

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ

**ΤΜΗΜΑ ΓΕΩΠΟΝΙΑΣ ΙΧΘΥΟΛΟΓΙΑΣ ΚΑΙ ΥΔΑΤΙΝΟΥ
ΠΕΡΙΒΑΛΛΟΝΤΟΣ**

ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ

Μεσογειακή Υδατοκαλλιέργεια

ΤΙΤΛΟΣ:

**Μελέτη επιπτώσεων διαφορετικών συχνοτήτων ήχου σε ενταντικά
εκτρεφόμενους ιχθύς**

Χρυσογέλου Φωτεινή

Βόλος 2023

Μελέτη επιπτώσεων διαφορετικών συχνοτήτων ήχου σε ενταντικά εκτρεφόμενους ιχθύς

Η παρούσα εργασία διαπιστώνει, μελετά και αναλύει τις επιπτώσεις ηχορυπαντών που επηρεάζουν σε επίπεδο ιστολογικής ανάλυσης υπατικά κύτταρα εκτρεφόμενων ιχθύων.

Επιπλέον ,εξετάζονται, διαχωρίζονται, μελετώνται και ερευνώνται τεχνητοί και φυσικοί ήχοι και καταμετράται η ένταση, αλλά και η εμβέλεια του ηχοτοπίου τους.

Επιπλέον, ερευνάτε η νομική, βιολογική, οικολογική και την επιστημονική ανάλυση των συνεχώς αυξανόμενων τεχνητών ήχων που επιβαρύνουν το θαλάσσιο περιβάλλον και που εντοπίζονται στο πεδίο, ιδιαίτερα σε περιοχές με έντονη θαλάσσια δραστηριότητα.

Effects of different sound frequencies on intensively farmed fish

The present study detects, studies analysis the impacts of noise pollutants that effect on liver tissue cells and DNA analysis of intensively farmed fish is occurred.

Furthermore, examines, categorizes, studies and researches artificial (manmade, machinery etc) and natural sounds but also measures the intensity and their sound range scape.

Finally, researching the jurisprudence, biological, ecological, the scientific analysis of the constant rising sound levels that cargo marine ecosystems and are located on field especially in areas with intense marine activities.

Τριμελής Εξεταστική Επιτροπή:

- 1) Ε. Γκολομάζου, Αν. Καθηγήτρια ,Ευζωία και Προστασία Ιχθύων, Τμήμα Γεωπονίας Ιχθυολογίας και Υδάτινου, Σχολή Γεωπονικών Επιστημών, Πανεπιστήμιο Θεσσαλίας , Επιβλέπουσα
- 2) Ε. Μαλανδράκης, Επικ. Καθηγητής, Εκτροφή Υδροβίων Οργανισμών ,Εργαστήριο Εφαρμοσμένης Υδροβιολογίας, Τμήμα Επιστήμης Ζωικής Παραγωγής, Σχολή Γεωπονικών Επιστημών, Πανεπιστήμιο Αθηνών , Μέλος
- 3) Π. Παναγιωτάκη, Καθηγήτρια, Υδατοκαλλιέργειες, Τμήμα Γεωπονίας Ιχθυολογίας και Υδάτινου, Σχολή Γεωπονικών Επιστημών, Πανεπιστήμιο Θεσσαλίας , Μέλος

ΕΥΧΑΡΙΣΤΙΕΣ

Θα ήθελα να εκφράσω τις ειλικρινείς μου ευχαριστίες σε όσους συνέλαβαν στο να φέρω σε πέρας την παρούσα Μεταπτυχιακή Διπλωματική Εργασία. Ιδιαίτερα θα ήθελα να ευχαριστήσω την επιβλέπουσα της εργασίας αυτής, κα Ε. Γκολομάζου για την βοήθεια της τόσο κατά την διεξαγωγή του πειράματος όσο και κατά τη συγγραφή της συγκεκριμένης εργασίας, αλλά και όλο το διδακτικό και γραμματικό προσωπικό που ήταν πάντα παρόν σε οποιαδήποτε στιγμή καθ'όλη τη διάρκεια της φοίτησης μου στο μεταπτυχιακό, αλλά και όσους χρειάστηκε να ενοχλήσω καθ'όλα τα στάδια της διεκπεραίωσης της μελέτης.

Επιπλέον, να ευχαριστήσω θερμά, τις θαυμάσιες βοηθούς του εργαστηρίου της comet assay, Μυρτώ Μανιάκη και Χριστίνα Παπαδούλη για την εκπληκτική θετική διάθεση τους να με βοηθήσουν, κατά τη διάρκεια του πειράματος και την έκδοση των αποτελεσμάτων.

Τέλος, θα ήθελα να ευχαριστήσω την μητέρα μου Κα Ιωαννίδου Κυρακή, η οποία με προέτρεψε να λάβω μέρος σε αυτό το μεταπτυχιακό και αποδείχθηκε ότι είχε δίκιο, μιας και κατά την φοίτηση μου διαπίστωσα για ακόμη μία φορά την απέραντη αγάπη μου για τη θάλασσα και τους θαλάσσιους οργανισμούς και τη δουλειά μου.

Θεσσαλονίκη 2023

CONTENTS

1. Introduction	6
1.1 About animals' welfare.....	6 - 17
1.2 Importance of water quality on fish welfare.....	17 - 18
1.3 Bioindicators.....	18 -22
1.4 Sound pollution	22
1.4.1 Sounds and humans, sounds and sea environment.....	22 - 31
1.4.2 Sound in water physics	31 - 34
1.4.3 Sounds in the sea	34- 36
1.4.4 Effect of sound pollution in economy (marine ecosystems)	36 - 38
1.4.5 Purpose and goal	38 - 40
2. Methods and Materials.....	40
2.1 Sound field experiment	40 - 42
2.2 Comet assay method	42 - 43
2.3 Protocols	43
2.3.1 Field experiment protocol	43 - 44
2.3.2 Laboratory conditions.....	44 - 46
3. Discussion and Results.....	47
3.1 Field experiment results.....	47 - 64
3.2 Comet assay results	65 - 77
3.3 Feeding behaviour.....	77 - 81
3.4 Estimating DNA damage	81 - 83
4. Conclusion	84
5. BIBLIOGRAPHIES	85 - 88

1. Introduction

1.1 About animals' welfare

Animals' welfare for the sustainable development on earth has promoted the idea of further investigation and research regarding animals' welfare as a crucial fact either for those animals that are under captivity or live in wild ([food.ec.europa.eu/animals/animal welfare-eu](http://food.ec.europa.eu/animals/animal-welfare-eu), 23/04/2023).

The well-being of fish and generally of all animals, refers to the painless and non-stressful living conditions, either in captivity or in wild([food.ec.europa.eu/animals/animal welfare-eu](http://food.ec.europa.eu/animals/animal-welfare-eu), 23/04/2023).

Regarding the welfare of breeding and under captivity animals these are all the recent directives that promote relative welfare facts:

Directive 98/58/EC – protection of animals kept for farming purposes.EC. 2002. A strategy for the sustainable development of European Aquaculture Commission of the European Communities COM (2002) 511 final, 26 pp., promotion of animal health and welfare as one of the three main objectives for the Sustainable Development of European Aquaculture ([food.ec.europa.eu/animals/animal welfare-eu](http://food.ec.europa.eu/animals/animal-welfare-eu), 23/04/2023).

Council of Europe – European Convention on the protection of animals kept for farming purposes: Recommendations for farmed fish (06/2006).

In 1992 FAWC (Farm animal welfare council, 1992) developed the 5 freedoms of animals in captivity welfare which include:

- ✓ Freedom from thirst and hunger
- ✓ Freedom from discomfort
- ✓ Freedom from pain, injury, or illness (or sickness)
- ✓ Freedom for abnormal expression behavior
- ✓ Freedom from fear and sorrow

All important, but the last freedom of fear and sorrow holds the highest position among others, showing that emotional welfare is far ahead from the others and may hold the key to preserving and repairing the already damaged ecosystems, from stressful and harmful human made intrusions.

In order to understand and study the wellbeing of animals we use several examination factors within their body or surrounding in which they live in.

Generally, the most common welfare indicators, both for animals in captivity and in wild can be categorized as follows:

- Indirect indicators such as oxygen, salinity, fish density (in tanks or aquariums).
- Biological indicators like health, behavior and feeding needs.
- External bioindicators that such as oxygen, food, or growth.
- Finally, laboratory holding hormones and health.

On the other hand, the most common stress assessment indices are:

- ✓ Hormonal: Cortisol, Catecholamines
- ✓ Biochemical: Glucose, Lactic acid
- ✓ Molecular: DNA distraction, Genetic expression
- ✓ Oxide/Earth: Muscle pH, blood pH
- ✓ Hematological: Hematocrit, Hemoglobin.

The need to preserve healthy animals is necessary for their well-being but also ours. The ranking of needs depends on how much each need affects the animal's ability to live its life. The ability to adapt to any new condition is vital for the wellbeing of animals. The cost of physical and psychological stress is of great importance since it affects the animals. Strong, frequent, and continuous strain (stress) significantly reduces animals' well-being. It is indeed well known that emotions are what ultimately determine the well-being of animals.

But what about fish? Do fish understand any of the impacts or changes of their environment?

Fish can feel pain, fear, and stress like any other animal. They have a central and autonomous nervous system, developed endocrine and sensory system, ability to learn and memorise. Which means that they can hear, feel, and see or sense, different stressful threats (Chao Peng et al 2015). Their main sensory organs are the eyes, their lateral line, their skin, and others follow. They can appear with diseases, illnesses, and sickness like many animals. We must reduce as much as possible the outbreak of diseases, the levels of mortality, aggressive behaviors, and conduct disorders in order to succeed the best welfare values and keep healthy animals. Even in wild pollution and contamination must be reduced if not zeroed (Chrysogelou 2020).

Research regarding the question whether there is any existence or non-existence of consciousness in fish has showed that they do have consciousness and have the ability to feel pain. They have sensory receptors and respond to painful and noxious stimuli (having a good response to the administration of analgesic substances). They have learning abilities that help them deal with difficult situations and show corresponding adaptability. Accordingly, there must be corresponding care for their well-being, and we are the most capable of providing them good and high standard life conditions (Qi Li et al 2022). After all they will end up in our body and can indeed contaminate our own organs.

On the other hand, it is of great interest keeping marine products healthy. Even in wild having them happy, meaning keeping welfare indicators high, can increase the nutrition value of products captured in open sea. Products of high decomposition rate and deterioration such marine products are even in a higher risk appearing such commercial damages and become less attractive. The last action can automatically be translated into lower income and less profits if their growth development does not prove welfare or excellent living conditions (Chrysogelou 2023).

Keeping fish healthy without stressing them in wild and in captivity is vital. Stressing them on the other hand, can increase strain and as a result can affect muscles tissues after death. Tissues of a stressed animal is harder, and fish is sold at a much lower value or not at all. Same goes for other marine products. If not in muscles, then in other organs of importance that could depend on their taste. Or else, the final products taste.

Environmental conditions also play a key role in marine life's welfare. All species, some more and some lesser, provide useful information about the environment they live in. Bioindicating through their organs some of the possible contamination or pollution threats of their environment (Daniel Lins et al 2021). They, in fact, show us whether, products from a specific area can or cannot be consumed.

According to a special Eurobarometer survey on the attitudes of Europeans to animal welfare in May 2021, 82% of respondents said the welfare of farmed animals should be better protected than it is now (food.ec.europa.eu/animals/animal-welfare-eu, 23/04/2023).

The first EU rules protecting farm animals date back to the 1970s. The 1998 directive for the protection of farmed animals established general standards for the protection of all animals kept producing food, wool, skin, fur or other farming purposes - including fish, reptiles and amphibians - and is based on the European Convention for the Protection of Animals kept for Farming Purposes of 1978 (www.eeu.europa.eu 29/05/2023).

EU rules on animal welfare reflect the so-called five freedoms:

- ✓ Clinical examination
- ✓ Vaccination
- ✓ Administration of high nutrition healthy food and medicine
- ✓ Screening according to body weight
- ✓ Screening according to body weight
- ✓ Careful stun before every action
- ✓ Non stressful transportation methods
- ✓ Collection of unhealthy nets (those that are not used and can harm species)
- ✓ Healthy and non-stressful pump distribution
- ✓ Low stressful killing methods).

Other EU rules set welfare standards for farm animals during stunning and slaughter, as well as for breeding conditions for specific animal categories such as calves, pigs and laying hens (www.eeu.europa.eu 29/05/2023).

In October 2018, MEPs adopted a new regulation on veterinary medicinal products to curb the use of medicines to compensate for poor conditions or to make animals grow faster (www.eeu.europa.eu 29/05/2023).

In line with the presentation of the new Farm to Fork strategy for a more sustainable agriculture, the European Commission is currently evaluating all EU legislation on the welfare of farmed animals (www.eeu.europa.eu 29/05/2023).

EU rules for the protection and welfare of animals during transport were approved in 2004. However, in a resolution adopted on 14 February 2019, Parliament called for better enforcement, sanctions, and reduced journey times (www.eeu.europa.eu 29/05/2023).

On 19 June 2020 MEPs set up an inquiry committee to investigate alleged breaches in the application of EU animal welfare rules during transport within and outside the EU (www.eeu.europa.eu 29/05/2023).

During a debate on 2 December 2020, MEPs asked Agriculture Commissioner Janusz Wojciechowski to ensure that EU farm policy improves animals' welfare during transportation. They called on the Commission to support the shortening of supply chains, to encourage the transport of meat instead of live animals and to guarantee imports comply with EU animal welfare standards (www.eeu.europa.eu 29/05/2023).

In April 2021, Parliament's inquiry committee found negative issues regarding the protection of animals during transport said the failure to enforce the current rules on animal welfare during transport is unacceptable and called for concrete action to correct the problem, including infringement procedures against member states that fail to enforce existing EU rules (www.eeu.europa.eu 29/05/2023).

The committee found systemic failures in the implementation of animal transport rules across the EU. The committee's report was adopted during a plenary session in January 2022 (www.eeu.europa.eu 29/05/2023).

On 10 June, in response to the European (www.eeu.europa 29/05/2023) citizens' initiative called End the Cage Age, which was signed by 1.4 million people from across the EU, MEPs called on the Commission to come up with a proposal to ban caged farming in the EU by 2027 (www.eeu.europa 29/05/2023).

In wild, on the other hand, things are easier (if measures taken) but even more complex. There, we need stricter measures on the levels of how much we can pollute and contaminate (although we are at the point of a complete zero level), as well as prohibitions on global plastic production and other unhealthy elements (carbon, phosphates etc.), which harm both species in wild and in captivity (Chrysogelou 2020). Since the environment plays a big role on any species welfare, then it is easy to understand the importance of keeping contamination and pollution levels on the lowest value if not at zero levels. This only means that having healthy and pollution free seas, we have the maximum product qualities. Keeping marine environment and water clean means automatically health in humanity (Valeriya Komyakova et al 2021).

The 500 wild birds naturally occurring in the EU are protected by the Birds Directive, whilst the Habitats Directive aims to ensure the conservation of rare, threatened, or endemic animal species and characteristic habitat types (www.eeu.europa 2/06/2023).

The EU Pollinators Initiative was launched in 2018 to tackle the decline of wild pollinating insects, especially bees. Parliament called for a further reduction of pesticides and more funds for research. In a report adopted in January 2018, EU Parliament had already said regional and local bees' varieties should be better protected (www.eeu.europa 2/06/2023).

In June 2021, when voting on the EU Biodiversity for 2030, MEPs called for an urgent revision of the EU Pollinators Initiative to include: a new EU-wide pollinator monitoring framework with robust measures, clear time-bound objectives, and indicators, including impact indicators and capacity building (www.eeu.europa 2/06/2023).

Whales and dolphins are protected from capture and killing in EU waters (L. Bittencourt et al 2017). In addition, the EU has always been a defender of the full implementation

of the moratorium on commercial whaling in place since 1986. An EU regulation bans the trade in seal products. There are also rules on trapping methods, prohibiting the use of leghold traps to catch wild animals in the EU and setting humane standards (www.eeu.europa 8/06/2023).

The EU implements and goes beyond the provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Cites) through its Wildlife Trade Regulations to ensure trade in wildlife products does not lead to species becoming endangered (www.eeu.europa 8/06/2023).

In June 2021, Parliament adopted its position on the EU Biodiversity Strategy for 2030, which is part of the EU Green Deal (www.eeu.europa 8/06/2023).

EU rules on keeping wild animals in zoos seek to strengthen their role in the conservation of biodiversity and set standards for protection measures, including appropriate accommodation for animals.

The EU has created a legal framework that regulates animal studies for the development of new medicines, for physiological studies and for testing of food additives or chemicals. The rules are based on the principle of the three R's:

- *Replacement (fostering the use of alternative methods)*
- *Reduction (trying to use fewer animals for the same objective)*
- *Refinement (efforts to minimize pain and suffering)* (www.fao 08/06/2023).

Animal testing on cosmetics and the marketing of such products is prohibited in the EU. In a resolution adopted in 2018, Parliament called for a global ban on animal testing for cosmetics. Parliament wants to go further. In September 2021, it called for more funding into alternative test methods so that the use of animals in research and testing can be phased out. To clamp down on the illegal trade in dogs and cats, Parliament called for an EU-wide action plan, tougher sanctions and mandatory registration in a resolution adopted on 12 February 2020. To address the concerns of Europeans who consider pets as part of their families, cat and dog fur has been banned in the EU since

2008. The legislation bans, the placing on the market and the import or export of cat and dog fur and of all products containing such fur (www.eeu.europa 08/06/2023).

Thanks to harmonized EU rules on travelling with pets, people are free to move with their furry friends within the European Union. The pet passport or the animal health certificate is the only requirement for dogs, cats, and ferrets to travel across EU borders, with certain exceptions.

Fishing in wild causes other problems on the other hand, for example when fish caught in wild are subject to very long and steep strain.

Netting causes:

- Increased muscle fatigue, rapid exhaustion
- Crushing together with the rest of the fish from their total weight
- Great pressure of the internal organs
- Injury from nets and hooks
- Suspended live or bait and food for other fish (www.fisheries.noaa.gov 11/06/2023).

Only in European Union, 12.3 million tons of fish are consumed per year. This can only prove the problems that appear while consuming unhealthy marine products and it must be of our high interest keeping healthier seas and marine products (*Abir Chahouri et al 2021*).

Europe union has lately accomplished to proceed to laws and legislations regarding animals. Accordingly, the basics are:

Article 13 of the Treaty on the Functioning of the EU stipulates that, as sentient beings, full regard should be paid to animals' welfare requirements on which:

Article 13 of the Treaty on the Functioning of the European Union

In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions

and customs of the Member States relating to religious rites, cultural traditions, and regional heritage (www.eeu.europa 11/06/2023).

And:

Legislation on the protection of farm animals covers all the different steps of production from farming itself, to transport and killing.

Farming activities are covered by five directives which impose minimum standards while transport and killing of animals are covered by regulations which set up similar requirements for all Member States (www.fao 11/06/2023).

Directives vs. regulations in the EU legal order

In the European Union's legal order, directives are applicable to Member States which have to transpose them into their own national legal system, with the possibility to go beyond the minimum rules laid down in an EU directive. By contrast, regulations are directly applicable to citizens and businesses, without being transformed into national laws. They usually establish common standards and restrict the possibility for the Member States to adopt standards other than those laid down in a regulation. Member States, however, still have to establish sanctions in their national legal order.

1. Directive 98/581: All farmed animals
2. Directive 2008/1193: Calves
3. Directive 2008/1204: Pigs
4. Directive 1999/745: Laying hens
5. Directive 2007/438: Chickens for meat production
6. Regulation 1/20059: Animal transport
7. Regulation 1099/200910: Killing of animals.

As a result of the above decisions future EU actions on animal welfare more actions about the issue includes complete the EU animal welfare strategy 2012-2015 and make the best of actions regarding their welfare, prioritize enforcement, also strengthen, and broaden dialogue with stakeholders, continue with better valorize animal welfare at global level (www.eeu.europa 11/06/2023).

The European Union has adopted in the last 40 years a very comprehensive and advanced set of legislation on animal welfare. The Union has developed animal welfare legislation quite extensively for farmed and laboratory animals. Some other areas of animal welfare remain under the sole competence of the Member States (www.eeu.europa 11/06/2023).

This body of EU legislation contributes to the sustainability of the EU food chain. Respecting production animals addresses the ethical concerns of citizens and consumers but also helps to develop production systems that are innovative and economically viable in the long term (www.eeu.europa 11/06/2023).

While Member States are primarily responsible for implementing EU rules in the field of animal welfare, the Commission has built a series of instruments to ensure a harmonized enforcement, through audits, training, scientific expertise, and advice (www.eeu.europa 11/06/2023).

Enforcement remains an important challenge since animal welfare is sometimes perceived as an economic hurdle rather than an opportunity for better and more efficient production (Chrysogelou 2023).

Against this background continuous stakeholders' dialogue, information and education are essential to create a positive dynamic for animal welfare and to improve enforcement through better understanding of the underlying animal welfare principles (Chrysogelou 2023).

Stakeholders' dialogue is also crucial to better integrate animal welfare within a general context of sustainable production, considering its integration within other societal issues such as public health, environment, and global competitiveness (Abir Chabuari et al 2022).

Scientific opinions and research also play an important role in providing the necessary understanding of the legislation as well as the innovations to make animal welfare an economic opportunity (Chrysogelou 2023).

The Union is one of the major world importers of food products and the globalization of the food market has an impact on the applicability of EU rules including those on animal welfare. The Union has so far been successful in advocating and promoting its food standards in the international arena, showing that consumers beyond EU borders also appreciate and value high quality standards. Such a policy should be therefore continued to consolidate the credibility of the EU food sectors in external markets (Chrysogelou 2023).

And all the above referring to animals in captivity. In wild there is Article 13 of Title II of the Lisbon Treaty. The EU has some of world's highest animal welfare standards. Also includes how the legislation protects wildlife, pets as well as farm and laboratory animals (Chrysogelou 2023).

The European Union has advocated animal welfare for more than 40 years and is widely recognized as a global leader, with some of the world's best animal welfare standards. EU rules have also positively influenced legislation in non-EU countries. They mainly concern farm animals (on the farm, during transport and at slaughter), but also wildlife, laboratory animals and pets (Chrysogelou 2023).

The Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals and more, but the problem with all is that they still need to cover bigger details and pay more attention on the implementation of their acts. Further and more detailed, but also more precise measures for the welfare of all beings should and must be carefully treated and not to be drowned up (Chrysogelou 2023).

All the previous written, are under formulation of legislative texts mainly of Scientific knowledge, EFSA's independent scientific opinions, studies to evaluate the effect of the new legislation in combination with the bureaucratic burden and the principle of subsidiarity and proportionality, consultation with stakeholders, online consultation with citizens (Chrysogelou 2023).

Still there are enormous threats that must be taken under consideration and contamination and pollution sources must be excluded. Artificial human sounds in a

not so quite world; where animals need to contact by making noises or cover other needs; is a new threat that needs to be taken under serious consideration. As already said, the need to have healthy seas and animals is humanities need too (Chrysogelou 2023).

1.2 Importance of water quality on fish welfare

Water composition is provenly a crucial factor of the wellbeing of species. Marine species that totally depend on water have even bigger reasons to live under non polluted or contaminated habitats (Kathryn A. Burns, Jonathan L. Smith 1981). The importance of keeping habitats pollution and contamination free, is known.

The sea, on the other hand, is not only the habitat of many species but also is of great financial importance to humanity. Marine related industries, such as the touristic, or shipping, depend exclusively on the sea (Chrysogelou 2023).

The past years the need for marine related products has increased rapidly making the sea and coastline, a competitive financial working field which is desirable for its commercial value (J.J. Cole et al 2014). Tourist industries, aquaculture, commercial shipping is only a few of the many industries that demand and claim a piece of sea (Chrysogelou F. 2023).

On the other hand, the increasingly sea connected industries have managed to extend their working territories but at the same time increased their impact on sea (Chrysogelou 2023).

Coastline areas have been under enormous pressure, and the stressful pollution results and contamination factors have increased and reached highest levels ever nowadays, these trash and pollutants make their existence even more visible and noticeable. Eutrophic (or else plankton bloom) areas of high non desirable elements, huge amounts of plastic or styrofoam are this kind of garbage that we can see and collect. But the problem is bigger, considering that all these materials can break in smaller invisible to necked eye particples that end up on every commercial product because fish and other species could feed on them, provide even bigger problems (Baptiste Vivier et al 2021).

Although, in some cases, pollution and contamination levels (or values) are secure (meaning within the positive restricted levels of values) yet that does not mean that they do not exist (Chrysogelou 2023).

Image 1: Field experiment area image, pollutants on field



(Photo by Chrysogelou 2022)

It is the basic physical and chemical environment of fish, which is influenced by several factors. Water quality relates to the welfare of fish. Fogginess, turbidity, oxygen levels, temperature, salinity, pH, levels of nitrites and ammonia, carbon dioxide levels are some of the more important factors that contribute to the wellness of fish and sea. Water examination of all above factors usually occurs by analyzing samples and microscopic analysis. In captivity these factors are easy to control. In wild it is on our disposal whether we will contribute to keep the environment clean and pollution and contamination free (Chrysogelou 2023).

Water plays a huge role on the life cycle of marine life. It is their habitat, and they are totally connected to it. Muscles tissues and other organs can be influenced and absorb elements that can be indeed harmful when eaten (Chrysogelou 2020).

