity of the stimulable cytotoxic complex from hemolymph of the Mediterranean mussel *Mytilus galloprovincialis*. Biochimica et Biophysica Acta 1361, 29–41.

Hubert, J., Campbell, J., van der Beek, J.G., den Haan, M.F., Verhave, R., Verkade, L.S., Slabbekoorn, H. (2018). Effects of broadband sound exposure on the interaction between foraging crab and shrimp-A field study. Envir. Poll. 243, 1923e1929

Humphris, S.E., Herzig, P.M., Miller, D.J, Alt, J.C., Becker, K. , Brown, D., Brügmann, G., Chiba, H., Fouquet, Y., Gemmell, J.B., Guerin, G. , Hannington, M.D., Holm, N.G., Honnorez, J.J. , Iturrino, G.J., Knott, R., Ludwig, R., Nakamura, K., Petersen, S., Reysenbach, A-L., Rona, P.A., Smith, S., Sturz, A.A., Tivey, M.K., Zhao, X. (1995). The internal structure of an active sea-floor massive sulfide deposit, Nature 377,713–716,

Hyne, R.V., and Maher, W.A. (2003). Invertebrate biomarkers: links to toxicosis that predict population decline. Ecotoxicology and Environmental Safety 54,366–374

International Marine Minerals Society. (2011). Code for Envir.Manag. Mar. Min. http://www.immsoc.org/IMMS_downloads/ 2011_SEPT_16_IMMS_Code.pdf.

International Seabed Authority. (2010a.) A Geological Model of Polymetallic Nodule Deposits in the Clarion Clipperton Fracture Zone, International Seabed Authority, Technical Study No. 6.

ISA. (2010b). International seabed Authority. Decision of the Assembly of the International Seabed Authority relating to the regulations on prospecting and exploration for polymetallic sulphides in the Area, ISBA/16/A/12/Rev.1, International Seabed Authority. (2010c). Regulations on prospecting and exploration for polymetallic sulphides in the area. In: International Seabed Authority (ed) ISBA/16/A/12/Rev.1. International Seabed Authority, Kingston, Jamaica, p. 49.

International Seabed Authority. (2013). Regulations on Prospecting and Exploration for Cobalt-rich Ferromanganese Crusts in the Area, ISBA/18/A/11 (27 July 2012), amended by ISBA/19/A/12 (25 July 2013) (Crusts Exploration Regulations)

International Seabed Authority. (2013b). Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area, ISBA/19/LTC/8, Kingston, Jamaica.

International Seabed Authority. (2014). Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area, ISBA/6/A/18 (13 July 2000), amended by ISBA/ 19/A/9; ISBA/19/A/12 (25 July 2013) and ISBA/20/A/9 (24 July 2014) (Nodules Exploration Regulations).

International Seabed Authority. (2011b) Environmental Management Needs for Exploration and Exploitation of Deep Sea Minerals. ISA Technical Study: No. 10. Nadi, Fiji

International Seabed Authority. (2011). Advisory Opinion of the Seabed Disputes Chamber on the responsibilities and obligations of States sponsoring persons and entities with respect to activites in the Area, ISBA/17/C/6.

Ioannou, C.C. (2017). Swarm intelligence in fish? The difficulty in demonstrating distributed and selforganised collective intelligence in (some) animal groups. Behav. Process. 141, 141– 151.

ISA. (2013a). Relating to an Application for the Approval of a Plan of Work for Exploration for Cobalt-rich Ferromanganese Crusts by China Ocean Mineral Resources Research and Development Association. International Seabed Authority. ISBA/19/C/n 2. Available at: http://www.isa.org.jm/files/documents/EN/19Sess/Council/ISBA-19C-2.pdf (Accessed February 06 2017).

ISA. (2013c). Report and Recommendations of the Legal and Technical Commission to the Council of the International Seabed Authority Relating to an Application for the Approval of a Plan of Work for Exploration for Cobalt-rich Ferromanganese Crusts by Japan Oil, Gas and Metals National Corporation. International Seabed Authority. ISBA/19/C/3. http://www.isa.org.jm/files/documents/EN/ 19Sess/Council/ISBA-19C-3.pdf (Accessed February 06 2017).

ISA. (2014). Status of Contracts for Exploration for Polymetallic Nodules, Polymetallic Sulphides and Cobalt-rich Ferromanganese Crusts. International Seabed Authority, Kingston, Jamaica.

http://www.isa.org.jm/en/scientific/exploration/contractors (Accessed February 06 2017).

ISA. (2018) Design of IRZs and PRZs in Deep-Sea Mining Contract Areas, International Seabed Authority, Kingston, Jamaica. pp. 1–8 Briefing Paper 02/2018.

Israeli-Weinstein, D., Kimmel, E. (1998). Behavioral response of carp (*Cyprinus carpio*) to ammonia stress. Aquaculture 81e93.

IUCN. (2006). Report of the interim independent scientists group (IISG) on mitigation measures to protect Western gray whales during Sakhalin II construction operations in 2006. Workshop convened by the IUCN, Vancouver, British Columbia. 3–5

Iwama, G.K. (1998). Stress in fish. Stress Life 851, 304e310.

Jaeckel, A. (2015). An environmental management strategy for the international seabed authority? The legal basis, Int. J. Mar. Coast. Law 30 1–27

Jaeckel, A., Gjerde, K.M., Ardron, J.A. (2017a). Conserving the common heritage of humankind – options for the deep seabed mining regime, Mar. Policy 78,150–157

Jaeckel, A.L. (2017b). The International Seabed Authority and the Precautionary Principle: Balancing Deep Seabed Mineral Mining and Marine Environmental Protection, Brill.

Jaeckel, A. (2019). Strategic environmental planning for deep seabed mining in the area. Mar. Policy.

Jain-Schlaepfer, S., Fakan, E., Rummer, J.L., Simpson, S.D., McCormick, M.I. (2018). Impact of motorboats on fish embryos depends on engine type. Conser. Phys. 6.

Jamieson, J.W., Clague, D.A., Hannington, M.D. (2014). Hydrothermal sulfide accumulation along the Endeavour Segment, Juan de Fuca Ridge, Earth Planet. Sci. Lett. 395,136–148.

Jankowski, P. (2012). NI43-101 Technical Report 2011: PNG, Tonga, Fiji, Solomon Islands, New Zealand, Vanuatu and the ISA (No. NAT008), Nautilus Minerals Inc., Canada, 2012.

Japan Times. (2017). Japan successfully Undertakes Large-scale Deep-sea Mineral Extraction. https://www.japantimes.co.jp/news/2017/09/ 26/national/japan-successfully-undertakes-large-scale-deep-sea-mineral-extraction/#.WddI9ltSyUn.(Accessed October 06 2017).

Jasny, M., Reynolds, J, Horowitz, C., Wetzler, A. (2005). Sounding the depths II: the rising toll of sonar, shipping and industrial ocean noise on marine life. Natural Resources Defense Council.

Jeffs, A., Tolimieri, N., Montgomery, J.C. (2003). Crabs on cue for the coast: the use of underwater sound for orientation by pelagic crab stages. Mar. Freshw. Res. 54, 841–845

Jenkins, C.N., Joppa, L. (2009). Expansion of the global terrestrial protected area system. Biol. Conserv. 142, 2166–2174.

Jiang, J., Zhou, Z., Dong, Y., Gao, S., Sun, H., Chen, Z., Yang, A., Su, H. (2017). Comparative analysis of immunocompetence between females and males in the sea cucumber *Apostichopus japonicus.* Fish Shell. Immun., 63, 438-443

Jing, G., Li, L.Y., Li, Y., Xie, L.P., Zhang, R.Q. (2006a). Purification and partial characterization of two acid phosphatase forms from pearl oyster (*Pinctada fucata*). Comp. Biochem. Phys. B. 143,229–235

Jing, G., Li, Y., Xie, L., Zhang, R. (2006b). Metal accumulation and enzyme activities in gills and digestive gland of pearl oyster (*Pinctada fucata*) exposed to copper. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 144,184–190.

Joint Nature Conservation Committee. Guidelines for minimising acoustic disturbing. (2004)

Jones, D.O.B., Kaiser, S., Sweetman, A.K., Smith, C.R., Menot, L., Vink, A., Trueblood, D., Greinert, J., Billett, D.S.M., Arbizu, P.M., Radziejewska, T., Singh, R., Ingole, B., Stratmann, T., Simon-Lledó, E., Durden, J.M., Clark, M.R. (2017) Biological responses to disturbance from simulated deep-sea polymetallic nodule mining. PLoS ONE 12(2): e0171750.

Jones, D.O.B., Durden, J.M., Murphy, K., Gjerde, K.M., Gebicka, A., Colaco, A., Morato, T., Cuvelier, D., Billet, D.S.M. (2019). Existing environmental management approaches relevant to deep-sea mining. Mar. Policy.

Juanes, F., Cox. K., Brenna. L. (2017). The effect of anthropogenic and biological noise on fish behavior and physiology: A meta-analysis. J. Acous.Soc. America 141, 3862

Juliani, C., Ellefmo, S.L. (2018). Resource Assessment of Undiscovered Seafloor Massive Sulfide Deposits on an Arctic Mid-Ocean Ridge: Application of grade and tonnage models, Ore Geology Reviews

Jumars, P.A. (1981). Limits to predicting and detecting benthic community responses to manganese nodule mining. Mar. Mining. 3,213–29.

Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, B. and Evans, H. (1997). Total and differential haemocytes count in western rock lobsters (*Panulirus cygnus* George) under post harvest stress. Mar. Freshw. Res. 48, 863-867.

Kaikkonen, L., Venesjärvi, R., Nygård, H., Kuikka, S. (2018). Rewiev: Assessing the impacts of seabed mineral extraction in the deep sea and coastal marine environments: Current methods and recommendations for environmental risk assessment. Mar. Poll. Bull.135, 1183–1197

Kaifu, K., Segawa, S., Tsuchiya, K. (2007). Behavioral responses to underwater sound in the small benthic Octopus *Octopus ocellatus*. J. Marine Acoust.Soc. Jpn. 34(4).

Kaiser, S, Smith, C.R., Arbizu, P.M. (2017). Editorial: Biodiversity of the Clarion Clipperton Fracture Zone. Mar Biodivers 47(2), 259–264.DOI:

Kane, A.S., Song., J., Halvorsen, M.B., Miller, D.L., Salierno, J.D., Wysocki, L.E., Zeddies, D., Popper, A.N. (2010). Exposure of fish to high intensity sonar does not induce acute pathology. J. Fish Biol. 76,1825-1840.

Kastelein, R.A., van der Heul, S., Verboom, W.C., Jennings, N., van der Veen, J., de Haan, D. (2008). Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. Mar. Environ. Res. 65, 369–377.

Kennedy, E.V., Holderied, M.W., Mair, J.M., Guzman, H.M., Simpson, S.D. (2010). Spatial patterns in reef-generated noise relate to habitats and communities: Evidence from a Panamanian case study. J. Exp. Mar. Biol. Ecol. 395, 85-92.

Kerstena, O., Smith, C.R., Vetter, E.W. (2017). Abyssal near-bottom dispersal stages of benthic invertebrates in the Clarion Clipperton polymetallic nodule province. Deep- Sea Research Part I 127, 31-40.

Kight, C.R., Swaddle, J.P. (2011). How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol. Lett. 14, 1052–1061.

Kim, R.E. (2017). Should deep seabed mining be allowed? Mar. Policy 82,134–137

Kojima, T., Suga, T., Kusano, A., Shimizu, S., Matsumoto, H., Aoki, S., Takai, N., Taniuchi, T. (2010). Acoustic pressure sensitivities and effects of particle motion in red sea bream *Pagrus major.* Fish Sci. 76,13–20

Kolasinski, J., Taddei, D., Cuet, P., Frouin, P. (2010). AChE and EROD activities in two echinoderms, *Holothuria leucospilota* and *Holoturia atra* (Holothuroidea), in a coral reef (Reunion Island, South-western Indian Ocean). J. Environ. Sci. Health. A. Tox Hazard Subst. Environ. 45,699–708

Koolhaas, J.M., Bartolomucci, a, Buwalda, B., de Boer, S.F., Flügge, G., Korte, S.M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Wöhr, M., Fuchs, E. (2011). Stress revisited: a critical evaluation of the stress concept. Neurosci. Biobehav. Rev. 35, 1291e1301

Kopecka-Pilarczyk, J. (2009). In vitro effects of pesticides and metals on the activity of acetylcholinesterase (AChE) from different tissues of the blue mussel, *Mytilus trossulus* L., J. Envir. Sci. Health, B: Pest. Food, Conts, Agric.Wastes, 45(1), 46-52

Koslow, J. A., Gowlett-Holmes, K., Lowry, J. K., O'Hara, T., Poore, G. C. B., Williams, A. (2001). Seamount benthic macrofauna off southern Tasmania: community structure and impacts of trawling. Mar. Ecol. Prog. Ser. 213, 111–125.

Kostyuchenko, L. (1973). Effects of elastic waves generated in marine seismic prospecting on fish eggs in the Black Sea. Hydrobiol. J. 9, 45–48.

Koschinsky, A., Heinrich, L., Boehnke, K., Cohrs, J.C., Markus, T., Shani, M., Singh, P., Stegen, K.S., Werner, W. (2018). Deep-sea Mining: Interdisciplinary Research on Potential Environmental, Legal, Economic, and Societal Implications. Critical Review. Int.Envir. Ass. Manag.t —14 (6), pp. 672–691

Krahforst, C.S. (2017). Impact of vessel noise on oyster toadfish (*Opsanus tau*) behavior and implications for underwater noise management. Ph.D. thesis, East Carolina University

Krasnov, S.G., Cherkashev, G.A., Stepanova, T.V., Batuyev, B.N., Krotov, A.G., Malin, B.V., Maslov, M.N., Markov, V.F., Poroshina, I.M., Samovarov, M.S., Ashadze, A.M., Lazareva, L.I., Ermolayev, I.K. (1995). Detailed Geological Studies of Hydrothermal Fields in the North Atlantic, 87. Geological Soc., London, pp. 43e64. Special Publications.

Kudriavtsev, I.V., Polevshchikov, A.V. (2004). Comparative immunological analysis of echinoderm cellular and humoral defense factors. Zh. Obshch. Biol. 65, 218–231.

Kunc, H.P., McLaughlin, K.E., Schmidt, R. (2016). Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proc. R. Soc. B Biol. Sci. 283, 20160839.

Kuşku, H., Yiğit, M., Ergün, S., Yiğit, Ü., Taylor, N. (2018). Acoustic Noise Pollution from Marine Industrial Activities: Exposure and Impacts.Aqu. Res. 1(4), 148-161.

Kusku, H., Ergün, S., Yilmaz, S., Güroy, B., Yigit, M. (2018). Impacts of Urban Noise and Musical Stimuli on Growth Performance and Feed Utilization of Koi fish (*Cyprinus carpio*) in Recirculating Water Condi-tions. Turkish Journal of Fisheries and Aquatic Sci-ences, 19(6).

Kuwada, H., Masuda, R., Shiozawa, S., Kogane, T., Imaizumi, K., Tsukamoto, K. (2000). Effect of fish size, handling stresses and training procedure on the swimming behavior of hatchery-reared striped jack: implications for stock enhancement. Aquaculture 185, 245e256.

La Bella, G., Cannata, S., Froglia, C., Ratti, S., Rivas, G. (1996). First assessment of effects of air-gun seismic shooting on marine resources in the central Adriatic Sea. Intern.Conf. Health, Safety Environ. Oil Gas Exp. Prod., pp. 227–238.

Ladich, F., Fay, R. (2013). Auditory evoked potential audiometry in fish. Rev. Fish Biol. Fish. 23, 317–364.

