



DEPARTMENT OF BIOLOGICAL AND  
ENVIRONMENTAL SCIENCES

# EFFECTS OF THE ANTIDEPRESSANT AMITRIPTYLINE ON MARINE MEIOFAUNA

With a novel methodology of examining activity



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## **Popular science summary**

Most of the chemicals humans use can end up in nature through sewage systems. The pollution of chemicals is predicted to increase in the future, posing risks to biodiversity and ecosystems. One chemical tested in this experiment was the antidepressant Amitriptyline (AMI). It was tested on meiofauna, which are medium sized animals and organisms that live in or on sediments and can barely be seen with the naked eye. The meiofauna was collected in the Gullmar fjord on the west coast of Sweden. The meiofauna was exposed to different levels of AMI for 30 days and 48 hours. This was done to see if the number of creatures and activity would be affected. A new method for examining meiofauna activity was also tested called the MTT assay, which colours samples purple if they are active. This was done because there is a need for new tools for testing possible effects on meiofauna.

AMI did not appear toxic to the meiofauna at 30 days or 48 hours exposure, showing little environmental risk. Small signs were seen however of negative effects on the abundance of two meiofauna groups at the lowest level of AMI, possibly due to stress as their activity appeared to increase as well. It is also uncertain how long AMI was available and if the meiofauna was exposed to the intended concentrations for the whole experiment. The new activity method worked and showed promise as a new tool for meiofauna in pollution research. In the future it is important to look more into the fate of AMI in sediments and its effects at low concentrations. The MTT assay also needs to be applied in other laboratories and with other meiofauna communities to further prove that it works.

## Abstrakt

Kemisk förorening av miljön är ett ökande problem som hotar jordens ekosystem. En viktig grupp av organismer är meiofauna som ofta förbises trots dess stora ekologiska betydelse och diversitet. En kemikalie som får alltmer uppmärksamhet som potentiell risk för meiofauna är antidepressiva medicinen Amitriptyline (AMI). AMI har affinitet för sediment och tidigare studier har observerat dess förmåga att negativt påverka meiofauna diversitet och abundans under miljömässigt relevanta koncentrationer. Denna studie syftade att undersöka toxiska effekterna av substansen i både lång- och korttids exponering av meiofauna samhällen, genom att bedöma effekter på både samhällsstruktur och aktivitet genom att utveckla och applicera en MTT analys.

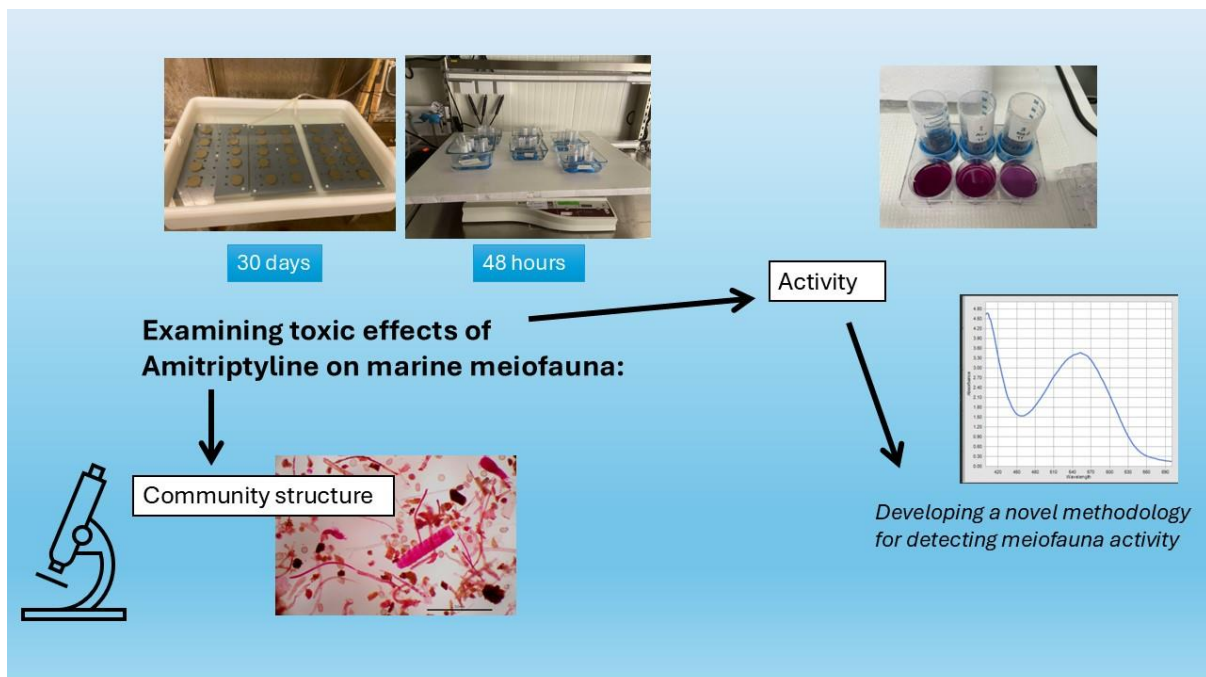
Resultatet visade att det fanns ingen signifikant toxisk effekt på samhällsstruktur eller aktivitet under de testade koncentrationerna (3–30 000 ng/g torrsvikt). Det fanns en liten indikation av möjliga negativa effekter på foraminiferer och nematoder vid den lägsta koncentrationen efter 30 dagar. MTT analysen visade sig vara ett lovande nytt ekotoxikologiskt mätverktyg av meiofauna och studien kunde etablera en grundläggande experimentdesign med upprepningsbara resultat. Vidare forskning behövs för att förstå exponeringsvägen av AMI i sediment och för att undersöka effekter vid låga koncentrationer. Dessutom behöver reproducerbarheten vidare testas för MTT analysen för att verifiera metoden, genom att applicera analysen på andra typer av meiofauna samhällen och i olika laboratorier.

## Abstract

Chemical pollution is an increasing problem threatening earth's ecosystems around the world. One important group of organisms is meiofauna that is often overlooked despite their great ecological importance and diversity. One chemical of emerging concern for meiofauna is the antidepressant Amitriptyline (AMI) that has affinity for sediments and previous studies have observed its ability to negatively affect meiofauna diversity and abundance at environmentally relevant levels. This study aimed to examine the toxic effects of the compound in both long- and short-term exposure on meiofauna communities, assessing effects on community structure and activity by developing and applying the MTT assay.

The findings showed that there was no significant toxic effect on the community structure or activity at the tested concentrations (3-30 000 ng/g dw). There was slight indication of possible negative effects on foraminifers and nematodes at the lowest concentration after 30 days. The MTT assay was deemed a successful possible new ecotoxicological tool, with an established experimental design and demonstrated repeatable stable results. Further research is needed to understand the exposure route of AMI in sediments and investigate effects at low concentrations. Additionally, the reproducibility of the MTT assay needs to be tested and verified, by applying it to other meiofauna communities.

## Graphical abstract



## **Keywords**

Meiofauna, Marine, Amitriptyline, MTT assay, Community, Gullmar fjord, Mesocosm.

## **Abbreviations**

**AMI:** Amitriptyline

**ANOSIM:** Analysis Of Similarity

**ANOVA:** Analysis of Variance

**DMSO:** Dimethyl Sulfoxide

**Dw:** Dry weight

**EC50:** Half maximal effective concentration

**LC50:** Lethal dose 50%

**Log K<sub>oc</sub>:** Base-10 logarithm of the organic carbon-water partition coefficient

**MIC:** Minimum inhibitory concentration

**MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

**NMDS:** Non-metric Multidimensional Scaling

**SD:** Standard deviation

**SIMPER:** Similarity Percentages

**TCA:** Tricyclic antidepressant

**Ww:** Wet weight

## Table of contents

Popular science summary.....	2
Abstrakt.....	3
Abstract.....	4
Graphical abstract.....	4
Keywords.....	5
Abbreviations.....	5
Table of contents.....	6
Table of figures.....	8
Table of tables.....	9
Acknowledgments.....	10
1. Introduction.....	11
1.1 The ecological threat of chemical pollution.....	11
1.2 Community Ecotoxicology.....	12
1.3 The hidden players in our oceans – meiofauna.....	13
1.4 Meiofauna in the field of ecotoxicology.....	15
1.5 The MTT assay.....	15
1.6 Chemical pollution in the marine environment.....	17
1.7 The antidepressant Amitriptyline.....	18
1.8 Aim.....	19
2. Methods.....	19
2.1 Chemical screening.....	19
2.2 Sediment sampling.....	21
2.3 Applying the MTT assay to meiofauna.....	22
2.3.1 Experimental development and optimization.....	22
2.3.2 Method verification and investigation of confounding factors.....	24
2.4 Amitriptyline toxicity assessment.....	27
2.4.1 Long term exposure.....	27
2.4.2 Short-term toxicity test.....	30
2.5 Statistical analysis.....	31
3. Results.....	32
3.1 MTT assay development.....	32
3.1.1 Optimization and development of the assay.....	32
3.1.2 Bacterial influence.....	32
3.1.3 Abiotic interactions and detection of viability.....	34

3.2 AMI effects on meiofauna activity.....	34
3.2.1 Effects on activity.....	34
3.3. AMI and effects on the community level.....	36
3.3.1 Community structure .....	36
3.3.2 Community similarity and dissimilarity between the treatments .....	38
4. Discussion.....	39
4.1 MTT assay development .....	39
4.1.2 Establishing the MTT assay’s applicability.....	39
4.1.3 The examination of possible confounding factors.....	40
4.2 Amitriptyline toxicity assessment .....	40
4.2.1 Examining effects on community structure.....	40
4.2.2 Assessing effects on activity by applying the MTT assay.....	41
4.2.3 Combining activity and community data.....	41
4.3 Limitations of the toxicity assessment .....	42
4.3.1 Uncertainty of sediment concentration and bioavailability .....	42
4.3.2 Stress induced from the experimental design .....	42
4.3.3 Meiofauna identification and sampling .....	43
4.4 Further research of the MTT assay needed .....	44
5. Conclusions.....	45
Bibliography .....	46
Supplementary information.....	56

## Table of figures

Figure 1. Planetary Boundary Diagram. ....	11
Figure 2. Ecotoxicological research at different levels of biological complexity. ....	12
Figure 3. Common meiofauna organisms. ....	14
Figure 4. The MTT assay. ....	16
Figure 5. Map of the Gullmar fjord and sampling site. ....	21
Figure 6. The different MTT incubation set-ups. ....	23
Figure 7. The mesocosm experiment. ....	28
Figure 8. Experimental set-up of the short-term toxicity test. ....	30
Figure 9. Examining bacterial activity in the MTT assay. ....	33
Figure 10. Prolonged MTT incubation. ....	34
Figure 11. Meiofauna activity after 30 days exposure of Amitriptyline (AMI). ....	35
Figure 12. Meiofauna activity after 48h Amitriptyline (AMI) exposure. ....	36
Figure 13. The relative abundance of each meiofauna taxa. ....	37
Figure 14. The most observed meiofauna organism groups. ....	37
Figure 15. A NMDS on meiofauna abundance. ....	38

### Supplementary

Figure S1. MTT assay absorbance spectrum. ....	59
Figure S2. Barplots of different univariate community measurements from the long-term Amitriptyline experiment. ....	60
Figure S3. Boxplots of each taxa group abundance from the long-term Amitriptyline (AMI) experiment. ....	61
Figure S4. Spearman correlation coefficients applied to meiofauna abundance and the activity measurements. ....	62

## Table of tables

Table 1. Monitoring data of AMI in sediment.....	18
Table 2. Antibiotics in the treatment mixture.....	25

### Supplementary

Table S1. Preliminary chemical priority list from the EU project group Contrast.....	56
Table S2. Summary of absorbance values for the different experimental set-ups.....	61
Table S3. ANOSIM test of significant dissimilarity.....	62
Table S4. SIMPER One-way analysis.....	63
Table S5. Meiofauna community data.....	63

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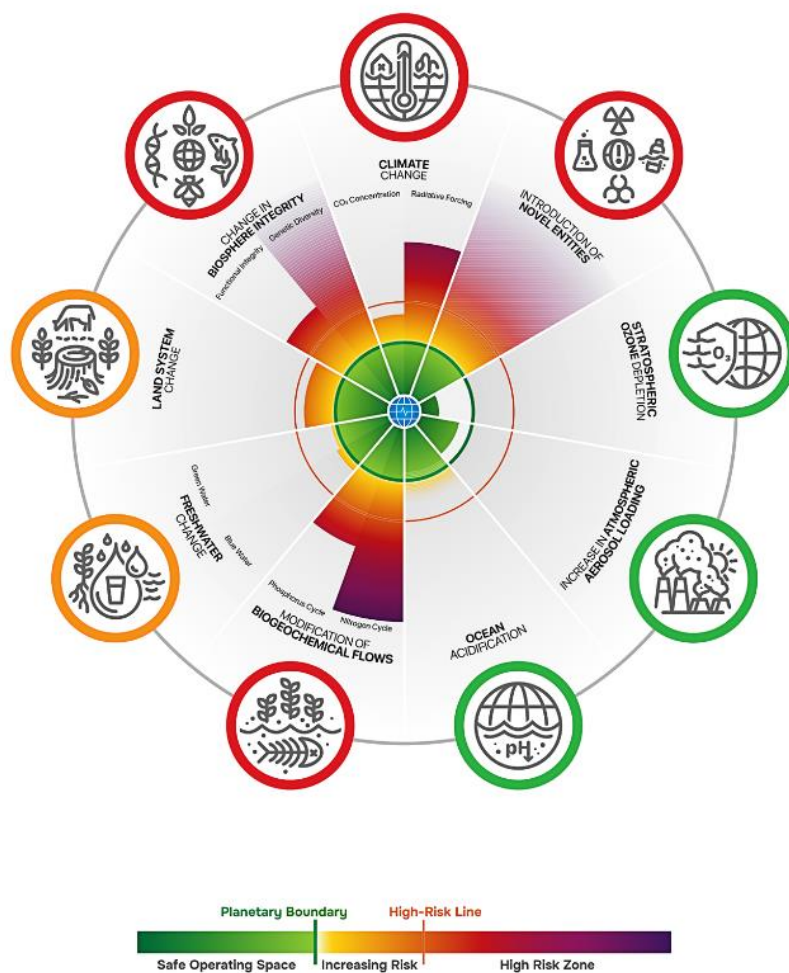
My project would not have been possible without the help from the staff at the Kristineberg Marine Research station, I want to say thanks to the boat crew and staff that helped me during my multiple visits. I also want to thank people from the Contrast group for giving me inspiration for relevant chemicals to test and for the opportunity to attend one of your workshops where I could try out some methodology and engage in other fun activities. I also want to thank Francesca Molinari for helping me out with taking down the experiment at Kristineberg and for giving me helpful meiofauna tips. Huge thanks also to Stefan Agrenius for helping me identify some mysterious organisms I would not have figured out on my own.

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# 1. Introduction

## 1.1 The ecological threat of chemical pollution

The industrialization of the 20<sup>th</sup> century has led to a substantial increase in the pollution of chemicals in our biosphere (Massard-Guilbaud & Mathis, 2017). Approximately 100 000 chemicals are on the market currently and from 2010 to 2050 chemical production is predicted to triple (EEA, 2018). This continuous production of new synthetic chemicals introduces compounds that never existed before to the environment and run the risk of being toxic both individually and together with other pollutants as mixtures (Naidu et al., 2021). This chemical burden has harmful implications for the health of ecosystems all around the world. Chemical pollution was first introduced as one of nine planetary boundaries in 2009 by Rockström et al, referred to as the Introduction of Novel Entities (Figure 1).