1.3 Bioindicators

One more important factor on the impact between fish products and their environment is the fact that they can be useful bioindicators (Eran Gefen et al 2003). Some parts of their body have the capability to keep or better store some chemical or natural elements or other bio factors, and according to the pathogenic or biological changes can give us useful information regarding their growth and their environment. (Roberto Chiarelli et al 2014). Additionally, a biomarker is a biological characteristic that is objectively measured and evaluated as an indicator of normal biological or pathological processes, or a response to a therapeutic intervention. Examples include patterns of gene

expression, levels of a particular protein in body fluids, or changes in electrical activity in the brain. Nevertheless, a feature that is determined objectively and can be considered as an indicator of a normal biological process, of the evolution of one disease or the typical response to a medicine or treatment can have the following properties:

- Biochemical
- Physiological
- Histological
- Morphological
- Biological

Indices measured within one organism or its products (Chrysogelou 2020).

Biomarkers are categorized depending on their ability towards their bioaccumulation such as:

1. Bioaccumulation markers
2. Biomarkers of exposure
3. Biomarkers of effect
4. Biomarkers of susceptibility (Chrysogelou 2020).

Bioaccumulation markers are indicators that indicate the exposure of an organism to some xenobiotic and to which degree (e.g., specific metabolites or compounds that interact with the biological system). Some of those elements that can be indicated are Al, Cd, Cu, Ga, Pb, Se, Zn, on gills, liver and muscle tissues and can be detected by inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES) and atomic absorption spectroscopy (AAS) (Giuseppe B , M O Bonaca 2019).

On exposure biomarkers, we have a biochemical response of an individual or an organism after exposure to one infectious agent or contaminant (J.J. Cole et al 2014). The main goal of metabolism of xenobiotics is to increase the hydrophilic property of the compound. Such metabolism shows the integration of the reactions of the biotransformation of phase I and phase II (Chrysogelou 2020).

According to biotransformation enzymes Phase I, is the major pathway for the biotransformation of lipophilic compounds and include oxidative, reductive, and

hydrolytic reactions where a polar group is introduced, rendering the xenobiotic molecule less active and more water-soluble and allowing it to be more readily excreted (J.J. Cole et al 2014). Most Phase I reactions are catalyzed within the microsomal mono oxygenase (MO) enzyme system and depend on the heme protein cytochrome P450 (cyt P450). Cyt P450 isozyme induction is triggered when specific xenobiotic compounds bind to the aromatic hydrocarbon (Ah) receptor protein complex and the HSP 90. Two are the main biotransformation enzymes of Phase I, Aryl hydrocarbon hydroxylase (AHH) and Ethoxy resorufin O- demethylase (EROD). These are sensitive biomarkers when measured in the livers of fish, especially when used in conjunction with other biomarkers such as CYP1A and CYP1A mRNA (Giuseppe B , M O Bonaca 2019).

Accordingly, biotransformation enzymes Phase II enzymes and co-factors involves the attachment (conjugation) of a polar molecule to the xenobiotic parent molecule. The resultant molecule is more water soluble, of higher molecular weight and is more effectively excreted (Giuseppe B , M O Bonaca 2019). It is Reduced and oxidized glutathione (GSH and GSSG) Glutathione S, transferases (GST) UDP-glucuronic transferases (UDPGTs) and total glutathione (Chrysogelou 2020).

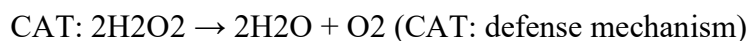
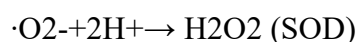
On the other hand, oxidative stress is in fact a disturbance of the balance between the production of active forms of oxygen (Reactive Oxygen Species, ROS) and the ability of one biological system to inactivate these toxic molecules and to repair the damage that is caused. ROS damages all components of the cell (proteins, lipids, DNA) (Chrysogelou 2020).

The Oxidative stress parameters are:

- Free radicals, such as the root hydroxyl ($\cdot\text{OH}$)
- Ions, such as hypochlorite anion (ClO^-)
- Free (root) radical combinations and ions, such as the anion superoxide ($\cdot\text{O}_2^-$)
- Molecules such as peroxide of hydrogen (H_2O_2).

Oxidative stress parameters

In order to maintain cellular homeostasis, it is necessary to establish a balance between creation and inactivation of ROS. Particularly the most common reaction that can take place on these cellular reactions are:



H₂O₂ από SOD activity: CAT liver (indicating oxidative damage)

Finally, the first defense line against oxidative stress combines SOD + CAT together (Chrysogelou 2020).

On exposure biomarkers can be defeated by non-enzymatic antioxidants mainly vitamins. In fact, they are biochemical indices of oxidative damage. Lipid peroxidation (LPOX) is the oxidation of polyunsaturated fatty acids as a consequence of oxidative stress aldehydes, acetone and malondialdehyde and these lipids mainly are Acyl-CoA oxidase, Methemoglobin, Nitric oxide synthase, Hypoxia inducible factor, Lipid hydroperoxide, Conjugated diene, Carbonyl proteins, 2-Keto-4-methiolbutyric acid, Oxidized proteins, DT-diaphorase (Catherine Desrosiers et al 2013).

Other exposure biomarkers are stress proteins or heat shock proteins (HSP). Stress proteins protect and regenerate cells in response to stress and harmful conditions. HSPs are those which respond (by increasing synthesis) to heat and other physical and chemical stresses, glucose-regulated proteins (GRPs) respond to oxygen or glucose deficiency, stressor-specific stress proteins include metallothionein's (MTs), heme oxygenase proteins or hematological parameters (Chrysogelou F. 2020). They mainly provide plasma enzyme activity by Erythrocyte counts, Hemoglobin content and Hematocrit (Catherine Desrosiers et al 2013).

Finally, exposure biomarkers can affect by immunological parameters with proliferating cell nuclear antigen (PCNA), calcium binding proteins (CBP), cell respiratory burst (RB) with total complementary activity of serum (TCAS) and neurotoxic parameters like cholinesterase (ChE) which are enzymes relevant to neural functions those with a high affinity for acetylcholine (AChE) and those with affinity for butyrylcholine (BChE). Accordingly, reproductive and endocrine parameters like

vitellogenin (VTG), genotoxic parameters. Genotoxic parameters refer to any changes to the genetic material of an organism caused by exposure to a contaminant DNA adducts. Mainly secondary DNA modifications that cause irreversible genotoxic events (J Evan Ward et al 2019)..

On biomarkers of effect there are measurable biochemical, physiological or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease (Eran Gefen et al 2003).

The main indices are the histopathology gross indices:

1. The hepatic somatic index (HIS)
2. The gonadosomatic index (GSI)
3. Visceral somatic index VSI
4. Condition factor (CF)
5. RNA: DNA ratio.

On susceptibility biomarkers, we have an organism's inherent or acquired ability to respond to exposure to a specific xenobiotic substance, including genetic factors and changes in receptors that alter an organism's susceptibility to this report (Carlos Navarro – Barranco et al 2020).

Identification of reliable biomarkers of fish welfare would greatly improve our knowledge regarding responses to stress (Chrysogelou 2020).

1.4 Sound pollution

1.4.1 Sounds and humans, sounds and sea environment

Soundscape, or else the landscape of sound, is an indicator towards the health of oceans. Marine acoustics is a useful tool to understand how noise effects on marine life and underwater ecosystems. On the other hand, which might be the minimum or maximum decibel levels a marine organisms can bear, and which are the minimum and maximum values of the sound frequencies that can tolerate? (Chrysogelou 2023).

Sound pollution or else sonic pollution has dramatically increased the past few years. Species and natural sounds in the sea are an important means of communication and are the primary way that many marine organisms gather and understand information about their environment, communicate by sending signals in long distances and many directions among each other. Aquatic animals use sound to find prey, locate mates and offspring, avoid predators, guide their navigation, and locate habitat, as well as listen and communicate or warn for danger etc. ([awiouline.org/content/ocean – noise](http://awiouline.org/content/ocean-noise) 04/07/2023).

For example, some common fish in the Mediterranean have certain muscles that produce sounds. *Argyrosomus regius* is one of them. This species has special "sound" muscles along their inner tissue surrounding, the abdomen, which is in direct contact with the urinary bladder, an organ that its main function is to control the buoyancy of the fish. These muscles produce special sounds that the fish uses. We do not know yet the actual use of this functionality, but we understand that it is of vital importance for its welfare and wellbeing.

Mainly anthropogenic sounds have proved to have negative effects on marine animals who constantly try to evolve and adapt new bio mechanisms to survive in always changing new conditions. Latest decades sound pollution values have increased due to fishing, shipping, over tourism and other tourism and non-tourism activities (regarding the high infrastructure development near coastline areas and much more) (www.awiouline.org 05/7/2023).

Studies on sound impact, regarding underwater ecosystems, do not show only that oceans are noisier but are making natural sounds (coming from the animals themselves) vanish (A. Chachouri et al 2021). It is well known in general terms, that soundscape, or else the landscape of sound, is an indicator towards the health of oceans (Chrysogelou 2023). Anthropogenic noise could become a major stressor for species facing multiple rapid changes and even more now than ever dominating underwater ecosystems crowding out species to deeper depths or even extinction (B. Geffroy, B. Sadoul 2019).

Sound pollution is not a fresh threat to species yet is still a threat. Anthropogenic actions cause sounds that increase stress levels to species. Sound itself can travel long distances

underwater, covering huge areas, and have effects on marine animals; by masking their ability to hear their prey or predators, finding their way, or connecting group members (Abir Chahouri et al, 2021).

During the pandemic and while the world stopped moving, scientists noticed and proved that there was a bloom of species in every coast ecosystem. The biggest number of species recordings was noted while nature wore its best showing us that nonhuman species appear when they listen to their familiar natural sounds. Even months like August or June when noise pollution is at its pick, the density of the biodiversity had been higher than ever since COVID 19 reduced tourism activities (Chrysogelou 2021).

Reduction of human noise during the COVID 19 pandemic brought positive impact on the marine ecosystems (Abir Chahouri et al ,2021).

Improving our lives by entering new technologies or AI on machineries that causes high decibel and high frequency sounds that animals cannot bear is indeed a problem that all species need to overcome (S. Elizabeth et al 2020). Living in complex environments such as oceans is even worse. Apart from the daily natural threats, species need to focus on their natural sounds avoiding confusing human made or AI (from machines) sounds which eventually will lead them to wrong conclusion that could and are proven to be lethal (Chrysogelou 2021).

In a silent world (which is not so silent but has ambient useful sounds for species) like the sea, sounds are important to survive (Chrysogelou 2023). Some species survival depends on sound and such pollution can be indeed lethal.



Graf 1: Recent progress in marine noise pollution: A thorough review (Abir Chahouri et al ,2021)

Generally, it is well known that small recreational boats can indeed span a wide range of frequencies and source levels (Graf 1), but the degree to which these impacts the soundscapes of shallow coastal habitats is poorly understood even up to now (Louise Wilson et al, 2022).

And this notice covers only a minor part of the bigger effect sound pollution provides within the marine environment. Imagine many sounds together in a bigger scale joined creating a huge hum. Living in a big city or near a highway can be stressful for humans (S. Elizabeth et al 2020). Living near a coast of a tourist financial depending on island, is a thousand times sound level higher. Excavators digging up the sand on coast in winter, building blocks making unhealthy human sounds, fixing and working on non-ecofriendly buildings, crowded and with high decibel values sounds and even more disturbing sounds that end up in the sea all year long. On the other hand, in high season things become even more difficult for underwater species. (Peter A. Todd et al 2019). Surviving in a high decibel environment can cause huge problems and make species survival unbearable (Chrysogelou 2020).

Humans living in urban areas are used to urban sounds. But still cannot accept intense sounds coming from certain machines. Such sounds can cause bigger damage to smaller animals. Using their sensory organs, e.g. their lateral line or other muscles/organs to understand and get information about the environment, a smaller animal can indeed be affected or even die by high sound exposure. Noise pollution affects behavior, physiological factors, and physiology itself on every species. Of course, it is known that

even for human species, intense noise exposure can cause serious psychological problems and ear loss. And what did evolution prove us through the centuries? Marine animals, thankfully, lost their biggest ear/hearing ability because they do not really need to expose themselves to any high stressful sounds. The past few years, they are threatened to lose their hearing sensory organs too (Chrysogelou 2023).

A basic example of the negative affect of sound pollution in animals is the effect of sounds on the ability of these animals to echolocate (Christine Ferrier – Pagesa et al 2021). Echolocation is connected to animals' sense or better it is used as a sonar. In fact, echolocation refers to the location of objects by reflected sound. In the quite natural underwater environment echolocation is used for several important biological activities. In a crowded environment studies showed that its use is zeroed out (I Van der 2022).

On the other hand, disastrous problems like sound pollution, need to be examined, understood, and solved. Natural soundscape is an essential property of any ecosystem, including coral reefs. Underwater noise pollution, from vessels and many other sound threats along with the rest contamination and pollution factors, causes major disturbances to reef organisms. The impacts of underwater noise pollution on the physiology and behavior of reef organisms must be studied. Guidelines must be presented, and strict measures must be taken under consideration. It is known that in 2030 there will be less fish and more plastic in the sea. Sound pollution is added in the list of pollutants affecting the species (Chrysogelou 2023).

Some years ago, and while the big industrial development started getting higher rates, no one thought the damages that these sounds were causing to all species (. While the word urbanization was officially part of our vocabulary, it was thought that the problem was solved. People living in the city along with their sounds, species living in the suburb or countryside without their annoying noises. Or at least there was a possibility for species to escape urban sounds, by moving on suburbs. And just when everything seemed to be working well, regarding the balance between nature and humanity, 17th century brings up a new financial promising income (Rae Zimmerman et al 2010). The new monster was called tourism. Reaching 20th century having spent more than 60% of our coastline areas investing in touristic industries, tourism is a fresh threat that causes irreversible damages to marine species. Convictions about the protection and

sustainable development ended up serving financial interests and zero any balance between humanity and nature, for example areas under Natura 2000 instead of being ecofriendly, or scientific friendly, turned out to be tourism friendly under the acceptance of Natura 2000 Body Managements and politicians. Activities such as diving, boat tripping etc. are proven lethal to the underworld along with over tourism activities bringing more problems. This recipe proved to be a slow speeding nuclear bomb causing harm rather than good. Sustainability does not come across with financial interests and the negative impacts are easily addressed and proved (Chrysogelou 2020).

Among the most dangerous commination's regarding underwater ecosystems, sound pollution keeps a high position which drives the attention of scientists and not unexcused. Human world, above the water or underneath, is indeed loud having low frequencies levels (Chrysogelou 2020).

Atmospheric pollution is not the only type of contamination that is harming living beings on the planet. According to the World Health Organization (WHO), it is one of the most dangerous environmental threats to health. And according to the European Environment Agency (EEA), noise is responsible for 12,000 premature deaths and 48,000 new cases of ischaemic heart disease every year (www.int 27/08/2019).

Drivers honking the horn, groups of workers drilling the road surface, aircraft flying over us in the sky. Noise and more noise. Cities have become the epicentre of a type of pollution, acoustics, which, although its invisibility and the fact that coronavirus crisis reduced it until almost yearn it, is severely damaging to human beings (Chrysogelou 2020).

Not all sound is considered noise pollution. The World Health Organization (WHO) defines noise above 65 decibels (dB) as noise pollution. To be precise, noise becomes harmful when it exceeds 75 decibels (dB) and is painful above 120 dB. As a consequence, it is recommended noise levels be kept below 65 dB during the day and indicates that restful sleep is impossible with nighttime ambient noise levels in excess of 30 dB (www.int 27/08/2019).

Causes of noise pollution

There are many sources of noise pollution, but here are some of the main ones:

Traffic noise

Traffic noise accounts for most polluting noise in cities. For example, a car horn produces 90 dB and a bus produces 100 dB.

Air traffic noise

There are fewer aircraft flying over cities than there are cars on the roads, but the impact is greater: a single aircraft produces 130 dB.

Construction sites

Building and car park construction and road and pavement resurfacing works are very noisy. For example, a pneumatic drill produces 110 dB.

Catering and night life

Bars, restaurants and terraces that spill outside when the weather is good can produce more than 100 dB. This includes noise from pubs and clubs.

Animals

Noise made by animals can go unnoticed, but a howling or barking dog, for example, can produce around 60-80 dB.

Not only does it hurt humans, but it is also bad for animals, too. According to the National Park Service (NPS) in the United States, noise pollution has an enormous environmental impact and does serious damage to wildlife. Experts say noise pollution can interfere with breeding cycles and rearing and is even hastening the extinction of some species (www.int 25/08/2019).

Effects of noise pollution on humans

Apart from damaging our hearing by causing — tinnitus or deafness —, constant loud noise can damage human health in many ways. Like any other pollutant noise can harm humans by causing:

Physical damage

Respiratory agitation, racing pulse, high blood pressure, headaches and, in case of extremely loud, constant noise, gastritis, colitis and even heart attacks.

Psychological damage

Noise can cause attacks of stress, fatigue, depression, anxiety and hysteria in both humans and animals.

Sleep and behavioural disorders

Noise above 45 dB stops you from falling asleep or sleeping properly. Remember that according to the World Health Organization it should be no more than 30 dB. Loud noise can have latent effects on our behavior, causing aggressive behavior and irritability.

Memory and concentration distraction or loss

Noise may affect people's ability to focus, which can lead to low performance over time. It is also bad for the memory, making it hard to study.

Interestingly, our ears need more than 16 hours' rest to make up for two hours of exposure to 100 dB (www.int 29/08/2019).

In recent years, the European Union has been promoting the use of new barriers that allow changes in sound waves, mainly for motorways and other forms of transport such as trains. Tests have been carried out in the Benelux and it seems that this is going to be extended to northern Europe. At the same time, work is being done on new, spongier, and quieter asphalts, which are also better for the environment (www.eeu.europa 30/08/2019).

Studies showed that anthropogenic noise can cause auditory masking, leading to cochlear damage, changes in individual and social behavior, altered metabolisms, hampered population recruitment, and can subsequently affect the health and service functions of marine ecosystems (H. Slabbekooru 2010). However, since different sampling methodologies and unstandardized measurements are used so far and the effects of noise on marine organisms are dependent on the characteristics of the species and noise investigated as it is up to now understood, it is difficult to compare the reported results (Sarah Piwetz et al 2015). Moreover, the scarcity of studies carried out with other species and with larval or juvenile individuals severely constrains the present understanding of noise pollution (J.F.D Sonzoa et al 2022). In addition, further studies are needed to reveal in detail the causes for the detected impacts. In this paper an attempt of understanding how loud ambient sounds is above the water and comparing them with air decibel levels and how species react on certain decibel level sounds is added on

getting to know better and understand more about how sound affects natural ecosystems and species (Chrysogelou 2023).

Sounds in the sea and their biological significances

Knowledge of the feature of sound is essential to a full understanding of the impacts of sound on marine organisms (Sarah Piwetz et al 2015). Frequency, wavelength, and intensity are the main parameters used to describe the characteristics of sound. One important feature of sound is its transmissibility in various mediums. Both the speed and the path upon which sound propagates are dependent on the characteristics of the medium through which it travels. In the sea, variations in the properties of seawater such as temperature, pressure, and salinity all have significant effects on the speed of sound propagation (Fillipo Ferrario et al 2015). As it travels through seawater with changing characteristics, the path that sound propagates changes as well due to refraction. The downward curving path found when sound travels in the thermocline is a typical example showing how the traveling path of sound is altered through refraction. As the sound travels downward in the thermocline, the water pressure increases gradually and leads to an upward alteration in the sound's traveling path below the bottom of the thermocline. In this circumstance, theoretically the sound may travel at this depth, also known as "the deep sound channel," without propagation loss (Chrysogelou F. 2023).

Propagation loss is the reduction of sound intensity with propagation due to absorption and scattering. In the sea, propagation loss is dependent on a variety of variables, such as the distance away from the sound source, the location of sound source, the number of particles suspended in the seawater, and the sound frequency. In the sea, the propagation of sound is also affected by reflection. Particularly when the water depth is less than the wavelength of the sound, a high propagation loss is expected (www.engineeringtoolbox.com 17/08/2023)

Noise may cause stress in animals, increase the risk of mortality by unbalancing predator-prey interaction, and interfere with sound-based orientation and communication, especially in reproductive contexts (L. Wilsona et al 2022). There is growing international concern regarding the impact of anthropogenic noise on marine

organisms (D Bagocius 2022). A number of studies have shown that the effects of anthropogenic sound on marine organisms can range from no influence on immediate death depending on the differences in the intensity and frequency of the noise and the distance from the noise source. However, the mechanisms underlying these effects are still poorly understood (Annebelle C. M. Kok 2021).

The first underwater noise pollution limits deliver on the Zero Pollution Action Plan (environment.ec.europa.eu 29/04/2023). The threshold values will contribute to set limits on where and for how long marine habitats can be exposed to underwater noise. Impulsive underwater noise, such as from oil and gas exploration, occurs in about 8 % of the EU's seas: it is particularly present in large areas of the Baltic, North and Celtic Seas, and the Mediterranean area. Maritime traffic is the main source of continuous underwater noise. With 27% of its area subject to shipping, the Mediterranean Sea sees the highest shipping traffic in the EU. This is followed by the Baltic Sea (19 % of the area). Overall, only 9% of the EU's Sea area has no shipping traffic. EU Member States will now need to take these threshold values into account when they update their marine strategies and eventually take actions in their programs of measures (www.eeu.europa 05/06/2023).

Accordingly, Brussels on the 5th of December 2022, new limits mean, that to be in tolerable status, no more than 20% of a given marine area, can be exposed to continuous underwater noise over a year. Similarly, no more than 20% of a marine habitat can be exposed to impulsive noise over a given day, and no more than 10% over a year (www.eeu.europa 05/06/2023).

Commissioner for the Environment, Oceans and Fisheries, Virginijus Sinkevičius, said: On the road towards COP 15 in Montreal, the EU is acting today to better protect marine life from underwater noise. The new, evidence-based noise pollution limits will help restore our seas, whilst steering us towards using them in a more sustainable way, in alliance with the life they host. They are based on the latest scientific advice available and were endorsed on 29 November by EU marine directors at their meeting under the trio Czech, French and Swedish Presidency of the Council (www.eeu.europa 05/06/2023).

1.4.2 Sound in water physics

Sound can be controlled by either reflecting it off a surface, diffusing it or by allowing it to be absorbed. Sound characteristics are echo and reverberation. Reverberation, in acoustics, is a persistence of sound, after a sound is produced. Reverberation is created when a sound or signal is reflected causing numerous reflections to build up and then decay as the sound is absorbed by the surfaces of objects in the space. In water reverberation is more complicated as mentioned. Echo, on the other hand, is a repeated sound that is caused by the reflection of the sound waves from the surface. The sound is heard more than once because of the time difference between the initial production of the sound waves and their return from the reflecting surface. Or a distinct, reflected sound wave from a surface (Chrysogelou 2023).

Moreover, sound is a pressure wave, but this wave behaves slightly differently through air compared to water (Table 2 and 3). Sound travels about 1500 meters per second in seawater. That's approximately 15 soccer fields end-to-end in one second. Sound travels much more slowly in air, at about 340 meters per second, only 3 soccer fields a second. Speed of sound in water at temperatures ranging 32 – 212 F (0 - 100°C) - Imperial and SI units (Table 1 & Graphic 1.2.1 and 1.2.2) (www.engineeringtoolbox.com 29/08/2019).

Speed of Sound in Water - imperial units (BG units) Temperature

Table 1: - t - (F), Speed of Sound in water - c - (ft/s) from engineeringtoolbox.com (page visit: 28/4/2019)

32	4603
40	4672
50	4748
60	4814
70	4871
80	4919
90	4960
100	4995
120	5049
140	5091
160	5101
180	5095
200	5089
212	5062

Graphic 1.2.1: Speed of sound in water regarding Temperature by engineeringtoolbox.com (page visit: 28/4/2019)

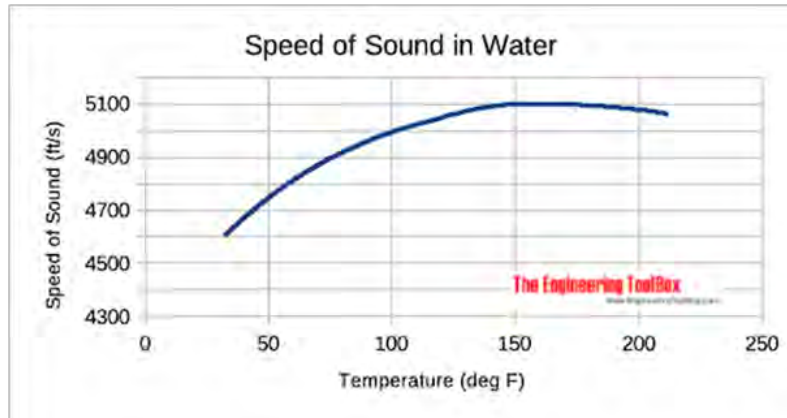
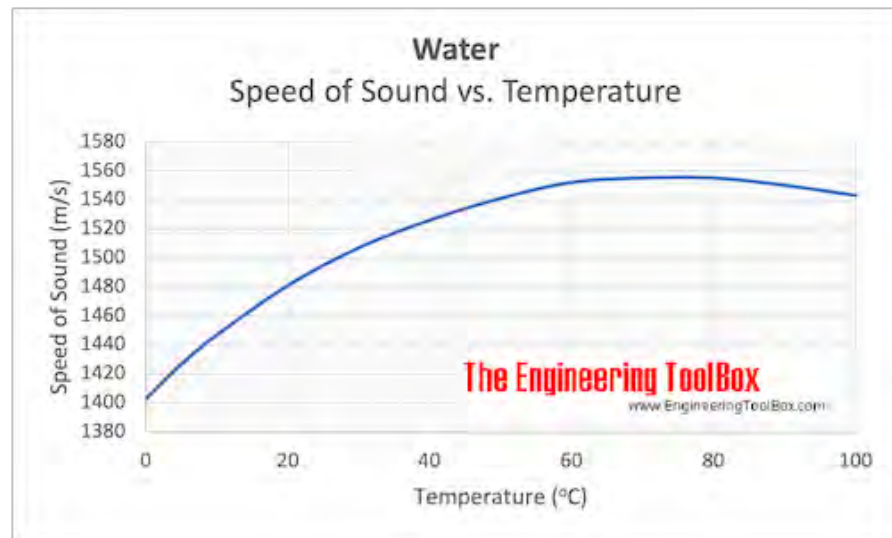


Table 2 and 3: Temperature - t - (°C), Speed of Sound- c - (m/s) Water & Sea Water values, & Seawater with salinity S = 35 (35.17 g/kg) 1 m/s = 3.28 ft/s ,by engineeringtoolbox.com (page visit: 28/4/2019)

Temperature °C
0
5
10
20
30
40
50
60
70
80
90
100

Water	Sea Water
1403	1490
1449	
1427	1490
1447	1522
1481	1546
1507	1563
1526	
1541	
1552	
1555	
1555	
1550	
1543	

Graphic 1.2.2: Speed and Velocity of Sound - Speed of sound on air, fluids, and solids.