Ladich, F., Schulz-Mirbach, T. (2013b). Hearing in Cichlid Fishes under Noise Conditions. Plos One 8(2) -e57588.

Lagardère, J.P. (1982). Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. Mar. Biol. 71, 177-185.

Lallier, L. E., Maes, F. (2016). Environmental impact assessment procedure for deep seabed mining in the area: independent expert review and public participation. Mar. Pol. 70, 212–219.

La Manna, G., Manghi, M., Perretti, F., and Sarà, G. (2016). Behavioral response of brown meagre (*Sciaena umbra*) to boat noise. Mar. Poll. Bull. 110(1):324-334.

Lambshead, P.J.D., Brown, C.J., Ferrero, T.J., Hawkins, L.E., Smith, C.R., Mitchell, N.J. (2003) Biodiversity of nematode assemblages from the region of the Clarion-Clipperton Fracture Zone, an area of commercial mining interest. BMC Ecology, 3:1

Lammers, M.O., Au, W.W.L., and Herzing, D.L. (2003). The broad band social acoustic signaling behavior of spinner and spotted dolphins. J. Acoust.Soc. America 105, 2493-2498.

Lankford, S.E., Adams, T.E., Cech, J.J. (2003). Time of day and water temperature modify the physiological stress response in green sturgeon *Acipenser medirostris*. Comp. Biochem. Physiol. Part A, Mol. Integr. Physiol. 135, 291e302.

Lanneau, D., Brunet, M., Frisan, E., Solary, E., Fontenay, M., Garrido, C. (2008). Heat shock proteins: essential proteins for apoptosis regulation. J. Cell Mol. Med. 12, 743-61.

Le, J.T., Levin, L.A., Carson, R.T. (2017). Incorporating ecosystem services into environmental management of deep-seabed mining. Deep-Sea Research II 137, 486-503.

Lecchini, D., Bertucci, F., Gache, C., Khalife, A., Besson, M., Roux, N., Berthe, C., Singh, S., Parmentier, E., Nugues, M.M., Brooker, R.M., Dixson, D.L., Hédouin, L. (2018). Boat noise prevents soundscapebased habitat selection by coral planulae. Sci. Rep. 8: 9283.

Lee-Dadswell, G.R. (2009). Theoretical Examination of the Absorption of Energy by Snow Crabs Exposed to Seismic Air-gun Pulses: Stage 2-Improvements to Model and Examination of Resonances. Tech. Rep. OEER Association

Leis, J.M., Siebeck, U., Dixson, D.L. (2011). How Nemo finds home: the neuroecology of dispersal and of population connectivity in larvae of marine fishes. Integr. Comp. Biol. 51, 826–843.

Leitner, A.B., Neuheimer, A.B., Donlon, E., Smith, C.R., Drazen, J.C. (2017). Environmental and bathymetric influences on abyssal bait-attending communities of the Clarion Clipperton Zone. Deep-Sea Research Part I 125, 65-80.

Le Moullac, G., Soyez, C., Saulnier, D., Ansquer, D., Avarre, J. C. and Levy, P. (1998). Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. Fish Shell. Immunol. 8, 621-629.

Lepage, O., Øverli, Ø., Petersson, E., Järvi, T., Winberg, S. (2000). Differential stress coping in wild and domesticated sea trout. Brain Behav. Evol. 56, 259–268

Levin, L. A., Etter, R.J., Rex, M.A., Gooday, A.J., Smith, C.R., Pineda, J., Stuart, C.T., Hessler, R.R., Pawson, D. (2001). Environmental influences on regional deep-sea species diversity. Ann. Rev. Ecol. Syst. 32: 51–93.

Levin, L.A., Mengerink, K., Gjerde, K.M., Rowden, A.A., Van Dover, C.L., Clark, M.R., Ramirez-Llodra, E., Currie, B., Smith, C.R., Sato, K.N., Gallo, N., Sweetman, A.K., Lily, H., Armstrong, C.W., Brider, J. (2016). Defining “serious harm” to the marine environment in the context of deep-seabed mining, Mar. Pol. 74, 245–259

Lewandowski, J.K. (2015). Transforming Conflict into Effective Action: A Case Study on the Effects of Anthropogenic Sound on Marine Mammals. PhD Thesis, George Mason University, Fairfax Virginia

Li, Y., Gu, Z., Liu, H., Shen, H., Yang, J. (2012). Biochemical response of the mussel *Mytilus coruscus* (Mytiloida: Mytilidae) exposed to *in vivo* sub-lethal copper concentraions. Chinese J. Ocea.Limn.30(5), 738-735.

Li, C., Ren, Y., Jiang, S., Zhou, S., Zhaoa, J., Wang R., Li, Y. (2018). Effects of dietary supplementation of four strains of lactic acid bacteria on growth, immune-related response and genes expression of the juvenile sea cucumber *Apostichopus japonicus* Selenka. Fish Shell. Immun. 74,69–75.

Lima,I.,Moreira, S.M.,Rendón-VonOsten, J.,Soares, A.M.V.M., Guilhermino, L.(2006). Biochemical responses of the marine mussel *Mytilus galloprovincialis* to petrochemical environmental contamination along the North-western coast of Portugal. Chemosphere 66 (7), 1230-1242.

Lindh, M.V., Maillot, B.M., Smith, C.R., Church, M.J. (2018). Habitat filtering of bacterial communities above polymetallic nodule fields and sediments in the Clarion-Clipperton Zone of the Pacific OceN. Envir.microb. rep.

Lin, W., Zhang, H., Beck, G. (2001). Phylogeny of natural cytotoxicity: cytotoxic activity of coelomocytes of the purple sea urchin, *Arbacia punctulata*. J. Exp. Zool. 290, 741–750.

Lin, H., Wang, X., Sun, X (2019). Effects of Noise Stress on the Pathological Sections of the Epinephelus Coioides and the Expression of Hsp70 Protein in the Liver. 2019 Intern. Conf. Biom. Sci. Inf. Syst.(ICBSIS 2019).

Lister, K.N., Lamare, M.D., Burritt, D.J.(2015). Pollutant resilience in embryos of the Antarctic sea urchin *Sterechinus neumayeri* reflects maternal antioxidant status. Aquatic Toxic.161, 61-72.

Lister, K.N., Lamare, M.D.,Burritt, D.J. (2016). Dietary pollutants induce oxidative stress, altering maternal antioxidant provisioning and reproductive output in the temperate sea urchin *Evechinus chloroticus.* Aquatic Tox. 177, 106-115.

Liu, Y., Jiang, X.L., Lu, Q., Guan, H.S. (2000). Effects of mannuronate polysaccharide on enzymes of *Penaeus chinensis* related with immune and hemolysis. J. Fish. China 24,549–553

Liu, S., Jiang, X., Hu, X., Gong, J., Hwang, H., Mai, K. (2004). Effects of temperature on non-specific immune parameters in two scallop species: *Argopecten irradians* (Lamarck 1819) and *Chlamys farreri* (Jones & Preston 1904). Aquac. Res. 35, 678–682.

Liu, Y., Kong, W.L., Jiang, G.L., Zhi, Q. (2008). Effects of two kinds of immunopolysaccharide on the activities of immunoenzymes in sea cucumber, *Apostichopus japonicus*. J. Fish. Sci. China. 15,787–793

Liua, Z., Ma, Y., Yang, Z., Lia, M., Liua, J., Bao, P. (2012). Immune responses and disease resistance of the juvenile sea cucumber *Apostichopus japonicus* induced by Metschnikowia sp. C14. Aquaculture 368-369 ,10-18.

Liu, M., Wei, Q.W., Du, H., Fu, Z.Y., Chen, Q.C. (2013). Ship noise-induced temporary hearing threshold shift in the Chinese sucker *Myxocyprinus asiaticus* (Bleeker, 1864). J. Appl. Ichthyol. 29, 1416–1422 © 2013 Blackwell Verlag GmbH ISSN 0175–8659

Livingstone, D. R. (1991) Organic xenobiotic metabolism in marine invertebrates. In *Advances in Comparative and Environmental Physiology*, eds. D. F. Houlihan, D. R. Livingstone and R. F. Lee. 7 (2), 45–185. Springer, Berlin

Livingstone, D.R., Lips, F., Garcia Martinez, P., Pipe, R.K. (1992). Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis*. Mar. Biol. 122, 265 – 276.

Livingstone, D.R. (2003). Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Rev. Med. Vet. 154, 427–430.

Ljungblad, D.K., Wursig, B., Swartz, S.L. and Keene, J.M. (1988). Observations on the behavioral responses of bowhead whales (*Balacena mysticetus*) to active geophysical vessels in the Alaskan Beaufort Sea. Artic 41,183-194.

Lodge, M. (2015). Deep Sea Mining: The New Resource Frontier? pp. 73–74. Outlook on the Global Agenda 2015.

Løkkeborg, S. (1991). Effects of a geophysical survey on catching success in longline fishing. ICES (CM) B: 40.

Løkkeborg, S., Ona, E., Vold, A., Salthaug, A. (2012). Sounds from seismic air guns: gear-and species-specific effects on catch rates and fish distribution. Can. J. Fish. Aquat. Sci. 69 (8), 1278-129

Lopes, C.L., Bastos, L., Caetano, M., Martins, I., Santos, M.M., Iglesias, I. (2019). Development of physical modelling tools in support of risk scenarios: A new framework focused on deep-sea mining. Sci. Tot. Environ. 650, 2294-2306

Lorenzon, S., Martinis, M., Ferrero, E.A. (2011). Ecological relevance of hemolymph total protein concentration in seven unrelated crustacean species from different habitats measured predictively by a density-salinity refractometer. J. Mar. Biol. 1–7.

Lossent, J., Lejarta, M., Folegotd, T., Clorennecd, D., Di Iorioc, L., Gervaisec, C. (2018). Underwater operational noise level emitted by a tidal current turbine and its potential impact on marine fauna. Mar. Poll. Bull. 131, 323–334

Lovell, J.M. (2003). The hearing abilities of the bass, *Dicentrarchus labrax*. In: Technical report commissioned by ARIA Marine Ltd. for the European Commission Fifth Framework Programme. Project Reference: Q5AW-CT-2001-01896.

Lovell, J.M., Findlay, M.M., Moate, R.M., Yan, H.Y. (2005). The hearing abilities of the prawn *Palaemon serratus*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 140, 89–100.

Lovell, T., Hastie, G., Hatch, L.T., Raustein, O., Southall, B.L., Tasker, M., Thomsen, F. (2009). Overview of the impacts of antropogenic underwater sound in the marine environment. In: OSPAR Biodiversity Series, London, pp. 134.

Luca, R.M., Gerlai, R. (2012). In search of optimal fear inducing stimuli: differential behavioral responses to computer animated images in zebrafish. Behav. Brain Res. 226, 66e76.

Luchtel, D., Martin, A., Deyrup-Olsen, I., Boer, H. (1997). Gastropoda: pulmonata. In: Harrison F, Kohn A (eds) Microscopic anatomy of invertebrates, vol 6 B. Wiley-Liss, New York, pp 459–718.

Lucke, K., & Siebert, U. (2009). Temporary shift in masked hearing thresholds in a harbor porpoise (*Phocoena phocoena*) after exposure to seismic airgun stimuli. J. Ac. Soc. America 125, 4060

Lundebye, A.K., Curtis, T.M., Braven, J., Depledge, M.H. (1997). Effects of the organophosphorous pesticide dimethoate on cardiac and acetylcholinesterase activity in the shore crab, *Carcinus maenus*. Aquat. Toxicol. 40, 23– 26

Lyamin, O.I., Korneva, S.M., Rozhnov, V.V., Mukhametov, L.M. (2011). Cardiorespiratory changes in beluga in re-sponse to acoustic noise. Doklady Biol.Sci. 440, 257–258.

Ma, Y., Liu, Z., Yang, Z., Li, M., Liu, J., Song, J. (2013). Effects of dietary live yeast Hanseniaspora opuntiae C21 on the immune and disease resistance against Vibrio splendidus infection in juvenile sea cucumber *Apostichopus japonicus*. Fish Shell. Immun.34, 66e73.

Ma, D., Fang, Q., Guan, S. (2016). Current legal regime for environmental impact assessment in areas beyond national jurisdiction and its future approaches. Environ. L. Kaikkonen et al. Mar. Poll. Bull. 135, 1183–1197 1195. Impact Assess. Rev. 56, 23–30.

Ma, W., Schott, D., Lodewijks, G. (2017a). Continuous Line Bucket Lifting Versus Pipe Lifting. J. Off. Mech. Arctic Engin. 139 / 051704-1

Ma, W., van Rhee, C., Schott, D. (2017b). Technological and Profitable Analysis of Airlifting in Deep Sea Mining Systems. Minerals.7, 143;

Ma, W., Echott, D., Van Rhee, C. (2018). Numerical calculations od environmentl impacts for deep sea mining activities. Sci. Tot. Environ. 652 996-1012.

Madsen, P.T. & Mohl, B. (2000). Sperm whales (*Physeter catodon* L. 1758) do not react to sounds from detonators. J. Acoust.Soc. Am. 107, 668-671

Madsen, P.T., Mohl, B., Nielsen, B.K., Wahlberg, M. (2002). Male sperm whale behaviour during exposures to distant seismic survey pulses. Aquatic Mammals. 28, 231-240

Magnhagen, C., Johansson, K., Sigray, P. (2017). Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: a field experiment. Mar. Ecol. Prog. Ser. 564, 115e125

Makogon, Y., Holditch, S., and Makogon, T. (2007). Natural gas-hydrates – a potential energy source for the 21st century. J. Petrol. Sci. Eng. 56, 14–31.

Malagoli, D., Casarini, L., Ottaviani, E. (2006). Monitoring of the immune efficiency of *Mytilus galloprovincialis* in Adriatic sea mussel farms. Invert. Surv. J. 3,1-3, ISSN 1824-307X Short Communication.

Malagoli, D., Casarini, L., Sacchi, S., Ottaviani, E. (2007). Stress and immune response in the mussel *Mytilus galloprovincialis*. Fish Shell. Immun. 23, 171–177

Malagoli, D., Casarini, L., Fiori, F., Ottaviani, E. (2008). Cytotoxic activity by the mussel *Mytilus galloprovincialis* and the Venus clam *Chamelea gallina* in the Adriatic sea. Invert.Surv. J.5, 50–53.

Malakoff, D. (2010). A push for quieter ships. Science 328, 1502–1503

Mallet, D., and Pelletier, D. (2014) Underwater video techniques for observing coastal marine biodiversity: A review of sixty years of publications (1952-2012). Fish.Res. 154, 44-62.

Malme, C.I. Miles, P.R., Clark, C.W., Tyack, P., and Bird, J.W. (1983). Investigations of the potential effects of underwater noise from petroleum industry activities on migrating gray whale behaviour (BBN Report No. 5366; NTIS PB86-174174). Report from Bolt Beranek and Newman Inc. For U.S. Minerals Management Service, Anchorage, A.K.

Manikandan, S., Srikumar, R., Jeya, Parthasarathy, N., Sheela Devi, R. (2005) Protective effect of *Acorus calamus* Linné on free radical scavengers and lipid peroxidation in discrete regions of brain against noise stress exposed rat. Biol. and Pharm.Bull.28, 2327–2330.

Mann, D.A., Lu, Z., Popper, A.N. (1997). A clupeid fish can detect ultrasound. Nature 389: 341

Mann, D.A., Higgs, D.M., Tavolga, W.N., Souza, M.J., Popper, A.N. (2001). Ultrasound detection by clupeiform fishes. J. Acoust.Soc.Am. 109,3048–3054

Maragos, J.E. (1993). Impact of coastal construction on coral reefs in the US-affiliated pacific islands. Coast. Manage. 21, 235– 269.