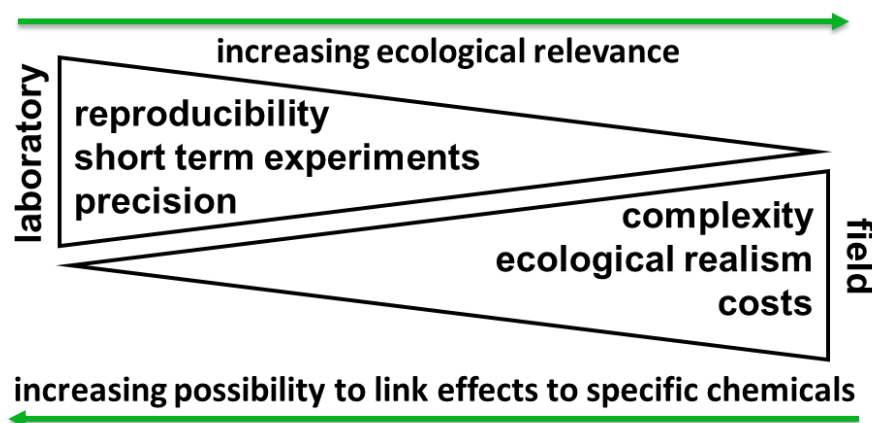
**Figure 1. Planetary Boundary Diagram.** An illustration of the nine planetary boundaries and their assessed risk level demonstrated by safe (green) to high risk (purple). Green represents a safe operating space where

healthy conditions can be maintained. Yellow and Orange symbolize increasing risk, indicating a possibility of causing damage if the boundary transgresses further. Red and Purple signifies high risk, with a high possibility of destabilizing the systems on earth. (Ceasar et al., 2024)

The chemical pollution boundary was deemed breached several years later, meaning that currently there is a great risk that chemical pollution will induce substantial change to our environment (Persson et al., 2022; Ceasar et al. 2024). One environmental change chemical pollution has been linked significantly to is biodiversity loss, a problem that continues to worsen (IPBES, 2019). Compared to other drivers of biodiversity loss chemical pollution has not been integrated well into biodiversity research (Sylvester et al., 2023), motivating further research into the area.

## 1.2 Community Ecotoxicology

The field of ecotoxicology began its research in the 1940s, then under the name of environmental toxicology (Rodríguez-Romero et al., 2021). The ecological perspective was not yet well established then, with a focus foremost on single model organisms in laboratory experiments. It was not until 1969 that the discipline got its name of ecotoxicology when ecology and toxicology were combined into one (Forbes & Forbes, 1994). The aim of the discipline is to examine and predict effects from chemical exposure on natural communities in realistic exposure scenarios (Chapman, 2002). Traditional ecotoxicology has however often focused solely on effects on single species, based on the “most sensitive species” concept, which have been criticized greatly over the years for being reductionistic (Cairns, 1986; Chapman, 2002; Schmitt-Jansen et al., 2008).



**Figure 2. Ecotoxicological research at different levels of biological complexity.** The different biological level of research in the field of ecotoxicology – laboratory and field experiments. Each green arrow symbolizes the benefits of each orientation as you move towards the other. Inside each triangle some summarizing terms are

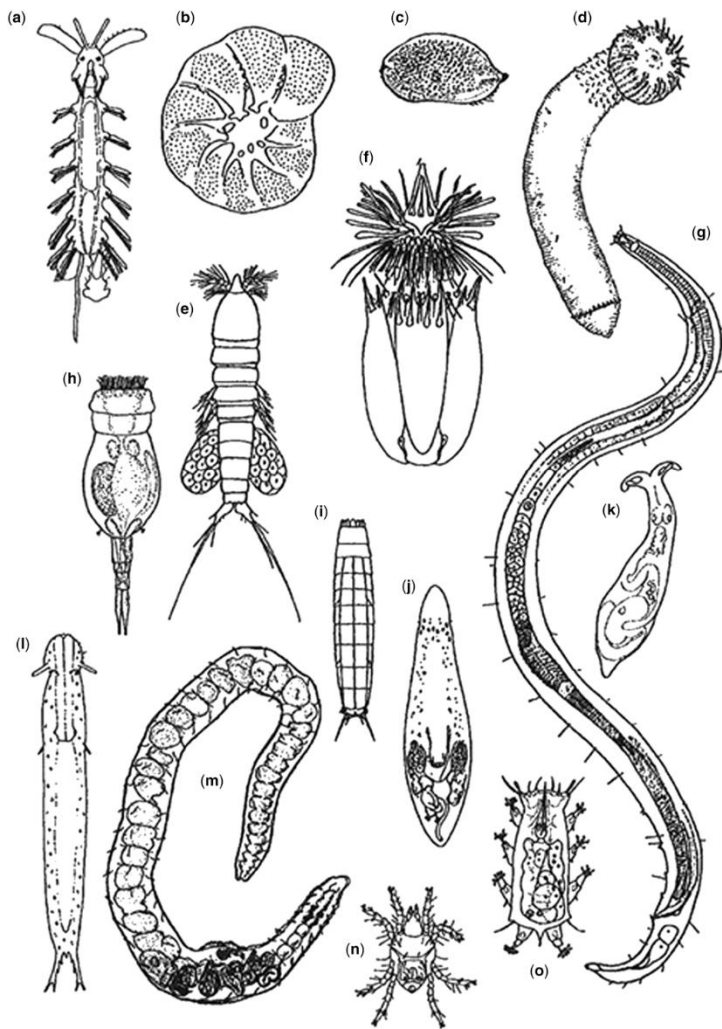
given for the two areas. (LibreTexts, 2023)

Due to the lack of ecological focus in the field of ecotoxicology, despite having “eco-” in its name, a subfield of community ecotoxicology advanced (Clements & Rohr, 2009).

Community ecotoxicology focuses on toxic effects on species abundance, diversity and community structure, increasing the biological complexity of the research (Figure 2). More complex experimental designs such as the micro- and mesocosms are often used as they are more ecologically realistic and can observe effects that single species tests would never be able to show (Chapman, 2002). Mesocosms can have many controlled variables but at the same time simulate the natural habitat of the organisms (Adams, 2003). However, these field semi-controlled experiments have their downfalls concerning statistical power and ability to establish causality, thus they should be considered complementary to the traditional laboratory experiment (Schmitt-Jansen et al., 2008).

### **1.3 The hidden players in our oceans – meiofauna**

Sediments around the world are packed with life, inhabiting small benthic organisms that are commonly called meiofauna, derived from their medium size compared to macro- and microfauna (Giere, 2009). Practically they are typically defined based on sieve mesh sizes, being able to pass through a 1 mm mesh but be retained by a 31  $\mu\text{m}$ , however the lower limit is still up for discussion (Ptatscheck et al., 2020). Meiofauna consists of invertebrates and protists that live in or on sediments, sometimes also referred to as meiobenthos. These meiobenthic creatures exist almost everywhere on earth and have great ecological importance in aquatic habitats as they provide essential ecosystem services (Giere, 2019), such as nutrient cycling and food production (Schratzberger & Ingels, 2018). In marine food webs they play an important role by influencing and connecting both lower and higher trophic levels with their ability to affect sediment stability and nutrient flows (Schratzberger & Ingels, 2018). They have even been linked to essential functions in the nitrogen and carbon cycle (Martínez et al., 2025).



**Figure 3. Common meiofauna organisms.** Represented by a) Polychaeta; b) Foraminifera; c) Ostracoda; d) Priapulida, e) Copepoda, f) Loricifera, g) Nematoda, h) Rotifer, i) Kinorhyncha, j) Turbellaria, k) Mollusca (Gastropoda), l) Gastrotricha, m) Oligochaeta, n) Halacaroidea, o) Tardigrada. (Urban-Malinga, 2013, adapted from Higgins & Thiel 1988).

Meiofauna biodiversity is represented by 24 different animal phyla, out of the total 35 phyla existing on earth (Balsamo et al., 2012), with some classical groups such as copepods and nematodes (Figure 3). Marine meiofauna communities are normally dominated primarily by nematodes which encompass many species at different trophic levels and roles of the food web (Haegerbaeumer et al., 2016). Foraminifers are another commonly dominating group but are often overlooked due to the focus on multicellular organisms in meiofauna research, since they are classified as protists and are unicellular (Giere, 2009). In marine and brackish areas dominating groups are usually harpacticoid copepods, kinorhynchs and ostracods (Chertoprud & Novichkova, 2023). Larval and polyp stages of some macrozoobenthos are also included in the meiofauna grouping normally referred to as the “pseudomeiobenthos”

(Giere, 2009; Chertoprud & Novichkova, 2023).

#### **1.4 Meiofauna in the field of ecotoxicology**

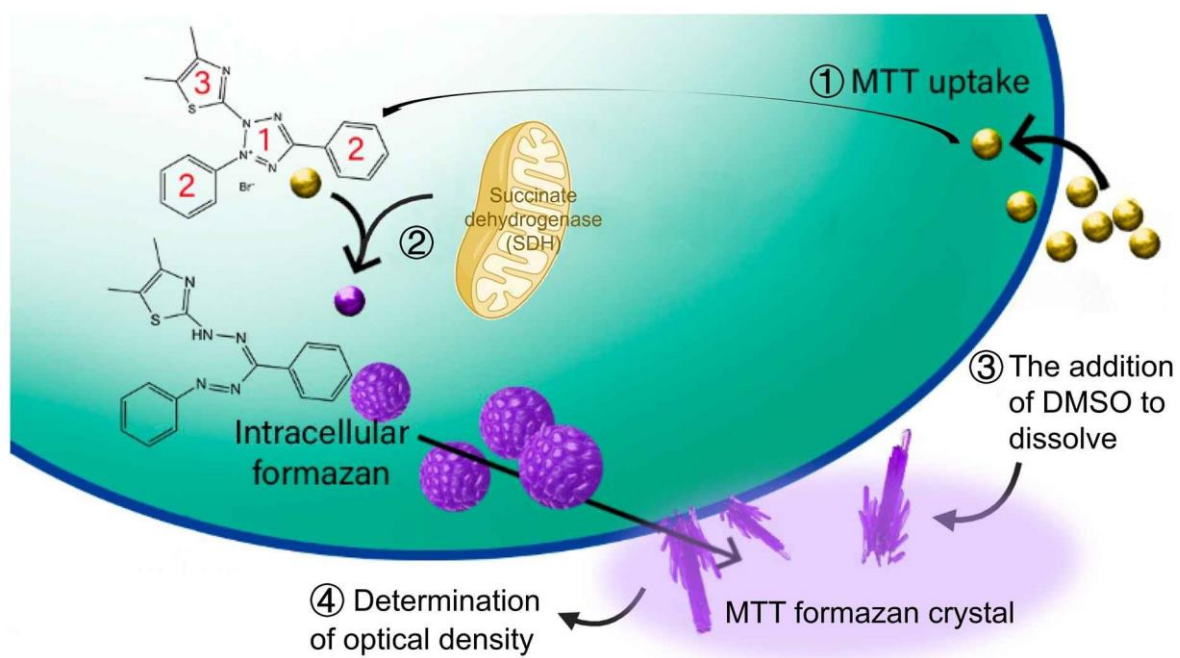
Despite their ecological importance, diversity and abundance, scientific research on meiofauna is lacking (Giere, 2009). Researchers and the public consider them, if they even do consider them, as irrelevant and uninteresting – the so called “Meiobenthos dilemma”. This refers to the common theme of smaller organisms like meiofauna being underrepresented in conservation agenda and biodiversity research, and the difficulty making them appeal to the public despite their ecological importance. One major challenge in studying meiofauna is their complexity and experience needed to identify to lower taxonomic ranks such as species and genus (Giere, 2009). A “taxonomic minimalistic” approach may instead be applied that identifies to higher levels of taxa such family and phyla. However, there is great value in studying meiofauna. They are, e.g. useful for assessing pollution (Schratzberger & Ingels, 2018) and can be used as bioindicators (Balsamo et al., 2012). Meiofauna studies from around the world show that pollution stress lowers the diversity of meiofauna (Gyedu-Ababio & Baird, 2006; Rubal et al., 2009; Gallucci et al., 2015). The stress induced by pollution has also been shown to cause cascading effects by affecting the resilience and health of ecosystems in marine sediments (Schratzberger & Ingels, 2018).

Meiofauna’s ability to rapidly respond to changes in the environment because of their relatively short generation time, steady reproduction and development make them excellent organisms to work with (Gyedu-Ababio & Baird, 2006). Not only are they practical to work with but they are considered a distinct structural unit of the benthic ecosystem with its own characteristics (Chertoprud & Novichkova, 2023). Therefore, meiofauna is considered ideal to use in toxicity assessments of chemicals. The status of available standardized toxicity tests for meiofauna is mainly focused on single species (Brinke et al., 2011), species of only one taxa group (ASTM International, 2000) or larger organisms like arthropods (US EPA, 1994). In biomonitoring a benthic community point of view is often applied, however the focus is foremost on macroinvertebrates (Grapentine, 2013). Thus, a need for more ecologically relevant standardized test procedures for meiofauna communities in ecotoxicology is evident.

#### **1.5 The MTT assay**

The MTT assay was first developed by Tim Mosmann in 1983 and has since then become a prominent and widely applied biological assay (Kamiloglu et al., 2020). The main usages

have been to examine cell viability and cytotoxicity (Stockert et al., 2012), with traditional applications on bacteria, fungi and animal cell lines (Grela et al., 2018). It is used as a rapid alternative for analyzing viability instead of cell counting, which can be quite time consuming (van Meerloo et al., 2015). The assay is based on the reduction of the MTT compound as an indication of active cells demonstrating activity and thus viability (Figure 4). The lipophilic MTT compound can pass through the cell membrane and be reduced into the product substance Formazan inside the cell, indicating mitochondrial function (Gilbert & Friedrich, 2017; Kamiloglu et al., 2020; Ghasemi et al., 2021). There is evidence that MTT can also be metabolized in other parts of the cell, thus not only representing mitochondrial function but also the function of other organelles (Ghasemi et al., 2021).



**Figure 4. The MTT assay.** The figure illustrates the different steps in the assay: 1) MTT enters the cell 2) MTT is reduced in metabolic active cells by the mitochondrial enzyme SDH to Formazan 3) The intracellular Formazan is dissolved by a solvent and extracted from the cell 4) The absorbance of the dissolved Formazan is measured for determining viability. (CD, n.d, adapted from Ghasemi et al., 2021).

The formation of the Formazan crystal can disrupt cells meaning that the assay is an endpoint assay (Grela et al., 2018). The metabolized formazan is insoluble in water and thus needs to be dissolved by a solvent prior to the spectrometric measurement (Kamiloglu et al., 2020). Dissolving Formazan results in a purple coloured sample with an absorbance intensity correlating to the viability of cells in the sample, since dead cells are believed to have

nonexistent or minimal metabolism of MTT (Gilbert & Friedrich, 2017; Braissant et al., 2020; Kamiloglu et al., 2020).

A novel approach applying the MTT assay on meiofauna was tested by Nooijer et al. in 2006 when the authors managed to stain foraminifers with MTT to distinguish between alive and dead individuals. The study microscopically observed that the organisms could ingest the compound and metabolize it within 24 hours. About 20 years prior, another study managed to apply the assay to nematodes observing effects with spectrometry (Comley et al., 1988), and other studies have followed with the aim of investigating antiparasitic treatments of different nematode species (Nayak et al., 2011; Mukherjee et al., 2016). However, no other studies applying the assay to other types of meiofauna organisms have been found and no study to my knowledge has applied the assay to meiofauna communities. This suggests further application of the assay on meiofauna organisms with the motivation to develop it into a possible tool applied to more biologically complex samples, i.e. communities. If the assay is deemed applicable it could act as a new ecotoxicological tool to assess toxic effects on meiofauna in future research.