Source: <https://www.engineeringtoolbox.com/>

According to an in vivo experiment that took place in a Mediterranean island port during May 2023, an old ferry boat's (meaning sound high) engine, along its course to the nearest port and while it was on the way to the programmed destination, reflected the sound wave at the same decibel levels for about an hour and more from zero point (departure port) to the 7th km (before arrival port) along the shore and had the same frequency and decibel levels to those of the starting point. Sound on air and above the water had the same sound wave level even in the distance of 7-8 km away both vertically directing and horizontal, again above the water and on air. All values were kept at same levels and sound seemed to have none changing decibel values even after some time. In the sketch and photo below, it is clearly shown how does this kind of sound reflects and affects the 7 km of coastline and underwater ecosystems (Image Sketch 3, 2023). The initial sound of the ferry boat has been recorded and has been stored due to data recording storage (Chrysogelou 2023).



Image Sketch 3: Sonic pollution for 7km along shore above the water and on air. (designed by Chrysogelou Foteini)

1.4.3 Sounds in the sea

Both natural and human made sounds retort in the water. What defines a natural and what a human made sound can easily explained. Natural sound mainly comes from the water itself by the wave movement, or from marine animals, and from natural events such as tectonic plates movement or cracks. On the other hand, a man-made sound is the one that humans create during their land or water activities which usually comes from structures or machines that help their living or work. Discussion or voice noises do not seem to reflect on fish although by experimenting individually voice or discussion sound (* low natural frequency sounds) is nearly lost in the water (Chrysogelou 2023).

The problem is that high frequency high decibel manmade sounds because of the high density of water travel faster and longer (deeper), confusing animals, or better disturbing their biological and ecological behavior. Their survival, on many occasions, depends on sound, as said, but even more, sound can have a negative impact on their physiology (Yue Man Yeung 2001). According to NOAA, sound can affect the hearing capability of animals, cause stress, dislodge species from their habitat, reflect negatively on the biological activities of fauna (www.nooa 29/09/2023).

Its speed and distance depend on the density (determined by its temperature, salinity, and depth) and the frequency of the sound is measured in hertz (Hz). Some sounds, particularly low-frequency ones, can cover vast distances, even across ocean basins (Chrysogelou F. 2023).

Depending on the density of the liquid element (temperature, salinity, and depth) and the sound frequency (Hz), sound has open access to travel with high speed in the water. Some sounds, particularly low-frequency ones, can cover vast distances, even across ocean basins. This capacity proves how threatened marine species are against human noise (Chrysogelou F. 2023).

Studies on how early fish can become sensitive to noise, showed that heart rates of embryonic clownfish were found to increase significantly with noise exposure (Simpson et al. 2005). Embryos as young as 3 days after fertilization exhibited this response. As embryos developed, their sensitivity to noise and to a broader spectrum of noise increased, up to the point of hatching. Not having symmetrical ear structures seems to hinder reef fish larvae from localizing sound well (Gagliano et al. 2008). These larvae had a more difficult time finding suitable settlement habitat and likely suffered and increased mortality as a result. Thus, the addition of anthropogenic underwater noise would probably further impact the survival of these fish. Different groups of trout from the same supplier varied in how they reacted to the same noise, suggesting there is a developmental or genetic component in sound sensitivity (Popper et al. 2007). Thus, preserving genetic diversity seems important in ensuring that enough individuals with good hearing ability are produced.

Results on sound stimulus effects on dusky damselfish behavior and cognition, suggest that anthropogenic noise applied through high-intensity music can increase anxiety and decrease memory retention in *S. fuscus*, suggesting the deleterious potential of noise for reef species (Jessica F.de Souza et al 2022).

It is clear, that our daily life has been improved by new technologies, and the farthest points have been brought closer by maritime traffic. These points are positive for people's lives, but they are sources of noise that negatively affect the marine ecosystem (Chahouri Abir et al 2022).

1.4.4 Effect of sound pollution in economy (marine ecosystems)

Noise levels are steadily rising, so ocean noise must be managed both nationally and internationally before any complete and irreversible damage occurs, regarding both biodiversity and marine ecosystem (Chrysogelou 2023).

It's an invisible threat, but noise pollution is a major — and often deadly — menace to ocean wildlife. Just as there's hardly a mountaintop free from the roar of airplanes overhead, there's virtually no place in the world's oceans where human sounds are not detectable (Valeriya Komyakova et al 2021). The loudest and most disruptive anthropogenic ocean sounds come from military sonar, oil exploration, industrial shipping, and the last several years by extended tourism industries (Chrysogelou 2023).

Naval sonar systems work like acoustic floodlights, sending sound waves through ocean waters for tens or even hundreds of miles to disclose large objects in their path. But this activity entails deafening sound. Even one low-frequency active sonar loudspeaker can be as loud as a twin-engine fighter jet at takeoff (www.nooa.gov 28/09/2023).

This onslaught of noise, which far exceeds the Navy's own safety limits for humans, can have a devastating effect on marine species — especially whales, who use their keen sense of hearing for almost everything they do. Sonar can displace whales from their preferred habitat and disrupts feeding, breeding, nursing, communication, navigation, and other behaviors essential to their survival. Most appallingly, sonar can directly injure whales — very often killing them — by causing hearing loss, hemorrhages, and other kinds of tissue trauma, or by driving them rapidly to the surface or to shore (I Van Der 2022).

Marine mammals are not the only animals that can be negatively affected by manmade sounds. Many marine species have been shown to be impacted by ocean noise to some degree. A cursory review of the literature on noise impacts revealed that at least 55 marine species have exhibited impacts from underwater noise in scientific studies. These impacts are not from nearby explosions, but more moderate noise. In cetaceans, the following 24 species have responded negatively to ocean noise: sperm whale, gray whale, bowhead whale, humpback whale, minke whale, Northern right whale, pygmy sperm whale, dwarf sperm whale, pilot whale, melon-headed whale, killer whale, beluga whale, Cuvier's beaked whale, Blainville's beaked whale, Gervais' beaked

whale, bottlenose whale, Risso's dolphin, common dolphin, bottlenose dolphin, humpback dolphin, white beaked dolphin, white-sided dolphin, Dall's porpoise, and harbor porpoise. At least 3 seal species have reacted negatively to underwater noise: elephant seal, gray seal, and harbor seal (Zhi-Tao Wang et al 2020). Regarding the effect on fish, the following 21 species have shown impacts to noise: pink snapper, goldfish, cod, haddock, rockfish, herring, sand eel, blue whiting, catfish, sea bass, thick lip mullet, horse mackerel, bluefin tuna, fathead minnow, toadfish, carp, gudgeon, perch, silver bream, trumpeter, and trevally (Annebelle C.M. Kok et al 2022). At least 5 invertebrate species (squid, giant squid, snow crab, lobster, and brown shrimp) and 2 marine turtle species (loggerhead turtle and green turtle) have shown negative responses to noise. Thus, it is hardly plausible that underwater noise would not affect marine biodiversity to some degree (Louise Wilson et al 2021). Noise is listed as an intrusion and one of the impacts that can result in a substantial loss of biodiversity over time in sensitive marine habitats (Warner 2008).

Low-frequency sounds are used by other marine vertebrates, including sharks and bony fish (Myrberg, 1972, 1978, 1980, 1990). Sharks are attracted to sources emitting such sounds as possible food indicators (Myrberg, 1978), and many species of fish use low-frequency sounds for communication (Demski et al., 1973). Pallid sturgeon *Scaphirhynchus albus* and shovelnose sturgeon, *S. albus* produce sounds during the breeding season (Carol E. Johnston & Catherine T. Phillips, 2003).

On the other hand, dolphins and other species tend to disappear in lower depths when strong sounds appear but if they manage to escape a speed boat or a ship fast enough, so they won't be killed (Chrysogelou 2023).

The impacts of noise can work cumulatively or synergistically with other environmental threats to cause, for instance, more by-catch (net entanglements), or ship strikes due to a failure to detect fishing gear or hear oncoming ships (M.A. Wale et al 2021). Already food-stressed animals, e.g., from chemical pollution or overfishing, may be unable to hear their prey because of anthropogenic ocean noise (Annebelle C.M. et al 2021). Different noise sources can also cause a magnified effect that may be greater than the sum of the individual noise sources, such as when seismic noise is superimposed on shipping noise (Chrysogelou 2023).

Biodiversity, in general, play an important role among the global health cycle. Loss of species means polluted water. Biodiversity and water quality are bounded and depend on each other. Loss of species means polluted ecosystems, and polluted ecosystems means zero human – marine biodiversity interaction, or better, death on both parties (Chrysogelou 2020).

Of course, there is also the importance of species itself. 75% of cancer drugs come from biodiversity. A great percentage of these species are marine species. Sponges, corals, and other reef organisms may be the next step to breaking down the shield that bacteria use to protect themselves from antibiotics. The sea may hold the key for new scientific discoveries to our welfare. But not if we don't keep it healthy, pollution & contamination free. The next cure could be hidden there. We rely on nature and species. Noise pollution is a threat that if not addressed the fastest, results can be fatal and irreversible both to nature and humanity (www.int 12/09/2019 and Chrysogelou F. 2020).

Biodiversity, play an important role among the global health cycle. On the other hand, loss of species means polluted water. Biodiversity and water quality are bounded and depend on each other. Loss of species means polluted ecosystems, and polluted ecosystems means zero human – marine biodiversity interaction, or better, death (Chrysogelou 2023).

1.4.5 Purpose and goal

Soundscape, sonic scape or else the landscape of sound, is an indicator towards the health of oceans. Marine acoustics is a useful tool to understand how noise effects on marine life and underwater ecosystems. On laboratory, tests about the impact of noise in fish can give us an image of how these sounds might affect species in wild (Chrysogelou 2023).

The purpose of this study is to understand and record the impact of sound in the sea firstly and then continue by explaining how three specific sounds can affect fish in tanks and in extend, in wild, more or less. Keeping in mind that in controlled environments, such us a laboratory there are no external noises but those of tanks or aquarium equipment, and fish usually after any transport live in quiet environments getting used

to only aquarium sounds or other sounds that can be heard within a room or a laboratory. Nevertheless, we want to examine the difference in intensity and frequency of extraneous sounds from anthropogenic activities, in underwater ecosystems and organisms. To understand the results of high and low frequency noise on species and observe the mobility and movement of the underwater biodiversity towards some sounds. This paper aims to propose an application using annotated data that were created for environmental conservatory purposes. The data collected are raw natural field noise data from diverse natural and artificial sounds coming from human made noises or natural noises. Along with comet assay experiment usage it might be easy to understand up to a point noise impact and its effects on marine organisms.

The prompt of proceeding to the following experimental attempt was first our scientific curiosity to understand the natural environment and then to take better look whether certain noises cause any further problems. Trying to record the pure decibel levels of sounds in nature without the interruption of artificial man-made sounds, gives us a bit of what is really happening on these ecosystems under real circumstances and how loud those ecosystems can be. On one hand, the goal was to see how loud some natural sounds are (of wave movement, wind, sea movements etc.) regarding the ecosystems, both above the water and air. Then to compare these prices and understand the difference between the loudness of these sounds when they effect the water and when air. The reason to do this was to use as much information about the way natural sounds act and impact on the ecosystems firstly and continue using this information by making an in vivo experiment on species (fish) in captivity and examine their stress levels on cells, using comet assay, on the other.

Regarding field experiment, it is useful to report that generally every bit of information on the effects of sound in nature is rather useful since species depend on sounds as mentioned before. It is also useful to understand and finally conclude on how much artificial sound pressure, these organisms can bare, since pollution levels have rapidly increased the past years.

Making on field experiments can give us a bit of what is really happening in natural environments and more answers on how these animals live and survive on these complex environments like the sea.

Especially for fish, which are included in our diet providing us with nutrition values, we understand that their welfare, both in wild and in captivity is of vital importance for humanity's health too. Nevertheless, for fish in captivity understanding how sounds

effect on them, can give us answers on how to create better products and might increase their nutritional and price value. Even in wild, pollution factors can cause apart from species extinction, less attractive and nutritional products too. In both cases, sustainability and welfare of marine products is the main interest of both experiments and studies regarding pollutants and contaminants in general.

In this work, besides revising the solutions available to obtain reproducible and reliable quantitative data, we developed a parallel experiment to understand how natural (ambient sounds) reflect on field, on coastline areas of a typical Mediterranean ecosystem, and to get a clear image on how loud natural world might be.

2. Methods and Materials

2.1 Sound field experiment method

According to the needs of this study two methods were used. On field and in the laboratory.

Three (3) random stations, sixteen, $15 + 1 \times 2$ (plus one test day) measurements on both air and above water, natural decibel level value along with external sound characteristics were recorded mid hours, mostly between 12:00pm 16:00pm time. Ten recordings each double level values (water, air) of eleven (11) seconds. Environmental factors were noted (temperature, wind, humidity levels and wind direction). Between measurements the weather conditions were almost the same. Eleven seconds are the least appropriate time to understand the decibel level impact since low tide and ebb tide were both on, within the measurements. Description of other environmental factors useful for this experiment were noted. Maximum, minimum, and average decibel values were recorded. Values were analyzed and corollaries were listed and analyzed. Accordingly, regarding the conclusions and results were discussed (Chrysogelou 2023).

Fields on the same perimeter (not far from each other and in almost 1km diameter, same station in fact but different spot). Depending on the weather and the less dangerous access the working field was chosen. A sandy beach, rocky beach, small gravel beach on the same perimeter. Recording of natural surroundings sounds, and their decibel levels were stored. Ten recorded decibel values each day. Comparisons were thought necessary (Chrysogelou F. 2023).

During the procedure all safety measures and licenses were taken under consideration and the equipment was a SONY HDR AS50 used to record some of the noises and the landscape, and a Decibel meter Radioshack 3302050 sound meter 126 - 130 dB, giving accurate results and numbers both on air and above the water. Recordings were filed as well as the values. No significant loss was noticed (almost 0,50 to 1,00decibel off) from above the water measurements when the instrument was covered, and the experiment continued accordingly. The experiment lasted 15 days all weather conditions regarding the month and season (Chrysogelou F. 2023).

Keeping in mind the physical and chemical status of sea water and the physics of sounds above it, it is rather understood that in this experiment only a first step to understand on how natural and artificial sound reflects the water and in extend species that live in it. And for this reason, keeping and recording just decibels was thought to be a good start to evaluate the way sound reacts above the few first meters of coast waters (Chrysogelou 2023).

The importance of natural noise decibel levels has important impact on marine organisms, especially in wintertime. Marine acoustics is a useful tool to understand how noise impacts marine life. According to the weather the decibel prices were restored. Conclusion notes about each day were also observed and all statements were restored. Decibel values were processed, and results were analyzed. Keeping in mind that pressure and depth as important factors that affect sound in water, we assume that decibels were recorded at standard atmosphere of sea level at average atmospheric pressure 1.013×10^5 Newtons per square meter (N/m² also known as the unit Pascal or Pa) because of the depth (Chrysogelou 2023).

2.2 Comet assay method

The second part is to experiment on tanks with fish in captivity. Three tanks of fish and three different sounds were applied, and different decibel level values sounds were placed in the tanks (on air). After one hour of the repeated sounds three fish of each tank were collected and comet assay (single cell gel) method was applied on each. The investigation of stress levels with comet assay application on fish cells was thought to be the best offering us the useful stressful level values results for fish in captivity.

Additionally, after 24 hours and again half an hour after feeding, another three fish from each tank were selected and once more comet was applied.

Comet assay (or single – cell gel) is a technique that allows detection of DNA damage at the level of isolates cells. Its name is due to the image of a comet which is formed in the case of a large area damage, during the exit of fragmented DNA from nucleus and cell and movement of to the rise. The comet consists of the head, which represents DNA that does not migrate out from the core and the tail, in which are the DNA fragments coming out of the nucleus and the cell body.

Additionally, comet assay utilizes single cells to measure DNA damage. First, cells are embedded into agarose and then placed into a slide. The slide is then immersed into lysis solution to break open the cell membrane. After the cells are lysed, DNA is denatured under neutral or alkaline conditions and run through electrophoresis. Or else Comet assay, also known as single cell gel electrophoresis (Images 3 & 2), is a sensitive technique for measuring DNA damage in single cells and has become well known in the field of toxicology and drug discovery. Comet assay is based upon the ability of denatured cleaved DNA fragments, or damaged DNA, to migrate out of the cell under electrophoresis creating a "comet tail", while the undamaged DNA remains within the cell membrane creating the "comet head." Comet assay is most run under alkaline conditions to detect single- and double-stranded DNA breaks but can also be run under neutral conditions to detect only double-stranded DNA breaks.

While performing comet assay utilizes single cells to measure DNA damage. First, cells are embedded into agarose and then placed into a slide. The slide is then immersed into lysis solution to break open the cell membrane. After the cells are lysed, DNA is denatured under neutral or alkaline conditions and run through electrophoresis. Cells are then stained with a fluorescent or chromogenic dye and visualized under a microscope. The resulting image obtained resembles a comet that includes a distinct head and tail. The comet head consists of undamaged DNA while the comet tail consists of the broken DNA strands (Images 2.1 & 2.2). Analysis of the comet tail can be performed by hand or with software.

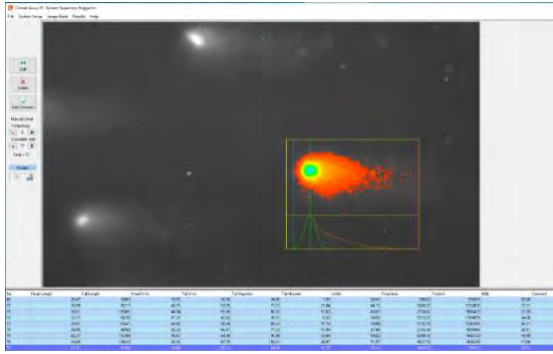


Image 2.1: Comet sample 1.1 Day 0

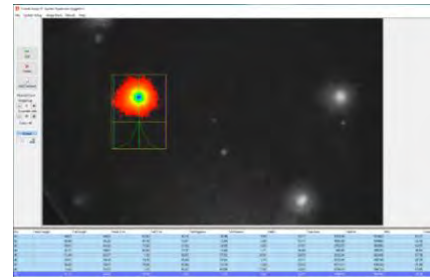


Image 2.2: Comet sample 1.3 Day 0

Photos by Christina Papadouli

2.3 Protocols

2.3.1 Soundscape field experiment protocol

According to the protocol this is the procedure that was followed:

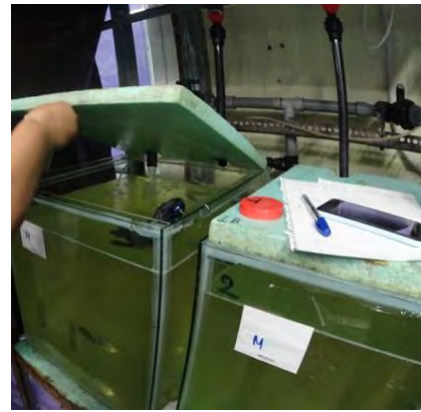
- Step 1: Choose field, the area must be quiet. 100% man-made sound free area (both artificial and natural man-made). Better a hidden easily accessible place. Access by land. Not by boat. This will help you record all natural biotic and non-biotic factors.
- Step 2: Select 3 – 4 different access and quality places.
- Step 3: Visit the field for at least 30 days (everyday) to ensure noises that are not accepted and can affect the procedure are escaped.
- Step 4: Choose and prepare the equipment.
- Step 5: Choose your decibel meter. Choose an electronic HQ with microphone extension. Cover it with any waterproof material (e.g., rubber one use surgery gloves) and check values both on air and above the water. Make sure you have plenty of checking tests.
- Step 6: Make at least 30, soundchecks both on air and water in each area during the day and on different time.
- Step 7: Above the water measurements double the tests.
- Step 8: After the checks choose the time between the experiment will be held. Mid-day was thought to be the best time because natural sounds (in our case) were at their pick. Values were more representative between 12:00 – 15:00.
- Step 9: Begin the process.
- Step 10: Choose specific arrival time and note the facts.
- Step 11: Observe the physical and meteorological factors.
- Step 12: Record the physical and meteorological factors.

- Step 13: Evaluate the starting time.
- Step 14: Begin the experiment.
- Step 15: Make 10 repeats of 11 seconds one after another. Make sure there isn't a big deviation gap between measurements.
- Step 16: Record average, lowest and highest values.
- Step 17: ALWAYS observe and record every useful detail.
- Step 18: Write (record your values on paper) all the details down.
- Step 19: In case of any unaccepted or failed attempt repeat the procedure from the start (all 10 of 11' each measurement).
- Step 20: Use any statistical program to proceed with the recording of your values (such as excel).
- Step 21: Statistical analysis, results and discussion following
 - Final step: Name, Date, Signature (Chrysogelou 2023).

** You may use scrap paper for your notes and avoid spending paper, giving a tree a chance to survive (Chrysogelou 2023).

2.3.2. Laboratory conditions

On the second stage a laboratory experiment and test held place. Three treatments with ten fish in captivity each (*Sparus aurata*), different mature stages and weight, kept on clean fish tanks. No 1 treatment, No 5 treatment and No 17 treatment. Fish were on non-feeding diet for at least two days before the experiment. One was kept sound clean (no treatment). No 1 treatment was applied on a real ferry boat field sound (Images 2.3.22 and 2.3.23 Treatment 1), No 5 treatment was applied under a punctuated continuous beep beep sound and one last treatment 17 ,without any sound. On noise/treatment tanks, the sound was applied on specific time and after 10' fish were fed and any behavioral reaction during their feeding was observed. 60' after their nutrition 3 three fishes were collected from each tank and comet applied on liver tissue. 24h later feeding and comet was repeated.



Images 2.3.22 Treatment 1 under ferry boat sound Day 0 & 2.3.23 Day 1

Fish weighting and feeding portions during experimental procedure:

Table 2.3.22: Sample weight (g) values

		Day 0		
Treatment 17	25g - 17.1sample	32g - 17.2 sample	22g - 17.3 sample	Day 0
Treatment 5	19g - 5.1 sample	24g - 5.2 sample	24 - 5.3 sample	Day 0
Treatment 1	15g - 1.1 sample	36g - 1.2 sample	22g - 1.3 sample	Day 0
		Day 1		
Treatment 17	30g - 17.4 sample	27g - 17.5 sample	27g - 17.6 sample	Day 1
Treatment 5	30g - 5.4sample	22g - 5.5 sample	24g - 5.6 sample	Day 1
Treatment 1	13g - 1.4 sample	27g - 1.5 sample	34g - 1.6 sample	Day 1

Table 2.3.23:Feeding portions on both days

	Feeding portions (g)		
	Total food weight (g)	Total food consumed (g) Day 0	Total food consumed (g) Day 1
Treatment 1	76,66 g	8,357 g	3,414g
Treatment 5	80,988 g	11,577g	6,227g
Treatment 17	75,508 g	6,941g	3.8g

Treatment 1 seemed to have an average effect on fishes that consumed 11,771g total portion, under normal sound conditions. Treatment 5 had the biggest consumption weighting 17,804.17th treatment had the lowest food consumption of total 10,741g, both days. The least food occurred on treatment 1 over Day 0. The more, occurred again in Day 0 and in treatment 5. Feeding (Tables 2.322 7 2.3.23) stopped by the moment of fish food saturation which was about two to four minutes long under treatment 1, more than five minutes on treatment 5, and less than three and a half on treatment 17. This can show that punctuated continuous beep sound had an effect on the feeding behavior considering as normal the behavior of the fish on treatment 17.

Physics and chemical parameters were standard, and the temperature was $\pm 21^{\circ}\text{C}$ with salinity levels at about 28 – 30 ‰, pressure on 1 ATM (almost 0,50meters of water on tanks), 1000 decimillibars is one decibar and approximately equal to 1 atmosphere and pH on basic levels 7 – 8.