Marine Mammal Commmission. (2007). Marine mammals and noise: A sound approach to research and management. A Report to Congress from the Marine Mammal Commission, Bethesda, MD.

Marrone, V., Piscopo, M., Romano, G., Ianora, A., Palumbo, A., Costantini, M. (2012). Defensome against toxic diatom aldehydes in the sea urchin *Paracentrotus lividus*. PLoS One 7, e31750.

Martinez-Porchas, M., Martinez-Cordova, L.R., Ramos-Enriquez, R. (2009). Cortisol and glucose: reliable indicators of stress? Pan. Am. J. Aquat. Sci. 4,158–178

Martin, W., Baross, J., Kelley, D., and Russell, M. J. (2008). Hydrothermal vents and the origin of life. Nat. Rev. Microbiol. 6, 805–814.

Massoullie, J., Pezzementi, L., Bon, S., Krejci, E., Valette, F.M. (1993). Molecular and cellular biology of cholinesterase. Prog. Neurobiol. 41, 31–91.

Masuda, N., Okamoto, N., Kawai, T. (2014). Sea-floor massive sulfide mining – its possibility and difficulties to emerge as a future business, in: C. Drebenstedt, R. Singhal (Eds.), Mine Planning and Equipment Selection, Springer, Germany, 2014, pp. 105–112.

Matozzo, V., Chinellato, A., Munari, M., Bressan, M., Marin, M.G. (2013). Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*? Mar.Poll. Bull.. 72 (1), 34-40

Matranga, V., Toia, G., Bonaventura, R., Müller, W. E.G. (2000). Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes. Cell Stress Chaperon. 5, 113-120

Matranga, V., Bonaventura, R., Di Bella, G. (2002). Hsp70 as a stress marker of sea urchin coelomocytes in short term cultures. Cell. Mol. Biol. 48,345-359.

Mazorra, M.T., Rubio, J.A., Blasco, J. (2002). Acid and alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 131, 241–249

McCauley, R.D., Jenner, M.N., Jenner, C., McCabe, K.A. & Murdoch, J. (1998). The response of humpback whales (*Megaptera novaeangilae*) to offshore seismic survey noise: preliminary results of observations about a working seismic vessel and experimental exposures. APPEA Journal 38, 692-707

McCauley, R.D., Fewtrell, J., Duncan, A.J., Jenner, C., Jenner, M.-N., Penrose, J.D., Prince, R.I.T., Adhitya, A., Murdoch, J., McCabe, K. (2000). Marine seismic surveys - a study of environmental implications. APPEA J. 40, 692–706

McCauley, R. D., Fewtrell, J., & Popper, A. N. (2003). High intensity anthropogenic sound damages fish ears. The Journal of the Acoustical Society of America, 113(1), 638–642.

McCauley, R.D., Kent, C.S. (2012). A lack of correlation between air gun signal pressure waveforms and fish hearing damage. Adv. Exp. Med. Biol. 730, 245–250

McCauley, R.D., Day, R.D., Swadling, K.M., Fitzgibbon, Q.P., Watson, R.A., Semmens, J.M. (2017). Widely used marine seismic survey air gun operations negatively impact zooplankton. Nat. Ecol. Evol. 1

McClain, C.R., Allen, A.P., Tittensor, D.P., Rex, M.A. (2012) Energetics of life on the deep seafloor. Proc. Nat. Acad. Sci. 109(38):15366–71.

McCormick, S.D., Shrimpton, J.M., Carey, J.B., O'Dea, M.F., Sloan, K.E., Moriyama, S., Björnssonc, B.Th. (1998). Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. Aquaculture 168, 221–235

McCormick, M.I., Makey, L., Dufour, V. (2002). Comparative study of metamorphosis in tropical reef fishes. Mar. Biol., 141, pp. 841-853

McCormick, M.I., Lönnstedt, O.M. (2013). Degrading habitats and the effect of topographic complexity on risk assessment. Ecol. Evol., 3, pp. 4221-4229

McCormick, M.I., Watson, S-A., Simpson, S.D., Allan, B.J.M. (2018a) Effect of elevated CO^2 and small boat noise on the kinematics of predator– prey interactions. Proc. R. Soc. B 285: 20172650.

McCormick, M.I., Bridie, J.M.A., Harding, H., Simpson, S.D. (2018b). Boat noise impacts risk assessment vessel a coral reef fish but effects depend on engine type. Sci.Rep. 8:3847.

McCormick, M.I., Fakan, E.P., Nedelec, S.L., Allan, B.J.M. (2019). EfFects of boat noise on fIsh fast-start escape response depend on engine type. Sci. Rep. 9:6554

McDonald, M.A., Hildebrand, J.A., Wiggins, S.M. (2006). Increases in deep ocean ambient noise in the Northeast Pacific West of San Nicolas Island, California. J. Acoustic. Soc. Amer. 120, 711– 718.

McCaig, A., Cliff, R.A., Escartín, J., Fallick, A.E., MacLeod, C.J. (2007). Oceanic detach-ment faults focus very large volumes of black smoker fluids, Geology 35.935–938.

McIntyre, A.D. (1995). Human impact on the oceans: the 1990s and beyond. Mar.Poll. Bull. 31, 147–151

McEwen, B.S. (2012). Brain on stress: howthe social environment gets under the skin. Proc. Natl. Acad. Sci. U. S. A. 109, 17180–17185

McKenna, M.F., Ross, D., Wiggins, S.M., Hildebrand, J.A. (2012). Underwater radiated noise from modern commercial ships. J. Acoust. Soc. Am. 131, 92–103

McLaughlin, K.J., Baran, S.E., Conrad, C.D. (2009). Chronic stress- and sex-specific neuromorphological and functional changes in limbic structures. Mol. Neurobiol. 40, 166–182

Melcón, M.L., Cummins, A.J., Kerosky, S.M., Roche, L.K., Wiggins, S.M., Hildebrand, J.A. (2012). Blue whales respond to anthropogenic noise. PLoS One 7, 1–6

Mendl, M. (1999). Performing under pressure: stress and cognitive function. Appl. Anim. Behav. Sci. 65, 221–244.

Mengerink, K.J., Van Dover, C.L., Ardron, J., Baker, M., Escobar-Briones, E., Gjerde, K., Koslow, J.A., Ramirez-Llodra, E., Lara-Lopez, A., Squires, D., Sutton, T., Sweetman, A.K., Levin, L.A. (2014). A Call for Deep-Ocean Stewardship. Vol.344. Science.

Mensinger, A.F., Putland, R.L., Radford, C.A. (2018). The effect of motorboat sound on Australian snapper *Pagrus auratus* inside and outside a marine reserve. Ecol. Evol.8:6438–6448.

Menzorova, N.I., Seitkalieva, A.V., Rasskazov, V.A. Enzymatic methods for the determination of pollution in seawater using salt resistant alkaline phosphatase from eggs of the sea urchin *Strongylocentrotus intermedius*. Mar. Poll. Bull. 79(1–2), 188-195

Merchant, N. D., Brookes, K. L., Faulkner, R. C., Bicknell, A. W. J., Godley, B. J., & Witt, M. J. (2016). Underwater noise levels in UK waters. Sci. Rep., 6, 36942.

Mero, J. L. (1977). Chapter 11 economic aspects of nodule mining. In Marine manganese deposits, ed. by G. P. Glasby vol. 15, 327–55. Elsevier Oceanography Series 15. Amsterdam: Elsevier

Mesa, M, G. (1994). Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. Trans. Am. Fish. Soc. 123, 786–793

Mickle, M.F., Harris, C.M., Love, O.P., Higgs, D.M. (2019). Behavioural and morphological changes in fish exposed to ecologically-relevant boat noises. Megan Can. J. Fish. Aquat. Sci.

MIDAS. (2016). Research Highlights. Managing Impacts of Deep Sea Resource Exploitation. http://www.eu-midas.net/

MIDAS. (2016) Implications of Midas results for policy makers: Recommendations for future regulations. 2016. http://www.eu-midas.net/

Miller, M.M., Morrison, J.H., McEwen, B.S. (2012). Basal anxiety-like behavior predicts differences in dendritic morphology in the medial prefrontal cortex in two strains of rats. Behav. Brain Res. 229, 280–288.

Miller, I., Cripps, E. (2013). Three dimensional marine seismic survey has no measurable effect on species richness or abundance of a coral reef associated fish community. Mar. Pollut. Bull. 77, 63–70

Miller, K.A., Thompson, K.F., Johnston, P., Santillo, D. (2018). An overview of seabed mining including the current state of development, environmental impacts, and knowledge gaps. Front. Mar. Sci. 4.

Mining Act (1992) and Regulation. Available at: http://www.mra.gov.pg/Portals/2/ Publications/MINING_ACT%201992.pdf (accessed 15.03.13).

Ministry of Economic Development. Current Permits NZ Petroleum and Minerals . Ministry of Economic Development. Available at: http://www.nzpam. govt.nz/cms/online-services/current-permits/ (accessed 15.03.13).

Miljutin, D., Miljutina, M., Arbizu, P., and Galéron, J. (2011). Deepsea nematode assemblage has not recovered 26 years after experimental mining of polymetallic nodules (Clarion-Clipperton Fracture Zone, Tropical Eastern Pacific). Deep Sea Res. Part I Oceanogr. Res. Pap. 58, 885–897.

Myrberg, Jr., A.A., Gordon, C.R., Klimley, A.P. (1978). Rapid withdrawal from a sound source by open-ocean sharks. J. Acoust. Soc. Am. 64, 1289–1297

Myrberg, A.J., Lugli, M. (2006). Reproductive behaviour and acoustical interactions. In: Ladich, F. (Ed.), Communication in Fishes Vol. 1. Science Publishers, Enfield, USA, pp. 149e176

Moalic, Y., Desbruyeres, D., Duarte, C.M., Rozenfeld, A.F., Bachraty, C., Arnaud-Haond, S. (2012). Biogeography revisited with network theory: retracing the history of hydrothermal vent communities. Syst. Biol. 61, 127e137

Monari, M., Matozzo, V., Foschi, J., Cattani, O., Serrazanetti, G.P., Marin, M.G. (2007). Effects of high temperatures on functional responses of haemocytes in the clam *Chamelea gallina*. Fish Shell.Immunol., 22, 98-114

Monecke, T., Petersen, S., Hannington, M.D. (2014). Constraints on water depth of massive sulfide formation: evidence from modern seafloor hydrothermal systems in arc-related settings, Econ. Geol. 109,2079–2101.

Monitoring Guidance for Underwater Noise in European Seas. Part II: (2014). Monitoring Guidance Specifications. JRC Scientific and policy reports. J. Res. Centre.

Monserrat, F., Guilhon, M., Correa, P.V.F., Bergo, N.M., Signori, C.N., Tura, P.M., De los Santos Maly, M., Moura, D., Jovane, L., Pellizari, V., Sumida, P.Y.G., Brandini, F.P., Turra, A. (2019).Deep-sea mining on the Rio Grande Rise (Southwestern Atlantic): A review on environmental baseline, ecosystem services and potential impacts. Deep-Sea Research Part I.

Montaño-Pèrez, K., Yepiz-Plascencia, G., Higuera-Ciapara, I., Vargas-Albores, F. (1999). Purification and characterization of the clotting protein from the white shrimp *Penaeus vannamei*. Comp. Biochem. Physiol. B 122, 381–387

Montgomery, J.C., Jeffs, A., Simpson, S.D., Meekan, M., Tindle, C. (2006). Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. In: Alan, J.S., David, W.S. (Eds.), Adv. Mar.Biol. Academic Press, pp. 143–196

Montie, E.W., Hoover, M., Kehrer, C., Yost, J., Brenkert, K., O'Donnell, T., Denson, M.R. (2017). Acoustic monitoring indicates a correlation between calling and spawning in captive spotted seatrout (*Cynoscion nebulosus*). PeerJ5: e2944; DOI10.7717/peerj.2944

Mooney, T.A., Hanlon, R.T., Christensen-Dalsgaard, J., Madsen, P.T., Ketten, D.R., Nachtigall, P.E. (2010). Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. J. Exp. Biol. 213, 3748–3759

Mooney, T.A., Samson, J.E., Schlunk, A.D., Zacarias, S. (2016). Loudness-dependent behavioral responses and habituation to sound by the longfin squid (*Doryteuthis pealeii*). J. Comp. Physiol. A 202, 489–501

Morato, T., Miller, P. I., Dunn, D. C., Nicol, S. J., Bowcott, J., and Halpin, P. N. (2016). A perspective on the importance of oceanic fronts in promoting aggregation of visitors to seamounts. Fish and Fish. 17, 1227–1233.

Morley, E.L., Jones, G., Radford, A.N. (2014). The importance of invertebrates when considering the impacts of anthropogenic noise. Proc. R. Soc. B 281, 20132683.

Mou, H.J., Jiang, X.L., Liu, S.Q., Guan, H.S. (1999). Effects of immunopolysaccharide on the activities of acid phosphatase, alkaline phosphatase and superoxide dismutase in *Chlamys farreri*. J Oce Univ Qingdao 25:463–468

Mueller-Blenkle, C., McGregor, P.K., Gill, A.B., Andersson, M.H., Metcalfe, J., Bendall, V., Sigray, P., Wood, D.T., Thomsen, F. (2010). Effects of pile-driving noise on the behaviour of marine fish. COWRIE Ref: Fish 06-08, Tech. Rep. 31st March 2010.

Munday, P.L., Dixson, D.L., McCormick, M.I., Meekan, M.G., Ferrari, M.C.O., Chivers, D.P. (2010). Replenishment of fish populations is threatened by ocean acidification. PNAS, 107, pp. 12930-12934

Mydlarz, L.D., Harvell, C.D. (2007). Peroxidase activity and inducibility in the sea fan coral exposed to a fungal pathogen. Comp. Biochem. Physiol. A 146:54–62.

Myrberg Jr., A.A. (1980). The effects of man-made noise on the behaviour of marine animals. Envir.Intern.16, 575–586.

Myrberg, A.J., Lugli, M. (2006). Reproductive behaviour and acoustical interactions. In: Ladich, F. (Ed.), Communication in Fishes Vol. 1. Sci. Publi., Enfield, USA, pp. 149e176.

Nabe-Nielsen, J., van Beest, F.M., Grimm, V., Sibly, R.M., Teilmann, J., Thompson, P.M. (2018) Predicting the impacts of anthropogenic disturbances on marine populations. Conservation Letters. 11: e12563. wileyonlinelibrary.com/journal/conl 1 of 8.

Naguib, M. (2013). Living in a noisy world: Indirect effects of noise on animal communication. Behaviour, 150, 1069–1084

Nautilus Minerals, (2008). Environmental Impact Statement. Available online at: http://www.cares.nautilusminerals.com (Accessed August 9, 2016).

Nautilus Minerals. (2011). Nautilus Granted Mining Lease. News Release Number 2011–2. Nautilus Minerals Inc. Available online at: http://www. nautilusminerals.com/irm/PDF/1009_0/NautilusGrantedMiningLease (Accessed December 11, 2017).

Nautilus Minerals. (2016a). Nautilus Obtains Bridge Financing and Restructures Solwara 1 Project Delivery. Press release dated August 22, 2016. Available online at: http://www.nautilusminerals.com/irm/PDF/1818_0/NautilusobtainsbridgefinancingandrestructuresSolwara1Projectdelivery (Accessed November 22, 2016).

Nautilus Minerals (2016b). How It Will All Work. Available online at: http:// www.nautilusminerals.com/irm/content/how-it-will-all-work.aspx?RID=433 (Accessed August 8, 2016).