## **1.6 Chemical pollution in the marine environment**

For a long time, the ocean was considered immune to pollution and thought to have limitless absorptive capacity (Cederqvist et al., 2019). However, in recent years chemical pollution and its effects on marine ecosystems have come to light (Naidu et al., 2021; Magnuson et al., 2022; Sylvester et al., 2023). Unfortunately, the toxic effects from emerging pollutants on marine meiofauna are not well studied, especially free-living invertebrates (Ishak et al., 2022). Another limiting factor to the knowledge on toxic effects to benthic organisms is that the standard ecotoxicological tests are mainly based on aqueous exposure levels assuming that the primary exposure route of toxicants is through water and is usually what common endpoints such as LC50 and EC50 are based on (Chapman, 2002). This can limit the knowledge of the toxic effects since other important exposure routes such as sediment may also be important to predict effects.

One group of chemicals gaining more and more attention is pharmaceuticals. The cause of concern has been raised due to their broad usage, wide detection in the environment and bioactive characteristics (Lahti & Oikari, 2012; Branchet et al., 2021). Observations of pharmaceuticals are mainly done in freshwater environments compared to marine (Branchet

et al., 2021). In the marine environment pharmaceuticals can act as sinks in the sediment by accumulating high concentrations compared to the water column (Magnuson et al., 2022) and can then be rereleased into the water (Merhaby et al., 2019). Aquatic organisms are subjected to immense risk since pharmaceuticals are considered pseudo-persistent leading to possible chronic exposure to the organisms (Magnuson et al., 2022). Many pharmaceuticals have been reported as chemicals of emerging concern, a new class of micropollutants detected at low levels in nature suspected to have potential risks to ecosystems (Real et al., 2015) and have usually not been regulated yet (Minguez et al., 2014b).

### 1.7 The antidepressant Amitriptyline

One chemical of emerging concern is the antidepressant Amitriptyline (AMI) (Real et al., 2015). Among pharmaceuticals, antidepressants are one of the most used globally (Imiuwa et al., 2024), acting on a signaling pathway that has been evolutionary conserved among many different animal phyla including both invertebrates and vertebrates that share similar serotonin receptors (Tierney, 2018). Tricyclic antidepressants (TCA) are a group of antidepressants where the compound AMI is prescribed the most for depression but also for a plethora of other medical conditions (Imiuwa et al., 2024). It has been on the market for depression since 1961, commonly sold as Elavil and was placed on WHO's list of essential medicines in 2019 due to its broad application (McClure & Daniels, 2021). The compound works by inhibiting serotonin and norepinephrine reuptake. However, the understanding of the mode of action of TCAs like AMI is limited and some studies show that they can also act by inhibiting other nontarget receptors.

The spread of AMI is worldwide and has been detected in both surface waters and wild animals (Imiuwa et al., 2024). Observations have likewise been done in sediments, however data for levels of the compound in sediment is scarce (Table 1).

**Table 1. Monitoring data of AMI in sediment.** The monitoring data is represented by different types of sediments and in different areas of the world.

Sediment type and location	AMI (ng/g dw)	Reference
Coastal sediment, Spain	<0.1–0.4	Maranho et al., 2015
Estuary sediment, Poland	0.7–25.1	Kucharski et al., 2022
Coastal sediment, Sweden	3.16-3.5	Norman Empodat

		Database
River sediment, Sweden	<0.035-0.94	Golovko et al., 2020
Fjord sediment, Norway	6.5–15.6	Magnusson et al., 2022

Concentrations of the compound have been detected from the lowest at <0.035 ng/g dw observed in Swedish river sediment and the highest concentration of 25.1 ng/g dw in sediment from the Baltic Sea near the coast of Poland. Since research has mainly focused on effects on non-target organisms from the other antidepressant group e.g. SSRIs, the possible non-target effects of AMI are not well documented (Minguez et al., 2014a). Previous studies have observed that the antidepressant may cause sublethal and lethal effects in aquatic organisms at environmentally relevant concentrations (Magnuson et al., 2022). Concerns have been raised about its possible toxic effects on benthic creatures in marine sediments, such as polychaetas and nematodes (Ishak et al., 2022; Magnuson et al., 2022). The toxic mode of action is believed to be primarily oxidative stress, since it has been linked to both cytotoxic effects and mitochondrial damage (Yang et al., 2014; Sehonova et al., 2017).

## 1.8 Aim

The aim of this project consists of two areas of interest: 1) To investigate the toxic effects of the antidepressant AMI on the activity and community structure of marine meiofauna, 2) To develop, optimize and verify the MTT assay applied to meiofauna communities to measure activity. The first area of interest focuses on analyzing toxic effects on the community level at environmentally realistic exposure levels as well as extreme levels. The chemical selected was selected based on the aim of testing the toxicity of a novel chemical of emerging concern. The first area of interest was then connected to the second area of interest where a novel activity assay is developed and applied to examine possible toxic effects on activity induced by AMI. Thus, the study aims to develop the MTT assay by establishing experimental design, optimal test conditions and by verifying its efficacy. The purpose of developing this method is to enable a possible new rapid tool in ecotoxicological research for assessing toxic effects on meiofauna communities.

## 2. Methods

### 2.1 Chemical screening

The chemical AMI used in the project was selected based on a preliminary priority list

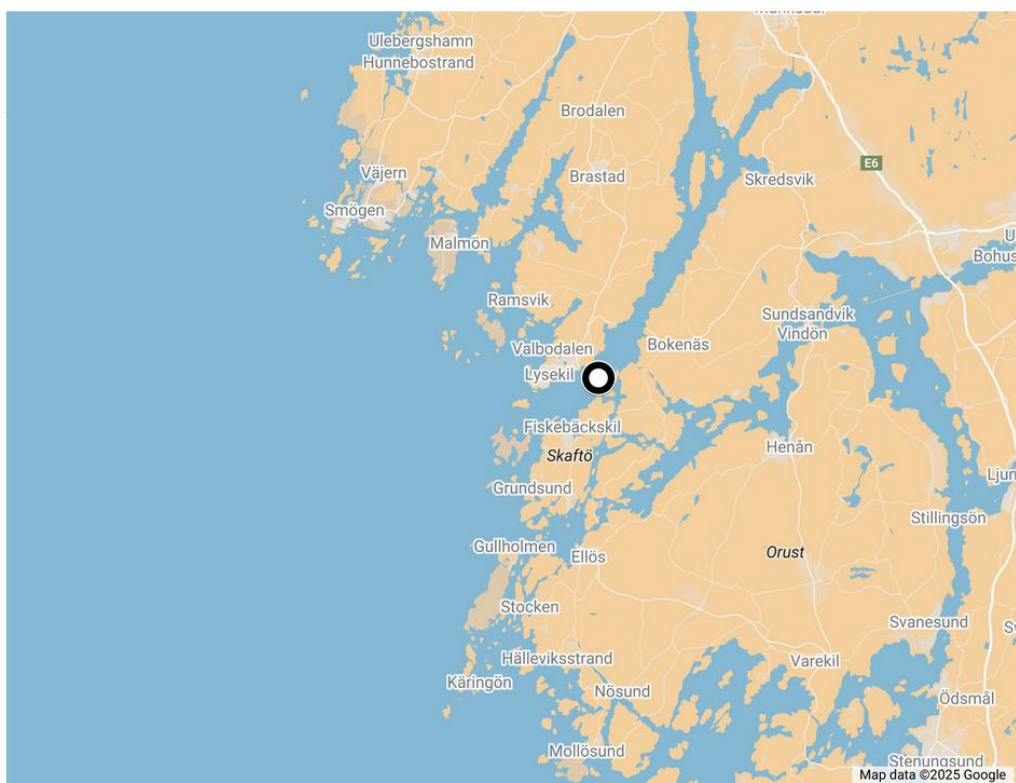
created by the EU project group Contrast for different chemicals of emerging concern (Table S1). The priority list was made available for this study in October 2024. Eight chemicals had already been pre-selected for the study based on the assessment of project group members, highlighting substances considered especially relevant for meiofauna. For these eight chemicals a selection scheme was applied based on different criteria to select a chemical for the long-term toxicity experiment. The first criteria applied was the lipophilicity of the compound indicating the possible sediment exposure risk to meiofauna, meaning that it needed to have a  $\text{Log } K_{ow} > 2$  to be selected. After this first elimination three different criteria were applied that concerned ecotoxicity, exposure and regulatory status of the substances. Data from the US EPA database and other ecotoxicological studies was used to assess ecotoxicity, using data for invertebrates, meiofauna, long term exposure, community studies and sediment exposure. There needed to be enough toxicity data to assess the risk of the chemical. There also needed to be enough different concentrations showing effects to base an experimental concentration range on.

Monitoring data for sediment was acquired from the Norman Empodat database to assess the exposure of the chemical, as well as from monitoring studies. The pollution data for all chemicals were quite scarce for sediment – therefore selection was based on the amount of available data, amount of different monitoring locations and sample occasions. This was done to enable the application of an environmentally relevant concentration range in the experiment. Moreover, the possibility for the chemical to bioaccumulate or persist in the environment were also factors that favored selection since it increased the concern of the chemical.

The regulatory status criteria considered the wider societal concern of the chemical. If the chemical was included in any restriction regulation or monitoring prioritization list in the EU, and if it was already banned. The so called “public debate” was also considered looking at scientific articles and debate articles to assess if the chemical was already being treated as a possible concern in the public debate. The selection favored chemicals that were not already restricted, banned or prioritized and not up for debate to be in the future. This was based on the project’s aim to test the toxicity of a novel chemical of emerging concern. An assessment of the delivery time was also considered for the selection due to the time constraints of the study. Ultimately this all resulted in the selection of the antidepressant AMI.

## 2.2 Sediment sampling

Sediment used for the various meiofauna experiments and MTT assay tests was collected from the Gullmar fjord situated on the west coast of Sweden ( $58^{\circ} 16.42\text{N}$ ,  $11^{\circ} 29.14\text{E}$ ) (Figure 5). Sampling was conducted in November and December of 2024, and in January and March of 2025. Gullmar is a sill fjord measuring 30 km in length and has a max depth of 118 m (Gothenburg University, 2023). The fjord has a unique, rich and interesting fauna due to its complex water exchange with Skagerrak, Kattegat and the Baltic Sea. The fjord's bottom water is usually renewed yearly and receives oxygen every spring (Hansson et al., 2000). The water exchange creates a strong stratification with a pycnocline around 15-20 m, separating lower saline water on top and higher saline water at the bottom around 34-35 psu at 50-60 m depth.



**Figure 5.** Map of the Gullmar fjord and sampling site. The sampling location is marked with a black circle in the Gullmar fjord. (Google Maps, 2025).

The fjord has been subject to research for many years, it has been studied since the 1830s and is one of the world's most studied marine areas (Harland et al., 2019; Gothenburg University, 2023). The Gullmar fjord is believed to not have been exposed to major sources of pollution from sewage or industry since the late 1960s, before this it has been greatly polluted with substances from a paper mill, a sulphite mill and other human activities (Nordberg et al.,

2000). Since 1983 it has been protected as a nature reserve and is also an EU Biomare reference area for marine research (Harland et al., 2019; Gothenburg University, 2023). Based on its protected status and duration free from substantial pollution it is considered a suitable reference area for sediment sampling.

An Olausson Box corer was used to gather the sediment at approximately 60 m depth. The sediment appeared to be well oxygenated and several macrofauna organisms could be observed in the sediment cores, e.g. brittle stars and sea urchins, indicating a healthy status of the sediment. The highest meiofauna abundance can normally be found at the first 0-2 cm layer of muddy sediments and decreases with depth (Giere, 2009). The first 1.5-2 centimeters of the sediment that was oxygenated was scraped off and then sieved through a 250  $\mu\text{m}$  sieve to remove larger objects and organisms. Meiofauna is known to have a patchy distribution which is important to consider when sampling them. At least three cores were sampled at each sampling event at different places of the sampling area, moving the boat slightly for each sediment core to avoid sampling the exact same place. Sediment not used directly after the sampling was transported back to Gothenburg and stored in a temperature-controlled room at 4 °C, in darkness and aerated.

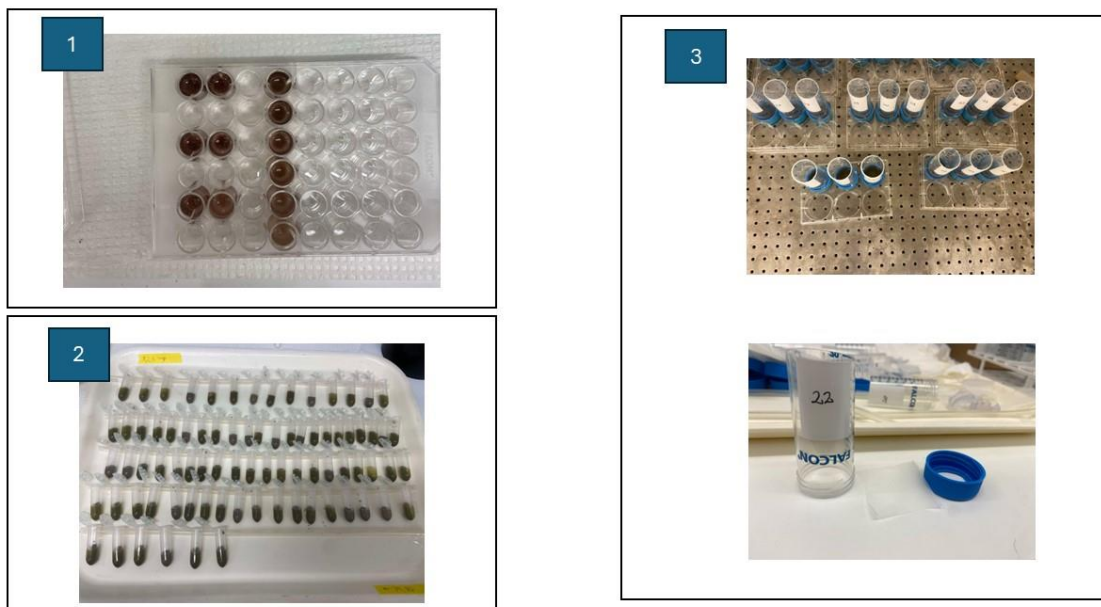
## **2.3 Applying the MTT assay to meiofauna**

### ***2.3.1 Experimental development and optimization***

Different incubation times and MTT concentrations were tested in the beginning according to various MTT guidelines and protocols (Gilbert & Friedrich, 2017; Grela et al., 2018; Benov, 2021). An incubation period of one to four hours adheres with the recommended MTT assay guidelines (Neufeld et al., 2018). However, in this more complex incubation scenario a much longer duration was also tested since it was expected that the compound would need more time to penetrate the cells of the meiofauna and be metabolized. Increasing incubation time is suggested as a method to increase the absorbance signal intensity (Gilbert & Friedrich, 2017). Thus 0.5, 1 and 20 h incubation times were tested. The assay was always protected from prolonged light exposure during the incubation and dissolution phase to avoid spontaneous reactions of MTT or Formazan. The concentration of MTT is another important factor, three different concentrations were tested (0.2, 0.5 & 1 mg/ml). The MTT (CAS: 298-93-1) stock was dissolved in pasteurized filtered seawater and stored in a fridge for a maximum of four weeks.

The assay's performance is greatly dependent on the complete solubilization of Formazan as well as the chemical stability of the dissolved solution (Benov, 2021). An DMSO solvent buffered with 8-800 mM ammonia was suggested by research to be the most ideal solvent of the MTT metabolite, stabilizing the product and resulting in a stable absorbance spectrum and limited background absorbance (Wang et al., 2012; Benov et al., 2021). Thus, a solvent of DMSO (CAS: 67-68-5) buffered with 50 mM ammonia (25%) (CAS: 1336-21-6) was used as a solvent, dissolving the sample for ten minutes. The pH of the dissolved sample can affect the absorbance spectrum and the stability of Formazan, which is less stable under acidic pH (Benov, 2021). The pH of all prepared solvents was measured with a pH stick indicating pH 8.

In terms of the practical experimental design different set-ups were tested to find the most suitable one (Figure 6).