Image 4 & 5 below : Comet assay method. Basic steps. In tube, dissolution of liver tissue with plastic equipment & smashing tissues with lancet after scalpel dissolution (by Chrysogelou Foteini 2023).



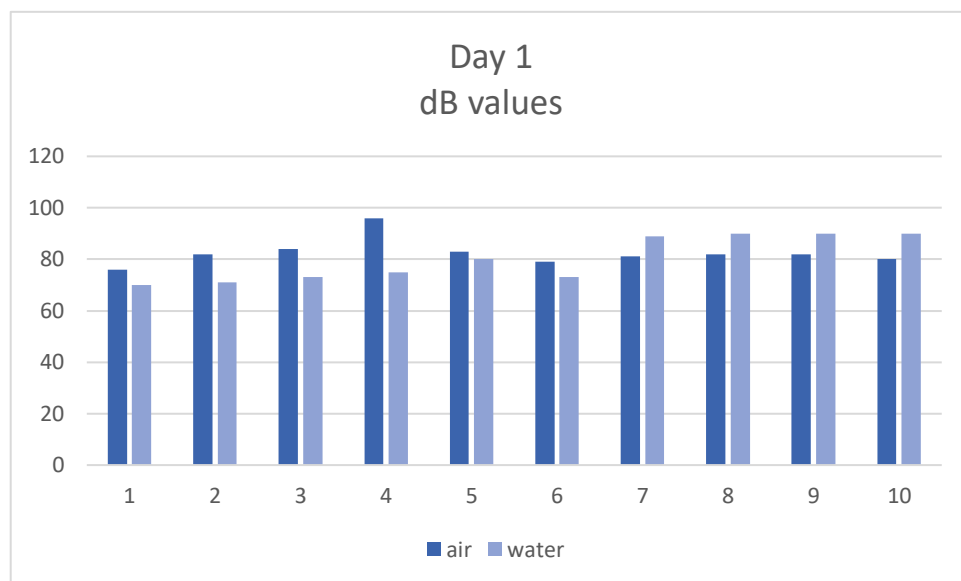
3. Discussion and results

3.1 Field experiment results

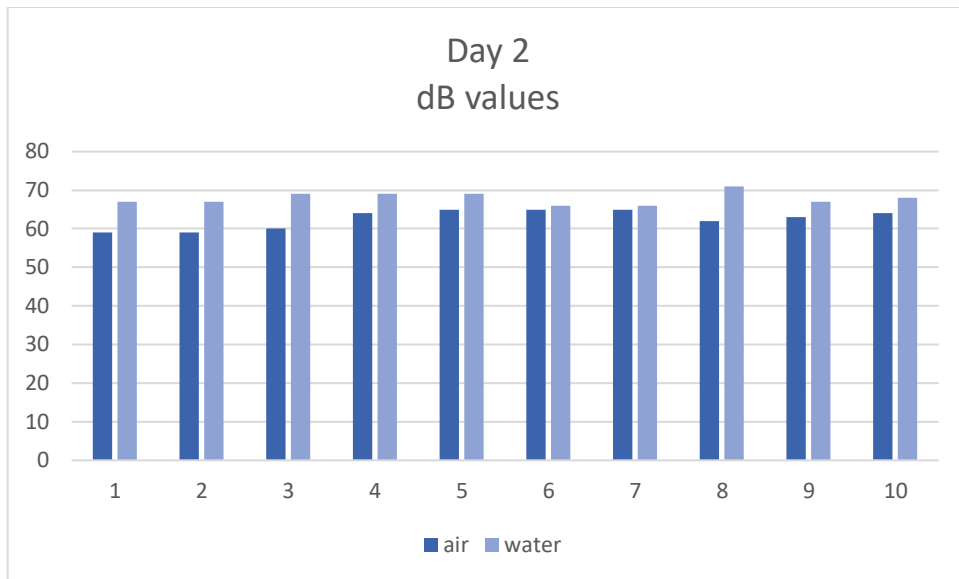
For this reason, meaning to understand how sound reflects on the sea the results of decibel levels of ambient sounds appeared to give the above values explaining exactly the difference of decibel levels and sound frequency. There are limits on how high or low sounds must be in order to be accepted by species. For example, you can have high decibel level sounds that species can accept (tolerate) but if their frequency is not within the physical affordable values then these sounds can harm. Otherwise, even the opposite can be harmful. Meaning low decibel values but high or even low frequency levels that

can harm. It is well known that animals can hear or not hear specific sounds that we (humans) either listen or not.

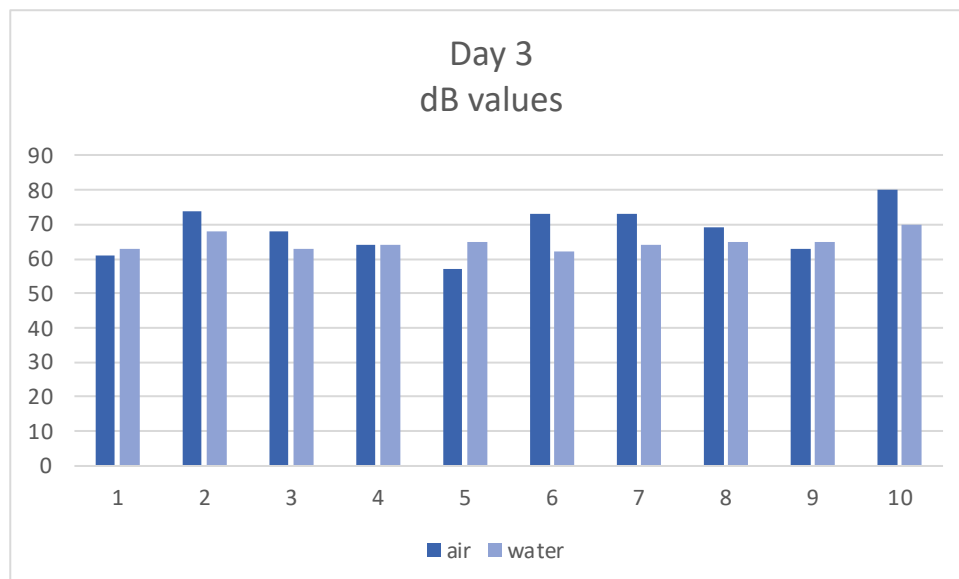
According to the graphics by an experiment in 2023 on the difference on decibel levels (Graf 3.1.1 – 3.1.16) of ambient sounds on air and above the water (up to 25 -30meters deep, coastline), by taking a first look, it was understood that there isn't any significant difference on decibel level sounds above the water and on air for ambient sounds (wind, currents, waves, rain etc). and value levels showed a regular distribution. As a matter of fact, in some cases results showed that values tend to be the same. The truth is, as said already, that there is some difference. Although small, yet there is one. In the cases that values differ, mostly on specific wind blow direction (always talking about the certain area) and others of certain wind direction. Even a light storm may not have impacted on sound levels in both means and continue keeping equal and steady values within the extended range.



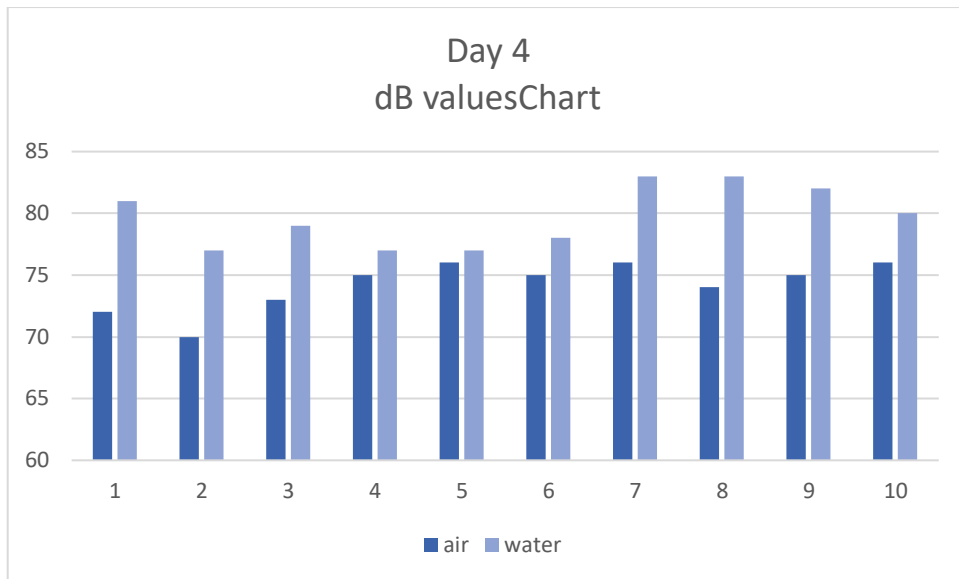
Graf 3.1.1.1 Comparing water and air dB values Day 1st



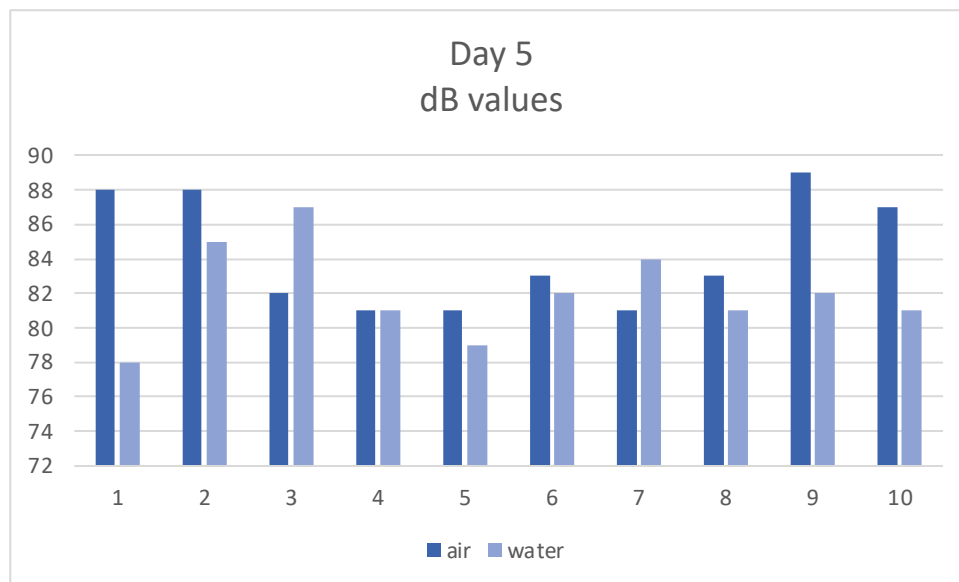
Graf 3.1.2 Comparing water and air dB values Day 2nd



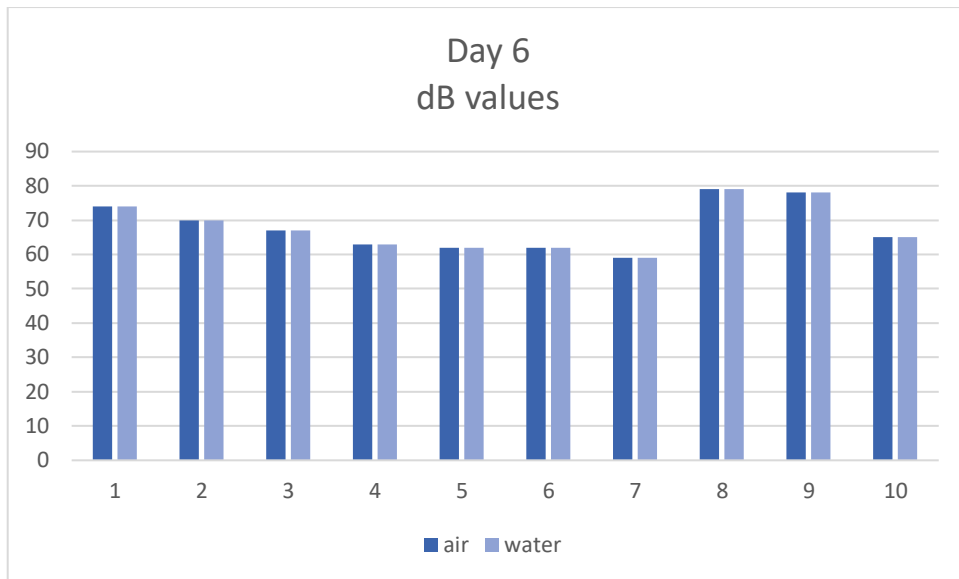
Graf 3.1.3 Comparing water and air dB values Day 3^d



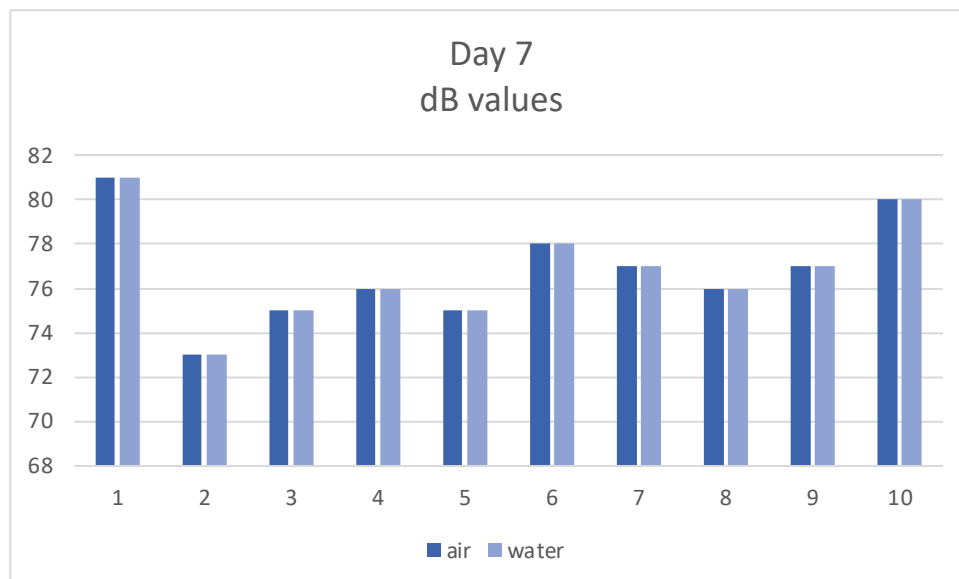
Graf 3.1.4 Comparing water and air dB values Day 4th



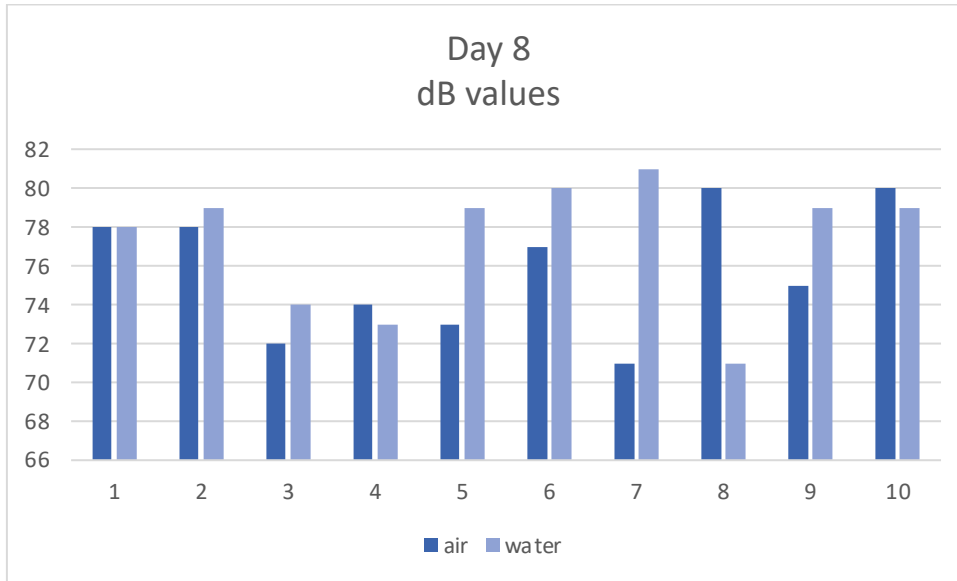
Graf 3.1.5 Comparing water and air dB values Day 5th



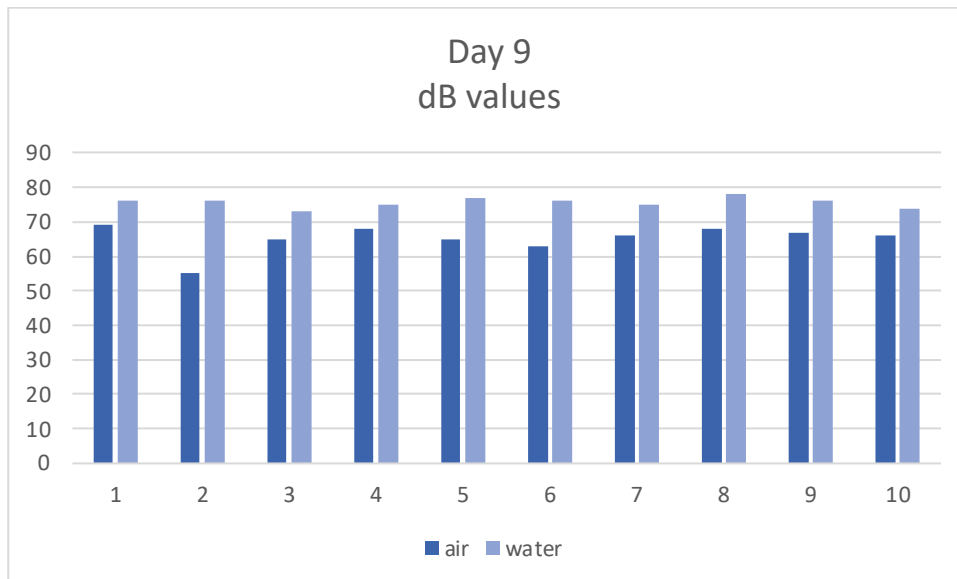
Graf 3.1.6 Comparing water and air dB values Day 6th



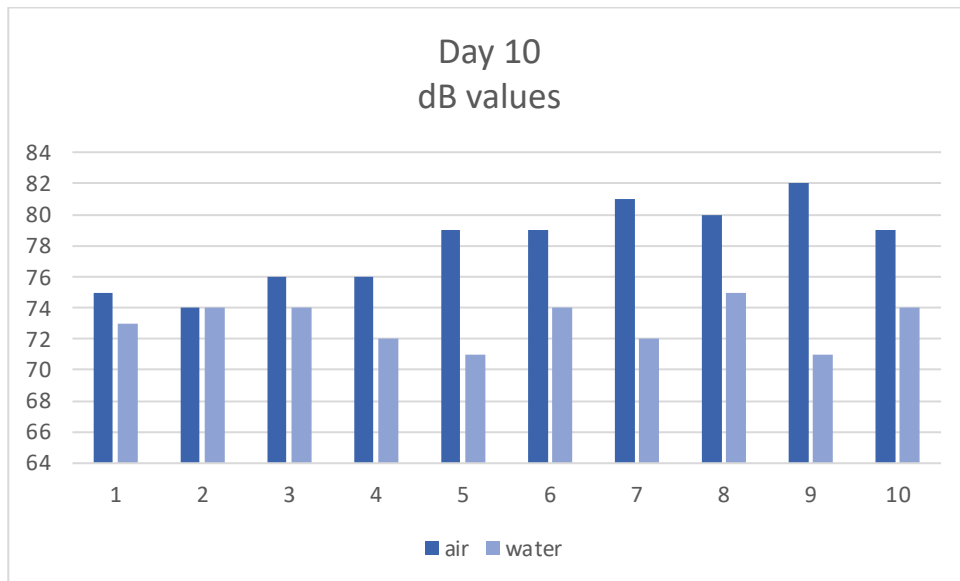
Graf 3.1.7 Comparing water and air dB values Day 7th



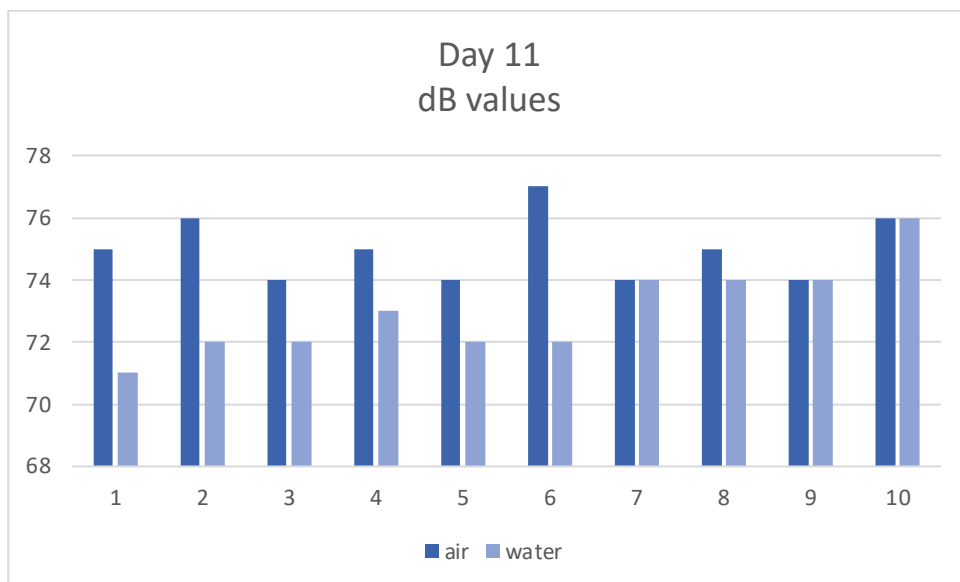
Graf 3.1.8 Comparing water and air dB values Day 8th



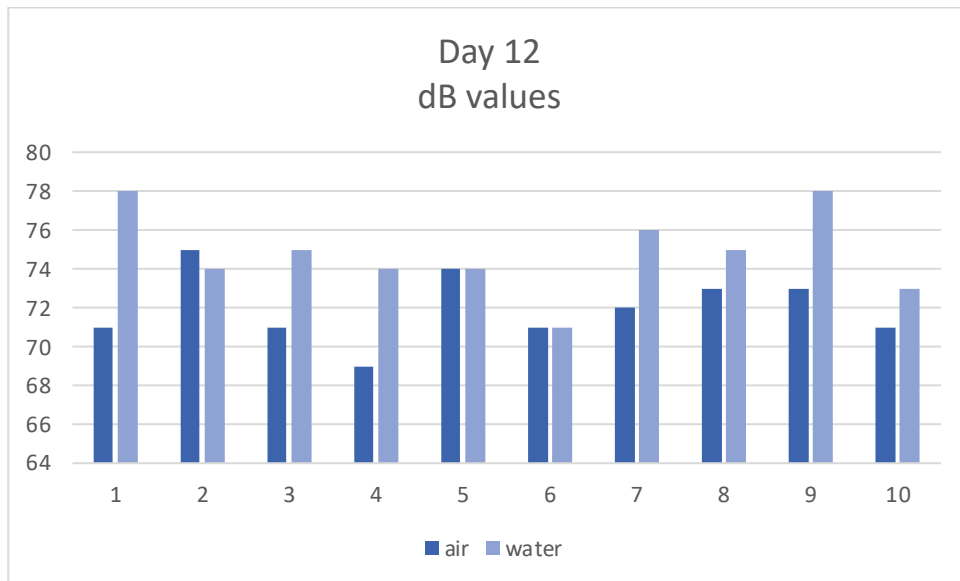
Graf 3.1.9 Comparing water and air dB values Day 9th



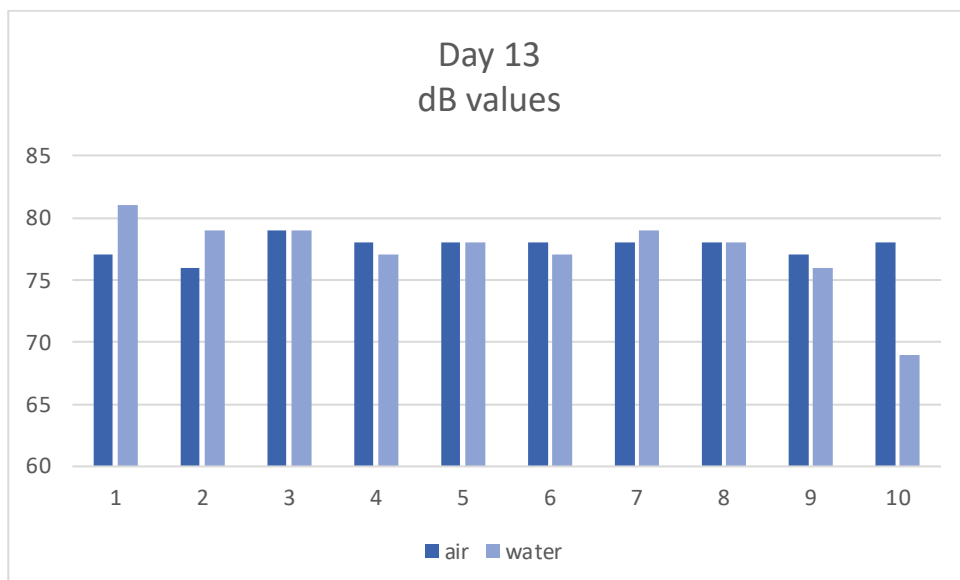
Graf 3.1.10 Comparing water and air dB values Day 10th