Nautilus Minerals (2017). Nautilus Minerals Seafloor Production Tools Arrive in Papua New Guinea. Press release April 3, 2017. Available online at: http://www.nautilusminerals.com/irm/PDF/1893_ 0/NautilusMineralsSeafloorProductionToolsarriveinPapuaNewGuinea (Accessed June 12, 2017).

Nawab, Z.A. (1984): Red Sea mining: a new era, Deep Sea Res. Part A Ocean. Res. Pap. 31,813–822

Nedelec, S.L., Radford, A.N., Simpson, S.D., Nedelec, B., Lecchini, D., Mills, S.C. (2014). Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. Sci. Rep. 4, 5891

Nedelec, S.L., Simpson, S.D., Morley, E.L., Nedelec, B., Radford, A.N. (2015). Impacts of regular and random noise on the behaviour, growth and development of larval Atlantic cod (*Gadus morhua*). Proc. R. Soc. B 282, 20151943

Nedelec, S.C., Mills, S.C., Lecchini, D., Nedelec, B., Simpson, S.D., Radford, A.N. (2016a). Repeated exposure to noise increases tolerance in a coral reef fish. Envir.Poll. 216, 428-436.

Nedelec, S.L., Campbell, J., Radford, A.N., Simpson, S.D., and Merchant, N.D., (2016b). Particle motion: the missing link in underwater acoustic ecology. Methods Ecol. Evol. 7, 836–842.

Nedelec, S.L., Radford, A.N., Pearl, L., Nedelec, B., McCormick, M.I., Meekan, M.G., Simpson, S.D. (2017a). Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Proc. R. Soc. B 284.

Nedelec, S.L., Mills, S.C., Radford, A.N., Beldade, R., Simpson, S.D., Nedelec, B., Côté, I.M. Motorboat noise disrupts cooperative interspecific interactions (2017b). Sci. Rep. 7: 6987.

Nelson, D., Johnson, R. (1972). Acoustic attraction of Pacific reef sharks: effect of pulse intermittency and variability. Comp. Biochem. Physiol. Part A Physiol. 42, 85e95

Neo, Y.Y., Seitz, J., Kastelein, R.A., Winter, H.V., ten Cate, C., Slabbekoorn, H. (2014). Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol. Conserv. 178, 65–73

Neo, Y.Y., Ufkes, E., Kastelein, R.A., Winter, H.V., ten Cate, C., Slabbekoorn, H. (2015). Impulsive sounds change European seabass swimming patterns: influence of pulse repetition interval. Mar. Pollut. Bull. 97, 111–117

Neo, Y.Y., Seitz, J., Kastelein, R.A., Winter, H.V., Ten Cate, C., Slabbekoorn, H. (2016). Noise impact on European sea bass behavior: temporal structure matters. In: The Effects of Noise on Aquatic Life II. Springer, pp. 763–76

Neo, Y.Y., Hubert, J., Bolle, L.J., Winter, H.V., Slabbekoorn, H. (2018). European seabass respond more strongly to noise exposure at night and habituate over repeated trials of sound exposure. Envir. Poll. 239, 367e374

Newell, R.C., Seiderer, L.J., Hitchcock, D.R. (1998). The impact of dredging works in coastal waters: a review of the sensitivity to disturbance and subsequent recovery of biological resources on the seabed. Oceanogr. Mar. Biol. Annu. Rev. 36, 127–178

Newell, R.C., Seiderer, L.J., Simpson, N.M., Robinson, J.E. (2004). Impacts of marine aggregate dredging on benthic macrofauna off the south coast of the United Kingdom. J. Coast. Res. 115–125

Ngo, H.T.T., Gerstmann, S., Frank, H. (2011). Subchronic effects of environment-like cadmium levels on the bivalve *Anodonta anatina* (Linnaeus 1758): II. Effects on energy reserves in relation to calcium metabolism. Toxic. Envir. Chem. 93, 1802-1814.

Nichols, T.A., Anderson, T.W., Širović, A. (2015). Intermittent Noise Induces Physiological Stress in a Coastal Marine Fish, PLOS ONE.

Nilsson, G.E., Ostlund-Nilsson, S., Penfold, R., Grutter, A.S. (2007). From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. Proc. R. Soc. B, 274, pp. 79-85

Niner, H.J., Ardron, J.A., Escobar, E.G., Gianni, M., Jaeckel, A., Jones, D.O.B, Levin, L.A., Smith, C.R., Thiele, T., Turner, P.J., Van Dover, C.L., Watling, L., and Gjerde, K.M. (2018) Deep-Sea Mining With No Net Loss of Biodiversity—An Impossible Aim. Front. Mar. Sci. 5:53.

NMFS (2016). Technical Guidance for Assessing the Effects of Anthropogenic Sound on Marine Mammal Hearing: Underwater Acoustic Thresholds for Onset of Permanent and Temporary Threshold Shifts. p. 178. Ed. by Resources O. O. P. U.S. Department of Commerce, Silver Spring, MD.

Normandeau Associates I. (2012) Effects of noise on fish, fisheries, and invertebrates in the U.S. Atlantic and Arctic from energy industry soundgenerating activities. A literature synthesis for the U.S. Dept. of the Interior, Bureau of Ocean Energy Management. Contract Number: M11PC00031, p. 153

Oebius, H.U., Becker, H.J., Rolinski, S., Jankowski, J.A. (2001). Parametrization and evaluation of marine environmental impacts produced by deep-sea manganese nodule mining. In: Deep Sea Res. Part II Top. Stud. Oceanogr. Environmental Impact Studies for the Mining of Polymetallic Nodules From the Deep Sea, vol. 48. pp. 3453–3467.

Outer Continental Shelf Lands Act. (OCSLA) (1953) (https://www.heritage.org/report/the-us-can-mine- the- deep-sea-sea-without-join-the-UN-convention-the-law-the-sea#_ftn6)

OGP/IAGC. Seismic Seurveys & Marine Mammals. Joint OGP/IAGC position paper.

Ojo, A. A., and Dharmadasa, I. M. (2017). Analysis of electrodeposited CdTe thin films grown using cadmium chloride precursor for applications in solar cells. J. Mater. Sci Mater. Electron. 28, 14110–14120.

OSPAR Commission. (2009). Overview of the impacts of anthropogenic underwater sound in the marine environment. OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic, Biodiversity and Ecosystems Series, Publication No. 441/2009, Richardson WJ (1995) Marine mammals and noise. Academic Press, San Diego, CA.

Ottaviani, E. (1984). Composition of the serum haemolymph of *Planorbis corneus* (Gastropoda, Pulmonata). Comp. Biochem. Physiol. B 78B, 227–239.

Ozbay, G., Riley, J.G. (2002). An analysis of refractometry as amethod of determining blood total protein concentration in the American lobster *Homarus americanus* (Milne Edwards). Aquac. Res. 33, 557–562.

Pagliara, P., Canicattì, C. (1993). Isolation of cytolytic granules from sea urchin amoebocytes. Eur. J. Cell Biol. 60, 179–184.

Palacios, E., Ibarra, A.M., Racotta, I.S. (2000). Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. Aquaculture 185, 353–371

Pan, L., Ren, J., Zheng, D. (2009) Effects of benzo(a)pyrene exposure on the antioxidant enzyme activity of scallop *Chlamys farreri*. Chin. J. Oc. Limn. 27 (1)43-53

Pancer, Z., Rast, J.P., Davidson, E.H. (1999). Origins of immunity: transcription factors and homologues of effector genes of the vertebrate immune system expressed in sea urchin coelomocytes. Immunogenetics 49, 773–786

Pancer, Z. (2000). Dynamic expression of multiple scavenger receptor cysteine-rich genes in coelomocytes of the purple sea urchin. Proc. Natl. Acad. Sci. U. S. A. 97, 13156–13161.

Pape, E., Bezerra, T.N., Hauquier, F., and Vanreusel, A. (2017). Limited Spatial and Temporal Variability in Meiofauna and Nematode Communities at Distant but Environmentally Similar Sites in an Area of Interest for Deep-Sea Mining. Front. Mar. Sci. 4:205.

Papoutsoglou, S.E., Karakatsouli, N., Batzina, A., Papoutsoglou, E.S. & Tsopelakos, A. (2008). Effect of music stimulus on gilthead seabream *Sparus aurata* physiology under different light intensity in a re-circulating water system. J. Fish Biol. 73, 980–1004.

Papoutsoglou, S.E., Karakatsouli, N., Batzina, A., Papoutsoglou, E.S., & Vasilikos, G. (2010). Common carp (*Cyprinus carpio*) response to two pieces of music (‘‘Eine

Kleine Nachtmusik’’ and ‘‘Romanza’’) combined with light intensity, using recirculating water system. Fish Physiol. Biochem. 36,539–554

Parisi, M. G., Mauro, M., Sarà, G., Cammarata, M. (2017). Temperature increases, hypoxia, and changes in food availability affect immunological biomarkers in the marine mussel Mytilus galloprovincialis. J. Comp. Physiol. B. 187, 1117–1126

Parrilla-Taylor, D.P., Zenteno-Savín, T. (2011).Antioxidant enzyme activities in Pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation.Aquaculture. 318 (3–4), 8 379-383

Parry, G.D., Heislers, S., Werner, G.F., Asplin, M.D., Gason, A. (2002). Assessment of Environmental Effects of Seismic Testing on Scallop Fisheries in Bass Strait. Marine and Freshwater Resources Institute (Report No. 50).

Parry, G.D., and Gason, A. (2006). The effect of seismic surveys on catch rates of rock lobsters in western Victoria, Australia. Fish. Res. 79, 272–284.

Parsons, E.C.M., Dolman, S.J., Jasny, M., Rose, N.A., Simmonds, M.P., Wright, A.J. (2009). A critique of the UK's JNCC seismic survey guidelines for minimising acoustic disturbance to marine mammals: best practise? Mar. Pollut. Bull. 58, 643–651.

Partridge, B.L., Pitcher, T.J. (1980). The sensory basis of fish schools: relative roles of lateral line and vision. J. Comp. Physiol. 135, 315– 325.

Pavlidis, M., Karantzali, E., Fanouraki, E., Barsakis, C., Kollias, S., Papandroulakis, N. (2011). Onset of the primary stress in European sea bass *Dicentrarhus labrax*, as indicated by whole body cortisol in relation to glucocorticoid receptor during early development. Aquaculture 315, 125e130

Paxton, A.B., Taylor, J.C., Nowacek, D.P., Dale, J., Cole, E., Voss, C.M., and Peterson, C.H. (2017). Seismic survey noise disrupted fish use of a temperate reef. Mar. Policy 78, 68-73.

Payne, R., Webb, D. (1971). Orientation by means of long range acoustic signaling in baleen whales. Ann. N. Y. Acad. Sci. 188, 110-141.

Payne, J.F., Andrews, C.A., Fancey, L.L., Cook, A.L., Christian, J.R. (2007). Pilot study on the effects of seismic air gun noise on lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci. (No. 2712).

Payne, J.F., Coady, J., White, D. (2009). Potential Effects of Seismic Air Gun Discharges on Monkfish Eggs (*Lophius americanus*) and Larvae. National Energy Board, Canada.

Peacock, Thomas. Is Deep-Sea Mining Worth It? Sci. Am. .318(5) ISSN: 0036-8733

Pearson, W.H., Skalski, J.R., Malme, C.I. (1992). Effects of sounds from a geophysical survey device on behavior of captive rockfish (Sebastes spp.). Can. J. Fish. Aquat. Sci. 49, 1343–1356.

Pearson, W., Skalski, J., Sulkin, S., and Malme, C. (1994). Effects of seismic energy releases on the survival and development of zoeal larvae of dungeness crab (*Cancer magister*). Mar. Environ. Res. 38,93–113.

Peck, M.A., Hufnagl, M. (2012). Can IBMs tell us why most larvae die in the sea? Model sensitivities and scenarios reveal research needs. J. Mar. Syst. 93, 77–93.

Pekkarinen, M., Suoranta, R. (1995). Effects of transportation stress and recovery and sample treatment on calcium and 175 Dis Aquat Org 65: 167–176, 2005 glucose concentrations in body fluids of *Anodonta anatina* (L). J. Shellfish. Res. 14,425–433.

Peña, H., Handegard, N.O., Ona, E. (2013). Feeding herring schools do not react to seismic air gun surveys. ICES J. Mar. Sci. 70, 1174–1180.

Peng, C., Zhao, X., Liu, G. (2015). Review: Noise in the Sea and Its Impacts on Marine Organisms. Int. J. Environ. Res. Public Health 2015, 12, 12304-12323;

Peng, C., Zhao, X., Liu, S., Shi. W., Han, Y., Guo, C., Jiang, J., Wan, H., Shen, T. & Liu, G. (2016). Effects of anthropogenic sound on digging behavior, metabolism, Ca2+/Mg2+ ATPase activity, and metabolism-related gene expression of the bivalve *Sinonovacula constricta*. Sci. Rep. | 6:24266

Perazzolo, L. M., Gargioni, R., Ogliari, P. and Barracco, M. A. A. (2002). Evaluation of some hemato-immunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. Aquaculture 214, 19–33.

Pérez, E.J.M. (2017). The environmental legal framework for the development of blue energy in Europe. In: The Future of the Law of the Sea. Springer, Cham, pp. 127–144.

Petersen, S., Monecke, T., Westhues, A., Hannington, M.D., Gemmell, J.B., Sharpe, R. et al., (2014). Drilling shallow-water massive sulfides at the Palinuro vol-canic complex, Aeolian island arc, Italy, Econ. Geol. 109, 2129–2157.

Petersen, S., Kratschell, A., Augustin, N., Jamieson, J., Hein, J.R., Hannington, M.D. (2016). News from the seabed-Geological characteristics and resource potential of Deep-sea mineral resources. Mar.Policy 70,175-187

Peterson, B.C., Booth, N.J. (2010). Validation of a whole-body cortisol extraction procedure for channel catfish (*Ictalurus punctatus*) fry. Fish. Physiol. Biochem. 36,661–665

Pfohl, R.J. (1965). Changes in alkaline phosphatase during the early development of the sea urchin, *Arbacia punctulata*. Exp. Cell Res. 39, (2–3), 496-503

Pfohl, R.J. (1975). Alkaline phosphatase of sea urchin embryos: Chromatographic and electrophoretic characterization. Develop.Biol. 44 (2), 333-345.

Phillips, B.T. (2017). Beyond the vent: New perspectives on hydrothermal plumes and pelagic biology. Deep–Sea Research Part II. 137, 480–485

Picciulin, M., Sebastianutto, L., Codarin, A., Farina, A., Ferrero, E.A. (2010). *In-situ* behavioural responses to boat noise exposure of *Gobius cruentatus* (Gmelin, 1789; fam. Gobiidae) and *Chromis chromis* (Linnaeus, 1758; fam. Pomacentridae) living in a Marine Protected Area. J. Exp. Mar. Biol. Ecol. 386, 125e132.

Picciulin, M., Sebastianutto, L., Codarin, A., Calcagno, G., Ferrero, E.A. (2012). Brown meagre vocalization rate increases during repetitive boat noise exposures: a possible case of vocal compensation. J. Acoust. Soc. Am. 132, 3118e3124.

Pickering, A.D. (1981). Introduction: the concept of biological stress. In: Pickering AD (ed) Stress and fish. Academic Press, New York, pp 1–7

Pierce, S.K., (1971). Volume regulation and valve movements by marine mussels. Comp. Biochem. Physiol. 39 A: 103-117.

Piercy J.J.B., Codling, E.A., Hill A.J., Smith D.J., Simpson S.D. (2014). Habitat quality affects sound production and likely distance of detection on coral reefs. Mar. Ecol. Prog. Ser. 516, 35– 47.