**Figure 6. The different MTT incubation set-ups.** 1) 48-well plate, 2) Open 2 ml Eppendorf tubes tilted on a plastic tube 3) Half 50 ml Falcon tubes with drilled holes in the lids, a 63 µm mesh square was screwed on together with the lid.

The 48-well plate method consisted of incubating sediment directly in the wells, then adding ammonia-DMSO, letting the samples suspend and then taking the supernatant for analysis. For the Eppendorf tubes the incubation was done by adding the sediment in the tubes together with the MTT. To dissolve the samples ammonia-DMSO was added to the tubes and then the tubes were vortexed. After the dissolution the samples were centrifuged at 2000g for 2 min at 20°C and the supernatant was taken for analysis. The Falcon tube set-up was prepared by

sawing 50 ml Falcon tubes in half, drilling a hole in the lid and cutting a square of 63  $\mu\text{m}$  mesh. The mesh was screwed together with the lid, acting as a sieve in the sample container where the sediment was added. The Falcon tubes were then placed in 6-well plates where they were first incubated with MTT and then transferred to new wells for the ammonia-DMSO dissolution. Finally, the remaining liquid in the well after the dissolution was taken for analysis. Different amounts of sediment (10 g and 5 ww) were also tested with the Falcon tube set-up to determine the optimal sediment weight.

In all the experimental set-ups absorbance was analyzed in 96-well plate with the Varioskan Flash Microplate reader. An absorbance spectrum (400-700 nm) was measured because it is advised to always define the measurement wavelengths for each new MTT assay since the absorbance peak can appear at different wavelengths depending on different factors such as which solvent was used (Grela et al., 2018). Based on the absorbance spectrum the wavelength for the absorbance peak and background absorbance were decided. The final absorbance was then calculated by accounting for the background absorbance to avoid possible sediment particle disturbance, applying the following equation:

$$Abs_{Final} = Abs_{Max} - Abs_{Background}$$

If the absorbance signal reached 6, which was the maximum absorbance reading of the plate reader, the sample was diluted until an absorbance signal  $<6$  could be detected (Gilbert & Friedrich, 2017). The final absorbance was then normalized based on its dilution. For all samples the wet weight (ww) of the sediment was noted and then used to normalize the final absorbance to 5 g ww.

### ***2.3.2 Method verification and investigation of confounding factors***

Assay tests were run with the different experimental set-ups to confirm the repeatability of the assay with the aim to observe absorbance peaks with low variation. When the optimal experimental set-up was decided the assay was repeated three times. Moreover, tests were done to verify that the assay measured what it was intended to measure, i.e. being able to detect activity differences between viable and less viable meiofauna. This was done by running the assay with live and dead meiofauna. The dead meiofauna was euthanized by either being submerged in Ethanol (96%) for 15 min and then boiled for 15 min, or only being boiled. Two different ways of euthanizing the organism were applied to find the most efficient method with the aim of observing low absorbance in the dead samples and a significant activity difference between live and dead samples.

The MTT assay has been applied greatly to bacteria throughout research (Grela et al., 2018; Benov, 2021). Meiofauna communities are intimately linked to bacteria (Giere, 2009), meaning that there would be a high risk of bacteria influencing the results of the assay. Different types of samples were run in the assay to test the possible effects from bacterial activity. These samples consisted of antibiotic treated sediment, whole sediment and dead meiofauna with unpasteurized seawater. The antibiotic treated sediment was exposed to a mixture of three antibiotics (Table 2), with the hypothesis to find a reduced activity in the treated samples if there was substantial bacterial presence. The antibiotic mixture consisted of Ciprofloxacin (CAS: 85721-33-1), Neomycin (CAS: 1405-10-3), and Oxacillin (CAS:1173-88-2) which all belong to three different antibiotic classes: fluoroquinolones, aminoglycosides and  $\beta$ -lactams.

**Table 2. Antibiotics in the treatment mixture.** The different antibiotics are displayed with their different minimum inhibitory concentrations (MICs) and ecotoxicity levels which were the basis for the final experimental concentration.

Antibiotic	Highest relevant MIC	Lowest relevant toxicity level	Experimental concentration
<i>Ciprofloxacin</i>	0.5 $\mu$ g/ml <i>Pseudomonas aeruginosa</i> (Liu et al., 2020)	1.1 $\mu$ g/ml EC50 <i>Daphnia magna</i> (Kim et al., 2010)	0.5 $\mu$ g/ml
<i>Neomycin</i>	13.7 $\mu$ g/ml <i>Streptococcus agalactiae</i> (Biedenbach et al., 2009)	34.1 $\mu$ g/ml EC50 <i>Moina macrocopa</i> (Park & Choi, 2008)	14 $\mu$ g/ml
<i>Oxacillin</i>	$\leq$ 4 $\mu$ g/ml Susceptible <i>Staphylococcus aureus</i> & <i>Staphylococcus lugdunensis</i> (CDC, n.d.)	61 $\mu$ g/ml EC10 <i>Raphidocelis Supcapitata</i> (Kusk et al., 2018)*	4 $\mu$ g/ml

\*No ecotoxicity data was found for Oxacillin in US EPA or in other scientific literature. Thus, ecotoxicity data for the compound Cloxacillin that differs only by one Cl atom was used instead.

Thus, the mixture chosen would be able to inhibit a wide range of different bacteria species based on their different mode of actions of affecting either DNA synthesis, cell wall synthesis or protein synthesis (Gupta & Datta, 2019; Pandey & Cascella, 2023). Moreover, they are also effective on both Gram-positive and Gram-negative bacteria. The exposure concentrations were decided based on the highest MIC and lowest ecotoxicity concentration for each antibiotic, preferably using endpoints based on relevant species if the data was

available. E.g. due to lack of data the toxicity level for Oxacillin was based on algae. The MIC value was then compared to the lowest ecotoxicity level to select an exposure concentration that would inhibit bacteria but at the same time did not cause toxic effects on the meiofauna. Ultimately the samples were treated with a mixture containing 0.5 µg/ml Ciprofloxacin, 14 µg/ml Neomycin and 4 µg/ml Oxacillin. The antibiotic stock was prepared in pasteurized filtered seawater. The samples were exposed to the antibiotic mixture prior to the MTT incubation, with an exposure time of at least 20 min before the addition of MTT and then remaining in the samples for the entire assay.

Another bacterial influence investigated was bacterial growth on dead meiofauna, which has been shown to be a possible food source for bacteria (Giere, 2009). This was done by euthanizing meiofauna by boiling them for 15 min, then submerging them in untreated seawater, assumed to have high bacterial content. The samples were then placed in room temperature overnight to enable bacterial growth. Another part of the bacteria influence testing was to run the MTT assay on whole sediment, by incubating 0.2 g ww sediment in Eppendorf tubes. The whole sediment consisted of sediment that had not been sieved through a 63 µm mesh, only presieved with a 250 µm mesh during the sampling. The aim of this was to determine if there were differences in bacterial content in whole versus sieved sediment. The hypothesis was that most of the bacteria would be sieved out from the samples with the finer sieving, thus resulting in a lower absorbance for the sieved samples.

It is also of importance to test the possible abiotic reduction of MTT since there could be Formazan formation without any alive cells present (Braissant et al., 2020). Any possible abiotic interference was tested by doubling the incubation period and running the assay with both live and dead (boiled) meiofauna. The meiofauna was boiled since a previous study has shown that MTT reduction can occur in dead organisms and boiling also helps denature any remaining enzymes in the sample that could also reduce the compound (Nooijer et al., 2006). The hypothesis was that any abiotic reduction of MTT should not increase over time and that the absorbance for live meiofauna would increase with the prolonged incubation or reach a maximum.

## **2.4 Amitriptyline toxicity assessment**

### ***2.4.1 Long term exposure***

#### **2.4.1.1 Experimental set-up**

A mesocosm experiment of 30 days was conducted to examine effects on activity and community structure from long term exposure. Amitriptyline hydrochloride (CAS 549-18-8) was purchased from Sigma-Aldrich. A 750 mg/ml stock was prepared with the compound in pasteurized filtered seawater, from which a ten-fold dilution series was applied to create four more stocks. The sediment was then spiked with final exposure concentrations of 3, 30, 300, 3000 and 30 000 ng/g dw based on a 25% dw fraction. The exposure concentrations were based on monitoring data (Table 1) and toxicity data (Calleja & Persoone, 1992; Nalecz-Jawecki & Sawicki, 2005; Ishak et al., 2022; Magnuson et al., 2022), representing both environmentally realistic levels but also extreme levels known to cause an effect.

The marine sediment collected in December was used for the long-term experiment. The sediment was brought to the lab directly after the sampling and homogenized by mixing it. Then the sediment was distributed into different bowls, one for each treatment, and spiked with AMI at the intended levels. A bowl was also prepared for the control replicates where seawater was added instead. From the control sediment samples were taken for meiofauna analysis (Samples T0), dry weight measurement and chemical analysis. Unfortunately, due to time constraints the dry weight and chemical analysis have not yet been analyzed. The dry weight of the sediment in this study is assumed to be 25% of the wet weight based on the experience of researchers that have worked in the area before.

From each sediment bowl five replicates of sediment cores were prepared in Petri dishes that were filled up to the edge with sediment, with approximative 30 g ww in each core. The sediment cores were then placed in random order in a container filled with seawater (Figure 7).



**Figure 7. The mesocosm experiment.** A total of 30 sediment cores submerged in seawater connected to a flow-through system from the Gullmar fjord. The mesocosm was covered by a blanket and was left there for 30 days. The temperature was set to 10 °C.

30 days later the sediment cores were removed from the water and the content was thoroughly mixed with a spoon to homogenize the content. Then samples for analysis were taken, 10g ww for meiofauna and 5 g ww for the activity assay. Additional samples were also taken for chemical analysis, however as previously mentioned these have not been analyzed yet.

#### **2.4.1.2 Meiofauna analysis**

Meiofauna analysis consisted of extraction and morphological analysis of the meiofauna organisms, this was done to all the treatments and the T0 samples taken prior to the experiment started. The weight was noted for all samples except T0 due to human error. The meiofauna extraction was done using the colloidal silica Ludox ® HS40, with a specific gravity of 1.1-1.3 g/cm<sup>3</sup> at 15 °. This method uses gravity to separate meiofauna individuals from the sediment particles and centrifugation is used to quicken the process (Rohal et al., 2018). Firstly, sediment from the sediment core was taken and then sieved in a 63 µm sieve and preserved in Ethanol (96%), stained with Rose Bengal and stored in a fridge. Later the samples were resieved, centrifuged and the supernatant was removed with a pipette. The Ludox was then added, and the samples were shaken on a multishaker to dissolve the pellet and then centrifuged to separate the meiofauna from the sediment. A visible layer of

meiofauna was then taken from the top layer of the supernatant with a Pasteur pipette and added to a new vial. The process of adding Ludox, shaking and centrifuging was repeated once more. The extracted content was sieved once again and then fixed in a mixture of Ethanol (96%), Glycol and Rose Bengal in plastic jars and stored in a fridge until further analysis.

For the analysis the samples were sieved and mixed with 10 ml water in a 50 ml Falcon tube. The samples were then subsampled by shaking the samples to resuspend the extracted meiofauna and then 0.5 or 1 ml was pipetted from the sample and added to a Petri dish. The Petri dish was then analyzed under the Leica stereomicroscope where the morphological identification of meiofauna took place. The meiofauna individuals were counted and divided into different morphological groups. At least 100 individuals were counted initially in total for each sample to ensure enough data for the extrapolation, thus for some samples several subsamples were taken to count 100 individuals.

In total there were 58 unique groups of organisms that were morphologically different to each other, each of these groups were then identified based on literature and consultation with researchers. In cases identification was not possible the group was simply referred to as Unknown. After the initial identification was done the first groups were combined into larger groups to a higher taxonomic level such as order and phyla. The abundance was then calculated by combining the data from all counted subsamples for each sample analyzed, applying the following equation:

$$\text{Total individuals in analyzed sample} = \text{Counted individuals} * \frac{\text{Subsample volume}}{\text{Sample volume}}$$

The results were used to determine both the relative and absolute abundance. The number of individuals were then further extrapolated to 5 g ww sediment for absolute abundance determination based on the sample's prior measured weight and then further to the whole sediment core (30 g ww sediment).

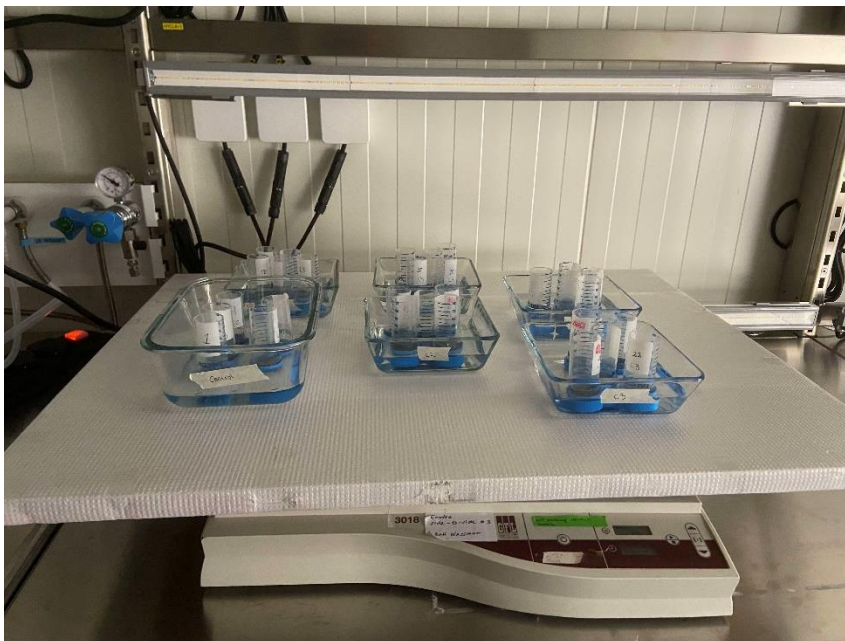
#### ***2.4.1.3 Activity analysis***

The MTT assay was applied to the sediment subsamples with the Falcon tube set-up (Figure 6) and followed the same experimental steps, with incubation and dissolution in 6-well plates. At the marine research center Kristineberg they were only equipped with a 545 nm absorbance filter for the Microplate reader. A first analysis was thus done at this wavelength,

and the samples were then transported back to Gothenburg. They were then stored in a temperature-controlled room at 4 °C overnight in darkness until further absorbance spectrum analysis. The day after the samples were analyzed with an absorbance spectrum of 400-700 nm, which was used for data analysis. Dissolved MTT samples with DMSO have previously been shown to remain quite stable over time, even for more than 24 hours (Comley et al., 1988), indicating the stability of the samples used in the study. Additionally, there did not appear to be a substantial difference in the absorbance readings when comparing the results.

#### ***2.4.2 Short-term toxicity test***

The short-term toxicity experiment was conducted with aqueous exposure by placing cut Falcon tubes in pans filled with AMI treatment or seawater for control (Figure 8). The pans were placed on a shake plate in a temperature-controlled room at 14 °C for 48 h.



***Figure 8. Experimental set-up of the short-term toxicity test. The experiment was conducted with water baths of the samples placed in pans on a shake table.***

The meiofauna was exposed to the same concentrations as in the long term mesocosm but in this case converted to aqueous concentrations (3, 30, 300, 3000 & 30 000 ug/l), assuming the density of the water to be 1 g/ml. A main stock of 6 mg/ml was prepared in pasteurized filtered seawater. From the main stock a serial dilution was applied for the intended concentrations by applying the stock into pans filled with pasteurized seawater and mixing the content, the tubes were then placed into the pans. The experiment was done with five replicates for each treatment and control. When the exposure ended the samples were rinsed

with pasteurized seawater and the MTT assay was conducted. No analysis of community structure was done in the short-term experiment.

Prior to the toxicity experiment, a control MTT assay test with untreated meiofauna was conducted to confirm that the meiofauna would survive the new experimental design and duration, which was deemed as a viable test design based on MTT assay results with relatively high absorbance results.