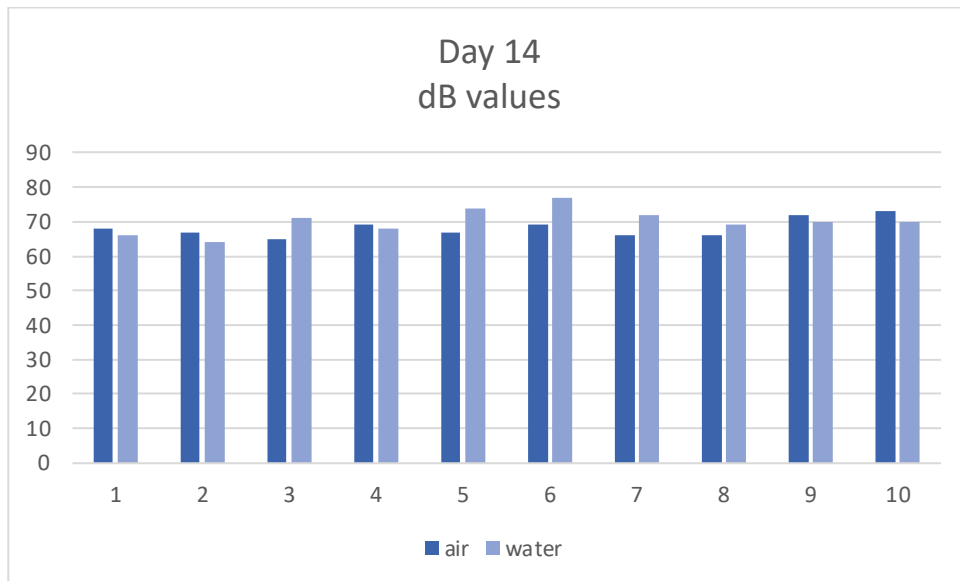
Graf 3.1.11 Comparing water and air dB values Day 11th



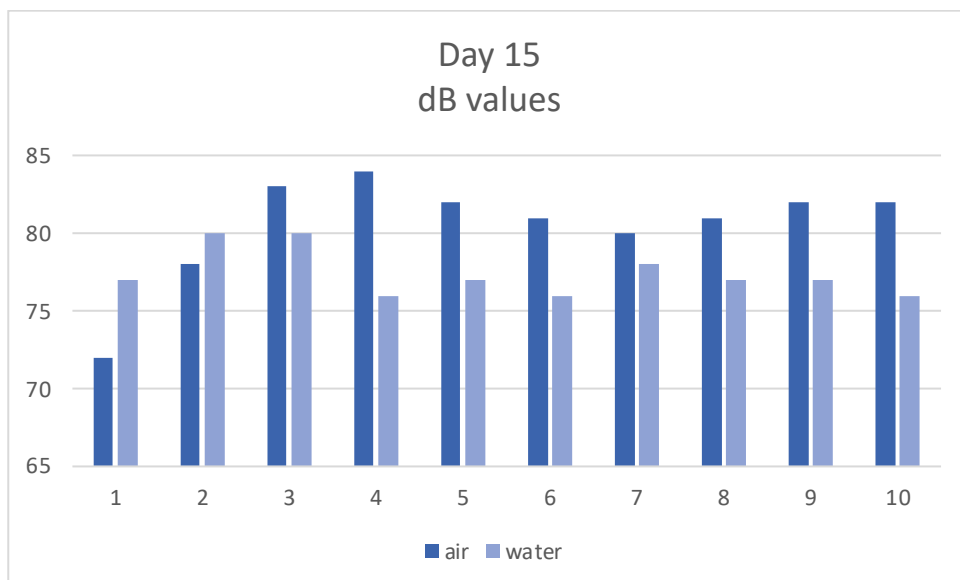
Graf 3.1.12 Comparing water and air dB values Day 12th



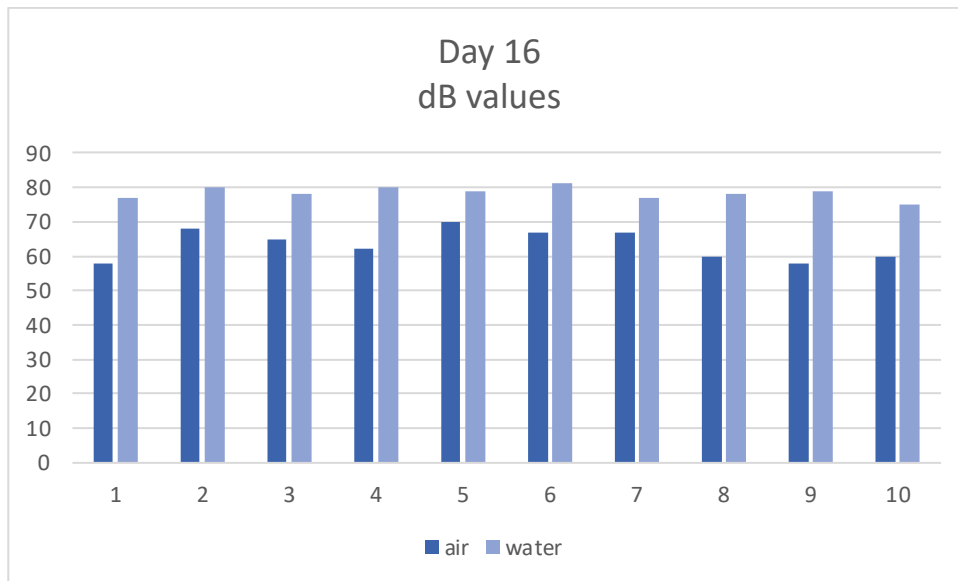
Graf 3.1.13 Comparing water and air dB values Day 13th



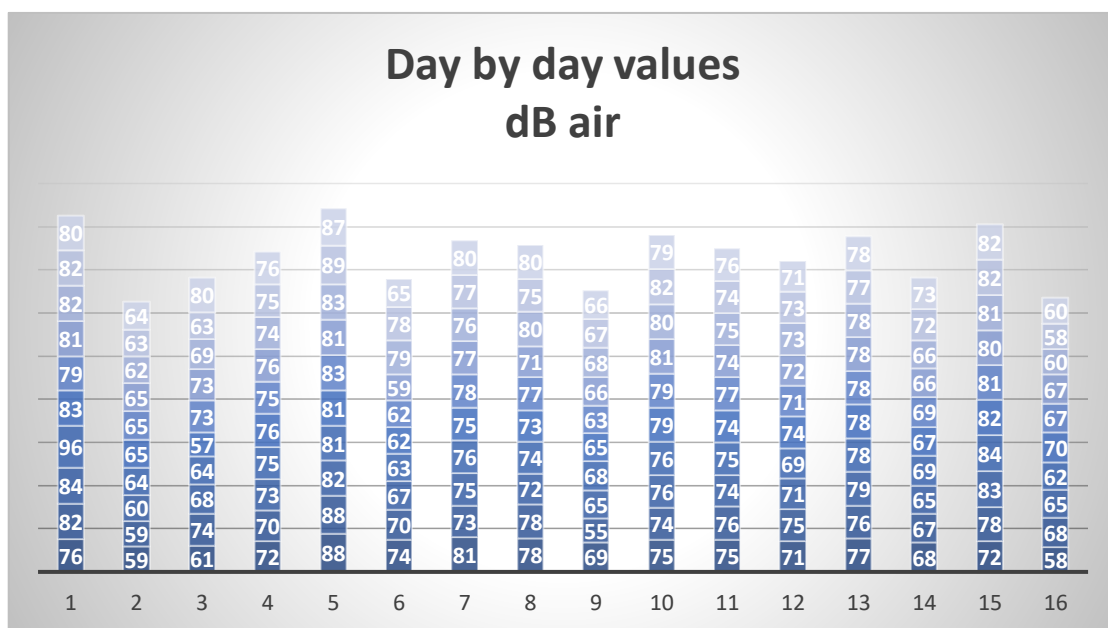
Graf 3.1.14 Comparing water and air dB values Day 14th



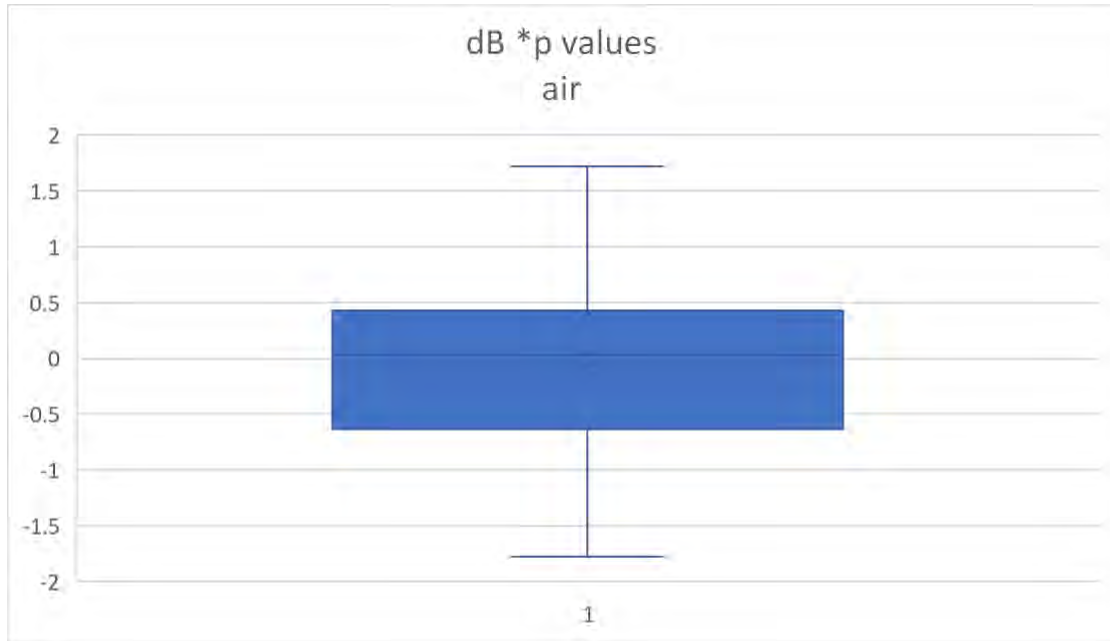
Graf 3.1.15 Comparing water and air dB values Day 15th



Graf 3.1.16th Comparing water and air dB values Day 16th



Graf 3.2 Record of day by day dB values on air

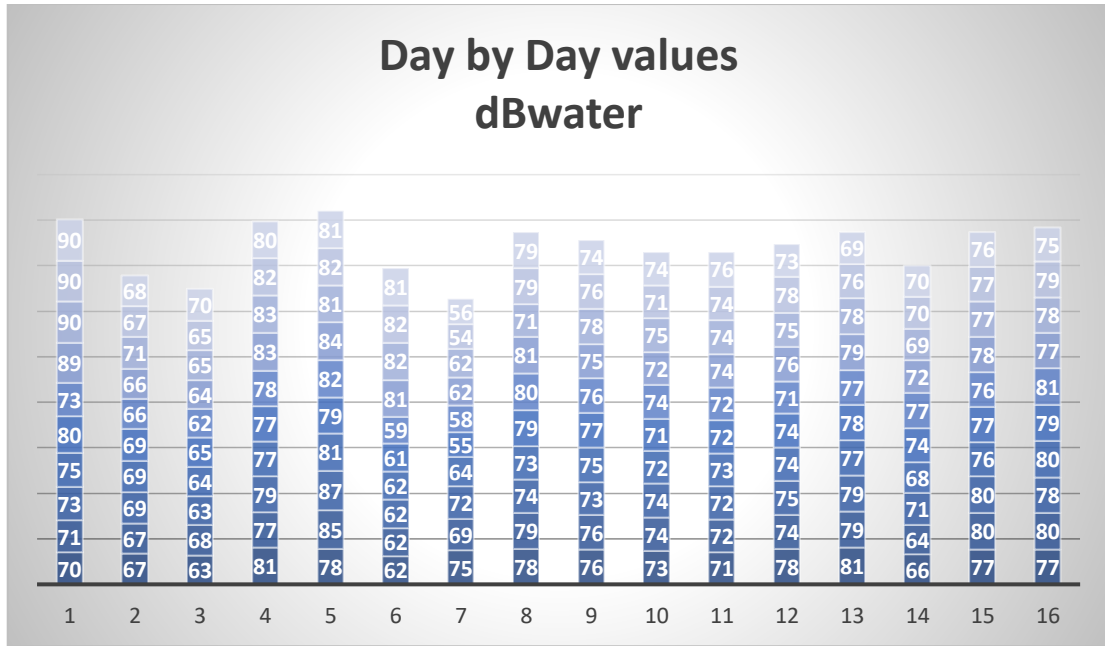


Graf 3.2.1 *p values on air / *p > 0,05

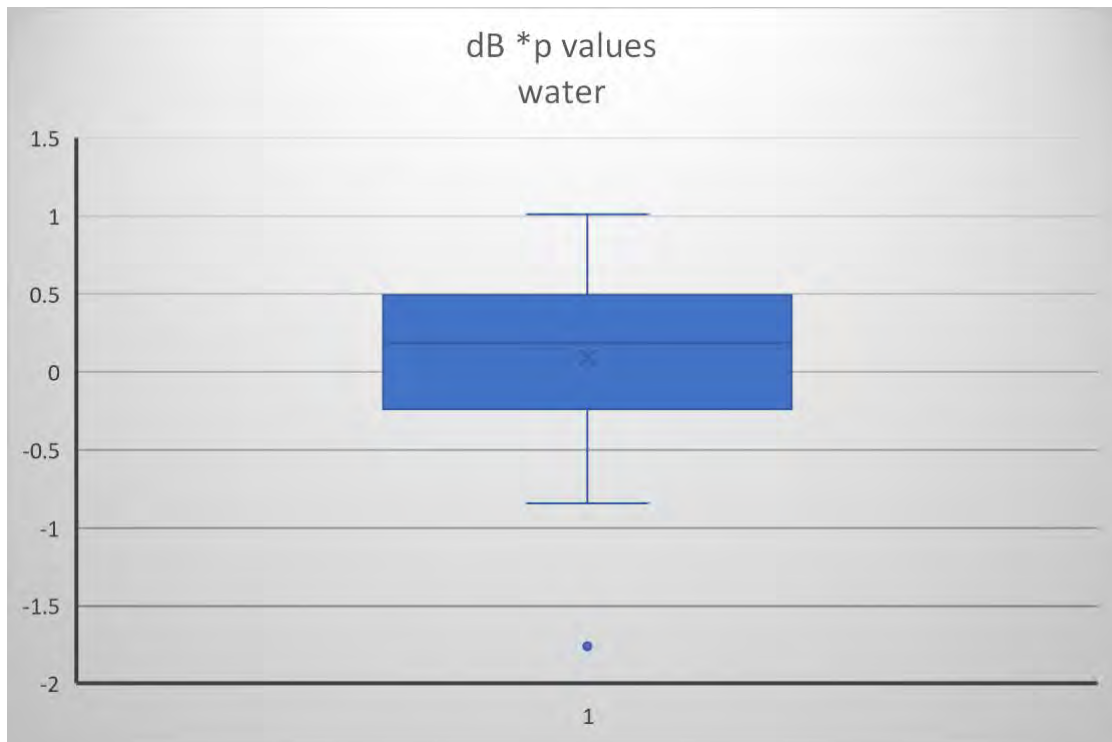
Table 3.3 Average STDV & MEDIAN values on air

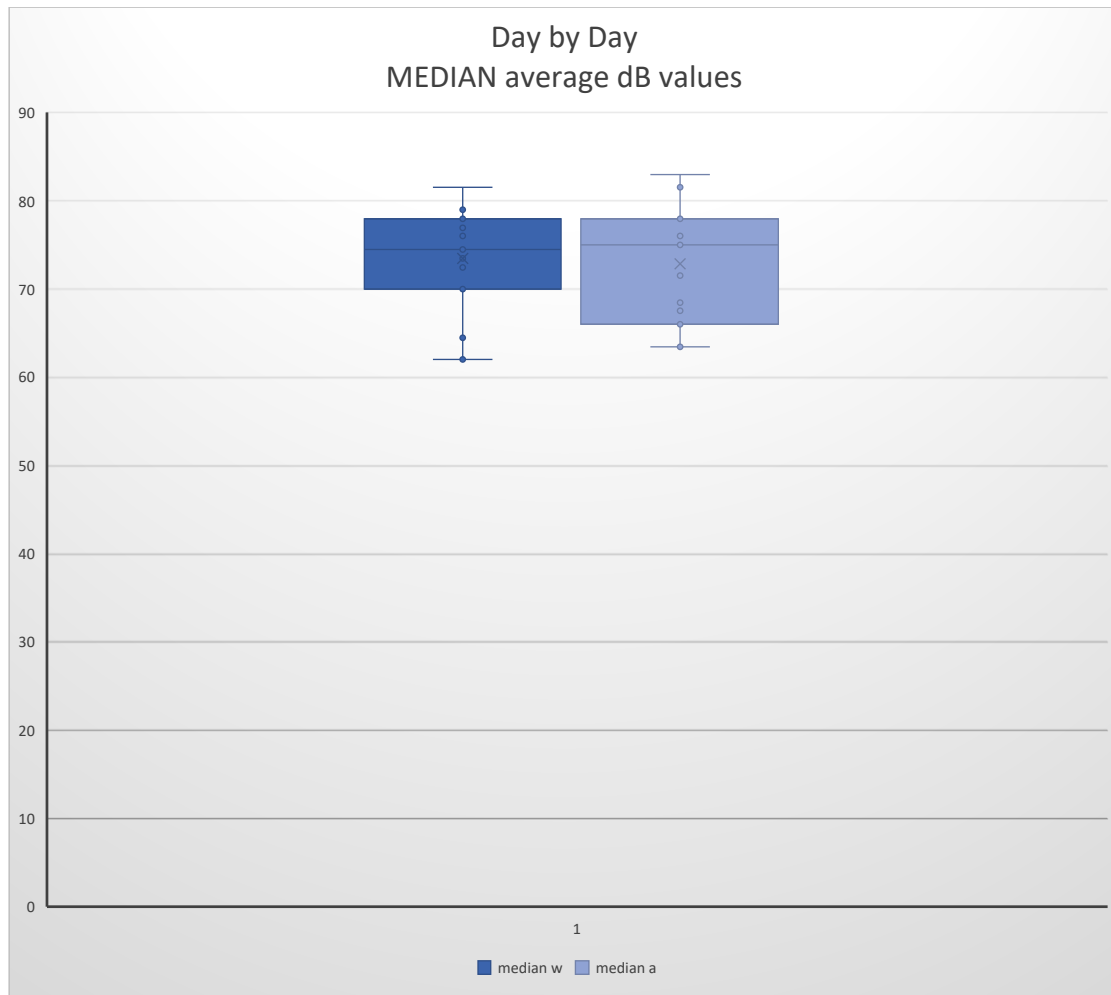
73,28125	median avg
3,3435919	stdv avg

Graf 3.4 Record of day by day dB values above the water

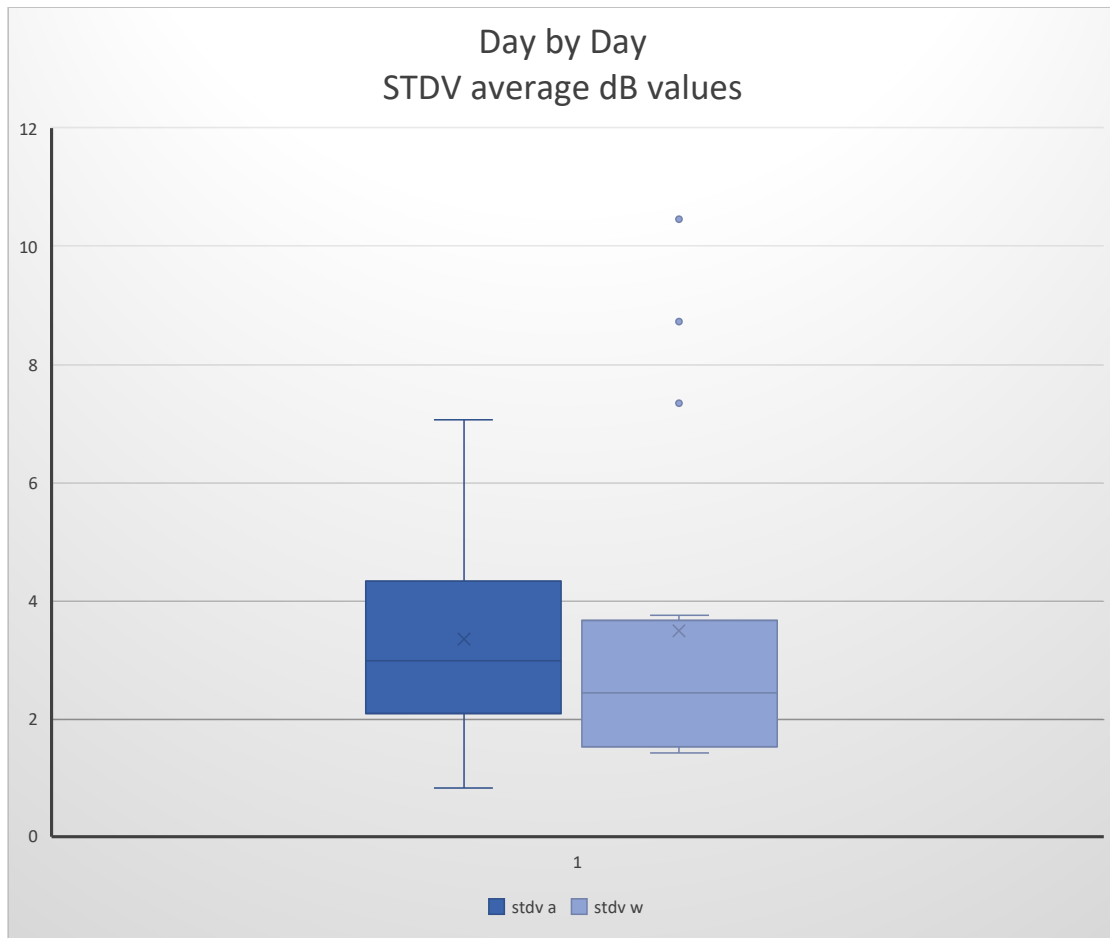


Graf 3.3 *p values above water / *p > 0,05





Graf 3.6 Comparing Day by Day MEDIAN water/air average values



Graf 3.5 Comparing Day by Day STDV water/air average values

Table 3.2.3 Average STDV & MEDIAN values on air

73,75	MEDIAN AVG
3,48967975	STDV AVG

All values proved that both air and underwater nature is indeed loud itself (Graf 3.2 & 3.3 & 3.4 & 3.5 & 3.6 & 3.7) to add more decibels that effect negatively on species physiology and biology. In some cases, each comparison had exactly the same value, showing that some sounds can emerge faster. Natural certain frequency and decibel level values sounds are normality to species. Adding new artificial man-made sounds can only bring more problems and bigger threats. It is another thing when low frequency sound like human talk enters the water and another when shipping industries use low frequency sounds to sonar. Separating sounds, the creation of these sound qualities categories was thought to be the best way to divide sound:

- ✓ Low frequency (low or high decibel) natural sounds such wind, waves, species sounds (or bio sounds: sounds coming from species through specific organisms and are used to connect or communicate, or to find prey or multiply etc, do not have a negative impact on organisms)
- ✓ High frequency (low or high decibel) natural sounds such as strong wind, waves, higher species sounds (or bio sounds: sounds coming from species through specific organisms and are used to connect or communicate, or to find prey or multiply etc, do not have a negative impact on organisms)
- ✓ Low frequency (low or high decibel) artificial manmade sounds, such as sonar machines or fishing boats, low sound music etc (they have effect organisms especially marine mammals)
- ✓ High frequency (low or high decibel) artificial manmade sounds, coming from ferry boats, fishing boats, loud decibel music, construction industries etc and are affecting organisms.

Additionally, what we understand is that natural sounds are connected to species and are understood and tolerated by species, giving them information that help them survive in their ecosystems. Yet few are known about species organs development towards high non-natural frequency and decibel sounds.

Research about the integration radioactivity and sound sensors the importance of gathering useful information to better understand the functionality of the marine environment through acoustic data (Sara Pensieri et al, 2018) notes the need for further details about the significant issues according to the threats that marine ecosystems go through and must be taken under serious consideration.

Additionally, the existence of both anthropogenic and high frequency clicks and marine mammal calls or quarks have been identified and examined in several papers by examining acoustic levels showing that animals not only are present on their habitat but live under a rather noisy environment.

Over tourism, climate crises, water level rises, pollution, contamination, and other threats are just a drop of what animals in the sea need to address in order to survive as if they had fewer threats behind to overcome by living in such environments. Daily they

need to cope to exist the natural dangers likewise wind, wave movement etc, making noises adds one more problem to be solved. For instance, sounds of small recreational boats spans a wide range of frequencies and source levels, but the degree to which this impacts the soundscapes of coastal habitats is poorly understood (Louise Wilson et al, 2022)._Others highlight that the broadband and highly variable sound emitted by small boats must be considered when evaluating anthropogenic impacts on coastal marine ecosystems worldwide (Louise Wilson et al, 2022). Generally, the impact of noise in the water is not much studied but is of extreme importance since marine species need sounds to survive and probably are much disturbed by artificial anthropogenic noises. Although there is a study that proved that boat sounds do not affect the breeding behaviors of *Sciaena umbra* (Marta Picciulin et al ,2022) in Natura 2000 area, yet it is rather on our common sense that noises depending on the frequency and decibel levels can be rather annoying and at some point, bring fatal results. Just because fish continue their biological cycles does not mean that there are not affected by annoying sounds. Even the fact that they run away in deeper depths shows that they are disturbed by boat noises indeed.