Pine, M. K., Jeffs, A. G., and Radford, C. A. (2012). Turbine sound may influence the metamorphosis behaviour of estuarine crab megalopae. PLOS ONE 7: e51790

Pinea, M.K., Hannay, D.E., Insleya, S.J., Hallidaya, W.D., Juanesa, F. (2018). Assessing vessel slowdown for reducing auditory masking for marine mammals and fish of the western Canadian Arctic. Marine Pollution Bulletin 135 290–302

Pinoni, S.A., Mañanes, A.A.L. (2004). Alkaline phosphatase activity sensitive to environmental salinity and dopamine in muscle of the euryhaline crab *Cyrtograpsus angulatus*. J. Exp. Mar.Biol.and Ecol. 307(1), 35-46

Pinsino, A., Della Torre, C., Sammarini, V., Bonaventura, R., Amato, E., Matranga, V. (2008). Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: a field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. Cell Biol. Toxicol. 24, 541–552.

Pipe, R.K., Porte, C., Livingstone, D.R. (1993). Antioxidant enzymes associated with the blood cells and hemolymph of the mussels *Mytilus edulis*. Fish Shellfish Immunol. 3, 221–233.

Pollack, I. (1975). Auditory informational masking. J. Acoust. Soc. Am. 57, S5.

Popper, A. N., and Fay, R. R. (1997). Evolution of the ear and hearing: issues and questions. Brain, Behaviour, and Evolution, 50, 213–221.

Popper A.N. (2003a) Effects of anthropogenic sound on fishes. Fisheries 28, 24–31.

Popper, A.N., Fewtrell, J., Smith, M.E., McCauley, R.D. (2003b). Anthropogenic sound: effects on the behavior and physiology of fishes. Mar. Technol. Soc. J. 37, 35–40.

Popper, A.N., Fay, R.R., Platt, C., Sand, O. (2003c). Sound detection mechanism and capabilities of teleost fishes. In: Collin, S.P., Marshall, N.J. (Eds.), Sens.Proc. Aqu.Envir. Springer, New York, pp. 3–38.

Popper, A.N., Fewtrell, J., Smith, M.E., McCauley, R.D. (2004). Anthropogenic sound: effects on the behaviour and physiology of fishes. Mar. Tech. Soc.J. 37, 35–40.

Popper, A.N., Smith, M.E., Cott, P.A., Hanna, B.W., MacGillivray, A.O., Austin, M.E., Mann, D.A. (2005). Effects of exposure to seismic airgun use on hearing of three fish species. J. Acoust. Soc. Am. 117, 3958– 3971.

Popper, A.N., Halvorsen, M.B., Kane, A., Miller, D.L., Smith, M.E., Song, J., Stein, P., and Wysocki, L.E. (2007). The effects of highintensity, low-frequency active sonar on rainbow trout. J. Acoust. Soc. Am. 122: 623–635.

Popper, A.N., Hastings, M.C. (2009a). The effects of human-generated sound on fish. Int. Zool. 4: 43-52

Popper, A.N., Hastings, M.C. (2009b). The effects of human-generated sound on fish. Int. Zool. 4, 43-52

Popper, A.N., Hastings, M.C. (2009c). Effects of anthropogenic sources of sound on fishes. J. Fish. Biol.75,455–498

Popper, A.N., Fay, R.R. (2011). Rethinking sound detection by fishes. Hear. Res. 273, 25–36.

Popper, A.N., Hawkins, A.D. (2012). The Effects of Noise on Aquatic Life. Springer

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., Coombs, S., Ellison, W.T., Gentry, R.L., Halvorsen, M.B., Løkkeborg, S., Rogers, P.H., Southall, B.L., Zeddies, D.G., Tavolga, W.N. (2014). ASA S3/SC1.4 TR-2014 Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report Prepared by ANSI-accredited Standards Committee S3/SC1 and Registered with ANSI. Springer International Publishing, New York, USA.

Popper, A.N., Gross, J.A., Carlson, T.J., Skalski, J., Young, J.V., Hawkins, A.D., Zeddies, D. (2016). Effects of exposure to the sound from seismic airguns on pallid sturgeon and paddlefish. PLoS One 11, e0159486.

Popper, N., Hawkins, A.D. (2018). The importance of particle motion to fishes and invertebrates. J. Acoust. Soc. Am. 143 (1).

Popper, A.N., Hawkins, A.D. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. Journal of Fish Biology. (2019)

Poulton, D.A., Porteus, C.S., Simpson, S.D. (2017). Combined impacts of elevated CO_2 and anthropogenic noise on European sea bass (*Dicentrarchus labrax*). ICES Journal of Marine Science. 74(4), 1230–1236.

Przeslawski, R., Brooke, B., Carroll, A.G., Fellows, M. (2018). An integrated approach to assessing marine seismic impacts: Lessons learnt from the Gippsland Marine Environmental Monitoring project. Ocean Coast. Manag.160,117–123

Prideaux, G., Prideaux, M. (2016). Environmental impact assessment guidelines for offshore petroleum exploration seismic surveys. Impact Assess. Proj. Apprais. 34, 33–43.

Purser, J., Radford, A.N. (2011). Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). PLoS ONE 6, e17478.

Purser, J., Bruintjes, R., Simpson, S.D., Radford, A.N. (2016). Condition-dependent physiological and behavioural responses to anthropogenic noise. Phys. Behav. 155, 157– 161.

Putland, R.L., Merchant, N.D., Farcas, A., Radford, C.A. (2017). Vessel noise cuts down communication space for vocalizing fish and marine mammals. Glob Change Biol.1–14. wileyonlinelibrary.com/journal/gcb © 2017 John Wiley & Sons Ltd |

Quade, M.J., Roth, J.A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. Vet. Immunol. Immunopathol; 58:239e48.

Rabeh, I., Telahigue, K., Bejaoui, S., Hajji, T., Chouba, L., El Cafsi, M., Soudani, N. (2019). Effects of mercury graded doses on redox status, metallothionein levels and genotoxicity in the intestine of sea cucumber Holothuria forskali. Chem. Ecol. 35, 204–218.

Radford, C., Jeffs, A., Tindle, C., Montgomery, J.C. (2008). Resonating sea urchin skeletons create coastal choruses. Mar. Ecol. Prog. Ser. 362, 37–4.

Radford C.A., Stanley J.A., Tindle C.T., Montgomery J.C., Jeffs A.G. (2010). Localised coastal habitats have distinct underwater sound signatures. Mar. Ecol. Prog. Ser. 401, 21-29.

Radford, A.N., Kerridge, E., Simpson, S.D. (2014). Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav. Ecol. 25, 1022e1030.

Radford, A.N., Lèbre, L., Lecaillon, G., Nedelec, S.L., Simpson, S.D. (2016). Repeated exposure to impulsive noise increases tolerance in a commercially important fish. Glob. Change Biol. 10, 3349 – 3360.

Rakhyun, E.K. (2017). Should deep seabed mining be allowed? Mar.Policy 82,134-137

Ramirez-Llodra, E., Shank, T. M., and German, C. R. (2007). Biodiversity and biogeography of hydrothermal vent species: Thirty years of discovery and investigations. Oceanography 20, 30–41.

Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith1, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., and Vecchione, M. (2010). Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. Biogeosciences, 7, 2851-2899.

Ramirez-Llodra, E., Tyler, P.A., Baker, M.C., Bergstad, O.A., Clark, M.R., Escobar, E., Levin, L.A., Menot, L., Rowden, A.A., Smith, C.R., Dover, C.L.V. (2011). Man and the last great wilderness: human impact on the deep sea. PLoS One 6, e22588.

Ramsay, J.M., Feist, G.W., Varga, Z.M., Westerfield, M., Kent, M.L., Schreck, C.B. (2006). Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio*. Aquaculture 258, 565-574.

Ramsay, J.M., Feist, G.W., Varga, Z.M., Westerfield, M., Kent, M.L., Schreck, K.B. (2009). Whole-body cortisol response of zebrafish to acute net handling stress. Aquaculture. 297 (1–4), 157-162.

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., Coppola, G., Geyer, M.A., Glanzman, D.L., Marsland, S., McSweeney, F.K., Wilson, D.A., Wu, C.-F., Thompson, R.F. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Memory 92, 135–138.

Ratcliffe, N.A., Rowley, A.F., Fitzgerald, S.W., Rhodes, C.P. (1985). Invertebrate immunity: basic concepts and recent advances. Intern. Rev. Cytol. 97, 183–350.

Rattner, B.A., Fairbrother, A. (1991). Biological variability and the influence of stress on cholinesterase activity. In: Mineau, P. (Ed.), Cholinesterase Inhibiting Insecticides, Their Impact on the Environment. Elsevier, Amsterdam, pp. 89–110

Read, J., Jones, G. & Radford, A. N. (2014). Fitness costs as well as benefits are important when considering responses to anthropogenic noise. Behav. Ecol. 25,4–7.

Reck, B. K., and Graedel, T. E. (2012). Challenges in metal recycling. Science 337, 690–695

Régnault, M.R., and Lagardère, J.-P. (1983). Effects of ambient noise on the metabolic level of *Crangon crangon* (Decapoda, Nanantia). Mar. Ecol. Prog. Ser. 11, 71-78

Reisinger, R. R., Keith, M., Andrews, R. D., and de Bruyn, P. J. N. (2015). Movement and diving of killer whales (*Orcinus orca*) at a Southern Ocean archipelago. J. Exp. Mar. Biol. Ecol. 473, 90–102

Ren, X., Pan, L., and Wang, L. (2015). Toxic effects upon exposure to benzo [a] pyrene in juvenile white shrimp *Litopenaeus vannamei*. Environ. Toxicol. Pharmacol. 39, 194–207.

Richardson, W. J., Greene, C. R. Jr., Malme, C. J., & Thomson, D. H. (1995). Mar.Mamm. and noise. San Diego, CA: Academic Press

Rickwood, C.J., Galloway, T.S. (2004). Acetylcholinesterase inhibition as a biomarker of adverse effect a study of *Mytilus edulis* exposed to the priority pollutant chlorfenvinphos. Aquat. Toxicol. 67, 45e56.

Rikans, L.E., Hornbrook, K.R. (1997). Lipid peroxidation, antioxidantprotection and aging. Biochim. Biophys. Acta 1362, 116–127.

Roberts, J. M., A. J. Wheeler, and A. Freiwald. (2006). Reefs of the deep: The biology and geology of cold-water coral ecosystems. Science 312: 543–547

Roberts, L., Cheesman, S., Breithaupt, T., and Elliott, M. (2015). Sensitivity of the mussel *Mytilus edulis* to substrate-borne vibration in relation to anthropogenically generated noise. Mar. Ecol. Prog. Ser. 538: 185-195

Roberts, L., Cheesman, S., Elliott, M., Breithaupt, T. (2016a). Sensitivity of *Pagurus bernhardus* (L.) to substrate-borne vibration and anthropogenic noise. J. Exp. Mar. Biol. Ecol. 474, 185–194

Roberts, L., Harding, H.R., Volley, I., Bruintjes, R., Simpson, S.D., Radford, A.N., Breithaupt, T., Elliot, M. (2016b). Exposure of benthic invertebrates to sediment vibration: from laboratory experiments to outdoor simulated pile-driving. Proc. Meet. Acoustics. Vol.27.

Roberts, L., Elliott, M. (2017). Good or bad vibrations? Impacts of anthropogenic vibration on the marine epibenthos. Sci. Tot. Environ. 595,255-268.

Robinson, S.P., Theobald, P.D., Hayman, G., Wang, L.-S., Lepper, P.A., Humphrey, V.F., Mumford, S. (2011). Measurement of Underwater Noise Arising from Marine Aggregate Dredging Operations.

Robinson, S.P.; Lepper, P. A. and Hazelwood, R.A. (2014). Good Practice Guide for Underwater Noise Measurement. Teddington, England, National Measurement Office, Marine Scotland, The Crown Estate, 95pp. (NPL Good Practice Guide No. 133)

Robledo, Y., Cajaraville, M.P. (1997). Isolation and morphofunctional characterization of mussel digestive gland cells *in vitro*. European J. Cell Biol. 72 (4), 262-269.

Rogers, P. H., and Cox, M. (1988). Underwater sound as a biological stimulus. In sensory biology of aquatic animals, pp. 131–149. Ed. by J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga. Springer-Verlag, New York.

Rolinski, S., Segschneider, J., Sündermann, J. (2001). Long-term propagation of tailings from deep-sea mining under variable conditions by means of numerical simulations. Deep-Sea Res. II Top. Stud. Oceanogr. 48, 3469–3485

Rolland, R. M., Parks, S. E., Hunt, K. E., Castellote, M., Corkeron, P. J., Nowacek, D. P., Kraus, S. D. (2012). Evidence that ship noise increases stress in right whales. Proc. Roy.Soc. B: Biolog.Sci.279(1737), 2363–2368.

Roman, J., Estes, J. A., Morissette, L., Smith, C., Costa, D., McCarthy, D., Smetacek, V. (2014). Whales as marine ecosystem engineers. Frontiers Ecol. Environ.12(7), 377– 385.

Romano, T.A., Keogh, M.J., Kelly, C., Feng, P., Berk, L., Schlundt, C.E., Carder, D.A., Finneran, J.J. (2004). An-thropogenic sound and marine mammal health: Measures of the nervous and immune systems before and after intense sound exposure. Canadian J. Fish. Aqu. Sci. 61: 1124–1134.

Romeo, R.D., McEwen, B.S. (2006). Stress and the adolescent brain. Ann. N. Y. Acad. Sci. 1094, 202–214.

Rona, P.A., Scott, S.D. (1993). Sea-floor hydrothermal mineralization: new perspectives. Econ. Geol. Bull. Soc. Econ. Geol. 88, 1935–1976.

Rona, P.A. (2008). The changing vision of marine minerals. Ore Geol. Rev. 33, 618–666.

Ross, N. W., Firth, K. J., Wang, A., Burka, J. F., Johnson, S. C. (2000). Changes in hydrolytic enzyme activities of naïve Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation, Dis. Aqu. Org.41, 43-51

Ross, D. (2005). Ship sources of ambient noise. IEEE J. Ocean. Eng. 30, 257–261.

Rotllant, J., Tort, L. (1997). Cortisol and glucose responses after acute stress by net handling in the sparid red porgy previously subjected to crowding stress. J. Fish Biol. 51, 21e28

Rouse, G., Wilson, N., Carvajal, J., and Vrijenhoek, R. (2016). New deep-sea species of Xenoturbella and the position of Xenacoelomorpha. Nature 530, 94–97.

Rowden, A. A., Schlacher, T. A., Williams, A., Clark, M. R., Stewart, R., Althaus, F., et al. (2010). A test of the seamount oasis hypothesis: seamounts support higher epibenthic megafaunal biomass than adjacent slopes. Mar. Ecol. 31, 95–106.

Rowe, S., Hutchings, J.A. (2006). Sound production by atlantic cod during spawning. Trans. Am. Fish. Soc. 135, 529e538.

Rowe, S., Hutchings, J. A., Skjæraasen, J. E., & Bezanson, L. (2008). Morphological and behavioural correlates of reproductive success in Atlantic cod *Gadus morhua*. Mar. Ecol. Progr. Series, 354, 257– 265.

Ryan, J.A., Hightower, L.E. (1996). Stress proteins as molecular biomarkers for environmental toxicology. Stress-Inducible Cellular Responses.77, 411-424. Ed. by U. Feige, R.I. Morimoto, I. Yahara and B. Polla, Birkhauser Verlag Basel/Switzerland.

Rzeznik, S.J., Flierl, G.R., Peacock, T. (2019). Model investigations of discharge plumes generated by deep-sea nodule mining operations. Ocean Engin.172, 684-696.