## **2.5 Statistical analysis**

Data analysis was conducted in R studio (Version 4.3.1) and Primer (Version: 7.0.24). The packages used in R studio for the analysis were: tidyverse (Wickham et al., 2019), multcomp (Hothorn et al., 2008), nlstools (Baty et al., 2015), GGally (Schloerke et al., 2024), vegan (Oksanen et al., 2025), Hmisc (Harrell, 2025), corrplot (Wei & Simko, 2024). Firstly, various data explorations were applied to visually examine possible patterns and the distribution of the data. Outliers were tested with the GraphPad's Grubb's test to test the significance of them. If an outlier was deemed significant statistical analyses were always done an additional time excluding this outlier to see if the result would differ without it. The normal distribution was tested with a Shapiro-Wilk test and followed if needed by data transformation.

To detect possible differences between samples an ANOVA followed by the Tukey test was applied. This was done to examine differences in absorbance for the MTT assay tests. When differences were compared to a control the Dunnett post hoc test was applied instead, e.g. the toxicity experiments. Univariate measurements for the community structure were done by examining taxa richness, evenness and applying the Shannon-Wiener diversity index. A correlation analysis was also conducted to find possible relationships between the different taxa abundances and their relation to the activity results on the data from the mesocosm experiment. This was done by applying the Spearman correlation coefficient to the analysis.

The multivariate methods are applied to have higher sensitivity compared to univariate methods in detecting changes at community level (Giere, 2009). To examine the effects on the community structure various multivariate methods were applied to the absolute abundance. The analysis was done on both transformed and untransformed abundance data to determine which resulted in the most apparent patterns and significance. Ultimately untransformed data was deemed the best fit, to avoid that rare taxa would dominate the analysis (Clarke & Gorley, 2015). A Bray-Curtis dissimilarity matrix was applied to the abundance data and was then followed by an NMDS, ANOSIM and SIMPER. The NMDS

was used to visualize clustering of the samples followed by statistical investigation of the dissimilarity in the ANOSIM analysis. The SIMPER then followed to identify which taxa contributed the most to any possible dissimilarity.

### **3. Results**

#### **3.1 MTT assay development**

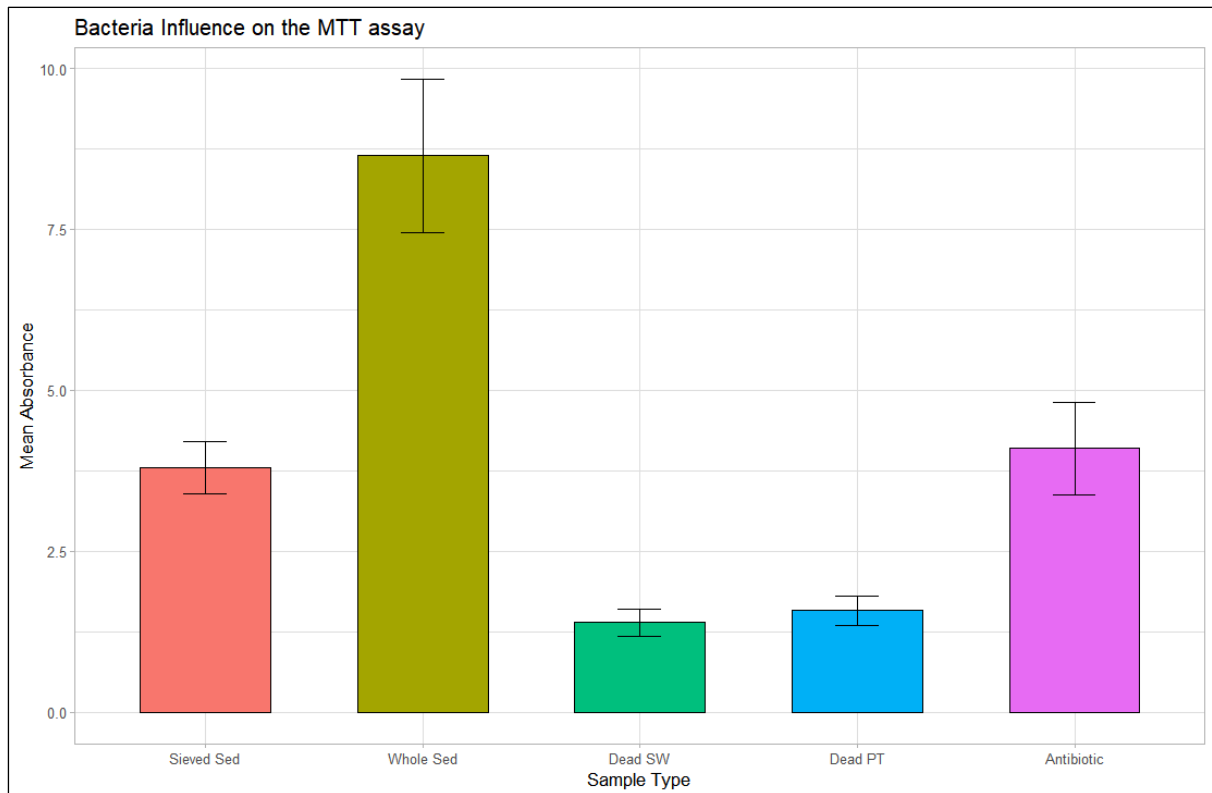
##### ***3.1.1 Optimization and development of the assay***

The MTT assay was applied with three different experiment set-ups to find the most suitable one (Figure 6; Table S2). Three MTT concentrations were tested using the 48-well plate set-up (1, 0.5 & 0.2 mg/ml), and no absorbance peaks could be detected. These concentrations were also tested at three different incubation times (0.5, 1 & 20h), with 20h achieving the highest absorbance. The highest absorbance ( $0.48 \pm 0.38$ ) was observed for the highest MTT concentration of 1 mg/ml with a light pink coloration of the samples, indicating incomplete dissolution of Formazan or limited MTT reduction.

The 1 mg/ml concentration was further tested by incubating meiofauna in Eppendorf tubes, this resulted in absorbance peaks and strong coloration of the samples indicating formation and extraction of Formazan. However, there was a great variation in the absorbance ( $4.38 \pm 2.12$ ), motivating further optimization of the test design. Ultimately MTT incubation in Falcon tubes were deemed the most optimal with a repeatable absorbance peak with low variation ( $3.08 \pm 0.26$ ). The absorbance had its peak around 550 nm and close to zero at 690 nm representing the background absorbance (Figure S1). Optimal sediment weight was also tested for the Falcon tube set-up, with 10 g ww ( $2.36 \pm 1.10$ ) and 5g ww ( $3.08 \pm 0.26$ ). 5 g ww sieved sediment had the lowest variation, deemed to be the most optimal weight for the Falcon tubes.

##### ***3.1.2 Bacterial influence***

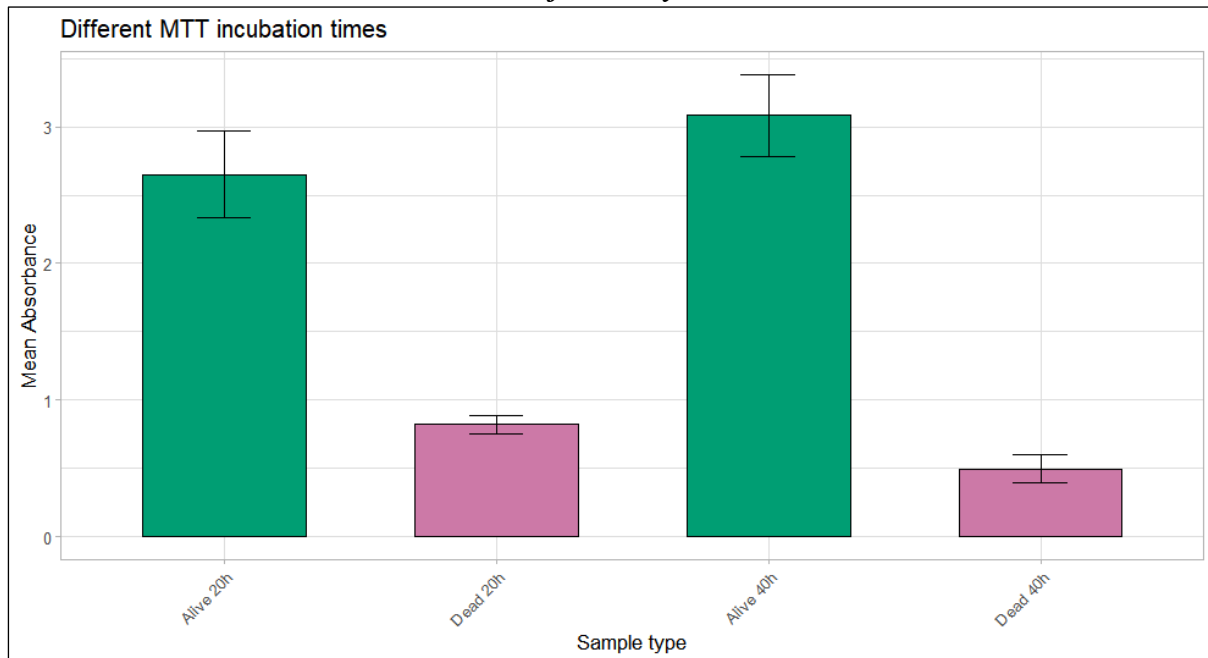
Another MTT assay test was done to test possible bacteria activity influencing the assay (Figure 9). All the samples were incubated in Falcon tubes except for the whole sediment that was incubated in Eppendorf tubes. The whole sediment had very high absorbance ( $8.64 \pm 1.19$ ), deviating significantly from the sieved sediment ( $p < 0.001$ ) and displayed quite high variation. There was no difference between the sieved sediment and the antibiotic treated sediment ( $p = 0.97$ ).



**Figure 9. Examining bacterial activity in the MTT assay.** Five different samples were tested: Sieved sediment, whole sediment, Dead meiofauna in Seawater (SW) and Dead meiofauna in pasteurized filtered seawater (PT) and antibiotic treatment. The bars display the mean absorbance of each sample with error bars representing the standard deviation. The absorbance values have been normalized to 5 g ww sediment.

The Dead SW consisted of unsieved and unpasteurized seawater, assumed to contain bacteria that could potentially grow on the dead meiofauna. There was no difference in absorbance between Dead SW and Dead PT ( $p=0.99$ ), indicating limited bacteria growth in the Dead SW samples. Both Dead SW ( $p=0.011$ ) and Dead PT ( $p=0.0023$ ) had a lower absorbance than the alive sieved samples. However, the absorbance values of the dead samples were still quite high (Dead SW:  $1.38\pm0.21$ , Dead Past:  $1.57\pm0.22$ ), motivating further investigation into the euthanization efficacy of the meiofauna and possible abiotic disturbance.

### 3.1.3 Abiotic interactions and detection of viability



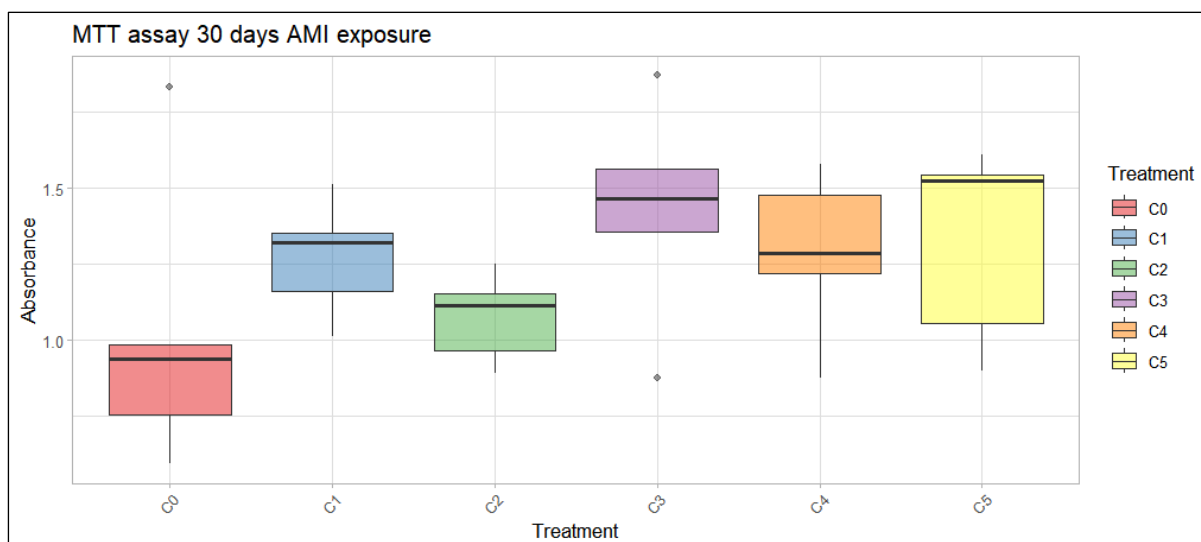
**Figure 10. Prolonged MTT incubation.** Activity measurement of normal and prolonged MTT incubation time together with live and killed meiofauna samples. The bars represent the mean absorbance of each sample together with error bars displaying the standard deviation. The absorbance values have been normalized to 5 g ww sediment.

The MTT assay was run with twice as long incubation time compared to the default time of 20h (Figure 10). The longer incubation period resulted in lower absorbance for the dead meiofauna ( $p = 0.038$ ) whereas the live meiofauna showed no difference ( $p=0.36$ ). In both incubation periods there was a clear difference between the alive and dead meiofauna (20h  $p=0.0013$  & 40h  $p<0.001$ ). Compared to previous euthanized meiofauna samples (Figure 9) there was a stronger difference to the live samples in this test. In this instance the meiofauna was euthanized by being submerged in Ethanol and then boiled, whereas previously they were only boiled. The dead meiofauna samples in this test also had a lower absorbance of  $0.82\pm 0.14$  (Figure 10) compared to  $1.58\pm 0.22$  in the previous test (Figure 9).

## 3.2 AMI effects on meiofauna activity

### 3.2.1 Effects on activity

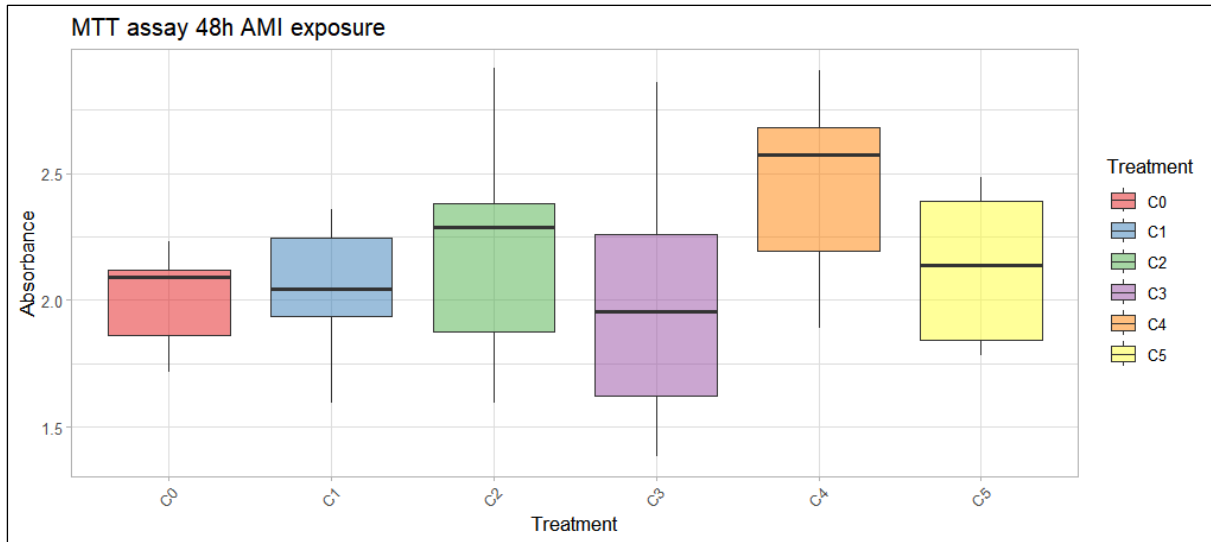
The activity measurements for the AMI experiments were done with the Falcon tube set-up that was proven to be the most optimal set-up prior to the toxicity experiments and was applied to both the long-term and short-term experiments. Overall, a visual increasing trend of the activity can be observed in the meiofauna exposed to AMI for 30 days in the mesocosm set-up (Figure 11).



**Figure 11. Meiofauna activity after 30 days exposure of Amitriptyline (AMI).** The boxplots signify the median as a thick line in the boxes and outliers are displayed as dots. Each box edge represents the first quartile at the bottom and the upper edge the third quartile, with the box itself displaying the middle 50% of the data. The whiskers illustrate the smallest and largest values within 1.5 times from the box. The absorbance has been normalized to 5 g ww sediment.

However, there was no statistical difference detected compared to the control. The control has one outlier that has quite a high absorbance and deviates from the rest of the samples. There was no apparent reason why the outlier deviated from the rest of the samples when analyzing the data further or examining lab notes. Additionally, the outlier was not significant ( $p > 0.05$ ). Comparing the absorbance of the controls ( $0.95 \pm 0.48$ ) to the previous tested alive meiofauna in the MTT assay development (Figure 9; Figure 10) with a total mean of  $3.11 \pm 0.60$  the controls from the AMI experiment appeared less active.

A short-term exposure experiment was done with the same concentrations as the long term (Figure 12). No statistical difference could be detected, indicating no effect on the meiofauna activity. Visually there is no apparent trend in the activity either.

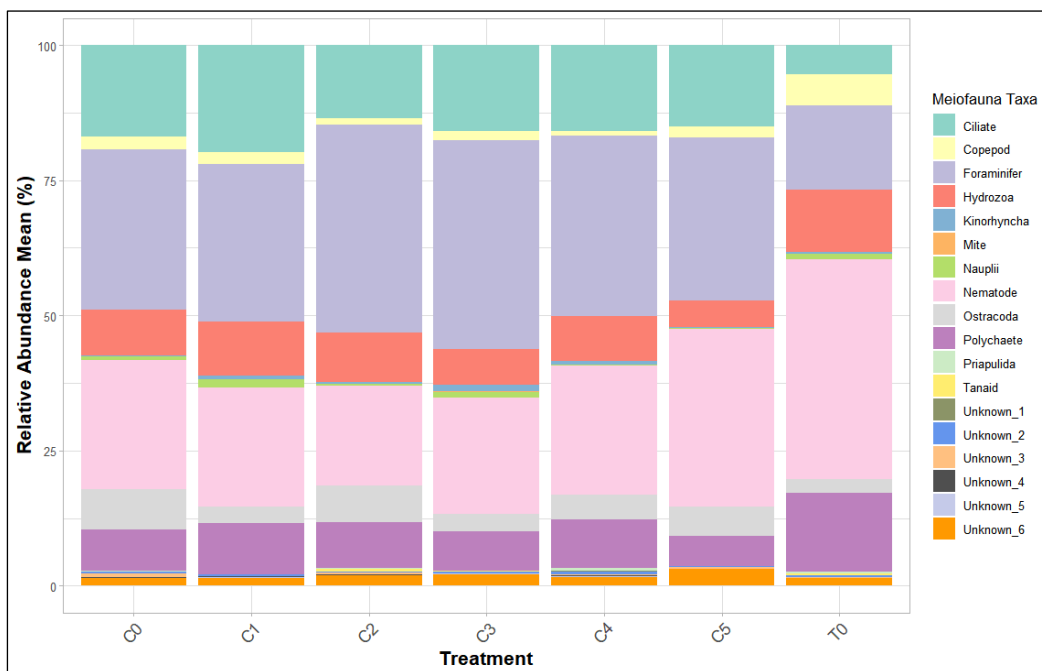


**Figure 12. Meiofauna activity after 48h Amitriptyline (AMI) exposure.** The boxplots display the median as the thick line in the boxes and outliers are displayed as dots. Each box edge represents the first quartile at the bottom and the upper edge the third quartile, with the box itself displaying the middle 50% of the data. The whiskers illustrate the smallest and largest values within 1.5 times from the box. Absorbance has been normalized to 5 g ww sediment.

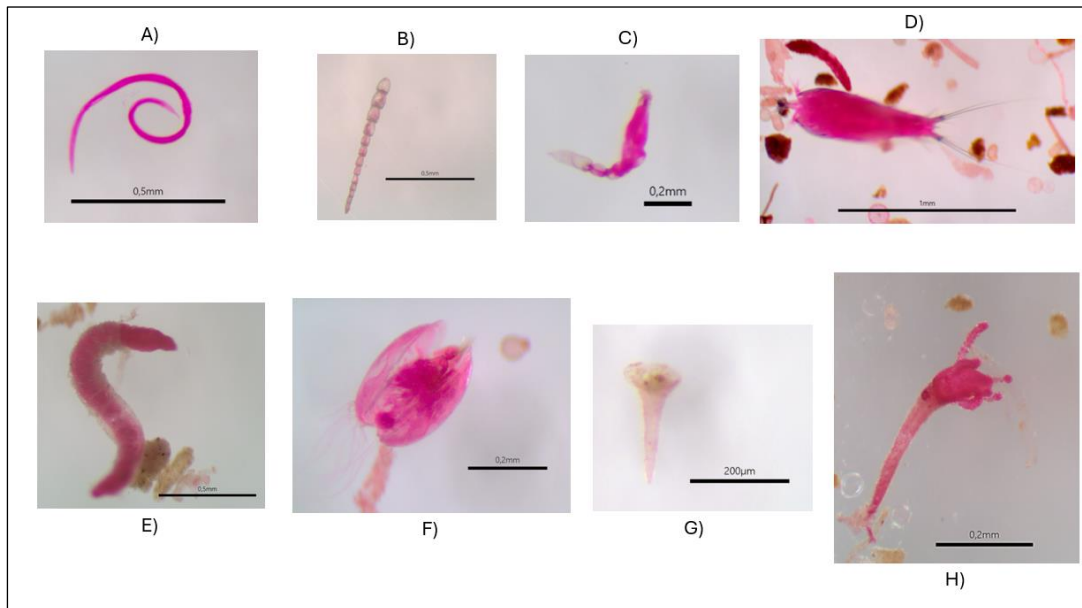
### 3.3. AMI and effects on the community level

#### 3.3.1 Community structure

There was a quite high meiofauna diversity in the samples overall (Figure 13) represented by 18 different taxa groups. Most of them were identified to the phyla level while some went down to class or order (e.g. tanaid and hydrozoa).



**Figure 13.** The relative abundance of each meiofauna taxa. Each taxa is illustrated with a colour bar, stacked on each other for AMI each treatment, control and T0 samples. Each bar represents the mean relative absorbance.



**Figure 14.** The most observed meiofauna organism groups. Example pictures of the top eight most observed meiofauna taxa groups, in random order. A) Nematoda, B) Foraminifera, C) Unknown 6, D) Harpacticoid (Copepoda), E) Polychaeta, F) Ostracoda, G) Ciliate & H) Hydrozoa.

Nematodes dominated in T0 (40.8%) and C5 (32.7%), the rest of the treatments and control was dominated by foraminifers with the highest relative abundance in C2 (38.5%). Overall nematodes and foraminifers were dominant in all the mesocosm samples (Figure 14). Other prominent taxa were ciliates, hydrozoa, polychaetes and an unidentified taxon called unknown 6. Some taxa were very rare, for example mite only appeared once in a C3 sample. Some other very rare taxa were Priapulida, tanaid, unknown 1, unknown 3, unknown 4 and unknown 5. This resulted in plenty of zero values in the data.

There was no significant difference in relative abundance, the only difference detected was when comparing C0 to T0. For example, there were more nematodes ( $p=0.0028$ ), copepods ( $p=0.0095$ ) and polychaetes ( $p=0.0023$ ) in the sediment prior to the experiment. On the contrast there were more foraminifers ( $p=0.0033$ ) and ciliates ( $p=0.00032$ ) when the experiment ended.

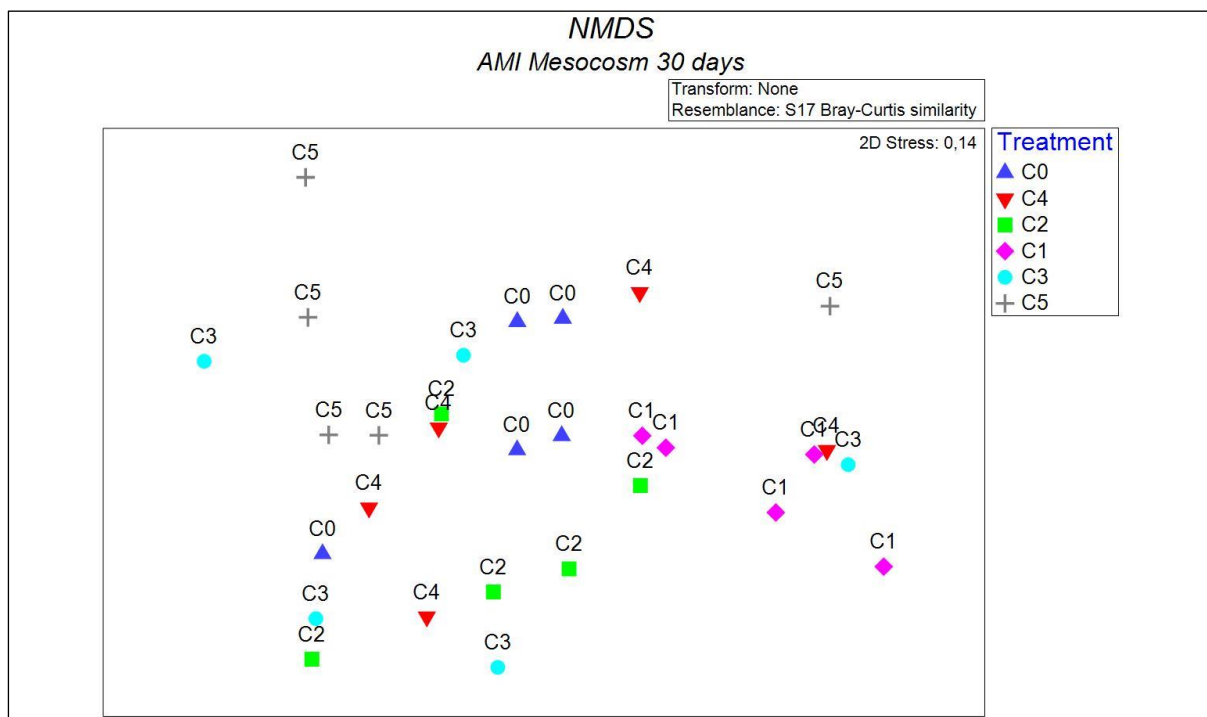
The absolute abundance was also examined for each taxa group and total abundance for each treatment (Figure S3; Table S5). The abundance was not calculated for T0 since no weight was noted for these samples making extrapolation not possible. There was a significant

outlier in the total abundance in treatment C5 for sample 18 ( $p < 0.05$ ), with much lower total abundance. No significant differences could be detected in total abundance or specific taxa abundance, even when excluding the C5 outlier.

Analysis of the community species richness, evenness and Shannon Weaver ( $H'$ ) diversity index was conducted however no significant differences could be observed for the treatments (Figure S2). Additionally, a correlation analysis with the Spearman correlation coefficient was applied, looking at taxa affecting other taxa and relations between taxa abundance and activity (Figure S4). No strong correlation could be detected. However, visually it appears as the abundance of the most prominent meiofauna taxa foraminifers and nematodes have a dip in the C1 treatment (Figure S3), simultaneously as the activity appears to increase (Figure 11).

### 3.3.2 Community similarity and dissimilarity between the treatments

The NMDS ordination analysis was applied to the absolute abundance data of the different AMI treatments (Figure 15).



**Figure 15.** A NMDS on meiofauna abundance. The NMDS was applied to non-transformed data with a Bray-Curtis similarity matrix applied to it. The abundance was compared based on treatment as the factor.

The most apparent clustering observed is C1 that is quite tight compared to the rest of the treatments. C0 and C5 also have quite tight clusters, however with quite deviating outliers. A dissimilarity between C0 and C1 could be detected in the ANOSIM analysis ( $p = 0.008$ ,  $R =$

0.60) (Table S3). A SIMPER analysis was applied to examine what meiofauna groups drove the dissimilarity, where foraminifers (24.9%) and nematodes (21.6%) had the highest contribution to the difference (Table S4). The average dissimilarity between C0 and C1 was 26.7%. An NMDS and ANOSIM analysis was also applied when excluding the previously mentioned outlier in sample 18 (C5), however this did not lead to any further significant dissimilarity or tighter clustering. Overall, no clear or strong dissimilarity was detected because of higher AMI exposure and in some of the treatments the variation was rather high, signified by the spread of the clusters.

## **4. Discussion**

### **4.1 MTT assay development**

#### ***4.1.2 Establishing the MTT assay's applicability***

The cut Falcon tube set-up was demonstrated to be the most optimal choice for the assay (Table S2), proven to have repeatable absorbance peaks and low variation compared to the other two set-ups with 5 g ww sediment in each sample. The suggested optimal MTT concentration and incubation time of 1mg/ml and 20-40h concurs with another study applying MTT to Foraminifers that could likewise observe reduction of MTT in the organisms at these conditions (Nooijer et al., 2006). A shorter incubation time with this set-up may also be possible to test further to make the assay more rapid. Previous research has shown that nematodes can reduce MTT within two hours exposed to half the concentration applied in this study (Nayak et al., 2011).

Moreover, the absorbance spectra observed (Figure A1) complied with the expected spectra for MTT assays with DMSO as the solvent (Neufeld et al., 2018; Babacan et al., 2022), confirming the detection of Formazan. When optimizing the MTT assay some of the most important factors are MTT concentration and incubation time (Ghasemi et al., 2021). The optimum conditions are when a maximum MTT reduction can be reached before inducing cell death from the MTT exposure. In this case a maximum MTT reduction seems to have been achieved at 20-40h of incubation (Figure 10), where there was no significant difference between the alive samples indicating that an incubation within 20-40h is appropriate.

The assay was further verified when it demonstrated the ability to distinguish between live and dead meiofauna, proven in two separate tests where the activity of the live meiofauna was significantly higher than the dead (Figure 9; Figure 10). This concurs with the few other studies that have applied the assay successfully to meiofauna organisms such as foraminifers

(Noojer et al., 2006) and nematodes (Nayak et al., 2011; Mukherjee et al., 2016). Overall, this confirms that the MTT assay was successfully developed and optimized to observe activity of meiofauna, indicating the ability of these organisms to ingest and reduce the MTT under the applied experimental conditions.

#### ***4.1.3 The examination of possible confounding factors***

No confounding bacterial activity was detected to substantially affect the assay measurement (Figure 9). This was demonstrated by the large difference in absorbance between sieved and whole sediment, indicating that most of the bacteria is removed during the finer sieving process. Additionally, there was no decreased absorbance in the antibiotic treatment of the sieved sediment, demonstrating little bacteria content to inhibit. The non detected difference in the antibiotic treated sediment may as well be due to the short time of exposure to the antibiotic mixture. However, despite that possibility, the difference between whole and sieved sediment still strongly indicates that there is little bacterial activity left in the sieved samples. Moreover, the risk of bacteria growing on dead meiofauna organism does not appear to be a problem in the assay, which showed no difference to the other dead samples.

The results demonstrated that there was no substantial abiotic interference in the assay, since the dead samples had a small decrease in absorbance at the prolonged incubation period (Figure 10). This indicates that there is an abiotic reduction of MTT, as expected, but it stopped and did not lead to any high absorbance differences which could mislead the results. Compounds that have the possibility to cause abiotic interference are mainly those with redox potential, e.g. vitamins, plant extracts, free radicals and metals (Gomez Perez et al., 2017; Grela et al., 2018). None of the compounds mentioned in literature as possible abiotic interference were assumed to exist in substantial amounts in the samples, which might explain the negligible abiotic influence. Additionally, the samples were rinsed with pasteurized filtered seawater before and after incubation, possibly rinsing away interfering compounds that remained in the samples.

## **4.2 Amitriptyline toxicity assessment**

### ***4.2.1 Examining effects on community structure***

Overall, there was quite a large variation in the abundance data of the organisms, possibly affecting the ability to detect significant differences and dissimilarities in the analysis. This study could not demonstrate any substantial negative effect on biodiversity because of long term AMI exposure. Negative effects on specific taxa abundance such as nematodes and

foraminifers were indicated by the dissimilarity analysis (Table S4) that showed a significant difference between the C1 treatment and control, driven primarily by these organism groups. Visually a decrease in both of their abundances exposed to C1 was observed (Figure A3), however it could not be statistically confirmed. A negative effect on nematode abundance due to AMI exposure has been observed previously at 0.4 and 40 ng/g (values converted from original aqueous concentration) (Ishak et al., 2022), concentrations relatively close to the C1 treatment of 3 ng/g dw.

In this study the abundance of both nematodes and foraminifers appeared to increase again in higher treatments (Figure A3), especially foraminifers, indicating possible recovery or acquired tolerance to the compound. Both foraminifers and nematodes are ecologically important groups of organisms and are useful as bioindicators (Balsamo et al., 2011), thus further research would be motivated to investigate effects further at these low levels. This is also motivated further by environmental relevance since the compound has been detected in natural sediments at these levels (Table 1).

#### ***4.2.2 Assessing effects on activity by applying the MTT assay***

Negative effects on the activity thus viability of the meiofauna were not significantly detected for either short term or long-term exposure (Figure 12; Figure 11). Despite this, an interesting weak trend of activity increasing with higher treatment was observed visually for the long-term experiment. The absorbance signal from the mesocosm experiment was quite weak compared to other MTT assay tests in the study. The low activity overall could be induced simply by mesocosm design, where perhaps the mesocosm induced resting conditions for the organisms. It was also demonstrated that some taxa were more or less sensitive to the mesocosm.

#### ***4.2.3 Combining activity and community data***

When combining the abundance data with the activity measurement, a possible pattern of increased activity (Figure 11) when the abundance decreased was observed for C1 when looking at foraminifer and nematode abundance (Figure A3). The activity measurement showed an increase in activity for the treatments compared visually to the control, but not statistically detected. There was, however, a substantial outlier in C0 for the activity, but could not be removed since it was not significant or explained by any possible mistake in the experiment. Without the outlier it would have been possible to detect a significant increase in activity caused by the higher AMI exposures. Thus, despite no statistical differences, the results point to a possible increased activity due to AMI exposure, which needs to be further

investigated. Increased activity may be a symptom of stress, since a way of compensating for toxic effects might be to increase metabolism (van der Oost et al., 2020). Furthermore, when connecting it with the possible decrease in abundance of foraminifers and nematodes, it motivates further investigation.

## **4.3 Limitations of the toxicity assessment**

### ***4.3.1 Uncertainty of sediment concentration and bioavailability***

A crucial factor to consider when assessing toxicity is the actual exposure time and bioavailability of a compound, since simply looking at the measured concentration might not show the entire exposure scenario (Giere, 2009). AMI is known to have a Log K<sub>oc</sub> of 2.3, indicating affinity to sediments, but was still assessed to have possible mobility by Costa Junior et al. (2022) and due to this considered an ecotoxicological risk. However, other studies have shown that AMI can decrease substantially in sediments 28 days after the addition of the compound with concentrations also used in this study (3 & 30 ng/g dw) (Magnuson et al., 2022). This implies that there may be rapid transformation, transportation or uptake of the compound in sediments over longer periods. Bioaccumulative tendencies were observed by Magnuson et al. (2022) in polychaetes, however the tendencies were quite negligible compared to the concentration of exposure. Thus, the other two most likely explanations for a rapid decrease of the compound are that it was transported away and that it was metabolized into a metabolite. Since no chemical analysis could be done for the study, there is no insight into the possible fate of the chemical in the sediment. Overall, the exposure concentrations given in this study should be taken with caution, assuming that the concentration most likely diminished.

### ***4.3.2 Stress induced from the experimental design***

One sign of stress in communities is a decrease in diversity and an increase in dominance among some species (Odum, 1985). Neither of these symptoms were detected in the treatments, but it was detected when comparing the control to T0 (Figure 13) which indicated stress induced because of the experimental design. Foraminifers have been shown to outcompete nauplii and young copepods because they can consume up food in forms of bacteria and microphytobenthos (Chandler, 1989). This may be the reason why Copepods were significantly more abundant in the T0 samples and less abundant in the control. Other organisms that appeared to be sensitive to the mesocosm were nematodes and polychaetes, whereas ciliates together with foraminifers appeared to thrive in the experimental set-up.

Furthermore, the overall activity for the mesocosm samples was much lower than the previous MTT assay tests. However, no explanation for the low activity could be determined based on the mesocosm conditions. If the study was repeated it would have been beneficial to as well measure the activity of the T0 samples and examine the water and sediment conditions.

#### ***4.3.3 Meiofauna identification and sampling***

The morphological meiofauna identification has its downfalls when it comes to uncertainty in the analysis, since in this study it was not done by a trained meiofauna expert. Additionally, important biological information may have been lost when identifying the taxa to order or higher taxonomic levels compared to going all the way down to the genus and species level (Giere, 2019). Some meiofauna could be identified down to the genus level, however no effects could be observed for these groups and a lot of the taxa were very rare. Furthermore, distinguishing between dead and alive organisms can be very difficult for some organism groups such as foraminifers that may just be dead shells resulting in inaccurate results of the number of individuals counted (Bernhard et al., 2006).

An alternative method for gaining more complex taxonomy information is metabarcoding, which has become more prominent in meiofauna research in recent years for identifying meiofauna organisms. However, metabarcoding runs a risk of underestimating biodiversity and there are not yet established genetic markers that are deemed representative for all meiofauna organisms (Giere, 2019; Mazurkiewicz et al., 2024). Many of the reference libraries are incomplete leading to a substantial number of organisms remaining unclassified or misidentified (Francesca et al., 2018) and the abundance may be miscounted compared to morphological identification due to multiple sets of genes in organisms (Hu, et al., 2025). Thus, using both methods and comparing the results to complement each other could have improved the meiofauna analysis, resulting in more comprehensive and accurate results.

The sediment sampling and subsampling for the stereomicroscope analysis could have introduced some unwanted bias. The mesh size of the sieve during the sediment sampling and sample preparations may have affected the results since the most representative mesh size for meiofauna is under great discussion, lacking a clear consensus yet (Ptatscheck et al., 2020). E.g. a mesh size of smaller than 44  $\mu\text{m}$  has been suggested to be more representative (Giere, 2009), in this study 63  $\mu\text{m}$  sieve was used. Additionally, the first sieving process with a 250  $\mu\text{m}$  mesh could have excluded larger meiofauna organisms.

The subsampling methodology during the morphological analysis could have also affected the results; possibly larger organisms were not able to be taken up with the pipette tip. E.g. juvenile brittle stars and other larger organisms were observed visually in the samples, but they were never observed in the subsamples analyzed. Likewise identifying dead organisms creates a bias towards organisms that are better preserved, meiofauna such as the soft bodied turbellaria and gastrotricha need to be alive for more accurate identification (Garaffoni et al., 2019) and they can be destroyed during the sieving process (Gerlach, 1971).

#### **4.4 Further research of the MTT assay needed**

It should be assumed that different kinds of meiofauna organisms contribute to the assay results to different extents depending on their ability to ingest MTT and reduce it in the respective experimental design. For example, more fast moving and active species like copepods and some nematodes have been shown to have almost two- or three-times higher metabolism compared to more sluggish organisms like ostracodes and other nematode species (Gerlach, 1971). Studies have confirmed that foraminifers and nematodes can reduce MTT in similar conditions as applied in this study (Nooijer et al., 2006; Nayak et al., 2011; Mukherjee et al., 2016). A link between certain meiofauna taxa and activity could not be correlated however in this study (Figure S4), thus not showing any evidence that a certain taxon would be dominating the assay results. However further studies should be done by conducting the assay with single species from different meiofauna groups. This would be important to avoid unwanted bias where the activity measurement is driven primarily by a few species and thus not representing the species of the whole community.

Overall, it is of great importance to prove the reproducibility of the assay, conducting the assay in other labs with other kinds of meiofauna communities and with other kinds of chemicals for toxicity assessments. Since there was no negative effect detected on activity from either short- or long-term exposure from AMI (Figure 11 & Figure 12) the assay could not be proven to detect toxicity of a compound in a toxicity experiment. However, its ability to do so is expected based on its ability to distinguish between living and dead meiofauna. There might be a need for fine tuning the assay further for increasing the sensitivity of sublethal effects.

Another important factor in the traditional MTT assays is the number of cells in each sample (Ghasemi et al., 2021). Since the aim of this study was not to quantify the viability the number of individuals in each sample was not counted. However, when it comes to

comparing activity between samples it would be beneficial to have approximately the same number of individuals in each sample. An even number of individuals was attempted by homogenizing the sediment prior to sample preparation; however, it is unknown if there were approximately same number of individuals in each sample.

## **5. Conclusions**

In this study I aimed to develop and test the applicability and validity of the MTT assay on meiofauna communities with the aim of detecting toxic effects. The MTT assay was proven to be a promising tool for distinguishing activity and thus viability among meiofauna, with repeatable results with low variation. A foundation of the experimental design was established and can be used for further development of the assay in future research. The most optimal assay design was deemed to be incubation of 1 mg/ml in cut Falcon tubes placed in a 6-well plate for 20-40h with a 50 mM ammonia buffered DMSO solvent. Confounding factors concerning bacteria and abiotic interactions were examined and no significant effect on the results were observed. Further application of the assay is needed to examine the reproducibility and if the assay is representative for most meiofauna organisms in different communities to avoid biased results.

Additionally, the study examined the toxic effects of the antidepressant AMI on the community structure and activity of marine meiofauna. No short-term toxic effects were detected on the activity after 48 hours of exposure. The long-term experiment indicated possible low toxicity for nematodes and foraminifers at 3 ng/g dw, possibly reducing their abundance and increasing their activity. The effects could not be proven statistically however so further research is needed to confirm the effects. The toxicity experiment may have limitations concerning the bioavailability and exposure of the compound. Further research is needed to assess the bioavailability and transformation of AMI in sediments to realistically assess its toxicity to benthic creatures.

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## R packages

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## Supplementary information

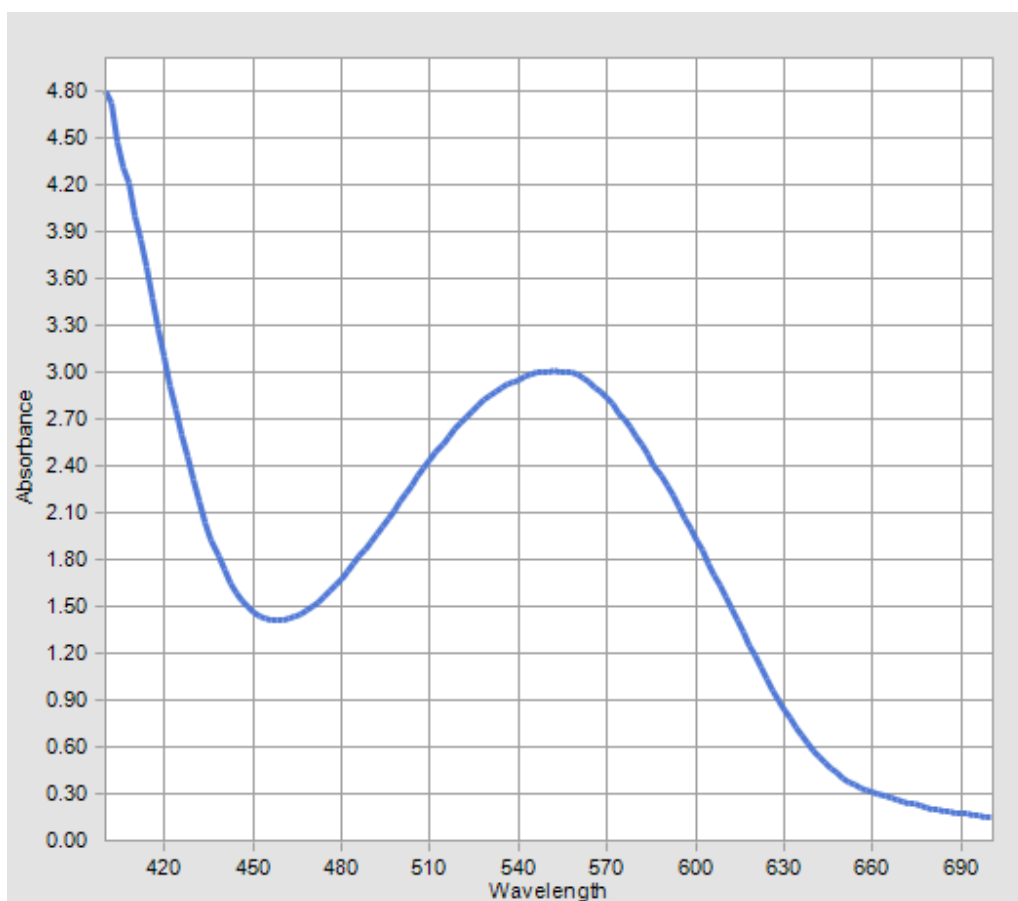
*Table S1. Preliminary chemical priority list from the EU project group Contrast. The preselected chemicals are in bold text which were used for the chemical screening.*

Compound
<b>N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6-PPD)</b>
2,4-Dihydroxybenzophenone

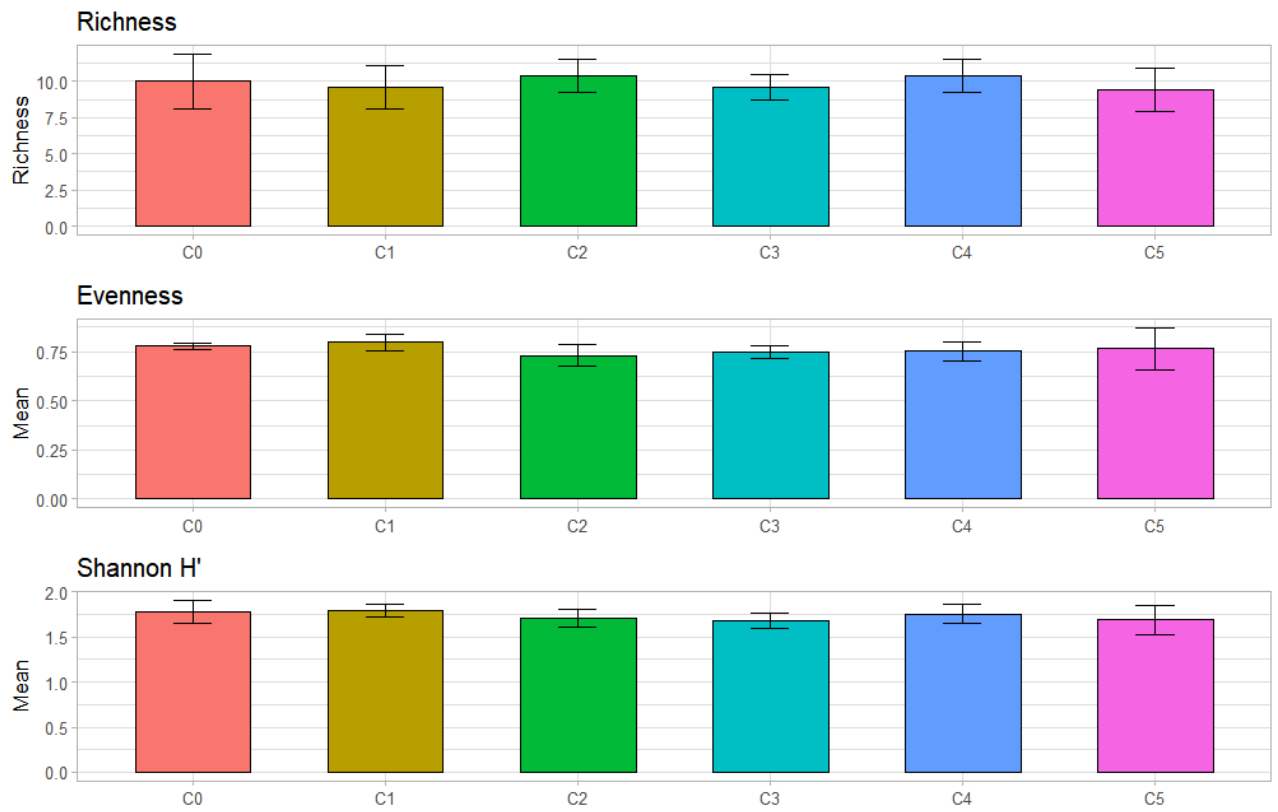
2-ethylhexyl diphenyl phosphate
Hexabromobenzene
Diclofenac
<b>Venlafaxine</b>
Trimethoprim
2-isopropylthioxanthone
Sulfamethoxazole
<b>Irbesartan</b>
N,N-Dimethyldodecan-1-amine
3,3,5-trimethylcyclohexyl salicylate
1,1'-Ethane-1,2- diylbis(pentabromobenzene) (aka DBDPE)
Tris(2-chloroisopropyl)phosphate
2,2'-Dihydroxy-4- methoxybenzophenone (18)
Pentabromotoluene
<b>Amitriptyline</b>
Octocrylene
4-cumylphenol
Enrofloxacin
1,3,5,7-Tetramethyl-1,3,5,7- tetravinylcyclotetrasiloxane
17alpha-ethinylestradiol
Ofloxacin
Citalopram
Estrone
Chloramphenicol
O-Desmethylvenlafaxin
<b>Tramadol</b>

2,2'-Methylenebis[phenol] (= 2,2' bisphenol F)
Tris(2-ethylhexyl) phosphate
Tris(2-butoxyethyl) phosphate
17alpha-ethinylestradiol
Ofloxacin
Estrone
Chloramphenicol
O-Desmethylvenlafaxin
2,2'-Methylenebis[phenol] (= 2,2' bisphenol F)
2-(2H-Benzotriazol-2-yl)-4-methylphenol
Mefenamic acid
Erythromycin
Octinoxate
Triclocarban
Oxolonic acid
Bumetizole
17beta-estradiol
1-(2,3-Dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl)ethan-1-one (traseolide)
2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol
Bis(2-ethylhexyl)hexanedioate
Carbazole
<b>3,4,5-Trimethylphenyl methylcarbamate</b>
Azacyclonol
Octabenzene
N,N-Dimethyl-1-octadecanamine
<b>Atorvastatin</b>
Acetaminophen
Bis(2,6-diisopropylphenyl)carbodiimide

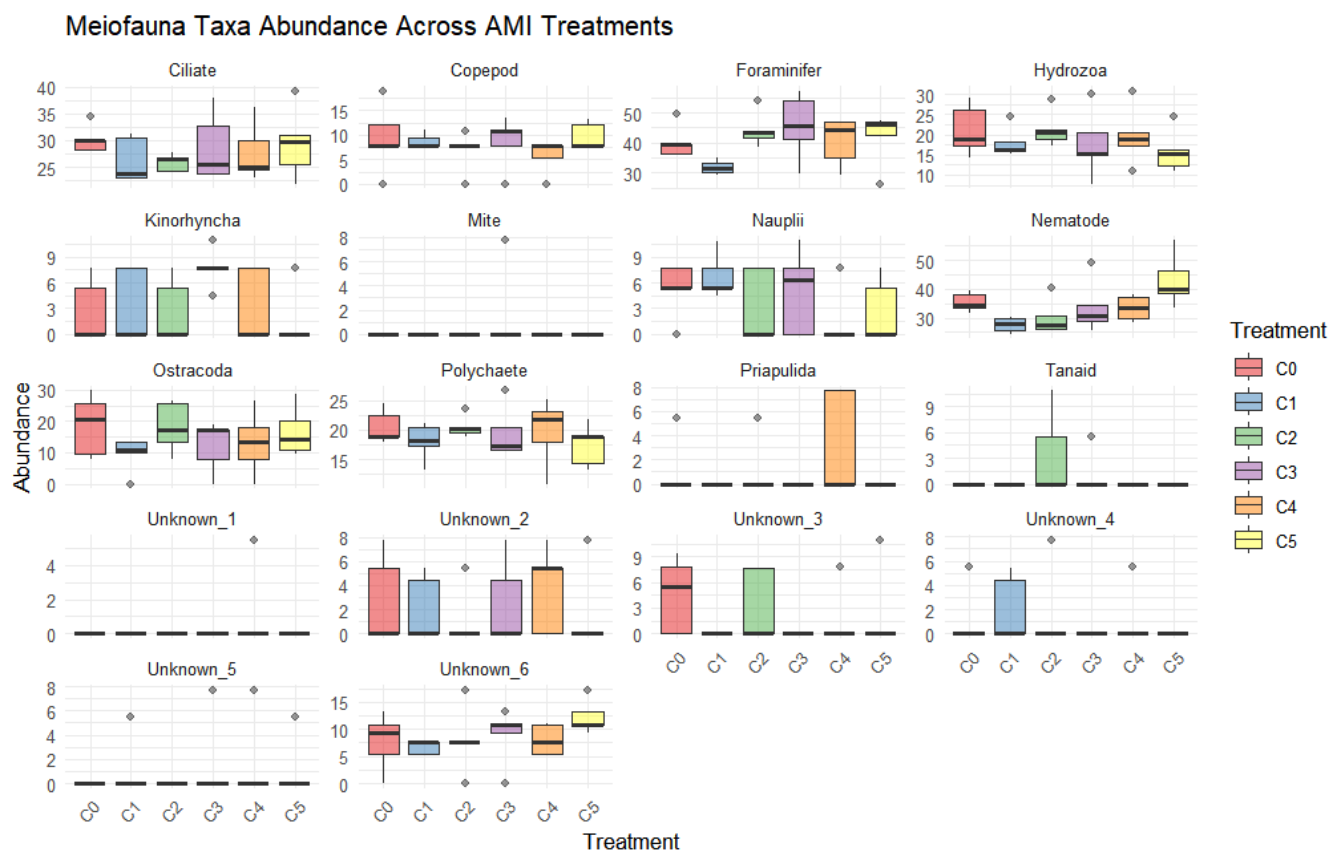
<i>N</i> -(1,3-dimethylbutyl)- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine-quinone (6PPD-quinone)
Teflubenzuron
Clarithromycin
Tributyl phosphate
Benzotriazole



**Figure S1. MTT assay absorbance spectrum.** The figure illustrates a typical absorbance spectrum observed repeatably for samples with the Falcon tube set-up. The absorbance spectrum was measured from 400-700 nm with the VarioskanFlash microplatereader.



**Figure S2. Barplots of different univariate community measurements from the long-term Amitriptyline experiment.** Species richness, evenness and the Shannon Weaver ( $H'$ ) diversity index was calculated for all the Amitriptyline treatments and control of the long-term mesocosm experiment. Each bar represents the mean value of each measurement with an error bar demonstrating the standard deviation. No statistical difference was detected between treatments and control.

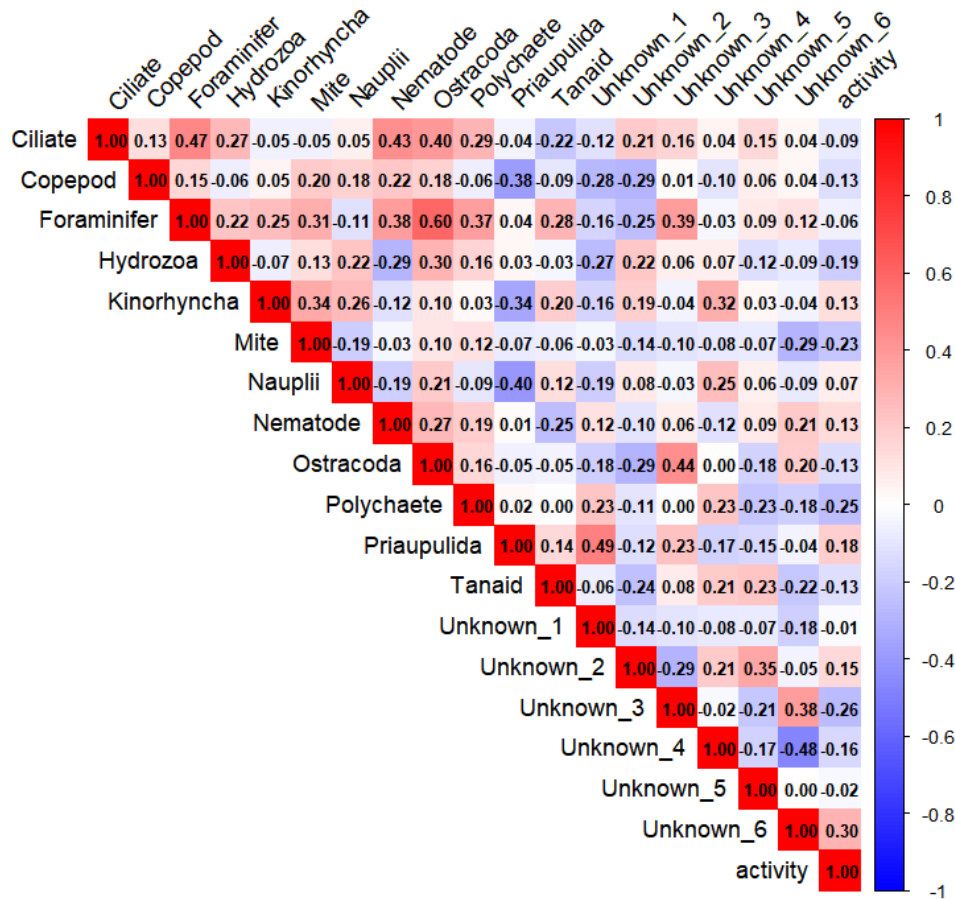


**Figure S3.** Boxplots of each taxa group abundance from the long-term Amitriptyline (AMI) experiment. The boxplots represent absolute abundance, with a square root transformation applied. The boxplots signify the median as a thick line in the boxes and outliers are displayed as dots. Each box edge represents the first quartile at the bottom and the upper edge the third quartile, with the box itself displaying the middle 50% of the data. The whiskers illustrate the smallest and largest values within 1.5 times from the box.

**Table S2.** Summary of absorbance values for the different experimental set-ups.

MTT incubation set-up (20h) with alive meiofauna	Absorbance mean and SD normalized to 5 g ww
48 well plate (n=3):	
- 1 mg/ml	0.48 ± 0.38
- 0.5 mg/ml	0.18 ± 0.04
- 0.2 mg/ml	0.18 ± 0.003
Eppendorf tubes (n= 10)	4.38 ± 2.12
Cut Falcon tubes (n=6)	
-5 g ww	3.08 ± 0.26

-10 g ww	2.36 ±1.10
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**Figure S4. Spearman correlation coefficients applied to meiofauna abundance and the activity measurements.** Each correlation coefficient value is each box comparing two variables at a time and showing the correlation strength and direction. A higher number signifies a positive correlation illustrated in red and the higher the number the stronger the correlation. Values below 0 to -1 are illustrated in blue, with a stronger negative correlation with values being close to -1. Due to weak correlation strengths no significance was tested.

**Table S3. ANOSIM test of significant dissimilarity.**

Groups	R statistic	Significance level (%)
C0-C1	0.604	0.8
C0-C2	0.068	27.8
C0-C3	0.096	23
C0-C4	-0.124	83.3
C0-C5	0.096	23

**Table S4. SIMPER One-way analysis.**

Group	Average dissimilarity	Taxa	Contribution (%)	Average Dissimilarity	Dissimilarity SD
C0 & C1	26.72	Foraminifer	24.86	6.64	1.55
		Nematode	21.59	5.77	1.91
		Ostracoda	14.27	3.81	1.31
		Ciliate	11.79	3.15	1.53
C0 & C2	22.09	Foraminifer	24.36	5.38	1.36
		Nematode	21.09	4.66	1.93
		Ostracoda	14.37	3.17	1.37
		Ciliate	10.33	2.28	1.42
C0 & C3	27.24	Foraminifer	30.44	8.29	1.66
		Nematode	17.88	4.87	1.46
		Ciliate	11.84	3.23	1.74
		Ostracoda	11.43	3.11	1.29
C0 & C4	22.90	Foraminifer	25.81	5.91	1.79
		Ostracoda	14.99	3.43	1.28
		Nematode	12.96	2.97	1.31
		Ciliate	12.82	2.94	1.57
		Hydrozoa	12.03	2.75	2.75
C0 & C5	25.01	Foraminifer	24.97	6.24	1.81
		Nematode	24.10	6.03	1.05
		Ostracoda	12.24	3.06	1.25
		Ciliate	11.53	2.88	1.28

**Table S5. Meiofauna community data.** The abundance data for each taxa group with mean value and standard deviation. For the T0 samples the relative abundance is given. The absolute abundance data is extrapolated to 30 g ww representing the whole sediment core. Activity is also given from the MTT assay measurements. No activity measurement was done for T0.

	C0	C1	C2	C3	C4	C5	T0 (%)
Ciliate	914±165	706±222	673±79	857±388	792±319	902±404	4.63±3.35
Copepod	125±141	77±27	59±42	95±68	42±27	101±58	5.40±1.86
Foraminifer	1631±474	1020±154	1986±548	2170±957	1693±616	1817±657	15.63±2.11
Hydrozoa	476±271	337±148	467±211	367±325	429±312	273±189	10.74±3.89
Kinorhyncha	18±27	24±32	18±27	64±36	24±33	12±26	0.52±0.39
Mite	0±0	0±0	0±0	12±27	0±0	0±0	0±0
Nauplii	36±25	52±41	24±33	44±50	12±27	18±26	1.09±0.89
Nematode	1270±239	770±142	939±414	1214±707	1133±291	1918±816	40.42±5.70
Ostracode	427±363	115±73	379±290	203±162	257±286	332±306	2.20±1.18
Polychaete	428±119	332±105	425±79	395±187	417±200	315±122	14.28±3.16
Priapulida	6±13	0±0	6±13	0±0	24±33	0±0	0.50±0.36
Tanaid	0±0	0±0	30±51	6±13	0±0	0±0	0.59±0.44
Unknown 1	0±0	0±0	0±0	0±0	6±13	0±0	0.34±0.15
Unknown 2	18±27	10±14	6±13	16±26	24±25	12±26	0.50±0.35
Unknown 3	35±38	0±0	24±33	0±0	12±27	24±53	0.47±0.21
Unknown 4	6±13	10±14	12±27	0±0	6±13	0±0	0.44±0.20
Unknown 5	0±0	6±13	0±0	12±27	12±26	6±13	0±0
Unknown 6	83±71	48±16	95±116	101±65	71±45	161±84	1.34±0.31
Total	5471±974	3505±535	5141±930	5556±1931	4953±1352	5890±1560	-
Activity	6.14±2.89	7.64±1.09	6.34±0.86	8.76±2.25	7.73±1.72	8.18±1.93	-