Comparing decibel level noises below the sea and taking a better look on the decibel levels of the ambient sounds we can understand that some values are almost the same both in nature and in wild. The problem is that ambient sound values are of different frequencies and most important familiar to animals and can be tolerated since organisms survived them for several thousand years. Manmade sounds on the other, probably are not adjustable to their organs and can cause certain irreversible damages. Sound stress might not affect their ability to breed but that does not mean that sound does not impact their lives.

In the information below there are few decibels that coastline sea ecosystems must face daily.

Artificial manmade sounds

Decibel levels of few artificial sounds

Additionally, another filed experiment comparing two anthropogenic common sounds occurred in a quiet laboratory room. One continuous beep sound and one punctuated beep in 0,40m distance from sound source (Chrysogelou 2023).

MIN	AVG	MAX
27	48	66
29	50	67
29	51	67
29	50	67
29	50	67
29	50	67
29	51	67
29	51	67
27	50	67
27	49	67

Table4.1: Decibel values of Continuous sound

MIN	AVG	MAX
31	40	50
30	36	41
35	77	42
30	39	49
29	39	52
28	39	54
28	39	65
28	39	77
24	40	62
33	40	56

Table4.2Decibel values of Punctuated sound

Both sounds are typical man-made sounds (Tables 4.2 & 4.1) that used daily around the globe but in much higher decibel levels. One of these two will is used on in vitro experiments keeping the same decibel levels on fish in tanks. These sounds were measured on air on field without other sound interruptions (Chrysogelou 2023).

Trawl moving sound (Table 3.3) from at least 30 to 35 meters distance. 30 seconds measurement on a shiny and not windy day (Chrysogelou 2023).

MIN	AVG MIN	AVG MAX	MAX
31	37	58	92

Table 4.3 Ferry boat punctuated repeatedly sound.

	DISTANCE 1	DISTANCE 2	DISTANCE 3
MIN	42	43	42
MAX	61	63	56
AVG	56	81	61

Table 4.4 Ferry boat leaving (port/land position).

	1st APPROACH	2nd APPROACH	3d APPROACH
MIN	92	78	44
MAX	167	141	76
AVG	85	96	66

Table 4.5, dB level value approaches, by Chrysogelou

Foteini 2023

1st approach starting engine, 2nd approach leaving, 3d about 60/100meters away from coast (Table 4.5).

Adding an unofficial observation in vivo watching experiment on field three notices were perceived and remarked and follow:

1. On human talk while having a loud conversation of three without moving on coast and without any sound distractions and after standing still for at list 11minutes until species approach, species of *Oblada melanura*, *Sparus aurata*, *Diplodus vulgaris*, *Lithognathus mormyrus* and *Diplodus sargus* do not seem to be disturb but on the contrary human talk sound seems to be attractive to them. They are rather disturbed by any movement rather than human sounds.
2. On the other hand, placing a Samsung Galaxy A13 near the surface playing loud beep - beep sounds scared a group of *Mugil cephalus* along a port.
3. Finally, although it was not planned but accidentally occurred, while on freezed position underwater and holding stones, a strong stone clatter (a bang, bang, bang, bang) did make a *Muraena helena* react having not seen the sound source.

All three experimental approached occurred on ideally environments without any artificial sounds surrounding the area and under perfect weather conditions.

Nevertheless, on the decibel field experiment, what was noticeable was the on-flood decibel levels were lower while on low tide higher than expected. Concluding the results showed that there are small but important decibel values differed from air to water giving us the answer that what is not annoying on air usually does not annoy above water and vice versa.

Adding at this point, that in a study of 2020 where a music festival occurred and decibel levels were recorded but also other sound factors were taken under consideration, and while underwater sound was elevated, scientists measured a significant elevation of toadfish plasma cortisol. Summarizing, this study concluded that during Ultra, scientists measured elevated sound pressure levels as well as a significant increase in plasma cortisol levels in toadfish while those held in tanks directly adjacent to the festival at

UMEH. They also found a signal of sound pollution in the adjacent Bear Cut channel, which may have affected wild organisms. To their knowledge, this is the first study to assess the impacts of sound pollution due to a coastal music festival and the results were not so positive.

Finally, our study should be interpreted as preliminary insight into the less papers among which scientists try to understand bio sounds, biological and ecological effects of sounds in the sea, soundscape and sonic pollution and its effects or impacts on fish and other marine organisms. In extend, share more knowledge through experimental process on field and on field and in the laboratory.



Images 5 & 6 Sampling after treatment



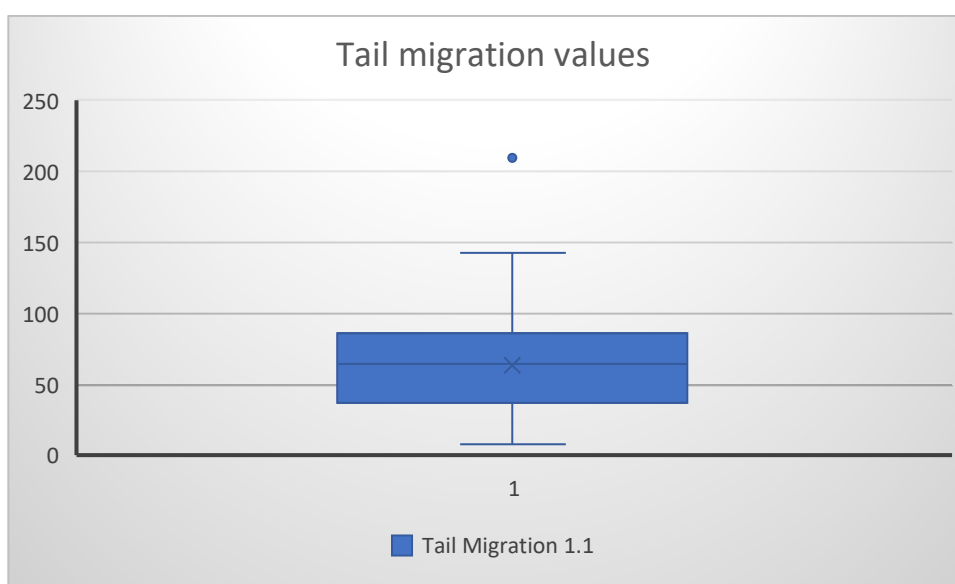
3.2 Comet assay results

Evaluation of DNA damage on fish liver under sound stress (Images 4 & 5)

Results

35,06325037	MEDIAN 1.1
64,06833334	STDV 1.1

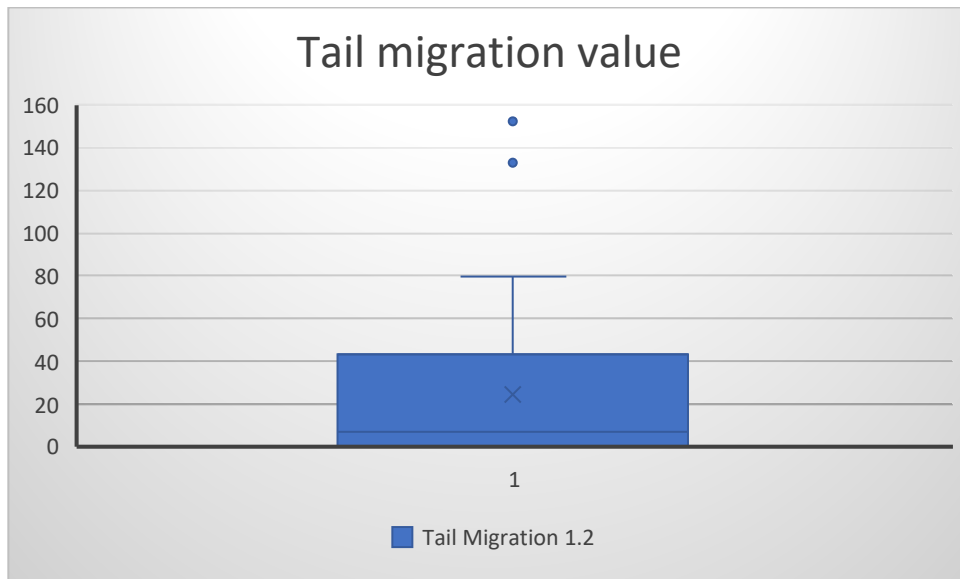
Table 3.2.1 MEDIAN and STDV values of tail migration, sample 1.1



Graf 3.2.1: Evaluation of DNA damage on liver tissue under sound stress

Table 3.2.2 MEDIAN and STDV values of tail migration, sample 1.2

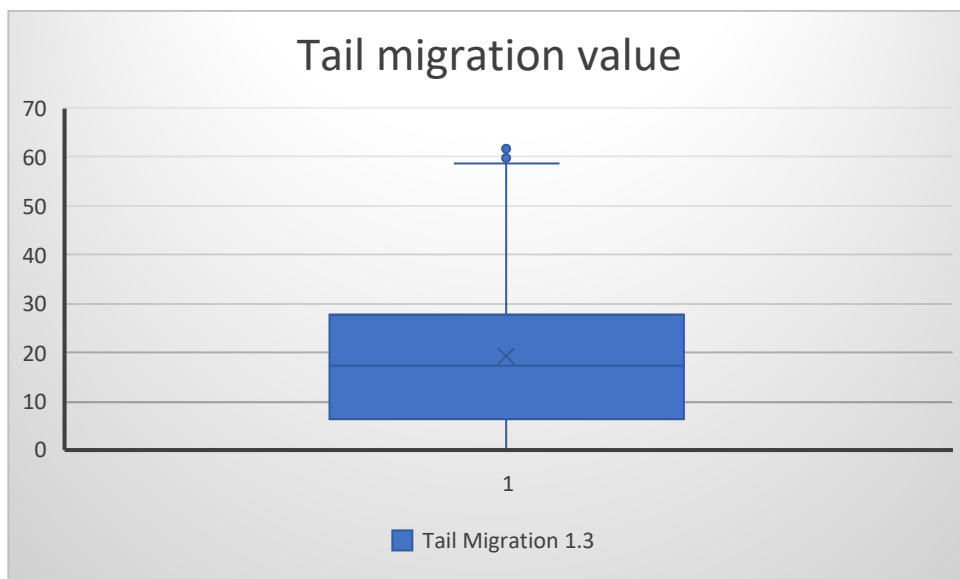
33,22425326	MEDIAN 1.2
6,911936377	STDV 1.2



Graf 3.2.2: Evaluation of DNA damage on liver tissue under sound stress

15,27549948	MEDIAN 1.3
17,14691909	STDV 1.3

Table 3.2.3 MEDIAN and STDV values of tail migration, sample 1.3



Graf 3.2.4: Evaluation of DNA damage on liver tissue under sound stress

Table 3.2.4 MEDIAN and STDV values of tail migration, sample 1.4

29,91624602	MEDIAN 1.4
51,57367912	STDV 1.4

Graf 3.2.4: Evaluation of DNA damage on liver tissue under sound stress

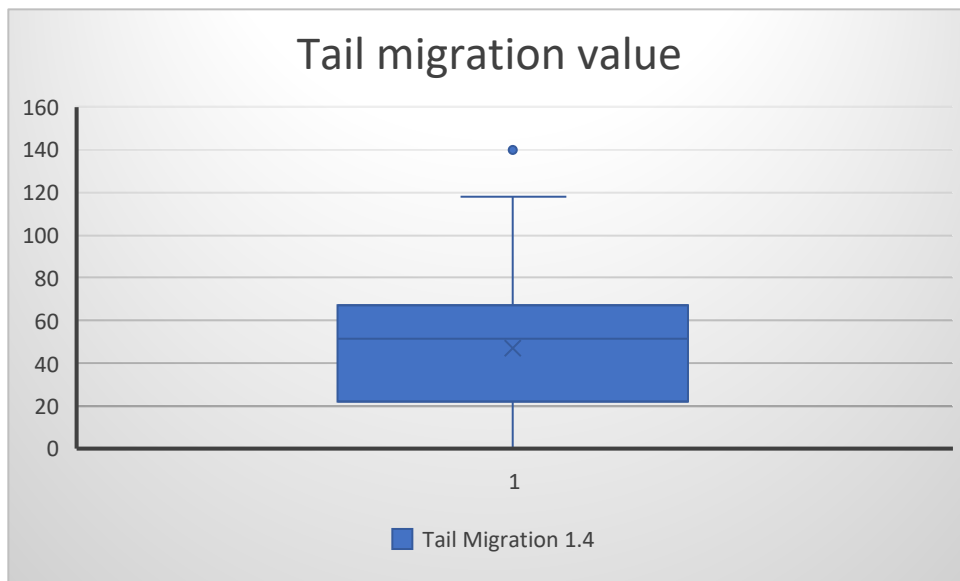


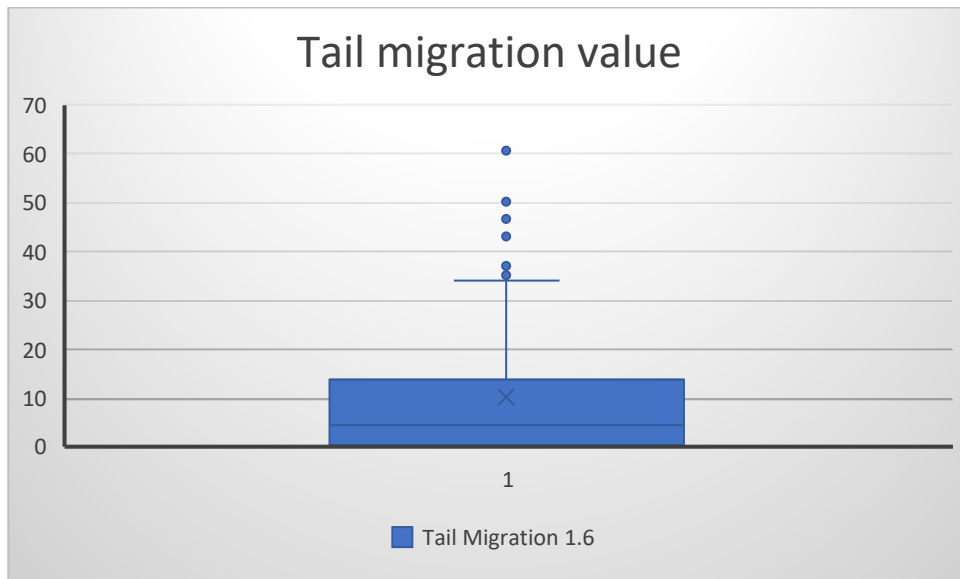
Table 3.2.5 MEDIAN and STDV values of tail migration, sample 1.5

10,25815843	MEDIAN 1.5
0,066460927	STDV 1.5

Table 3.2.6 MEDIAN and STDV values of tail migration, sample 1.6

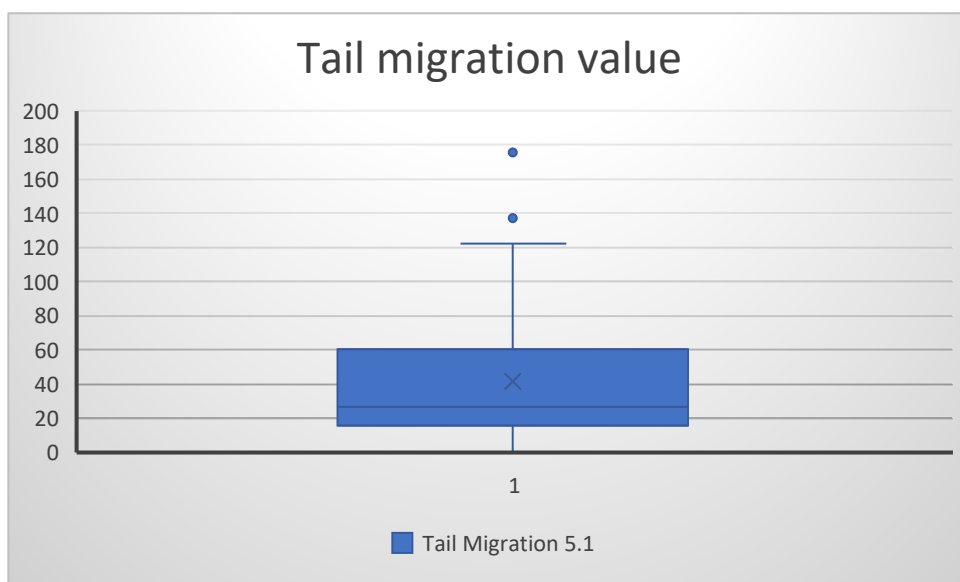
13,21184646	MEDIAN 1.6
4,386421163	STDV 1.6

Graf 3.2.6: Evaluation of DNA damage on liver tissue under sound stress



36,86190868	MEDIAN 17.1
26,71729254	STDV 17.1

Table 3.2.7 MEDIAN and STDV values of tail migration, sample 5.1



Graf 3.2.7: Evaluation of DNA damage on liver tissue under sound stress

Table 3.2.8 MEDIAN and STDV values of tail migration, sample 5.2

26,14449033	MEDIAN 5.2
6,779014524	STDV 5.2

Graf 3.2.8: Evaluation of DNA damage on liver tissue under sound stress

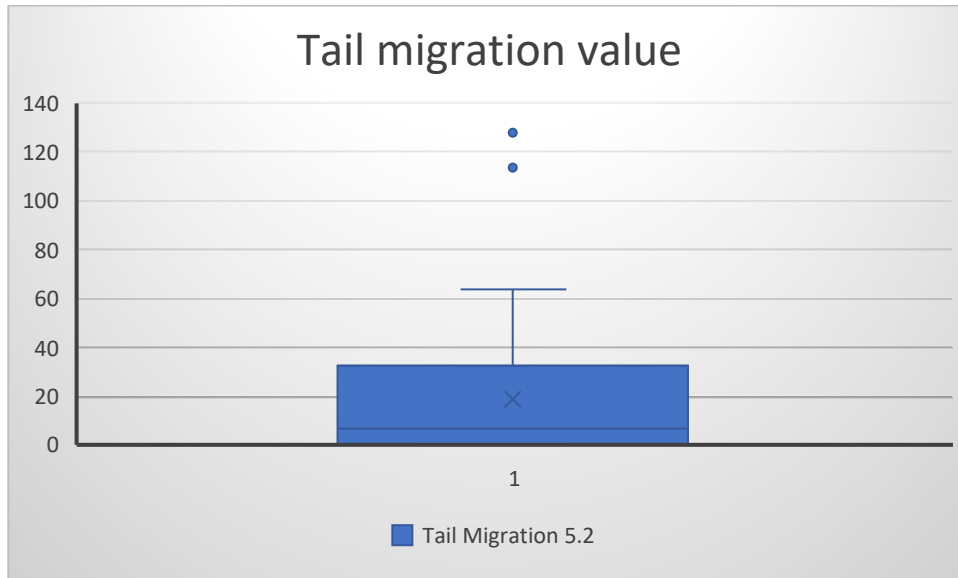
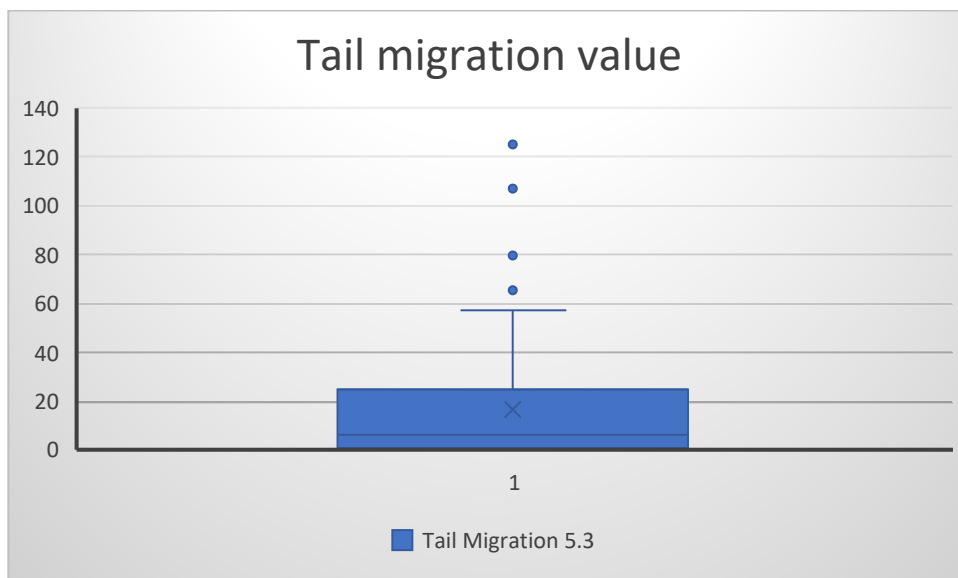


Table 3.2.9 MEDIAN and STDV values of tail migration, sample 5.3

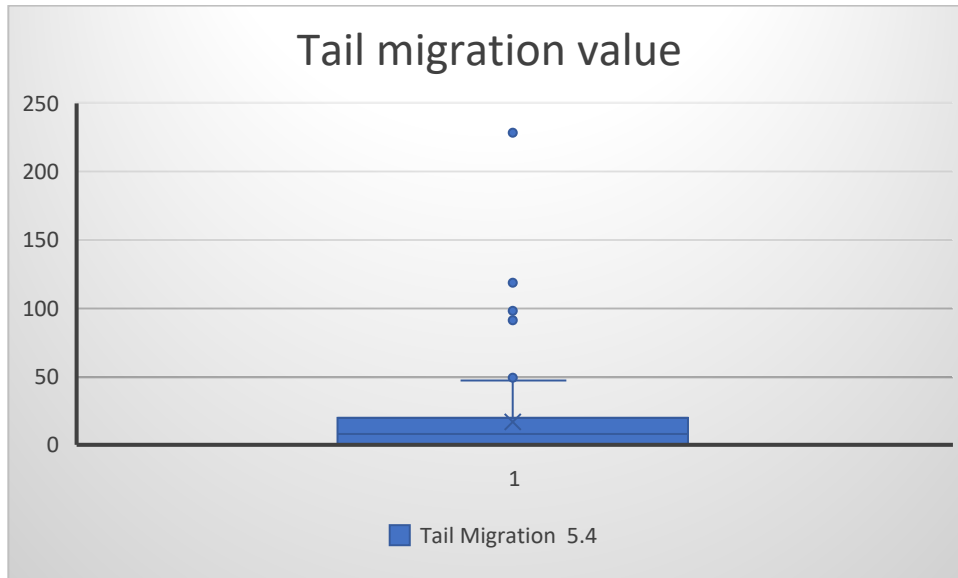
22,24556403	MEDIAN 5.3
6,114405257	STDV 5.3



Graf 3.2.9: Evaluation of DNA damage on liver tissue under sound stress

30,33889447	MEDIAN 5.4
7,842389351	STDV 4

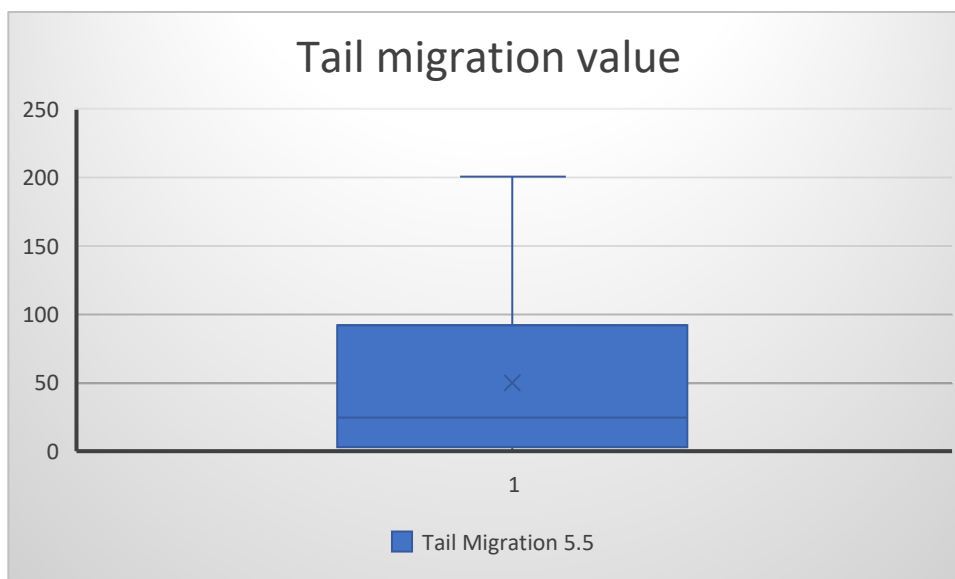
Table 3.2.10 MEDIAN and STDV values of tail migration, sample 5.4