Sabet, S.S., Wesdorp, K., Campbell, J., Snelderwaard, P., Slabbekoorn, H. (2016). Behavioural responses to sound exposure in captivity by two fish species with different hearing ability. Animal Behav. 116, 1-11.

Samson, J.E., Mooney, T.A., Gussekloo, S.W.S., Hanlon, R.T. (2014). Graded behavioral responses and habituation to sound in the common cuttlefish *Sepia officinalis*. J. Exp. Biol. 217, 4347–4355.

Sánchez, A., Pascual, C., Sánchez, A., Vargas-Albores, F., Le Moullac, G. and Rosas, C. (2001). Hemolymph metabolic variables and immune response in

Litopenaeus setiferus adult males: the effect of acclimation. Aquaculture 198, 13-28.

Sandström, A., Eriksson, B.K., Karås, P., Isus, M., Schreiber, H. (2005). Boating and navigation activities influence the recruitment of fish in a Baltic Sea archipelago area. A J. Hum. Environ.34, 125–130.

Santovito, G., Piccinni, E., Cassini, A., Irato, P., Albergoni, V. (2005). Antioxidant responses of the Mediterranean mussel, *Mytilus galloprovincialis,* to environmental variability of dissolved oxygen. Comp. Biochem. Physiol. C 140, 321–329.

Santulli, A., Santulli, A., Modica, A., Messina, C., Ceffa, L., Curatolo, A., Rivas, G., Fabi, G., D'Amelio, V. (1999). Biochemical responses of European sea bass (*Dicentrarchus labrax* L.) to the stress induced by off shore experimental seismic prospecting. Mar. Pollut. Bull. 38, 1105–1114.

Sarà, G., Dean, J.M., D'Amato, D., Buscaino, D., Oliveri, A., Genovese, S., Ferro, S., Buffa, G., Lo Martire, M., Mazzola, S. (2007). Effect of boat noise on the behaviour of bluefin tuna *Thunnus thynnus* in the rspb.royalsocietypublishing.org Proc. R. Soc. B 284: 20171627 7 mediterranean sea. Mar. Ecol. Prog. Ser. 331, 243– 253.

Savary, C.A., Lotzová, E. (2001). Phylogeny and ontogeny of NK cells. In: Lotzová E, Heberman RB (eds), Immunobiology of natural killer cells, vol 1, FLA: CRC Press, Boca Raton, pp 45–61, 1986.

Sarlin, P.J., Philip, R. (2011). Efficacy of marine yeasts and baker's yeast as immunostimulants in *Fenneropenaeus indicus*: a comparative study. Aquaculture 321,173e8

Scaps, P., Demuynck, S., Descamps, M., Dhainaut, A. (1996). Biochemical and enzymatic characterisation of an acetylcholinesterase from *Neiris diversicolor* (Annelida, Polychaeta): comparison with the cholinesterases of Eisenia fetida (Annelida, Oligochaeta). Biol. Bull. 190, 396–402.

Schillaci, D., Arizza, V., Parrinello, N., Di Stefano, V., Fanara, S., Muccilli, V., Cunsolo, V., Haagensen, J.J., Molin, S. (2010). Antimicrobial and antistaphylococcal biofilm activity from the sea urchin *Paracentrotus lividus*. J. Appl. Microbiol. 108, 17–24.

Scholik, A.R., and Yan, H.Y. (2001). Effects of underwater noise on auditory sensitivity of a cyprinid fish. Hearing Research 152, 17-24.

Scholik, A.R., and Yan, H.Y. (2002). Effects of boat engine noise on the auditory sensitivity of the fathead minnow, *Pimephales promelas*. Environ. Biol. Fish. 63: 203-209

Schulz, H.D., Zabel, M., 2006. Marine Geochemistry. Springer.

Schwarz, A.L., and Greer, G.L. (1984). Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds. Can. J. Fish. Aquat. Sci. 41(8), 1183-1192

Schulte, P.M. (2014). What is environmental stress? Insights from fish living in a variable environment. J. Exp. Biol. 217, 23e34.

Schoening, T., Jones, D.O.B., Greinert, J. (2017). Compact-Morphology-based polymetallic Nodule Delineation. Scientific RepoRts | 7: 13338 |

Schwarz, A.L., Greer, G.L. (1984). Responses of Pacific herring, *Clupea harengus pallasi*, to some underwater sounds. Canadian J. Fish. Aquatic Sci. 41, 1183–1192.

Sebastianutto, L., Picciulin, M., Costantini. M., Ferrero, E.A. (2011). How boat noise affects an ecologically crucial behaviour: the case of territoriality in *Gobius cruentatus* (Gobiidae). Environ. Biol. Fishes. 92: 207–215.

Secretariat of the Pacific Community (SPC). (2013). Deep sea minerals and the green economy, in: E. Baker, Y. Beaudoin (Eds.), Secretariat of the Pacific Community, Vol. 2.

Seewald, J.S., Reeves, E.P., Bach, W., Saccocia, P.J., Craddock, P.R., Shanks W.C. (2015). Submarine venting of magmatic volatiles in the Eastern Manus Basin, Papua New Guinea, Geochim. Cosmochim. Acta 163 178–199, http://dx.

Seitkalieva, A.V., Menzorova, N.I. and Rasskazov, V.A. (2016). Application of different enzyme assays and biomarkers for pollution monitoring of the marine environment. Environ. Monit. Assess. 188, 1-13.

Shafir, S., Tom, M., Ovadia, M., Lubzens, E. (1992). Protein, vitellogenin and vitellin levels in the hemolymph and ovariesduring ovarian development in *Penaeus semisulcatus* (de Haan). Biol. Bull. 183, 394–400

Shannon, G., McKenna, M.F., Angeloni, L.M., Crooks, K.R., Fristrup, K.M., Brown, E., Warner, K.A., Nelson, M.D., White, C., Briggs, J., McFarland, S., Wittemyer, G. (2016). A synthesis of two decades of research documenting the effects of noise on wildlife. Biol. Rev. 91, 982e1005.

Sharma, R. (2011). Deep-sea mining: economic, technical, technological and environmental considerations for sustainable development. Mar. Technol. Soc. J. 45 (5), 28–41.

Shi, W., Han, Y., Guan, X., Rong, J., Du, X., Zha, S., Tang, Y., Liu, G. (2019). Anthropogenic Noise Aggravates the Toxicity of Cadmium on Some Physiological Characteristics of the Blood Clam *Tegillarca granosa*. Front. Physiol. 10:377.

Sierra-Flores, R., Atack, T., Migaud, H., Davie, A. (2015). Stress response to anthropogenic noise in Atlantic cod *Gadus morhua* L. Aquac. Eng. 67, 67e76.

Silva, P.I.M., Martins, C.I.M., Engrola, S., Marino, G., Øverli, Ø., Conceicao, L.E.C. (2010). Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: evidence for coping styles. Appl. Anim. Behav. Sci. 124, 75–81.

Simon-Lledó, E., Bett, B.J., Huvenne, V.A.I., Schoening, T., Benoist, N.M.A., Jones, D.O.B. (2019). Ecology of a polymetallic nodule occurrence gradient: Implications for deep-sea mining. Limnol. Oceanogr. 9999, 2019, 1–12

Simons, R.D., Siegel, D.A., Brown, K.S., (2013). Model sensitivity and robustness in the stimation of larval transport: a study of particle tracking parameters. J. Mar.Syst. 119–120, 19–29

Simontacchi, C., Poltronieri, C., Carraro, C., Bertotto, D., Xiccato, G., Trocino, A., Radaelli, G. (2008). Alternative stress indicators in sea bass *Dicentrarchus labrax*, L. J. Fish Biol. 72, 747e752

Simpson, S.D., Meekan, M., Montgomery J., McCauley, R., Jeffs, A. (2005). Homeward Sound. Science 308, 221.

Simpson, S.D., Meekan, M.G., Larsen, N.J., McCauley, R.D., and Jeffs, A. (2010). Behavioral plasticity in larval reef fish: orientation is influenced by recent acoustic experiences. Behav. Ecol. 21(5): 1098–1105.

Simpson, S.D., Radford, A.N., Tickle, E.J., Meekan, M.G., and Jeffs, A.G. (2011). Adaptive avoidance of reef noise. PLoS ONE. 6, e16625

Simpson, S.D., Purser, J., Radford, A.N. (2015). Anthropogenic noise compromises antipredator behaviour in European eels. Glob. Change Biol. 21, 586– 593.

Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C., Chivers, D.P., McCormick, M.I., Meekan, M.G. (2016). Anthropogenic noise increases fish mortality by predation. Nat. Commun. 7, 10544.

Sink, T.D., Kumaran, S., Lochmann, R.T. (2007). Development of a whole-body cortisol extraction procedure for determination of stress in golden shiners, *Notemigonus crysoleucas*. Fish Physiol. Biochem. 33,189–193

Sivle, L.D., Kvadsheim, P.H., Ainslie, M.A., Solow, A., Handegard, N.O., Nordlund, N., and Lam, F.P.A. (2012). Impact of naval sonar signals on Atlantic herring (*Clupea harengus*) during summer feeding. ICES J. Mar. Sci. 69(6): 1078-1085.

Skalski, J.R., Pearson, W.H. and Malme, C.I. (1992). Effects of sounds from a geophysical survey device on catch-per-unit-effort in a hook-and-line fishery for rockfish (Sebastes spp.). Can. J. Fish. Aquat. Sci. 49(7),1357-1365.

Skilbrei, O., Holst, J. (2009). Vertical movements of “escaped” farmed Atlantic salmon (*Salmo salar* L.) da simulation study in a western Norwegian fjord. ICES J. Mar. Sci. 66, 278e288.

Slabbekoorn, H., Ripmeester, E.A. (2008) Birdsong and anthropogenic noise: implications and applications for conservation. Mol. Ecol. 17, 72 – 83.

Slabbekoorn, H., Bouton, N., van Opzeeland, I., Coers, A., ten Cate, C., Popper, A.N. (2010). A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419e427.

Slabbekoorn, H. (2016). Aiming for progress in understanding underwater noise impact on fish: complementary need for indoor and outdoor studies. The Effects of Noise on Aquatic Life II. Springer, New York, pp. 1057–1065

Slabbekoorn, H., Ripmeester, E.A.P. (2008) Birdsong and anthropogenic noise: implications and applications for conservation. Mol. Ecol. 17, 72 – 83.

Slotte, A., Hansen, K., Dalen, J., Ona, E. (2004). Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fish. Res. 67, 143–150.

Smith, V. (1981). The echinoderms. In: Ratcliffe, N., Rowley, A. (Eds.), Invertebrate Blood Cell. Academic Press, New York, pp. 513–562.

Smith, V. J., Swindlehurst, R. J., Johnston, P. A. and Vethaak, A. D. (1995). Disturbance of host defense capacity in the common shrimp, *Crangon crangon,* by exposure to harbour dredge spoils. Aquat. Toxicol. 32, 43-58.

Smith, L.C., Chang, L., Britten, R.J., Davidson, E.H. (1996). Sea urchin genes expressed in activated coelomocytes are identified by expressed sequence tags. Complement homologues and other putative immune response genes suggest immune system homology within the deuterostomes. J. Immunol. 156, 593–602

Smith, V.J. (2001) Immunology of invertebrates: cellular. Wiley, NewYork.

Smith, C. R., and A. W. J. Demopoulos. (2003). Ecology of the deep Pacific Ocean floor, p. 179–218. In P. A. Tyler [ed.], Ecosystems of the world: Ecosystems of the deep ocean. Elsevier

Smith, M. E., Kane, A. S., & Popper, A. N. (2004a). Noise-induced stress response and hearing loss in goldfish (*Carassius auratus*). J. Exp. Biol.,207(3), 427–435.

Smith, M., Kane, A., Popper, A. (2004b). Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hypothesis hold water? J. Exp. Biol. 207, 3591–3602.

Smith, M.E., Coffin, A.B., Miller, D.L., and Popper, A.N. (2006). Anatomical and functional recovery of the goldfish (*Carassius auratus*) ear following noise exposure. J. Exp. Biol. 209: 4193-4202.

Smith, C.R., De Leo, F.C., Bernardino, A.F., Sweetman, A.K., Arbizu, P.M. (2008). Abyssal food limitation, ecosystem structure and climate change. Trends Ecol Evol. 23(9):518–28.

Smith, C.R., Paterson, G.P., Lamshead, J., Glover, A., Rogers, A., Gooday, A.J., Kitazato, H., Sibuet, M., Galeron, J., Menot, L. (2008b). Biodiversity, Species Ranges, and Gene Flow in the Abyssal Pacific Nodule Province: Predicting and Managing the Impacts of Deep Seabed Mining. ISA Technical Study No. 3. International Seabed Authority, Kingston, Jamaica, p. 38.

Smith, K. L., Ruhl, H. A., Bett, B. J., Billet, D. S. M., Lampitt, R. S., and Kaufmann, R. S. (2009). Climate, carbon cycling, and deep-ocean ecosystems. Proc. Natl. Acad. Sci. U.S.A. 106, 19211–19218.

Sogard, S.M. (1997). Size-selective mortality in the juvenile stage of teleost fishes: A review. Bull. Mar. Sci. 60, 1129–1157

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., and White, P. (2016). Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rpt. 6: 20540.

Solé, M., Baena, M., Arnau, S., Carrasson, M., Maynou, F., Cartes, J.E. (2010). Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables. Environ. Int. 36, 202-211.

Solé, M., Lenoir, M., Durfort, M., López-Bejar, M., Lombarte, A., van der Schaar, M., and André, M. (2013a). Does exposure to noise from human activities compromise sensory information from cephalopod statocysts? Deep Sea Research Part II: Topical Studies in Oceanography 95, 160-181

Solé, M., Lenoir, M., Durfort, M., López-Bejar, M., Lombarte, A., and André, M. (2013b). Ultrastructural damage of *Loligo vulgaris* and Illex coindetii statocysts after low frequency sound exposure. PLoS ONE 8(10): e78825.

Solé, M., Lenoir, M., Fortuño, J.M., Durfort, M., van der Schaar, M., André, M. (2016). Evidence of Cnidarians sensitivity to sound after exposure to low frequency underwater sources. Sci. Rep. | 6:37979 |

Song, J., Mann, D.A., Cott, P.A., Hanna, B.W., Popper, A.N. (2008). The inner ears of northern Canadian freshwater fishes following exposure to seismic air gun sounds. J. Acoust. Soc. Am. 124, 1360–1366

Sonawane, S.M., Sonawane, M.R. (2018). Effect of heavy metal Copper sulphate on Glycogen activity of Bivalve *L. marginali*. IOSR J. Pharm.www.iosrphr.org (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219 8, 21-27.

Sørensen, J. G., Kristensen, T. N., and Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 6, 1025-1037.

Southall, B. L., Bowles, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. L., Greene, Jr., C. R., Kastak, D., Ketten, D., Miller, J., Nachtigall, P., Richardson, W., Thomas, J., Tyack, P (2007). Marine mammal noise exposure criteria: Initial scientific recommendations. Aquatic Mammals, 33, 411–521.

Speed, S.R., Baldwin, J., Wong, R.J., Wells, R.M.G. (2001). Metabolic characteristics of muscles in the spiny lobster, *Jasus edwardsii*, and responses to emersion during simulated live transport. Comp. Biochem. Physiol. 128, 435–444.

Spiga, I., Caldwell, G.S., and Bruintjes, R. (2016). Influence of pile driving on the clearance rate of the blue mussel, *Mytilus edulis* (L.). Proc. Mtgs. Acoust. 27(1): 040005.

Spiga, I., Aldreda. N., Caldwella, G.S. (2017). Anthropogenic noise compromises the anti-predator behaviour of the European seabass, *Dicentrarchus labrax* (L.). Mar. Poll.Bull.122, 297–305

Stadler, J.H., Woodbury, D.P. (2009). Assessing the effects to fishes from pile driving: application of new hydroacoustic guidelines. Inter-Noise

Stanley, J. A., Van Parijs, S. M., & Hatch, L. T. (2017). Underwater sound from vessel traffic reduces the effective communication range in Atlantic cod and haddock. Scientific Reports, 7(1), 14633.

Stöber, U., Thomsen, F. (2019). Effect of impact pile driving noise on marine mammals: A comparison of different noise exposure criteria. J. Acoust.Soc. Am.145, 3252;

Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F., Dupon, S. (2013). Digestion in sea urchin larvae impaired under ocean acidification. Nature Climate Change 3,1044-1049.

Sun, Y., Song, Y., Zhao, J., Chen, J., Yuan, Y., Jiang, S., & Zhang, D. (2001). Effect of drilling noise and vibration on growth of carp (*Cyprinus carpio*) by cut-fin marking. Marine Fisheries Research/Haiyang Shuichan Yanjiu 22, 62–68.

Sun, H., Wang, Y., Wang, H. (2006). Activities and properties of esterases in haemolymph of scallop *Chlamys farreri*. J. Fish. Sci. China.

Suresh. P.G., , M.K., Mohandas, A. (1993). Haemolymph phosphatase activity levels in two fresh-water gastropods exposed to copper. Sci. Tot. Environ. 134, (2) 1265-1277.

Sverdrup, A., Kjellsby, E., Krüger, P., Fløysand, R., Knudsen, F., Enger, P., Serck-Hanssen, G., Helle, K. (1994). Effects of experimental seismic shock on vasoactivity of arteries, integrity of the vascular endothelium and on primary stress hormones of the Atlantic salmon. J. Fish Biol. 45, 973–995

Taboada, S., Kenny, N.J., Riesgo, A., Wiklund, H., Paterson, G.L.J., Thomas, J., Dahlgren, G., Glover, A.G. (2017). Mitochondrial genome and polymorphic microsatellite markers from the abyssal sponge Plenaster craigi Lim & Wiklund, 2017: tools for understanding the impact of deep-sea mining. Mar. Biodiv.

Tasker, M.L., Amundin, M., Andre, M., Hawkins, A., Lang, W., Merck, T., Scholik-Schlomer, A., Teilmann, J., Thomsen, F., Werner S., Zakharia, M. (2010) MARINE STRATEGY FRAMEWORK DIRECTIVE Task Group 11 Report Underwater noise and other forms of energy. Joint Report Prepared under the Administrative Arrangement between JRC and DG ENV (no 31210 – 2009/2010), the Memorandum of Understanding between the European Commission and ICES managed by DG MARE, and JRC's own institutional funding.JRC Scientific and Technical Reports.

Takaya, Y., Yasukawa, K., Kawasaki, T., Fujinaga, K., Ohta, J., Usui, Y., Nakamura, K., Kimura, J.I., Chang, Q., Hamada, M., Dodbiba, G., Nozaki, T., Iijima, K., Morisawa, T., Kuwahara, T., Ishida, Y., Ichimura, T., Kitazume, M., Fujita, T., & Kato, Y. (2018). The tremendous potential of deepsea mud as a source of rare-earth elements. Sci. Rep. 8, 5763.

Teague, J., Allen, M.J., Scott, T.B. (2018). The potential of low-cost ROV for use in deep-sea mineral, ore prospecting and monitoring. Ocean Engin. 147,333–339

Telahiguea, K., Rabeh, I., Hajji, T., Trabelsi, W., Bejaoui, S., Chouba, L., El Cafsi, M., Soudani, N. (2019). Effects of acute mercury exposure on fatty acid composition and oxidative stress biomarkers in *Holothuria forskali* body wall. Ecotoxicol. Environ. Saf. 169, 516–522.

Terhune, J.M., Friars, G.W., Bailey, J.K. & O'Flynn, F.M. (1990). Noise levels may influence Atlantic salmon smolting rates in tanks. J. Fish Biol. 37, 185–197.

Testa, J.W., Mock, K.J., Taylor, C., Koyuk, H., Coyle, J.R., Waggoner, R. (2012). Agentbased modeling of the dynamics of mammal-eating killer whales and their prey. Mar. Ecol.Progr.Ser.466, 275–291.

Thatje, T., Marsh, L., Roterman, C., Mavrogordato, M., and Linse, K. (2015). Adaptations to hydrothermal vent life in Kiwa tyleri, a New Species of Yeti Crab from the East Scotia Ridge, Antarctica. PLoS ONE 0:e0127621.

Thiel, H., Schriever, G., Bussau, C., and Borowski, C. (1993). Manganese nodule crevice fauna. Deep Sea Res. Part I Oceanogr. Res. Pap. 40, 419–423.

Thiel, H., Tiefsee-Umweltschutz, F. (2001). Evaluation of the environmental consequences of polymetallic nodule mining based on the results of the TUSCH Research Association. Deep-Sea Res. II Top. Stud. Oceanogr. 48, 3433–3452.

Thistle, D. (2003). "The deep-sea floor: an overview," in Ecosystems of the World, Vol. 28, ed P. A. Tyler (Amsterdam: Elsevier), 5–39.

Thomsen, F., Lüdemann, K., Kafemann, R. and Piper, W. (2006). Effects of offshore wind farm noise on marine mammals and fish, biola, Hamburg, Germany on behalf of COWRIE Ltd. Available from: http://offshorewind.co.uk/Downloads/BIOLAReport06072006FINAL.pdf

Thomsen, F., McCully, S.R., Wood, D., White, P., Page, F. (2009). A Generic Investigation Into Noise Profiles of Marine Dredging in Relation to the Acoustic Sensitivity of the Marine Fauna in UK Waters: PHASE 1 Scoping and Review of Key Issues. Aggregates Levy Sustainability Fund/Marine Environmental Protection Fund (ALSF/MEPF), Lowestoft, UK (61 pp., Aggreg. Levy Sustain. FundMarine Environ. Prot. Fund ALSFMEPF Lowestoft UK).

Thomson, R., Sporcic, M., Foster, S., Haddon, M., Potter, A., Carroll, A., Przeslawski, R., Knuckey, I., Koopman, M., Hartog, J. (2014). Examining Fisheries Catches and Catch Rates for Potential Effects of Bass Strait Seismic Surveys. Hobart and Canberra, CSIRO and Geoscience Australia, p. 84

Thurber, A. R., Jones, W. J., and Schnabel, K. (2011). Dancing for food in the deep dea: bacterial farming by a new species of yeti crab. PLoS ONE 6: e26243.

Tidau, S., Briffa, M. (2019). Anthropogenic noise pollution reverses grouping behaviour in hermit crabs. Anim. Behav. 151,113e120

Tiele, T., Bourrel, M. (2016). The common heritage of mankind as a means to assess and advance equity in deep seabed mining, Mar. Policy

Todd, S., Stevick, P., Lien, J., Marques, F., Ketten, D. (1996). Behavioural effects of exposure to underwater explosions in humpback whales (*Megaptera novaeangliae*). Can. J. Zool. 74, 1661–1672

Tomanek, L., Zuzow, M.J., Ivanina, A.V., Beniash, E., Sokolova, I.M. (2011). Proteomic response to elevated PCO2 level in eastern oysters, *Crassostrea virginica*: evidence for oxidative stress.J. Exp. Biol., 214, 1836-1844

Torreilles, J., Guérin, M.C., Roch, P. (1997). Perioxidase-release associated with phagocytosis in *Mytilus galloprovincialis* haemocytes. Dev. Comp. Immunol. 21, 267–275.

Towbin, H., Staehelin, T., Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U. S. A. 76, 4350-4354.

Tracey, S.R., Hartmann, K., Hobday, A.J. (2012). The effect of dispersal and temperature on the early life history of a temperate marine fish. Fisheries Oceanogr.21, 336–347.

Treweek, J. (2009). Ecological Impact Assessment. John Wiley & Sons.

Triki, Z., Grutter, A.S., Bshary, R., Ros, A.F.H. (2016), Effects of short-term exposure to ectoparasites on fish cortisol and haematocrit levels. Mar. Biol. 163,187

Tsujii, K., Akamatsu, T., Okamoto, R., Mori, K., Mitani, Y., Umeda, N. (2018). Change in singing behavior of humpback whales caused by shipping noise. PLoS ONE 13(10): e0204112.

Tyack, P.L. (2008) Implications for marine mammals of large-scale changes in the marine acoustic environment. J. Mammal. 89(3),549–558

UNCLOS, United Nations Convention on the Law of the Sea. Opened for signature 10 Dec 1982 and entered into force 16 Nov 1994; 1833 UNTS 397, 1982

Underwood, A.J. (1992). Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. J. Exp. Mar. Biol. Ecol. 161, 145–178.

United Nations. (1982). United National Convention on the Law of the Sea, part IX.

United Nations. (2015). Department of Economic and Social Affairs, Population Divi- sion, World Population Prospects: The 2015 Revision, New York, United Nations.

United Nations Environment Programme (2013a). Environmental Risks and Challenges of Anthropogenic Metals Flows and Cycles. A Report of theWorking Group on the GlobalMetal Flows to the International Resource Panel eds E. van der Voet, R. Salminen, M. Eckelman, G. Mudd, T. Norgate, and R. Hischier.

United Nations Environment Programme (2013b). Metal Recycling: Opportunities, Limits, Infrastructure. A Report of the Working Group on the Global Metal Flows to the International Resource Panel, edsM. A. Reuter, C. Hudson, A. van Schaik, K. Heiskanen, C. Meskers, and C. Hagelüken.

UNEP. (2014). Wealth in the Oceans: Deep Sea Mining on the Horizon? United Nations Environment Programme. Available at: http://www.unep.org/pdf/GEAS_May2014_DeepSeaMining.pdf (Accessed February 06 2017).

UNGA, United Nations General Assembly. (1970). A/RES/25/2749(XXV) (17 December 1970).

Urick, R. J. (1983). Principles of underwater sound, McGraw-Hill, New York. Xiii, 423 p

Valbonesi, P., Sartor, G., Fabbri, E. (2003). Characterization of cholinesterase activity in three bivalves inhabiting the North Adriatic sea and their possible use as sentinel organisms for biosurveillance programmes. Sci. Total Environ. 312, 79-88.

Vargas-Albores, F., Yepiz-Plascencia, G. (2000). Beta glucan binding protein and its role in shrimp immune response. Aquaculture 191, 13–21.

Van Dover, C. L., C. J. Berg, and R. D. Turner. (1988). Recruitment of marine invertebrates to hard substrates at deep-sea hydrothermal vents on the East Pacific rise and Galapagos spreading center. Deep-Sea Res. Part A Oceanogr. Res. Pap. 35: 1833–1849.

Van Dover, C.L. (2000). The Ecology of Deep-sea Hydrothermal Vents. Princeton University Press, Princeton, New Jersey.

Van Dover, C. L., German, C. R., Speer, K. G., Parson, L. M., and Vrijenhoek, R. C. (2002). Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295, 1253–1257.

Van Dover, C.L. (2010). Mining seafloor massive sulphides and biodiversity: what is at risk? ICES J. Mar. Sci. 68, 341–348.

Van Dover, C.L. (2011a). Mining seafloor massive sulphides and biodiversity: what is at risk? ICES J. Mar. Sci. 68, 341e348

Van Dover, C. (2011b). Tighten regulations on deep-sea mining. Nature 470, 31–33.

Van Dover, C.L. (2014). Impacts of anthropogenic disturbances at deep-sea hydrothermal vent ecosystems: a review. Mar. Environ. Res. 102, 59–72

Van Dover, C.L., Ardron, J.A., Escobar, E., Gjerde, K.M., Jaeckel, A., Jones, D., Levin, L.A., Niner, H., Pendleton, L., Smith, C.R., Thiel, T., Turner, P.J., Watling, L., Weaver, P.P.E. (2017). Biodiversity loss from deep-seabed mining, Nat. Geosci. http://dx.doi.org/10.1038/ngeo2983 (online June 26, 2017)

Van Dover, C.L., Arnaud-Haond, S., Gianni, M., Helmreich, S., Huber, J.A., Jaeckel, A.L., Metaxas, A., Pendleton, L.H., Petersen, S., Ramirez-Llodra, E., Steinberg, P.E., Tunnicliffe, V., Yamamoto, H. (2018). Scientific rationale and international obligations for protection of active hydrothermal vent ecosystems from deep-sea mining. Mar. Policy 90, 20-28.

Vanreusel, A., Hilario, A., Ribeiro, P.A., Menot, L., Arbizu, P.M. (2016). Threatened by mining, polymetallic nodules are required to preserve abyssal epifauna. Sci. Rep. 6, srep26808.

Vasconcelos, R.O., Amorim, M.C.P., and Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. J. Exper. Biol. 210(12), 2104-2112.

Vazzana, M., Cammarata, M., Cooper, E.L., Parrinello, N. (2002). Confinement stress in sea bass (*Dicentrarchus labrax*) depresses peritoneal leukocyte cytotoxicity, Aquaculture 210, 231e243.

Vazzana, M., Celi, M., Genovese, L., Corrias, V., Quinci, E.M., De Vincenzi, G., Maccarrone, V., Cammilleri, G., Mazzola, S., Buscaino, G., Filiciotto, F. (2016). Are mussels able to distinguish underwater sounds? Assessment of the reactions of *Mytilus galloprovincialis* after exposure to lab-generated acoustic signals. Compar. Bioch. Phys, A 201, 61-70.

Vazzana, M., Celi, M., Arizza, V., Calandra, G., Buscaino, G., Ferrantelli, V., Bracciali, C., Sará, G. (2017). Noise elicits hematological stress parameters in Mediterranean damselfish (*Chromis chromis*, perciformes): A mesocosm study. FishShell.Immun. 62, 147e152

Vazzana, M., Celia, M., Chiaramonte, M., Inguglia, L., Russo, D., Ferrantelli, V., Battaglia, D., Arizza, V. (2018). Cytotoxic activity of *Holothuria tubulosa* (Echinodermata) coelomocytes. Fish Shellfish Immunol. 72, 334–341.

Veillette, J., and others. (2007). Ferromanganese nodule fauna in the tropical North Pacific Ocean: Species richness, faunal cover and spatial distribution. Deep-Sea Res. Part I Oceanogr.Res. Pap. 54: 1912–1935.

Vera, L.M., Montoya, A., Pujante, I.M., Calduch-Giner, J.A., Mancera, J.M., Moliner, J., Sánchez-Vázquez, F.J. (2014). Acute stress response in gilthead sea bream (*Sparus aurata* L.) is time-of-day dependent: physiological and oxidative stress indicators. Chronobiology Int. 31, 1051e1061

Verlecar, X.N., Jena, K.B., Chainy, G.B.N. (2007). Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. Chem.-Biol. Interact., 167 (2007), pp. 219-226

Vermeij, M.J.A., Marhaver, K.L., Huijbers, C.M., Nagelkerken, I., Simpson, S.D. (2010). Coral larvae move toward reef sounds. PLoS One 5, e10660

Vetter, B.J., Murchy, K.A., Cupp, A.R., Amberg, J.J., Gaikowski, M.P., Mensinger, M.P. Acoustic deterrence of bighead carp (*Hypophthalmichthys nobilis*) to a broadband sound stimulus. J. Great Lakes Res. 43,163-171.