Graf 3.2.10: Evaluation of DNA damage on liver tissue under sound stress

Table 3.2.11 MEDIAN and STDV values of tail migration, sample 5.5

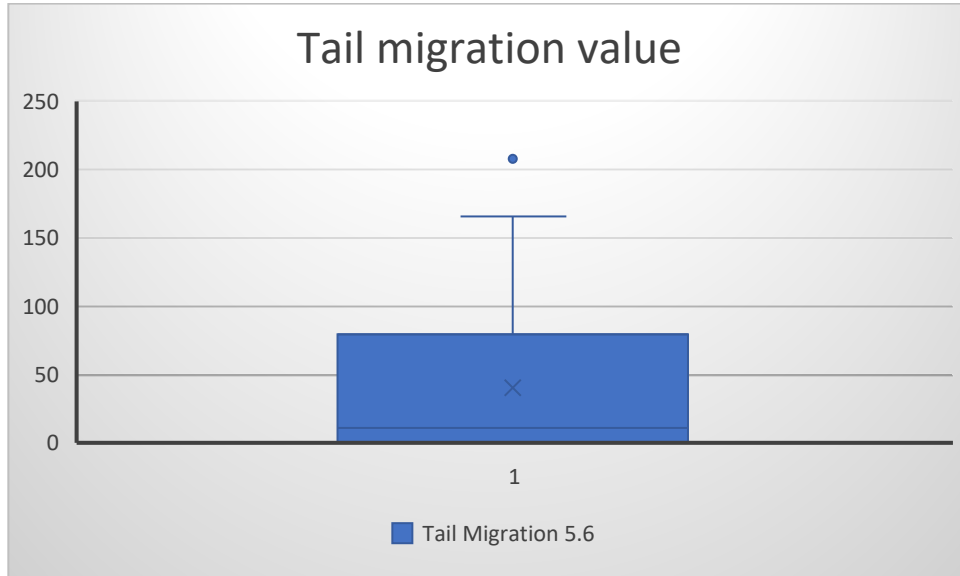
56,03680912	MEDIAN 5.5
24,19177732	STDV 5.5



Graf 3.2.11: Evaluation of DNA damage on liver tissue under sound stress

55,16929655	MEDIAN 5.6
11,16543569	STDV 5.6

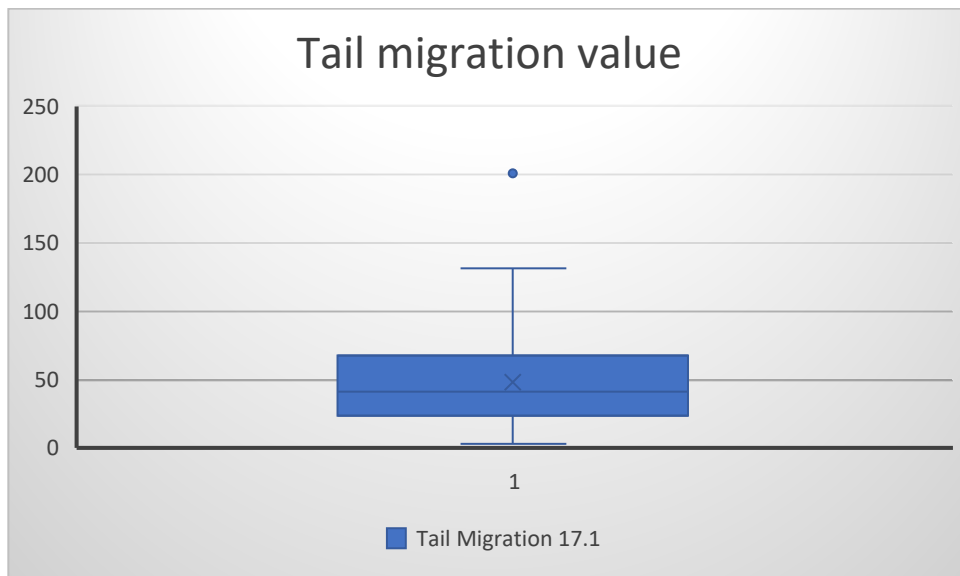
Table 3.2.12 MEDIAN and STDV values of tail migration, sample 5.6



Graf 3.2.12: Evaluation of DNA damage on liver tissue under sound stress

31,7465749	MEDIAN 17.1
41,33869641	STDV 17.1

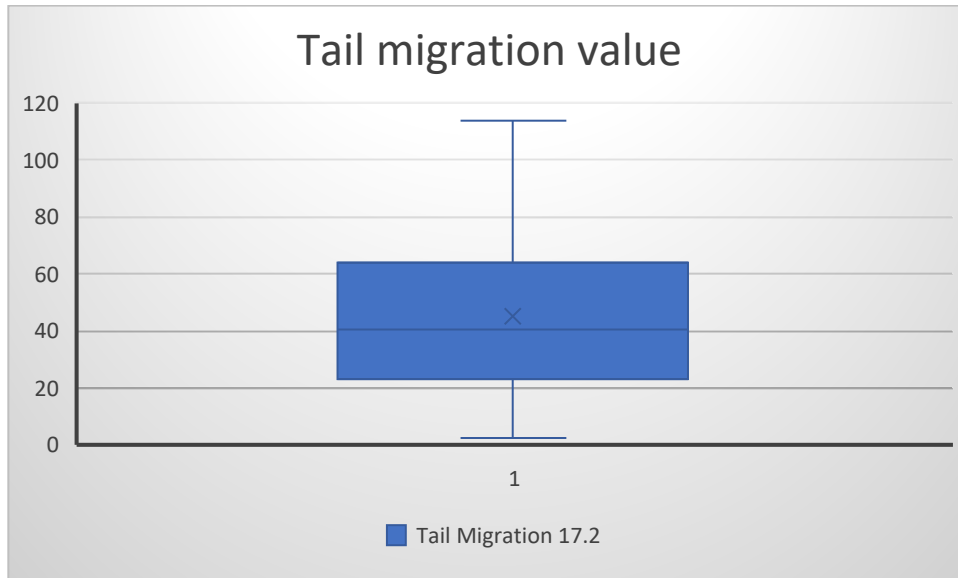
Table 3.2.13 MEDIAN and STDV values of tail migration, sample 17.1



Graf 3.2.13: Evaluation of DNA damage on liver tissue under sound stress

25,590105	MEDIAN 17.2
40,40824344	STDV 17.2

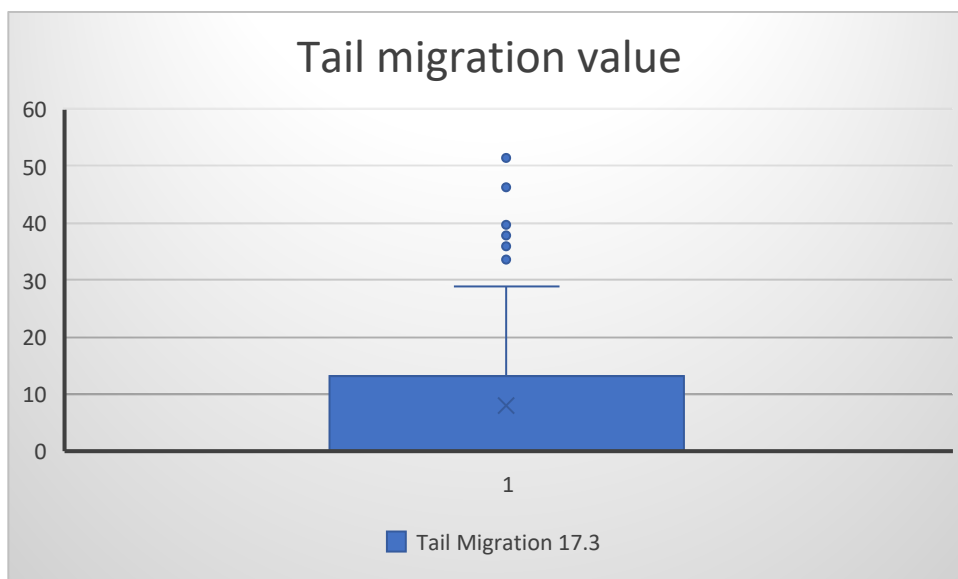
Table 3.2.14 MEDIAN and STDV values of tail migration, sample 17.2



Graf 3.2.14: Evaluation of DNA damage on liver tissue under sound stress

13,70119289	MEDIAN 17.3
0	STDV 17.3

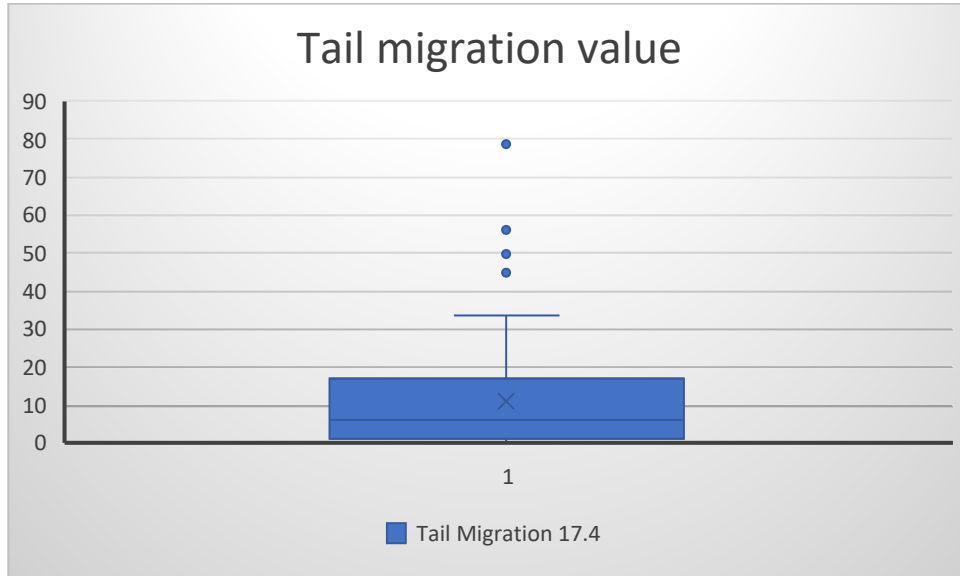
Table 3.2.15 MEDIAN and STDV values of tail migration, sample 17.3



Graf 3.2.15: Evaluation of DNA damage on liver tissue under sound stress

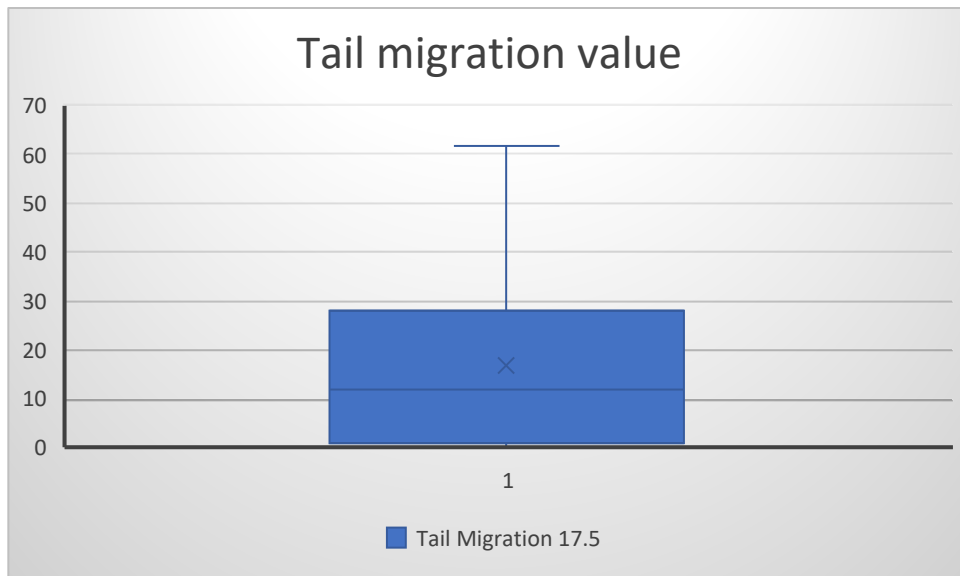
14,74537528	MEDIAN 17.4
5,981483403	STDV 17.4

Table 3.2.16 MEDIAN and STDV values of tail migration, sample 17.4



Graf 3.2.16: Evaluation of DNA damage on liver tissue under sound stress

Graf 3.2.17: Evaluation of DNA damage on liver tissue under sound stress

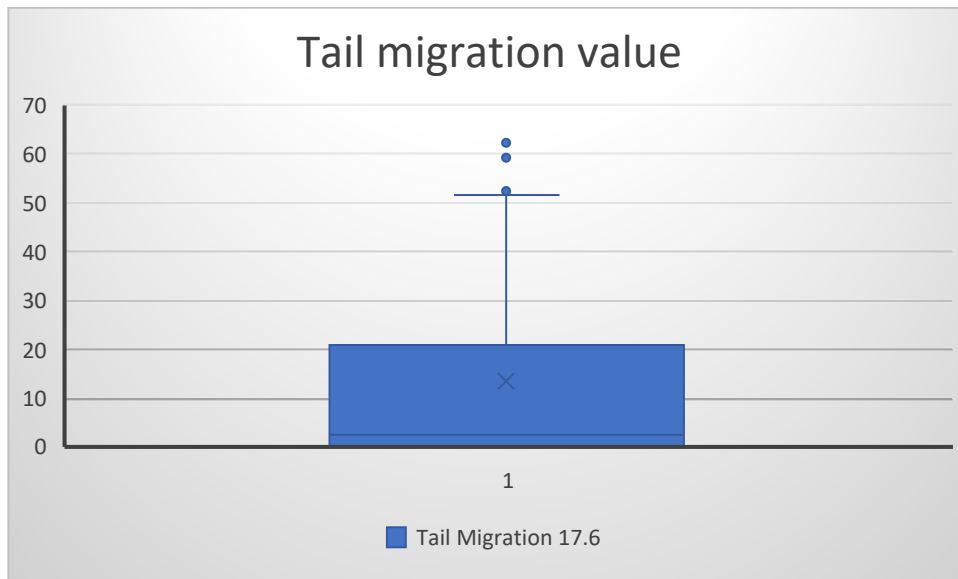


16,95421894	MEDIAN 17.5
11,6971231	STDV 17.5

Table 3.2.17 MEDIAN and STDV values of tail migration, sample 17.5

Table 3.2.18 MEDIAN and STDV values of tail migration, sample 17.6

19,66931599	MEDIAN 17.6
2,525515215	STDV 17.6



Graf 3.2.18: Evaluation of DNA damage on liver tissue under sound stress

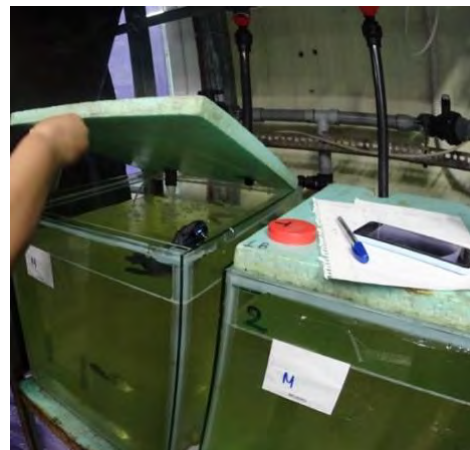
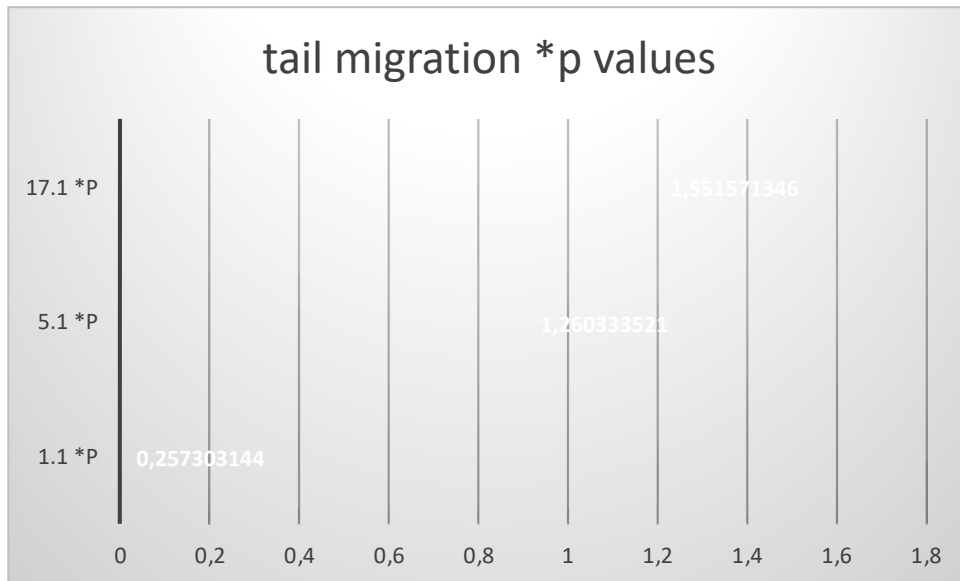
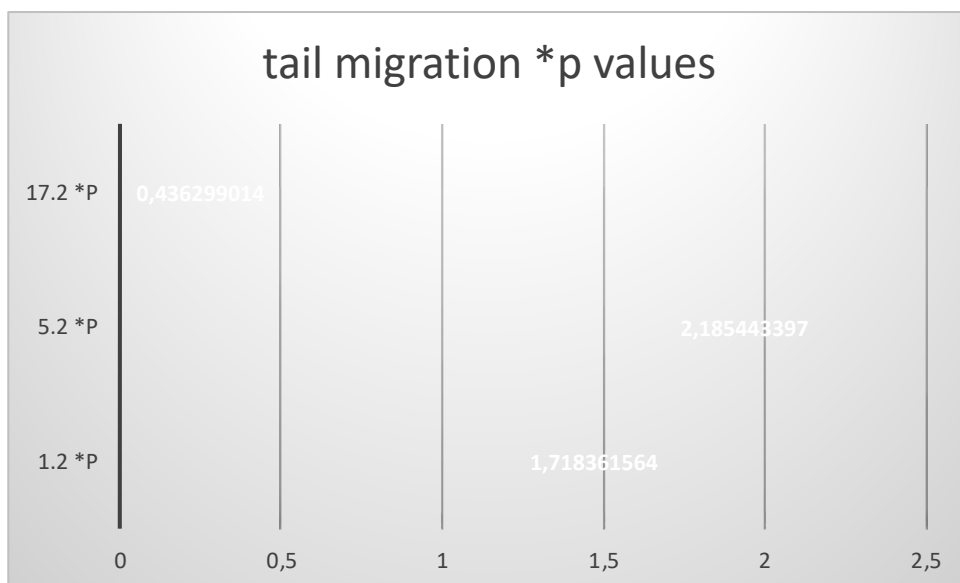


Image 3.3, Treatment 1 Feeding under sound, by Chrysogelou Foteini

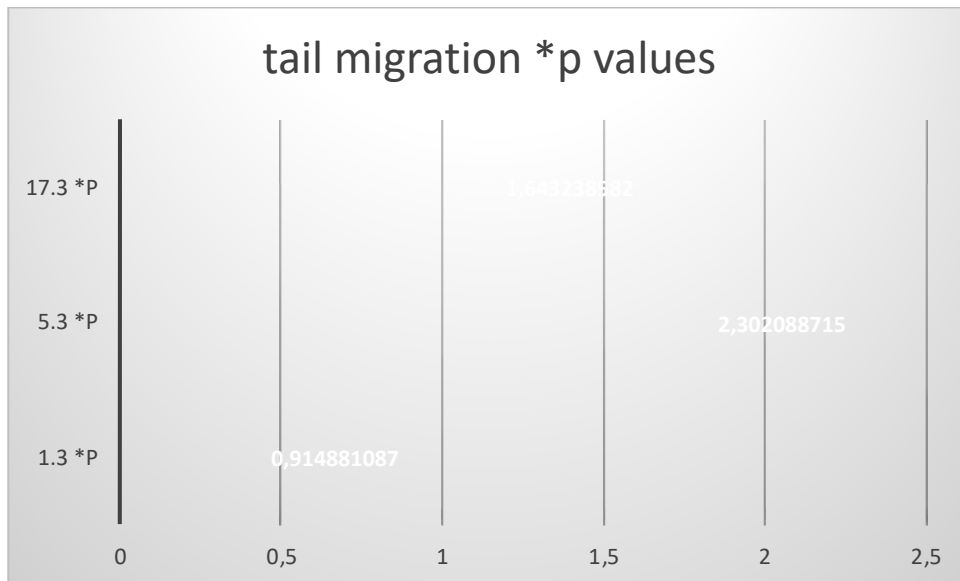
Graf 3.2.19 Comparing *p values of tail migration damage



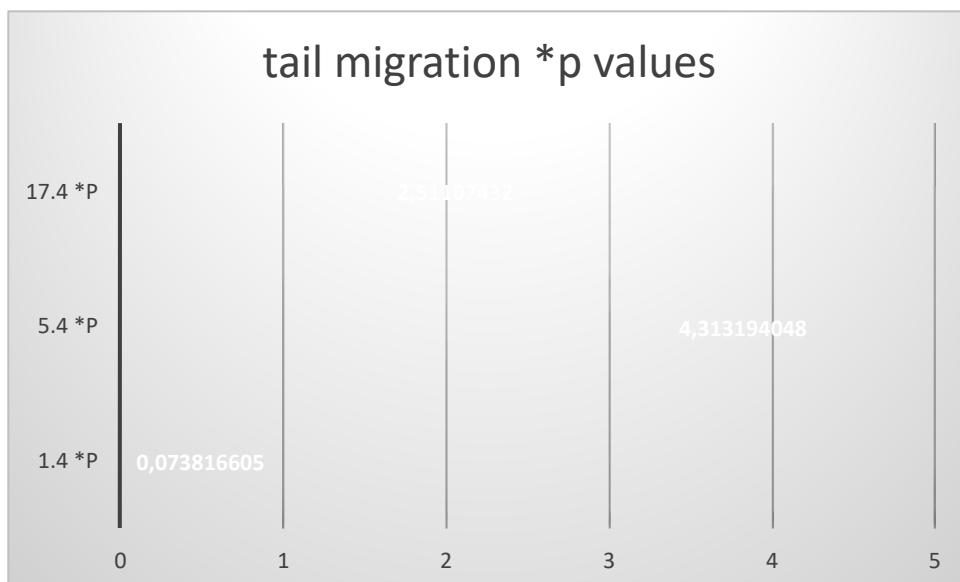
Graf 3.2.20 Comparing *p values of tail migration damage



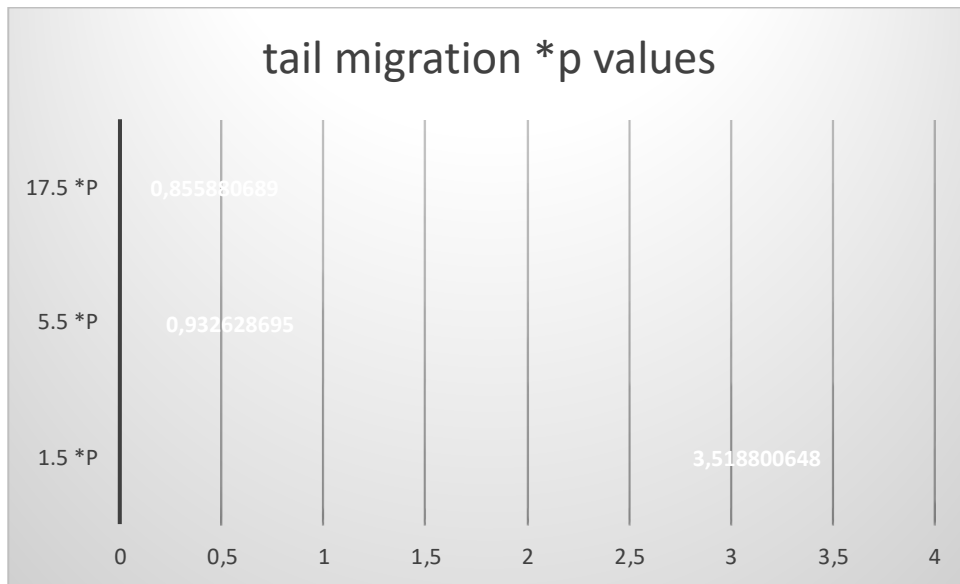
Graf 3.2.21 Comparing *p values of tail migration damage



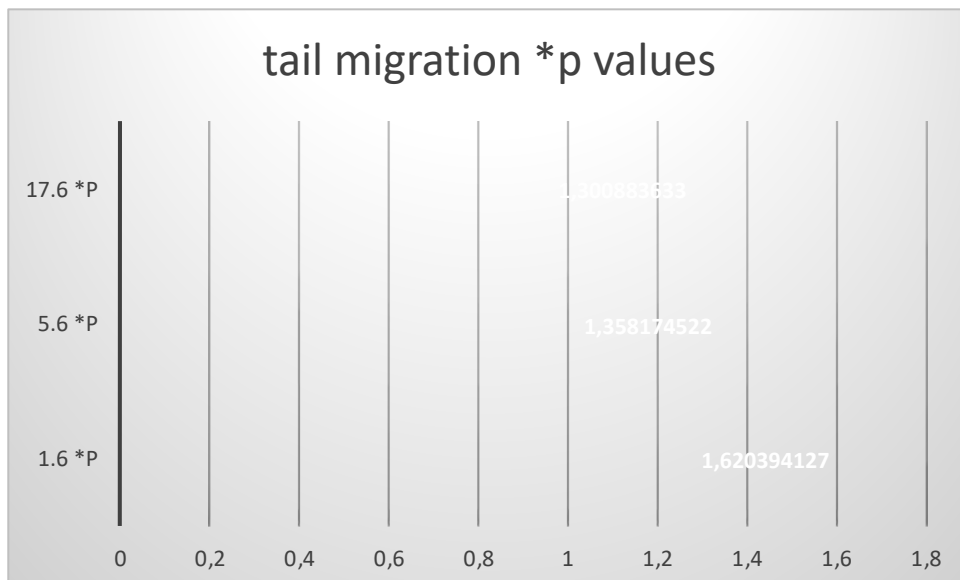
Graf 3.2.22 Comparing *p values of tail migration damage



Graf 3.2.23 Comparing *p values of tail migration damage



Graf 3.2.24 Comparing *p values of tail migration damage



*p values for all samples *p < 0,05

3.3 Feeding behaviour

During the experimental process behavioral observation analysis were recorded and behavioral facts were noted (Image 3.3). Along with the expected behavior some peculiar excessive behavior was observed. Accordingly, we can categorize these attitudes as follow:

Day 0

According to observations, while performing the experiments, different behaviors were noticed throughout the procedure. Taking under consideration the laboratory and aquarium sounds (noise) which in some cases were reaching noise decibel pollution levels, fishes have been noticed to approach different behavioral attitudes towards their feeding and manners. Before proceeding with the rest information, we need to note once more than decibel levels are not the only factor that needs to be noticed when trying to understand what makes a sound to become noise while reaching pollution levels.

Beginning with treatment 17, the ten fish that lived in, did not seem to reflect with the fact that they had two days of total no feeding diet. Of course, they had the typical anxious behavior, but when their feeding started, it took them time to understand that food was dropping in, and they appeared only after some seconds to start eating. When individuals started realizing the food, they mostly started eating it not from the surface but from the middle of the tank and from the bottom. Only a few were swimming to get some from the surface. Generally, their movements were calm, they seemed not stressed at all, moving slowly without tension, and looking confident that they would eventually eat. The control tank had the biggest food waste portion left in the tank after the ten minutes feeding and observing process. Lastly, we need to add that when their feeding repletion levels reached the highest point with the least food portion on tank comparing with the other two, fish disappeared swimming to the back of the tank.

Continuing with treatment 5 tank, that was observed, we noticed that fish were stressed by the first few seconds of the immersed sound. They started to swim anxiously and stressfully some making nervous rounds driving circles around themselves and when food started entering the tank apart from their nervousness some aggressive behavior towards them was observed (going after one another's tails and biting them in some cases). Almost half of fish seemed to adapt a violent behavior towards another. Of course, almost all of them moved on the surface, trying to eat as much as they could, and after only a few seconds of feeding, they stopped and started spitting back the food out of their mouths. Generally, even after feeding and while the sound was still on, fish seemed to continue this behavior and kept their movements of anxiety levels high until the point of repletion when they started decreasing their mobility levels. In this tank fishes were not observed to having any calmness during the observation procedure.

Regarding the last treatment no 1, a continuous ferry boat engine sound was submerged for another hour. Within the first seconds of the sound impact, fish started swimming faster and along the hall tank. Some making rapid rounds and some reaching up and down the tank. By the moment the first pellets were in the tank fish started eating greedily. Some were climbing (swimming on top of others). There were not any aggressive behaviors noticed on this tank. Fish looked like they were anxious about whether they were going to be fed ever again or not. Their behavior had steady rapid and quick movements even after minutes. It seemed that there was a steady anxious behavior during the procedure and that only after feeding fish seemed to absorb a calmer but still vivid behavior (notice by Miss Myrto Maniaki).

Concluding about the general behavior of the 30 fish (ten in each tank), we would have the following total observations. Accordingly, we would notice that on treatment 17, were not physically stressed at all on treatment 5, with the punctuated continuous beep sound was the most stressed with aggressive behavior, and no 1 with the ferry boat had all the characteristics of treatment 5 but without the aggressiveness and with a bit lower nervousness levels.

Following within the progress of the experiment, treatment 17, escapes any comment because there were not any differences noticed in fish behavior. Thus, fish respond exactly like Day 0. There was not any aggressiveness between individuals, neither any stress behavior nor anxiety, not even nervousness while feeding apart the obvious typical feeding behavior of an animal knowing that will eat eventually. This means that fish got used to any air environment sound and accepted the noise while growing. At this point, it is good to note that fish were at an early growing stage. Lengths in centimeters have been mentioned in another paragraph. On treatment 17 (control tank), there are none, nonnormal (within these certain conditions) behavior among individuals.

On the other hand, even after 24 hours, treatment 5 that was forced with sound stress under sound effect, fish were a bit anxious while watching us approaching the tank, but probably because of habitual interest. The main conclusion for this tank is the fact once more, that all behaviors remained the same apart the fact that time had a negative impact on their increased greediness. Fish ate faster and still looking for food (pellets) much time after their 10 minutes of feeding time. Treatment 5 ate the most. 29,381g were fed

on these fish giving this tank an advantage of food portion of almost 8-9g weightier from the other two.

Finally, on treatment 1, fish seemed to have lost their ability to approach the surface by the time the first pellets fell in the water and only after a while (few seconds) realized that food was entering the tank. Individuals did not look less nervous or anxious. They did not even swim slower. They only looked a bit confused and as if they did not understand their environment. Looking like they were a bit disoriented much alike fish in an unknown to them environment.

Lastly, summing the observations, it is obvious that all sounds had an impact on fish. The problem is that in fish tanks or fish in captivity in general, individuals are born and raised under specific and controlled conditions. In wild, there are totally different forces, factors, and environmental parameters that need to be examined before analyzing the harm or good of a sound. The important evidence that can indeed help us understand whether if one sound is noise or just sound and if it is harmful or harmless, in a long-term basis flow over the examination of all parameters, such as decibel levels, frequency levels etc. On the other hand, it is well known, evolution rule explains that when things change and get worse, species migrate to other locations or dive deeper. But this attitude towards difficulties is adapted only if fish are lucky. Otherwise, only the strongest species survive. The rest are doomed to extinct – die.

Image 3.3: Control tank No 17 under zero sound (sounds only come from the room)



by Chrysogelou Foteini 2023

3.4 Estimating DNA damage

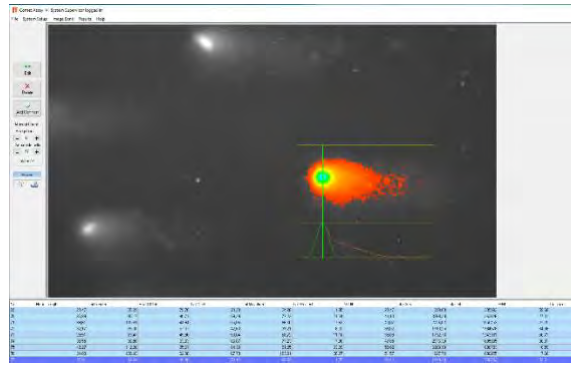
Estimate DNA damage in single cells through microscopy, the analysis of sound pollution damages induced by high and of different frequency sounds.

In the results of the DNA damage that was quantified at the single-cell level by computing the displacement between the genetic material within the nucleus, typically called “comet head”, and the genetic material in the surrounding part of the cell, considered as the “comet tail” and finally the examination of tail migration, was found that almost all parties were damaged keeping higher scores on the punctuated sound that is, on treatment No5.

Based on the technique of microgel electrophoresis, comet assay gave certain numbers describing the shape and the moment of the DNA visualized damage (Image 3.5) upon observing agarose gels.

In this study analysis of the main parts of Tail Fluorescence Percentage are introduced, thus being the most important features considered upon evaluating this assay. All comets are oriented with head on the left and tail on the right. Comet assay method in general terms, provides fully automatic, semi-automatic and manual methods to segment comets based on intensity thresholds defined by the user. Head and tail profiles are visualized directly on the images (Images 3.3,3.4,3.5).

Image 3.5 Comet data image ,photo by Christina Papadouli



Typically, several intensity/morphological features are evaluated in a Comet Assay analysis report. Percent DNA [%]: percentage between Sum Intensities of the object (i.e., head or tail) and Sum Intensities of the entire comet are mainly analyzed and used as the perfect factor in this study.

Additionally, results showed (Graphics and Tables 3.59 to 3.79) that none of the sample's media and mean prices were close to each other, and none of the sample's skewness were close to zero explaining the absence of normality on the tests.

Furthermore, all samples appeared to have DNA damages and showed stress reactions. In some cases, a complete DNA damage occurred, and prices reached tail migration highest levels. Moreover, p value of N prices both days were compared (Tables 3.79 & 3.80), and these results were discovered.

Table 3.79: Tail migration values for 18samples both days

	tail migration samples avg		tail migration samples avg	
1.1	62,75507		48,85986	1.4
1.2	40,69205		10,96339	1.5
1.3	20,99143		13,11438	1.6
5.1	42,55669		21,62536	5.4
5.2	25,49146		58,58763	5.5
5.3	20,96178		52,71968	5.6
17.1	48,16556		13,7331	17.4
17.2	45,02329		19,61145	17.5
17.3	19,70566		20,07914	17.6

Table 3.80: *p values ND0 & ND1

*p	0,25730314	1.1
*p	1,26033352	5.1
*p	1,55157135	17.1
*p	1,71836156	1.2
*p	2,1854434	5.2
*p	0,43629901	17.2
*p	0,91488109	1.3
*p	2,30208872	5.3
*p	1,64323858	17.3
*p	0,07381661	1.4
*p	4,31319405	5.4
*p	2,51107432	17.4
*p	3,51880065	1.5
*p	0,93262869	5.5
*p	0,85588069	17.5
*p	1,62039413	1.6
*p	1,35817452	5.6
*p	1,30088363	17.6

4. Conclusions

According to the statistical results (Tables 3.80 and Graphics 3.81 – 3.87) of N average values, normality tests and statistical process showed that all *p values are more than 0.05, $p \geq 0,005$ at all times and Ho (zeroed probability factor) was accepted. DNA damage appears among samples and there is homogenous in variation concluding that non-unchanged stage occurred within them. Since all TTESTs showed relative normality and normal distribution giving us the Ho hypothesis, there was 50% normality distribution that makes us conclude that there was also a median comparison and most of the times values were almost close.

According to the tests and diagrams, treatment 17 had the lowest *p values indicating that samples had the least DNA damage between days. Accordingly, the highest value between all samples was at Day 0 treatment and in 1.1s with an estimating value almost 210value and biggest value on Day 1 and treatment and in 5.4s having almost 228.5value. Peculiar similarity was shown on samples by having the same lowest damage value on treatments 1.4s,1.5s,1.6s,5.4s,5.5s,5.6s,5.2s,17.3s on lowest among all samples value at 0,132921853value. Lowest skewness between all samples closer to 0,05 value was on Day 1 treatment and on sample number 1.4s. Finally, the lowest N average on 17.4s.

Continuing, we understand that treatment 17 had the lowest DNA damage generally. Between day samples we observed that on treatment 1 and 17 on Day zero, DNA samples had the less damage while on treatment 5 the less damage occurred on Day 1. Between samples the biggest damage difference occurred between samples 17.1s and 17.4s while the second keeps a highest record value between two. Meaning that Day 1 had the biggest damage time influence.

Nevertheless, Day 0 most affected samples 5.5s and 5.4s while Day 1 affected Treatment 17 samples.

5. Bibliographies

- ✓ Abir Chahouri Nadia Elouahmani, Hanan Ouchene (2021) Recent progress in marine noise pollution: A thorough review, Aquatic System Laboratory: Marine and Continental Environment, Faculty of Sciences Agadir, Department of Biology, Ibn Zohr University, Agadir ,Volume 291,Part 2, Chemosphere, Earth Science,Elsevier (DOI: [10.1016/j.chemosphere.2021.132983](https://doi.org/10.1016/j.chemosphere.2021.132983))
- ✓ Louise Wilson, Matthew K. Pineb, Craig A.Radforda, Leigh (2022) Small recreational boats: a ubiquitous source of sound pollution in shallow coastal habitats, Volume 174, Marine Pollution Bulletin,Elsevier (DOI: [10.1016/j.marpolbul.2021.113295](https://doi.org/10.1016/j.marpolbul.2021.113295))
- ✓ Jessica F.de Souzaa, Mayara, M. Silveira, Heloisa H .A. Barcellos, Leonardo J. G.Barcelloscde, Ana C.Luchiarria November (2022) Sound stimulus effects on dusky damselfish behavior and cognition Volume 126, Elsevier (doi: [10.1016/j.marpolbul.2022.114111](https://doi.org/10.1016/j.marpolbul.2022.114111))
- ✓ Annebelle C.M. Kok, Lisa Bruil, Benoit Berges, Serdar Sakinan, Elisabeth Debusschere, Jan Reubens, Dick de Haan, Alain Norro, Hans Slabbekoorn (2021) An echosounder view on the potential effects of impulsive noise pollution on pelagic fish around windfarms in the North Sea, Volume 290, Environmental Pollution, Science Direct (doi.org/10.1016/j.envpol.2021.118063)
- ✓ Louise Wilson, Rochelle Constantine, Tane van der Boon, Craig A. Radford (2022) Using timelapse cameras and machine learning to enhance acoustic monitoring of small boat sound (<https://doi.org/10.1016/j.ecolind.2022.109182>)
- ✓ Zhi-Tao Wang, Tomonari Akamatsu, Peng-Xiang Duan, Lu Zhou, Jing Yuan, Jiao Li, Pei-Yu Lei, Yu-Wei Chen, Yi-Ning Yang, Ke-Xiong Wang, Ding Wang (2020) Underwater noise pollution in China's Yangtze River critically endangers Yangtze finless porpoises (*Neophocaena asiaorientalis asiaorientalis*), Volume 262, Environmental Pollution, Science Direct (<https://doi.org/10.1016/j.envpol.2020.114310>)
- ✓ M.A. Wale, R.A. Briers, K. Diele (2021) Marine invertebrate anthropogenic noise research – Trends in methods and future directions, Marine Pollution Bulletin, Volume 173, Part A, Article Number 112958 , Springer Nature (<https://doi.org/10.1016/j.marpolbul.2021.112958>)
- ✓ H Slabbekoorn, N Bouton, I van Opzeeland (2010) A noisy spring: the impact of globally rising underwater sound levels on fish,Trends in Ecology 7 Evolution,Cell press (DOI: [10.1016/j.tree.2010.04.005](https://doi.org/10.1016/j.tree.2010.04.005))
- ✓ NJ Edmonds, CJ Firmin, D Goldsmith (2016) A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species, Marine pollution bulletin, Volume 108, Science Direct (<https://doi.org/10.1016/j.marpolbul.2016.05.006>)

- ✓ R Williams, AJ Wright, E Ashe, LK Blight (2015) Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management, volume 114, Issue 2, Science Direct, Marine Pollution Bulletin (DOI: [10.1016/j.ocecoaman.2015.05.021](https://doi.org/10.1016/j.ocecoaman.2015.05.021))
- ✓ Sarah Piwetz, David Lundquist, Bernd Wursig (2015) Chapter two – Humpback Dolphin (Genus *Sousa*) Behavioural responses to human activities, Volume 72, page 17-45, Science Direct (doi.org/10.1016/bs.amb.2015.08.007)
- ✓ L Bittencourt, IMS Lima, LG Andrade, RR Carvalho (2017) Underwater noise in an impacted environment can affect Guiana dolphin communication, National Library of Medicine, Pub Med, Abstract (DOI: [10.1016/j.marpolbul.2016.10.037](https://doi.org/10.1016/j.marpolbul.2016.10.037))
- ✓ NCF van Geel, D Risch, A Wittich (2022) A brief overview of current approaches for underwater sound analysis and reporting, Marine Pollution Bulletin, Volume 178, Science Direct (doi.org/10.1016/j.marpolbul.2022.113610)
- ✓ D Bagočius, A Narščius (2022) The underwater sound levels of Lithuanian marine harbour, Marine Pollution Bulletin, X- MOL, Abstract ([doi: 10.1016/j.marpolbul.2022.114335](https://doi.org/10.1016/j.marpolbul.2022.114335))
- ✓ I van der Knaap, E Ashe, D Hannay, AG Bergman (2022) Behavioral responses of wild Pacific salmon and herring to boat noise, Marine Pollution Bulletin, Volume 234 Science Direct (DOI: [10.1016/j.marpolbul.2021.113257](https://doi.org/10.1016/j.marpolbul.2021.113257))
- ✓ B Mishachandar, S Vairamuthu (2021) Diverse ocean noise classification using deep learning, Applied Acoustics, Volume 181, Science Direct (doi.org/10.1016/j.apacoust.2021.108141)
- ✓ SV Wang, A Wrede, N Tremblay, J Beermann (2022) Low-frequency noise pollution impairs burrowing activities of marine benthic invertebrates, Environmental Pollution, Volume 310, Science Direct (DOI: [10.1016/j.envpol.2022.119899](https://doi.org/10.1016/j.envpol.2022.119899))
- ✓ M Sèbe, P Scemama, A Choquet, JL Jung (2022) Maritime transportation: Let's slow down a bit, Science of The Total Environment, Volume 811, (<https://doi.org/10.1016/j.scitotenv.2021.152262>)
- ✓ Qi Li (李琪) Dingding Xie (谢丁丁) Rui Tang (唐锐) Dajing Shang (尚大) and Zhichao Lv (吕志超) (2022) Improving sound diffusion in a reverberation tank using a randomly fluctuating surface, Chinese Physics B. Volume 31, No 6, IOP SCINECE, Chinese physical society (DOI: [10.1088/1674-1056/ac3812](https://doi.org/10.1088/1674-1056/ac3812))
- ✓ Warner, R. (2008) Protecting the diversity of the depths: environmental regulation of bioprospecting and marine scientific research beyond national jurisdiction. Ocean Yearbook. 22: 411-443 (DOI: [10.1163/221160008X00172](https://doi.org/10.1163/221160008X00172))
- ✓ Simpson, S.D., Yan, H.Y., Wittenrich, M.L., and Meekan, M.G. (2005) Response of embryonic coral reef fishes (Pomacentridae: Amphiprion spp.) to noise, Marine ecology progress series, Inter – Research Science Publisher, Volume 287, pages 201 – 208 (<https://doi.org/10.3354/meps287201>)

- ✓ Simpson, S.D., Meekan, M.G., Jeffs, A., Montgomery, J.C., McCauley (2008) Settlement-stage coral reef fish prefer the higher-frequency invertebrate-generated audible component of reef noise, *Animal behaviour*, Volume 75, Issue 6, Science Direct (<https://doi.org/10.1016/j.anbehav.2007.11.004>)
- ✓ Gagliano, M., Depczynski, M., Simpson, S.D., and Moore, J.A.Y. (2008) Dispersal without errors: symmetrical ears tune into the right frequency for survival, Volume 275, Issue 1634, The Royal Society Publishing (<https://doi.org/10.1098/rspb.2007.1388>)
- ✓ 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 high-intensity, low-frequency active sonar on rainbow trout, Volume 122, Issue 1, *The Journal of Acoustical Society of America* (DOI: [10.1121/1.2735115](https://doi.org/10.1121/1.2735115))
- ✓ Numair Masud, Laura Hayes, Davide Crivelli, Stephen Grigg and Jo Cable (2020) Noise pollution: acute noise exposure increases susceptibility to disease and chronic exposure reduces host survival, Volume 7, Issue 9, *Royal Society Open Science* (doi.org/10.1098/rsos.200172)
- ✓ Jessica F.de Souzaa, Mayara M.Silveira, Heloisa H.A.Barcellos, Leonardo J.G.Barcellos, Ana C.Luchiari (2022) Sound stimulus effects on dusky damselfish behavior and cognition, *Marine Pollution Bulletin*, Volume 184, Science Direct (doi.org/10.1016/j.marpolbul.2022.114111)
- ✓ Christine Ferrier-Pagès, Miguel C.Lealbl, Ricardo Calado, Dominik W.Schmid, Frédéric Bertuccide, David Lecchinief Denis Allemanda, (2021) Noise pollution on coral reefs? — A yet underestimated threat to coral reef communities, *Marine Pollution Bulletin*, Volume 165, Science Direct (DOI: [10.1016/j.marpolbul.2021.112129](https://doi.org/10.1016/j.marpolbul.2021.112129))
- ✓ Benjamin Geffroy, Bastien Sadoul (2019) Measuring cortisol, the major stress hormone in fishes, Volume 94, Issue 4, *Journal of Fish Biology*, Wiley on line Library (doi.org/10.1111/jfb.13904)
- ✓ Skarsoulis, E.K., Piperakis, G.S., Orfanakis, E., Papadakis, P., (2016) Prediction of shipping noise in the Eastern Mediterranean Sea, In 45th International Congress and Exposition on Noise Control Engineering, Volume 11, Issue 2, *Physics*, MDPI (doi.org/10.3390/jmse11020290)
- ✓ Louise Wilson, Matthew K. Pine, Craig A. Radford, (2022) Small recreational boats: a ubiquitous source of sound pollution in shallow coastal habitats, Volume 174, *Marine Pollution Bulletin*, Science Direct (doi.org/10.1016/j.marpolbul.2021.113295)
- ✓ Marta Picciulin, Matteo Zucchetta, Chiara Facca, Stefano Malavasi, (2022) Boat-induced pressure does not influence breeding site selection of a vulnerable fish species in a highly anthropized coastal area, Volume 180, *Marine Pollution Bulletin*, Science Direct (doi.org/10.1016/j.marpolbul.2022.113750)

- ✓ Chao Peng, Xinguo Zhao, and Guangxu Liu (2015) Noise in the Sea and Its Impacts on Marine Organisms, Volume 12, Issue 10, MDPI (doi.org/10.3390/ijerph121012304)
- ✓ Halit Kuşku, Murat Yiğit, Sebahattin Ergün, Ümüt Yiğit, Nic Taylor (2018) Acoustic noise pollution from marine industrial activities: exposure and impacts, Volume 1, Issue 4, Aquatic research, Dergi Park, AKADEMIK (DOI: [10.3153/AR18017](https://doi.org/10.3153/AR18017))
- ✓ Anthony D. Hawkins, Arthur N. Popper (2016) A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates, Volume 74, Issue 3, Pages 635 – 651, ICES Journal of Marine Science, Journal Du Conseil (doi.org/10.1093/icesjms/fsw205)
- ✓ Onyeogulu Tochukwau Rowland (2018) Sound pollution effect on fish migration rate in a tank experiment, Volume VI, Issue 11, page 64, Scientific Research Journal (Scirj Volume VI, Issue II, February 2018 Edition ISSN: 2201-2796)
- ✓ S. Shafiei Sabet (2018) Effects of sound pollution on predatory behaviour strategies in aquatic animals, Volume 31, Issue 2, Veterinary Researches and Biological Products ([10.22092/VJ.2017.114830.1342](https://doi.org/10.22092/VJ.2017.114830.1342))
- ✓ Maria C. Cartolano, Igal Berenshtein, Rachael M. Heuer, Christina Pasparakis, Mitchell Rider, Neil Hammerschlag, Claire B. Paris, Martin Grosell, M. Danielle McDonald (2020) Impacts of a local music festival on fish stress hormone levels and the adjacent underwater soundscape, Volume 265, Part A, Environmental Pollution Science Direct (DOI: [10.1016/j.envpol.2020.114925](https://doi.org/10.1016/j.envpol.2020.114925))
- ✓ Abir Chahouri , Nadia Elouahmani , Hanan Ouchene (2022) Recent progress in marine noise pollution: A thorough review, Volume 291, Part 2, Cemosphere, Science Direct (DOI: [10.1016/j.chemosphere.2021.132983](https://doi.org/10.1016/j.chemosphere.2021.132983))
- ✓ Halit Kusku (2020) Acoustic sound–induced stress response of Nile tilapia (*Oreochromis niloticus*) to long-term underwater sound transmissions of urban and shipping noises, Volume 27, Environmental Science and Pollution, Springer Link (DOI: [10.1007/s11356-020-09699-9](https://doi.org/10.1007/s11356-020-09699-9))
- ✓ Chrysogelou Foteini (2023) Theory of sounds. Noise pollution, Version 1, Mendeley Data, Science Direct (MaRes ORG , Private Research Publications, In Charge) ([doi:10.17632/pgyhf6src5.1](https://doi.org/10.17632/pgyhf6src5.1))
- ✓ Chrysogelou Foteini (2023) Soundscape. The impact of natural (ambient) sounds above the water and on air, Version 1, Mendeley Data, Science Direct (MaRes ORG , Private Research Publications, In Charge) ([doi:10.17632/p3hky78kd.1](https://doi.org/10.17632/p3hky78kd.1))
- ✓ Chrysogelou Foteini (2020) Artificial coral and non coral Mediterranean Reefs. A threat or a solution? Version 1, Mendeley Data, Science Direct (MaRes ORG , Private Research Publications, In Charge) ([doi:10.17632/r3cvbepfhf.1](https://doi.org/10.17632/r3cvbepfhf.1))

- ✓ Chrysogelou Foteini (2020) Hypothetical artificial reef. Contribution of mussels farming to the development and conservation of species. Mussels and aquaculture history. Negative environmental impacts. ARDA Conference. Research Society. International Conference on Marine Science and Aquaculture (ICMSA-22)-04th October 2022, London , Version 1, Mendeley Data, Science Direct (MaRes ORG , Private Research Publications, In Charge) ([doi:10.17632/52hn82967m.1](https://doi.org/10.17632/52hn82967m.1))

Internet Links

- ✓ <https://awionline.org/content/ocean-noise> (2019 – 2023)
- ✓ https://food.ec.europa.eu/animals/animal-welfare_en (2023)
- ✓ <https://www.eea.europa.eu/> (2019 – 2023)
- ✓ https://www.engineeringtoolbox.com/sound-speed-water-d_598.html (2019 – 2023)
- ✓ <https://www.fisheries.noaa.gov> (2016 – 2023)
- ✓ www.int (2023)
- ✓ www.fao (2010 – 2023)