Vidal-Liñán,L.,Bellas, J.,Etxebarria,N.,Nieto,O.,Beiras, R.(2014). Glutathione S-transferase, glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour areas. Sci. Tot Environ. 470–471, 107-116

Vidal-Liñán, L., Bellas, J., Soriano, J.A.,Concha-Graña, E., Muniategui, S.,Beiras,R.(2016). Bioaccumulation of PCB-153 and effects on molecular biomarkers acetylcholinesterase, glutathione-S-transferase and glutathione peroxidase in *Mytilus galloprovincialis* mussels. Environm.Poll. 214 -891.

Vidal, O., Rostom, F., François, C., Giraud, G. (2017). Global trends in metal consumption and supply: the raw material–energy nexus. Elements 13, 319–324.

Vioque-Fernández, A., Alves de Almeida, E., López-Barea, J. (2007). Esterases as pesticide biomarkers in crayfish (*Procambarus clarkii*, Crustacea): Tissue distribution, sensitivity to model compounds and recovery from inactivation Comp. Bioch. Phys. C 145,404–412

Virkar, R.A. (1966). The role of free amino acids in the adaptation to reduced salinity in the sipunculid *Golfingia gouldii*. Comp. Biochem. Physiol., 18,617-624.

Voellmy, I.K., Purser, J., Flynn, D., Kennedy, P., Simpson, S.D., Radford, A.N. (2014) Acoustic noise reduces foraging success in two sympatric fish species via different mechanisms. Anim. Behav. 89, 191– 198.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N. (2014b). Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS One 9, e102946

Volkmann, S.E., and Lehnen, F. (2017). Production key figures for planning the mining of manganese nodules. Mar. Geor. Geot.

Wagner, T. P., and Raymond, T. (2015). Landfill mining: case study of a successful metals recovery project. Waste Manag. 45, 448–457.

Wakefield, J.R., Myers, K. (2016). Social cost benefit analysis for deep-sea minerals mining. Mar. Policy

Wale, M.A., Simpson, S.D., Radford, A.N. (2013) Noise negatively affects foraging and antipredator behaviour in shore crabs. Anim. Behav. 86, 111 – 118.

Wale, M.A., Briers, R.A., Bryson, D., Hartl, M.G.J., and Diele, K. (2016). The effects of anthropogenic noise playbacks on the blue mussel *Mytilus edulis*. Marine Alliance for Science & Technology for Scotland (MASTS) Ann.Sci. Meeting, 19-21 http://www.masts.ac.uk/media/36069/2016-abstracts-gen-sci-session-3.pdf

Walsh, E.P., Arnott, G., Kunc, H.P. (2017) Noise affects resource assessment in an invertebrate. Biol. Lett. 13: 20170098.

Wang, F.Y., Yang, H.S., Gao, F., Liu, G.B. (2008a). Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. Comp Biochem. Physiol. A Mol. Integr. Physiol. 151,491–498

Wang, F.,Yang, H., Gabr, H.R.,Gao, F.(2008b). Immune condition of *Apostichopus japonicus* during aestivation. Aquaculture,Volume 285, Issues 1–4, 7 December 2008b, Pages 238-243.

Wang, F., Yang, Y.H., Xiaoyu, W., Kun, X. (2010). Antioxidant enzymes in sea cucumber *Apostichopus japonicus* (Selenka) during aestivation. J. Mar.Biol. Association of the United Kingdom.

Wang, R. (2011). (The precautionary principle in maritime affairs, WMU Journal of Maritime Affairs 10 (2) 143)

Wang, Y., Hu, M., Cheung, S.G., Shin. P.K.S., Lu, W., Li, J. (2012). Immune parameter changes of hemocytes in green-lipped mussel *Perna viridis* exposure to hypoxia and hyposalinity. Aquaculture 356–357, 22-29.

Wang, J., Zhao, L., Liu, J., Wang, H., Xiao, S. (2015). Effect of potential probiotic Rhodotorula benthica D30 on the growth performance, digestive enzyme activity and immunity in juvenile sea cucumber *Apostichopus japonicus*. Fish Shell. Immun. 43, 330e336

Wardle, C.S., Carter, T.J., Urquhart, G.G., Johnstone, A.D.F., Ziolkowski, A.M., Hampson, G., Mackie, D. (2001). Effects of seismic air guns on marine fish. Cont. Shelf Res. 21, 1005–1027

Wartzok, D., Ketten, D.R. (1999). Marine mammal sensory systems. In Biology of Marine Mammals (Eds J Reynolds, S Rommel), pp. 17 – 175. Washington, DC: Smithsonian Institution Press.

Waye-Barker, G.A., McIlwaine, P., Lozach, S., Cooper, K.M. (2015). The effects of marine sand and gravel extraction on the sediment composition and macrofaunal community of a commercial dredging site (15 years post-dredging). Mar. Pollut. Bull. 99, 207–215.

Webster, F.J., Wise, B.S., Fletcher, W.J., and Kemps, H. (2018). Risk Assessment of the potential impacts of seismic air gun surveys on marine finfish and invertebrates in Western Australia. Fisheries Research Report No. 288 Department of Primary Industries and Regional Development, Western Australia. 42pp

Webb, P. (1986). Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). Can. J. Fish. Aquat. Sci. 43, 763–771.

Wedding, L.M., Reiter, S.M., Smith, C.R., Gjerde, K.M., Kittinger, J.N., Friedlander, A.M., Gaines, S.D., Clark, M.R., Thurnherr, A.M., Hardy, S.M., Crowder, L.B. (2015). Managing mining of the deep seabed: Contracts are being granted, but protections are lagging. Science 349(6244): 144–145.

Wedemeyer, G.A., Barton, A.B., McLeay, D.J. (1990). Stress and acclimation. In: Schreck, C.B., Moyle, P.B. (Eds.), Meth Fish Biol. American Fisheries Society, Bethesda, Maryland, pp. 451e489.

Wei, Z., Yi,L., Xu, W., Zhou, H., Zhang, Y., Zhang, W., Mai, K. (2015). Effects of dietary nucleotides on growth, non-specific immune response and disease resistance of sea cucumber *Apostichopus japonicas*. Fish Shell.Immun. 47 (1), 1-6

Wei, C.A., Lin, T.H., Chen, R.D., Tseng, Y.C., Shao, Y.T. (2017). The effects of continuously acoustical stress on cortisol in milkfish (*Chanos chanos*). Gen. Comp. Endocrinol. (2017).

Weilgart, L. S. (2007). The impacts of anthropogenic ocean noise on cetaceans and implications for management. Canadian J. Zool. 85(11), 1091–1116.

Weilgart, L. (2018). The impact of ocean noise pollution on fish and invertebrates. Oceancare & Dalhousie University.

Weinstock, M. (2011). Sex-dependent changes induced by prenatal stress in cortical and hippocampal morphology and behaviour in rats: an update. Stress 14, 604–613.

Weir, C.R., Dolman, S.J. (2007). Comparative Review of the Regional Marine Mammal Mitigation Guidelines Implemented During Industrial Seismic Surveys, and Guidance Towards a Worldwide Standard, J. Intern. Wildlife Law Pol. 10:1, 1-27

Wells, R.M.G., Pankhurst, N.W. (1999). Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. J. World. Aquac. Soc. 30,276–284.

Wendelaar Bonga, S.E. (1997). The stress response in fish. Physiological reviews, 77(3), 591-625.

Wen, B., Gao, Q., Dong, S., Hou, Y., Yu, H., Li, W. (2016). Effects of dietary inclusion of benthic matter on feed utilization, digestive and immune enzyme activities of sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 458,1–7.

West, A.D., Stillman, R.A., Drewitt, A., Frost, N.J., Mander, M., Miles, C., Langston, R., Sanderson, W.G., Willis, J. (2011). WaderMORPH – a user-friendly individualbased model to advise shorebird policy and management. Met. in Ecol. and Evol. 2, 95–98.

Wheelock, C.E., Phillips, B.M., Anderson, B.S., Miller, J.L. (2008). Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). Rev. Environ. Contamin. Toxicol. 195, 117-178.

Wilson, B., Dill, L.M. (2002). Pacific herring respond to simulated odontocete echolocation sounds. Can. J. Fish. Aquat. Sci. 59, 542e553.

Williams, R., Clark, C. W., Ponirakis, D., & Ashe, E. (2013). Acoustic quality of critical habitats for three threatened whale populations. Animal Conservation, 17(2), 174–185.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., Clark, C.W., Cullis-Suzuki, S.,Dakin, D.T., Erbe, C., Hammond, P.S., Merchant, N.D., O'Hara, P.D., Purser, J., Radford, A.N., Simpson, S.D., Thomas, L., Wale, M.A.(2015). Impacts of anthropogenic noise on marine life: publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17– 24.

Willis, J. (2007). Could whales have maintained a high abundance of krill? Evolut. Ecol. Res. 9, 651–662.

Willis, J. (2011). Modelling swimming aquatic animals in hydrodynamic models. Ecol. Modell. 222, 3869–3887.

Winston, G.W., Di Giulio, T.D. (1991). Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat Toxicol 19:137–161. 5.

Wittke, M, Renwrantz, L. (1984). Quantification of cytotoxic hemocytes of *Mytilus edulis* using a cytotoxicity assay in agar. J. Invert. Path. 43, 248-253.

Wolfrum, R. Common heritage of mankind. In Max Planck Encyclopaedia of Public International Law[Online],2009.Availableat:⟨http://opil.ouplaw.com/view/10.1093/law:epil/9780199231690/law-9780199231690-e1149?Rskey=1EmERR& result=1&prd=EPIL⟩ (Accessed October 2017).

Wong, B.B.M., Candolin, U. (2015) Behavioral responses to changing environments. Behav. Ecol. 26, 665– 673.

Woodbury, D., Stadler, J. (2008). A proposed method to assess physical injury to fishes from underwater sound produced during pile driving. Bioacoustics 17,289–291

Woodley, C.M., Peterson, M.S. (2003). Measuring responses to simulated predation threat using behavioral and physiological metrics: The role of aquatic vegetation. Oecologia 136, 155–160. pmid:12684858

World Health Organization. (2011). Burden of disease from environmental noise: quantification of healthy life years lost in Europe. Geneva, Switzerland: World Health Organization.

Wright, A.J., Soto, N.A., Baldwin, A.L., Bateson, M., Beale, C.M., Clark, C., Deak, T., Edwards, E.F., Fernández, A., Godinho, A., Hatch, L.T., Kakuschke, A., Lusseau D., Martineau, D., Romero M.L., Weilgart, L.S., Wintle, B.A., Notarbartolo-di-Sciara, G., Martín, V.C. (2007). Anthropogenic noise as a stressor in animals: a multidisciplinary perspective. Intern. J. Compar. Psych. 20, 250–273.

Wright, D.J., Heyman, W.D. (2008). Introduction to the special issue: marine and coastal GIS for geomorphology, habitat mapping, and marine reserves. Mar. Geod. 31, 223–230.

Wysocki, L.E., Ladich, F. (2005). Effects of noise exposure on click detection and the temporal resolution ability of the goldfish auditory system. Hear. Res. 201, 27–36.

Wysocki, L.E., Dittami, J.P., and Ladich, F. (2006). Ship noise and cortisol secretion in European freshwater fishes. Bio. Conserv. 128, 501-508.

Wysocki, L.E., Davidson, J.W. III, Smith M.E., Frankel A.S., Ellison T.E., Mazik P.M., Popper A.N. & Bebak J. (2007). The effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 272, 687– 697.

Wu, T., Ma, J., Chen, S., Sun, Y., Xiao, C., Gao, Y., Wang, R., Poudrier, J., Dargis, M. and Currie, R. W. (2001). Association of plasma antibodies against the inducible Hsp70 with hypertension and harsh working conditions. Cell. Stress Chaperones 6,394-401.

Wu, H., Li, D., Zhu, B., Du, Y., Chai, X., Murata, Y. (2013). Characterization of acetylcholinesterase from the gut of sea cucumber *Stichopus japonicus*. Fish Sci 79:303–311

Xinhai, Z., Xiaomei, X.U., Xinbing, TU., Wenpeng, W., Yougan, C. (2016) Preliminary Exploration of Underwater Noise Impact on Japanese Eel (*Anguilla Japonica*) Elvers. IEE/OES China Ocean Acoustics symposium.

Xue, Q., Renault, T. (2000). Enzymatic Activities in European Flat Oyster, *Ostrea edulis*, and Pacific Oyster, *Crassostrea gigas*, Hemolymph. J. Invert. Path.76,155–163.

Yang, K., Scott, S.D. (2002). Magmatic degassing of volatiles and ore metals into a hydrothermal system on the modern sea floor of the eastern Manus back-arc basin, western Pacific, Econ. Geol. 97 1079–1100.

Yang, G., Tian, X., Dong, S., Peng, M., Wang, D. (2015). Effects of dietary Bacillus cereus G19, B. cereus BC-01, and Paracoccus marcusii DB11 supplementation on the growth, immune response, and expression of immune-related genes in coelomocytes and intestine ofthe sea cucumber (*Apostichopus japonicus* Selenka). FishShell. Immun. 45, 800e807

Yao, C.L., Somero, G.N. (2013). Thermal stress and cellular signaling processes in hemocytes of native (*Mytilus californianus*) and invasive (*M. galloprovincialis*) mussels: cell cycle regulation and DNA repair. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165, 159–168

Yesson, C., Clark, M. R., Taylor, M. L., and Rogers, A. D. (2011). The global distribution of seamounts based on 30 arc seconds bathymetry data. Deep Sea Res. Part I Oceanogr. Res. Pap. 58, 442–453.

Yunwei, D., Tingting, J., Shuanglin, D. (2007). Stress responses to rapid temperature changes of the juvenile sea cucumber (*Apostichopus japonicus* Selenka). J. Ocean Univ. China. 6(3) 275-280.

Tian, X., Dong, S., Dong, Y.(2012). Growth, metabolism and immune responses to evisceration and the regeneration of viscera in sea cucumber, *Apostichopus japonicus*. Aquaculture, 358–359, 50-60

Zhang, M., Wang, L., Guo, Z. Y., and Wang, B. J. (2004). Effect of lipopolysaccharide and *Vibrio anguillarum* on the activities of phosphatase, superoxide dismutase and the content of hemocyanin in the serum of *Fenneropenaeus chinensis*. Mar. Sci. 28, 22-25.

Zhang, Q., Ma, H., Mai, K., Zhang, W., Liufu, Z., Xu, W. (2010). Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, *Apostichopus japonicus*. Fish Shell. Immun. 29(2) 204-211

Zhamoida, V., Grigoriev, A., Ryabchuk, D., Evdokimenko, A., Kotilainen, A.T., Vallius, H., Kaskela, A.M. (2017). Ferromanganese concretions of the eastern Gulf of Finland–environmental role and effects of submarine mining. J. Mar. Syst. 172, 178–187.

Zhao, W., Liang, M., Zhang, P. (2010). Effect of yeast polysaccharide on the immune function of juvenile sea cucumber, *Apostichopus japonicus* Selenka under pH stress. Aquacult Int 18,777–786

Zhao, Y.,Ma, H.,Zhang, W., Ai, Q.,Mai, K.,Xu, W.,Wang, X., Liufu,Z. (2011). Effects of dietary β-glucan on the growth, immune responses and resistance of sea cucumber, *Apostichopus japonicus* against *Vibrio splendidus* infection. Aquaculture 315(3–4), 21 269-274

Zhao, Y., Zhang, W., Xu, W., Mai, K., Zhang, Y., Liufu, Z. (2012). Effects of potential probiotic Bacillus subtilis T13 on growth, immunity and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. Fish Shell.Immunol. 32 750

Zhou, W., Huang, X., & Xu, X. (2018). Changes of movement behavior and HSP70 gene expression in the hemocytes of the mud crab (*Scylla paramamosain*) in response to acoustic stimulation, Mar. and Fresh. Beh. and Phys.

Fondo Sociale Europeo, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale"