Università di Genova

DIPARTIMENTO DI SCIENZE DELLA TERRA,
DELL'AMBIENTE E DELLA VITA (DISTAV)

Master's degrees class in Science and Technology for the Environment and the Territory
LM/75

Master's Degree in

MARINE BIOLOGY AND ECOLOGY

Tyre Wear Particles: a threat to the marine plankton food web

Master thesis of:

Giuditta Lastrico

Matricola n. 5423574

Tutors:

Prof. Marco Faimali
Dr. Rodrigo Almeda García
Dr. Chiara Gambardella
Dr. Jessy Medhy Manu Le Du-Carrée

Cotutor:
Prof. Mariachiara Chiantore

ACADEMIC YEAR 2024-2025

To all those who cross the sea,
especially those who are forced to flee
from suffering and injustice,
may the sea be gentle to you
and protect you along your journey.

Abstract (EN)

Tyre wear particles (TWP), generated by road traffic, are a major source of microplastics in the environment. Recent research has shown that leachates from TWP contain a complex mixture of chemicals that can adversely affect marine biota, including the first levels of marine trophic web. In this study we assessed the acute toxicity of TWP leachates on various phytoplankton species (*Rhodomonas salina*, *Isochrysis galbana*, *Chrysotila elongata*) and on early larval stages of echinoderms (*Paracentrotus lividus*, *Arbaxia lixula*) using microplates. We hypothesize that the toxicity of the TWP leachates differs among species and that microplate-based assays yield comparable results to glass bottle incubations. Leachates were prepared by incubating 1 g/L of micronized (<250 µm) TWP in seawater for 72 hours. Bioassays involved exposing test organisms to a range of TWP leachate concentrations and control treatments. Microalgae concentration and larval growth and malformations were measured after 24 and 48 hours of exposure, respectively. Our results show that TWP leachates negatively impacted both phytoplankton and zooplankton by significantly inhibiting algal growth and sea urchin larval development. Particularly, *R. salina* was the main affected by the leachate with high sensitivity even at low concentration. Comparison with previous bottle incubation studies revealed that microplate assays are suitable for assessing TWP toxicity in both phytoplankton and echinoderm embryos. Overall, our findings highlight the detrimental effects of TWP pollution on marine planktonic web, underscoring the urgent need to reduce TWP emissions.

Abstract (IT)

Le particelle rilasciate dall'usura degli pneumatici (TWP), generate dal traffico, sono una delle principali fonti di microplastiche nell'ambiente. Recenti studi hanno mostrato come i lisciviati derivati da TWP contengono diverse sostanze chimiche che possono avere impatti sugli organismi marini, inclusi i primi livelli della rete trofica marina. In questo studio viene valutata la tossicità del lisciviato di TWP su diverse specie di organismi fitoplanctonici (*Rhodomonas salina*, *Isochrysis galbana*, *Chrysotila elongata*) e sui primi stadi larvali di organismi zooplanctonici (*Paracentrotus lividus*, *Arbaxia lixula*). I test di tossicità sono stati eseguiti utilizzando un nuovo approccio metodologico, ovvero le micropiastre invece che bottiglie di vetro. Il lisciviato è stato preparato incubando 1 g/L di TWP micronizzate (<250 μm) in acqua di mare per 72 ore. I saggi sono stati condotti esponendo gli organismi a diverse concentrazioni di TWP. Dopo 24 e 48 ore di esposizione sono state misurate rispettivamente la concentrazione delle microalghe e la crescita e malformazione delle larve. I risultati mostrano che i lisciviati di TWP impattano negativamente sia il fitoplancton che lo zooplancton inibendo significativamente la crescita algale e lo sviluppo delle larve di riccio di mare. In particolare, *R. salina* è risultata essere la specie più impattata dal lisciviato con una alta sensibilità anche alle basse concentrazioni. Confronti con precedenti studi che hanno utilizzato le bottiglie per l'incubazione rivelano come le micropiastre possono essere utilizzate per valutare la tossicità delle TWP sia per il fitoplancton che per gli embrioni di echinodermi. I risultati di questa tesi evidenziano gli effetti dell'inquinamento di TWP sulla rete planctonica marina, sottolineando la necessità urgente di ridurre le emissioni di queste particelle.

Index

1. INTRODUCTION

1.1. Plankton ecological role

Marine ecosystems are essential to the health of the planet, providing critical services such as climate regulation, carbon sequestration, oxygen production, erosion control and sustenance for species all around the world (Barbier, 2017). An important component and fuel for the engine of the ocean is plankton. Plankton are organisms of different sizes, the majority in the order of millimeters, who drift on the water current. This term includes different organisms that can be broadly categorized into phytoplankton and zooplankton depending on the feeding mode. Planktonic organisms, such as microalgae, invertebrate larvae crustaceans and jellyfish, play a key role in aquatic ecosystems and it is essential to evaluate their health to better understand physical and biological processes involved in the interconnected marine system (Daly & Smith, 1993; Fenchel, 1988).

Phytoplankton, unicellular photosynthetic organisms, are essential for the marine ecosystems because of their essential contributions to the food web and oxygen production, producing almost half of Earth's primary production (Falkowski, 1994). As primary producers, they form the base of the marine food web, providing essential nutrients for a wide range of marine organisms, from small zooplankton to large fishes and marine mammals. The amount of phytoplankton in the ocean depends on "bottom-up" mechanisms regulated by nutrients and sunlight (Fontúrbel & Castaño-Villa, 2011) or "top-down" mechanisms regulated by copepods or other grazers (Prowe et al., 2012). Moreover, microalgae contribute to the global carbon cycle by absorbing carbon dioxide during photosynthesis and sequestering it by falling as marine snow in ocean depth; actually, 40% of all anthropogenic carbon dioxide has entered the oceans (Brierley, 2017; Naselli-Flores & Padisák, 2023). Their adaptability to diverse environments also plays a vital role in maintaining ecosystem stability and biodiversity, while their growth patterns affect the health of coastal and open ocean habitats.

Zooplankton are animals with a wide range of dimensions, from micrometric foraminifera to jellyfish with tentacles one or two meters long. They represent a dominant component of the marine life, playing a key function in the transfer of energy to higher trophic levels in marine food web. Their diversity influences the health of the ecosystem, and they are also important in the carbon cycle especially being part of the biological carbon pump, the main mechanism that transport organic carbon to the deep ocean. Specifically, zooplankton contribute to the biological carbon pump both with their faecal pellet, death or with vertical migration as shown in Figure 1 (Nowicki et al., 2022).

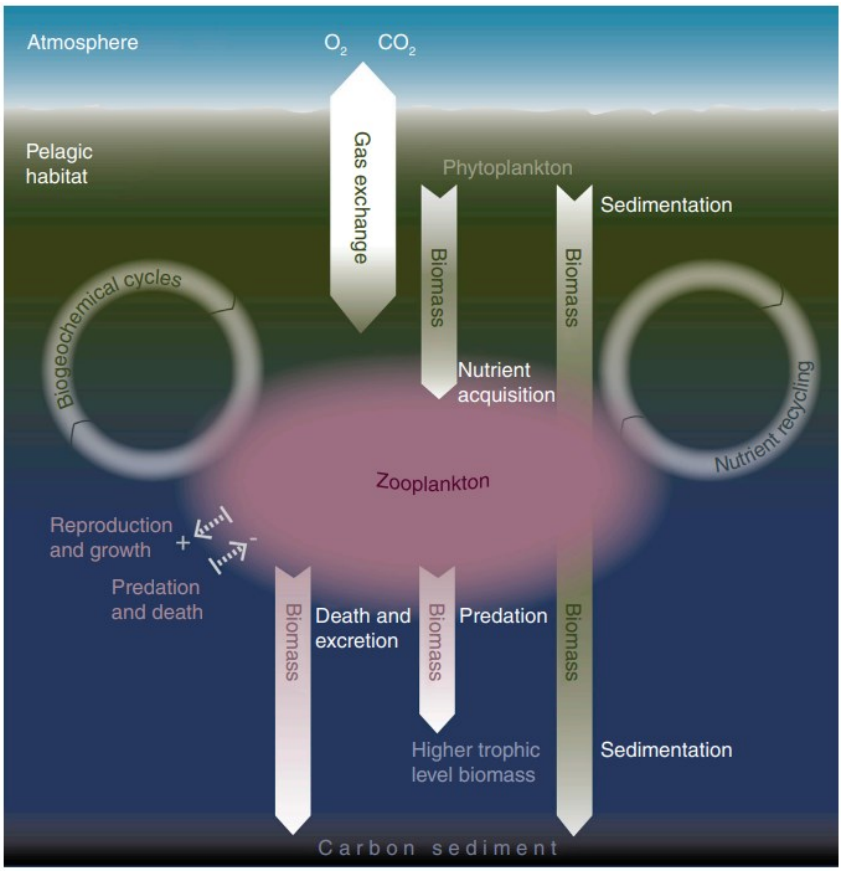


Figure 1: Schematic representation of the roles of phytoplankton and zooplankton in carbon transportation, nutrient cycling and food chains. The dashed arrows on the edge of the zooplankton community represent increase and decrease of biomass and abundance by various processes.
Image drafted by Steve Smart, University of St. Andrews (Brierley, 2017).

Plankton can be classified as holoplankton or meroplankton based on their life cycle strategy. Holoplankton are organisms that spend their entire life cycle as drifting organisms in the plankton, while meroplankton are organisms that are part of the plankton only during certain stages of their cycle, typically benthic invertebrate larvae (Brierley, 2017). Meroplanktonic larvae play a crucial role in species dispersal, and the recruitment, population genetics, and genetic diversity of marine benthic populations are decisively influenced by the fitness and dynamics of their larval stages (Ayata et al., 2011; Silberberger et al., 2016; Thorson, 1950).

Organisms like sea urchins, which have planktonic larval stages, are important because of their role in the benthic communities. They are important herbivores and their grazing activity help to maintain the balance between algae and other benthic organisms on the seafloor, influencing the structure of marine habitats like kelp forests and coral reefs (Dang et al., 2020; Pearse, 2006). Sea urchin larvae are crucial to the dynamics of marine ecosystems, particularly in the context of benthic-pelagic coupling and ecosystem stability. These larvae are an integral part of the marine food web, serving as a food source for various predators, including small fish, cnidarian and other invertebrates (Pennington et al., 1986). Reviews of field and laboratory data confirm that predation is a significant

source of mortality for the eggs, embryos, and larvae of marine invertebrates like sea urchins (Morgan, 2020; Young, 1987). In addition to predation, development and survival of sea urchin larvae depend on availability of planktonic food sources, such as microalgae, which underscores the interconnection between various marine organisms and the health of marine ecosystems. Sea urchin larvae are also sensitive to environmental factors like temperature (Wangensteen et al., 2013), acidity (Passarelli et al., 2017), and pollution (Bielmyer et al., 2005) making them indicators of ocean health and climate change impacts (Sartori et al., 2023).

1.2. Pollution: a planetary crisis

1.2.1. Plastic pollution

Despite the importance of marine ecosystems, multiple threats are affecting marine habitats with impacts on different organisms and human well-being. Among these anthropogenic stressors, pollution is a key component of the triple planetary crisis. Plastic pollution is one of the main challenges of this century (Carpenter & Smith, 1972; Stefatos et al., 1999). Every year hundreds of tons of plastic are produced, reaching 413.8 Mt of global primary production in 2023, and most of them turned out to be waste (Geyer, 2020). Even though only 2% of plastic debris in the ocean is generated from activities at sea (Boucher & Friot, 2017), an important quote of plastic debris ends up in the ocean, which is considered to be the ultimate sink for all plastic within the environment (Horton & Dixon, 2018; Jambeck et al., 2015).

Due to mechanical stress and physicochemical and biological processes, plastic debris is subject to fragmentation in different environments, forming microplastics (MPs, plastics < 5 mm) and nanoplastics (NPs, plastics < 1 um) (Frias & Nash, 2019; Wayman & Niemann, 2021). The time required for plastics to degrade in the environment is estimated to be on the order of hundreds to thousands of years. MPs can be classified as primary microplastics when manufactured for the purpose of being added to other products and secondary microplastics if created by the fragmentation and degradation of macroplastics. Most microplastics found in the environment are secondary microplastics; a major source of these microplastics is the abrasion of tyres against road surfaces. All the main sources of microplastics in marine environment are shown in Figure 2 (Environment, 2021).

Once MPs enter the marine environment, for example through rivers (Campanale et al., 2020), they are extremely difficult and expensive to remove. They are proven to harm different freshwater and marine animals (Balestrieri et al., 2022; Gallitelli et al., 2021; Guzzetti et al., 2018) and plants (Rillig et al., 2019). The harmful effects of MPs in animals are associated with entanglement or ingestion that can potentially expose them to hazardous additives contained in the plastic or attached pathogens.

When ingested, MPs can be accumulated and distributed in gastrointestinal tract, gills and muscles leading to lethal and sublethal effects like reduced growth, modifying biological activity (Egbeocha CO et al., 2018). Moreover, floating plastic transports invasive alien species and disease affecting local habitats (Rech et al., 2016). MP are not only pervasive in water but also they accumulate in the seafloor and present in in soils (Corradini et al., 2019), sediments (Dekiff et al., 2014), atmosphere (Chen et al., 2020) and the human body (Leslie et al., 2022; Ragusa et al., 2021).

1.2.2. Toxicity of plastic leachates

Thousands of chemicals commonly referred to as "plastic additives" are added during the manufacturing of plastics to enhance their properties. Many of these additives are not chemically bonded to the polymer matrix and they can leach into the environment (Hahladakis et al., 2018). For this reason, plastics not only pose a direct threat through entanglement and ingestion but also release potentially hazardous chemicals through leaching, which can negatively impact marine organisms at various trophic levels. The chemical leachates released from plastics, comprising a complex mixture of additives and other pollutants, can be toxic to marine species, influencing their survival, growth, and reproduction. Additives may represent a high percentage of the final plastic materials (in some cases up to 50%) and are used to meet safety standards in electronic materials and prevent ignition, like flame retardants (including polybrominated diphenyl ether) make plastics more flexible (as polyesters and phthalates), reduce degradation from UV rays (UV-stabilizers) and heat (phenols and arylamines), protect from chemical degradation, or impart a desired colour (Table 1), (Pfaendner, 2006; Turner, 2016).

Table 1: Common additives used for plastics.

Additive	**Purpose**
Calcium carbonate	Filler: generally used for cost reduction as much cheaper than polymer
Pigments	Give the plastic a colour. Generally for aesthetic properties
Glass fibre	Reinforcement: increased strength and stiffness
Flame retardants	Increased fire resistance
Heat stabilisers	Increased resistance to heat exposure
Light stabilisers	Increased resistance to light exposure
Plasticisers	Process aid which reduces viscosity
Foaming agents	Lightness and stiffness

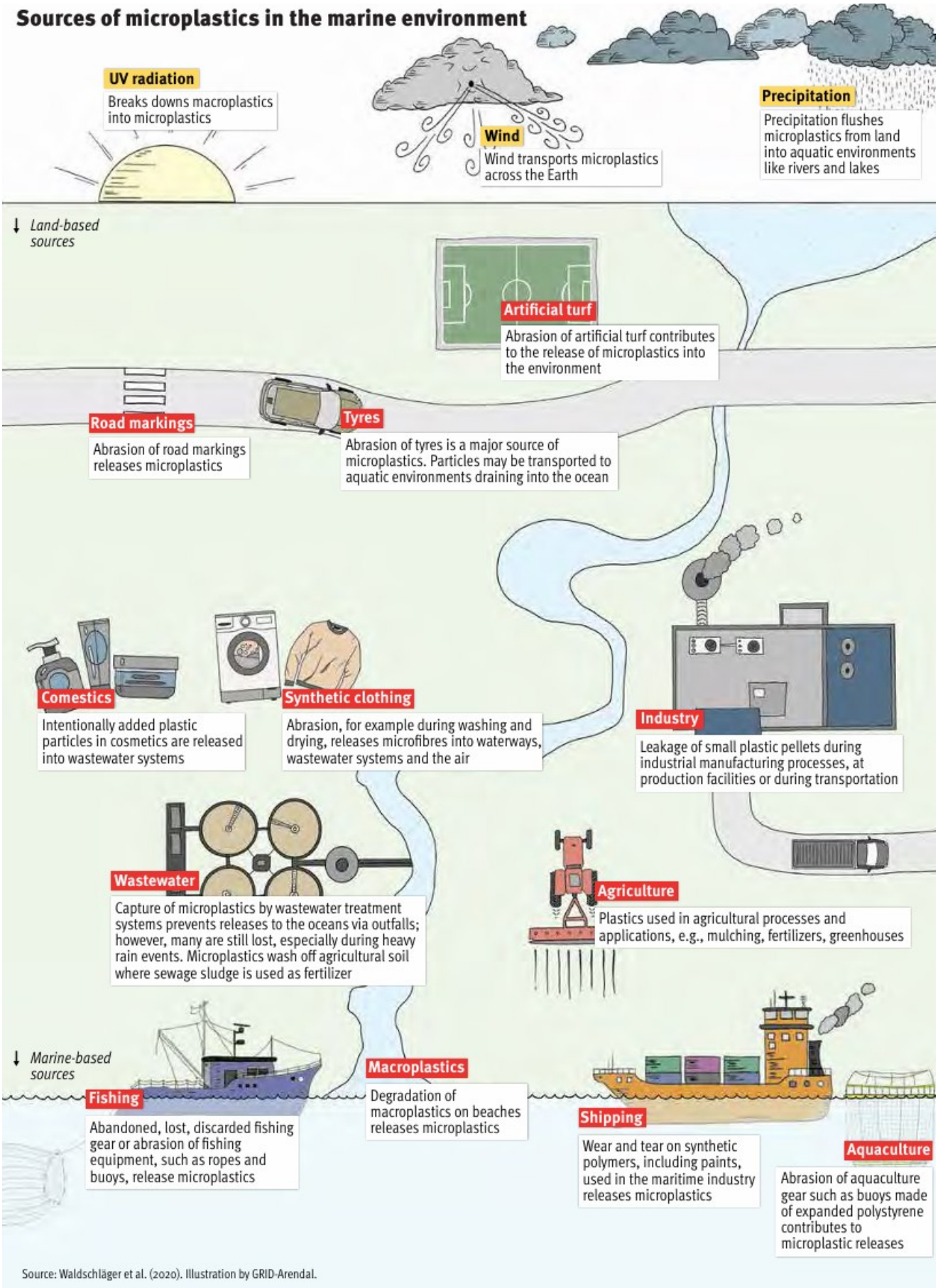


Figure 2: Sources of microplastics in the marine environment. (Environment, 2021)

Even if plastic pollution and its effect on ecosystems are studied, the impacts of plastic leachates on biota have been little explored so far. More than 65% of the plastic leachate literature have been published since 2020 (Delaeter et al., 2022). In the last 2 decades, research on plastic leachates has intensified, in parallel with the increasing use of plastics and awareness of environmental pollution and health risks. Since plastic additives are persistent pollutants, ubiquitous in marine environments and released continuously, especially during the process of fragmentation, the origin, nature and impact of plastic leachates is of upmost importance for the future of plastic pollution research.

Leaching is the desorption of chemicals into the surrounding environment, including the additives added during the manufacturing and the chemicals absorbed and adsorbed to plastic polymers during weathering. With this consideration, it is possible to define primary and secondary leachates; the first one indicates molecules, added to the polymer during the manufacturing process, and the second one indicates adsorbed molecules to plastic polymers through hydrophobic and electrostatic interactions, and non-covalent bounding such as van der Walls forces. This terminological distinction is particularly relevant in the context of the research conducted on the effects of the molecules released by non-weathered plastics (also called virgin plastics) and weathered plastics (Figure 3) because in the second case plastic particles may have attracted other contaminants and particles to their surface like heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) (Kedzierski et al., 2018).

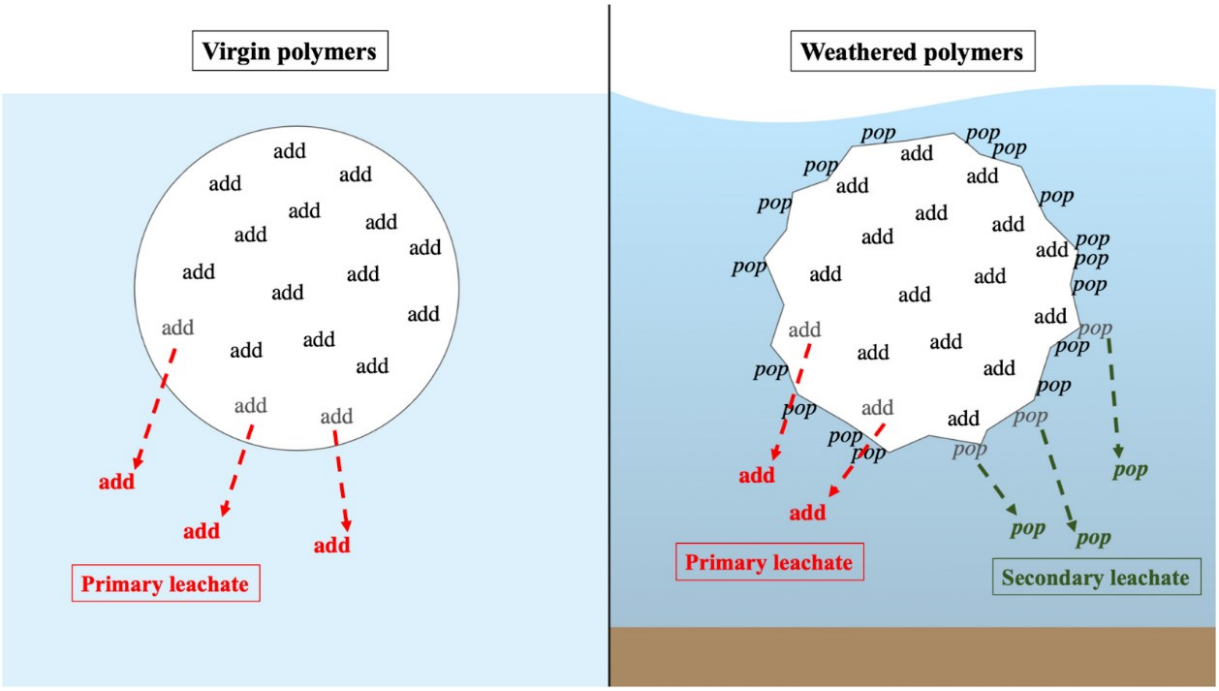


Figure 3: Composition of plastic leachates of virgin and aged, i.e. that have stayed in the environment, polymers. Add: plastic additives, pop: persistent organic pollutants. (Delaeter et al., 2022)

A wide range of organisms have been found to be sensitive to plastic leachates even if the ecotoxicity of leachate were tested in most of the studies from pristine plastic, not weathered. Future studies should focus on environmental plastics because weathering increases the acute toxicity of plastic on organisms like Ferrari et al. (2024) studied with pellets leachates on sea-urchin larvae and (Lv et al., 2024) for TWPs on microalgae.

1.2.3. Tyre wear particle: an abundant and high concern type of MPs

Actually, 98% of the losses of primary MPs are generated during land-based activities. The main pathway is road runoff (tyres, road markings and pellets incidents on land) (66%) followed by wastewater treatment systems (25%) and by wind transfer (7%) (Boucher & Friot, 2017). Since traffic volume is expected to grow and pollution issues are related to plastic, attention is focused on non-exhaust emissions resulting from tyre wear, brake wear, as well as the resuspension of road dust (Piscitello et al., 2021). Tyre Wear Particles (TWP; Figure 4a) are small debris, mostly black particles <200 µm (Wagner et al., 2018), which represent an important part of non-exhaust vehicle emissions. TWP are generated by friction between tires and the road, specifically due to shear and friction forces, originating from tyre slip relative to the road in the process of steering, braking and driving (Figure 4b). The annual TWPs released by tire wear can reach 6.1 million tons, and recent statistics indicate that tyre emissions account for about 5–10 % of the total global major sources of MPs (Kole et al., 2017). In the environment, pure tyre wear particles are rarely found as road pavement materials tend to also agglomerate within the tyre material; thus, the emitted particles are generally referred to as Tyre and Road Wear Particles (TRWP). However, in scientific literature the term ‘tyre wear’ is often used even if the heterogeneous aggregates of ‘tyre and road wear particles’ are meant (Baensch-Baltruschat et al., 2020).

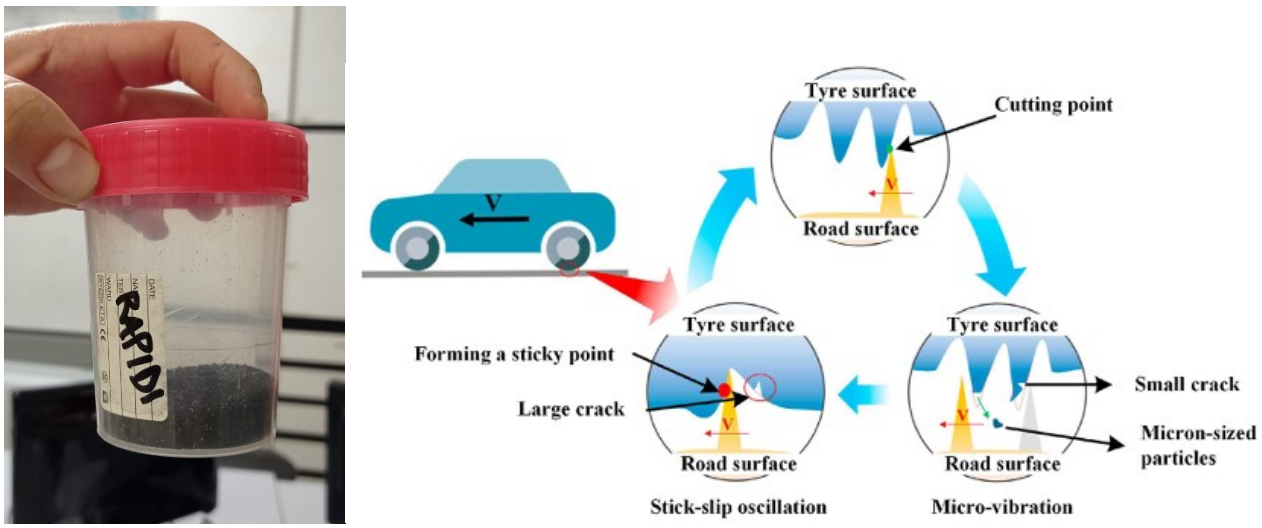


Figure 4a e 4b: TWP used in the experiment (left) and formation mechanisms (right)

TRWP are composed of a complex mixture of tyre tread material (e.g. synthetic and natural rubber, silica, oil, carbon black, sulphur compounds, zinc oxide), road pavement material (e.g. polymer modified bitumen), road marking particles, brake wear particles and other airborne elements that commonly deposit on pavements. Even if they are composed of both synthetic (like butadiene rubber and styrene butadiene rubber) and natural polymers (like natural rubber), they are classified as MP and can be divided into coarse and fine TWP. It has been reported that there are a variety of factors influencing physical and chemical properties of TWP (Zhang et al., 2023). At the point of emission, TWP may become suspended in air or deposit on road surfaces and nearby soil leading to potential environmental health problems. As TWP have lower density compared to other road derived particles or suspended particulate matter it can be assumed that TWP have a high potential to remain in suspension and be transported over longer distances, while TWP heteroaggregates with a higher density would be subjected to sedimentation. The physical characteristics of the emitted particles, and particularly their size, may be important determinants of their environmental fate. This illustrates the need to investigate aggregation processes of TWP in more detail (OECD, 2021).

Surface runoff, stormwater, wind, drainage systems, wastewater effluent, and atmospheric deposition may disperse or flush emitted TWP into nearby water streams leading this polluting particles into aquatic ecosystems (Parker-Jurd et al., 2021).The small size of TWP allows them to easily be ingested by plankton, potentially causing physical harm or internal damage and can be a threat due to the reduction of food intake with loss of energy and vitality. Even if the effects of TWP in the aquatic environment have been examined primarily focusing on the leachate fraction, TWP can also be ingested by various organisms, such as zooplankton with harmful effects. The first observation of rubber ingestion by planktonic copepods was published by Wik & Dave (2009) with other few studies till nowadays including other organisms (Koski et al., 2021; Redondo-Hasselerharm et al., 2018). Specifically Koski et al. (2021) found effects on two species of copepods, also showing the influence of feeding mode and food concentration on response to microplastic. Once in the water, aging processes like mechanical stress, oxidation (if TWP are not deposited in sediments), microbial colonization and degradation, may change the properties of TWP and enhance leaching of a wide spectrum of substances. Major effects may be connected to leachates since studies have reported non-significant difference in toxicity between leachates alone or TWP (Thomsen et al., 2024). Effects of TWP leachates can be various (from sub-lethal to lethal), affecting different planktonic organisms, including phytoplankton (Capolupo et al., 2020; Page et al., 2022) and zooplankton (i.e. rotifers (Shin et al., 2022), copepods (Bejgarn et al., 2015; Koski et al., 2021; Yang et al., 2022), echinoderms (Calle et al., 2025), mussels (Thomsen et al., 2024), fish (Sartori et al., 2023; Tian et al., 2021; Wik et al., 2009)).

The community structure of zooplankton may be altered due to the different resistance of zooplankton to TWP (Li et al., 2023). Furthermore, the physical properties of TWP can disrupt the behaviour of plankton affecting the community. For example, the particles may accumulate in the water column, altering light penetration and thus influencing the growth of light-dependent phytoplankton species. This can further change the dynamics of plankton communities, potentially favouring certain species while harming others. These disruptions caused by TWP can reduce biodiversity in plankton populations and have cascading effects throughout the ecosystem, affecting fish, invertebrates, and other aquatic organisms that depend on plankton as a food source. Moreover, in a perspective of higher temperature caused by climate change, studies on TWP toxicity are imperative since a positive correlation between particles emission or exacerbation of toxic effects and temperature was found in various research (Li et al., 2024; Zhang et al., 2023).

1.3. Model organisms in ecotoxicology

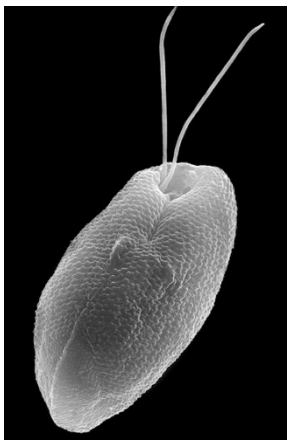
Model organisms are critical tools in ecotoxicology, as they allow scientists to investigate the potential effects of pollutants in a controlled and reproducible manner. These organisms are chosen for their characteristics making them suitable for monitoring the impact of substances in natural environments. When selecting model organisms for ecotoxicological testing, characteristics that must be considered are the organism's ecological relevance, sensitivity to pollutants, and ease of culture and maintenance under laboratory conditions. For studies on plastic leachates, microalgae and sea urchin larvae are particularly valuable and recommended due to their ecological significance since they are at the first levels of the trophic chain and their sensitivity to environmental changes.

1.3.1. Microalgae

Microalgae are a fundamental component of marine ecosystems, forming the base of the food chain and contributing significantly to oxygen generation and primary production in the ocean, contributing with 45-50 Pg C per year (Field et al., 1998). These tiny, photosynthetic organisms are essential for the overall health of marine environments. Microalgae are highly sensitive to various pollutants, including those released by plastics, such as plasticizers and other chemicals. Their rapid growth and division rates make them ideal for testing the acute and chronic toxicity of pollutants, as any effects can be observed within short periods (Lu et al., 2021). Furthermore, microalgae are sensitive to a range of chemical contaminants, which makes them suitable for detecting even low concentrations of toxins in water (Chung et al., 2007). Indeed, microalgae are commonly used in tests that evaluate the effects of chemical leachates, including plastic leachates, on cellular processes, such as photosynthesis, growth, and reproduction (Nam et al., 2022).

Cryptophyta

Rhodomonas salina (Wislouch) D.R.A.Hill & R.Wetherbee, 1989 (Figure 5) is a unicellular flagellate algae belonging to the group of Cryptophytes. Cells are ovoid and flattened in shape with an anterior groove. At the edge of the groove there are two slightly unequal flagella used for locomotion. The genus *Rhodomonas* is widely distributed in aquatic environments, and its members are an essential part of the planktonic community; they are fast-growing microalgae with red colour (caused by the antenna pigment phycoerythrin 545). *R. salina* has many attributes of interest for aquaculture, such as the lack of a cell wall, small size, massive outdoor growth and high protein, lipid, and polyunsaturated fatty acid (PUFAs) content (Dunstan et al., 2005). This species has been reported as a high-quality diet for copepods (Dayras et al., 2020) and different life stages of bivalves (Fernández-Reiriz et al., 2015; González-Araya et al., 2012; Tremblay et al., 2007).



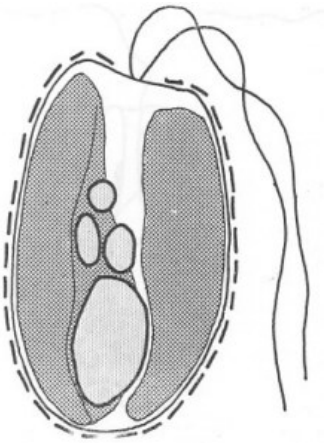
Scientific classification	
Domain	Eukaryota
Kingdom	Chromista
Phylum	Cryptophyta
Class	Cryptophyceae
Order	Pyrenomonadales
Family	Pyrenomonadaceae
Genus	*Rhodomonas*
Species	*R. salina*

Figure 5: Scanning electron micrograph (SEM) of marine R. salina *(downloaded from afloimages.com) and scientific classification.*

Haptophytes

Haptophyta is a diverse group of marine and freshwater microalgae. They can have an oval or spherical shape and can be distinguished by the presence of a unique structure called haptonema, a hair-like appendage that is distinct from flagella. The haptonema can be involved in various functions such as movement, feeding, or attachment to surfaces. They are an important source of food for small zooplankton and other marine organisms. Haptophytes are also involved in the production of dimethylsulfoniopropionate (DMSP), a compound that can be converted to dimethyl sulfide (DMS), which plays a role in cloud formation and has a potential impact on climate regulation (Larsen & Beardall, 2021).

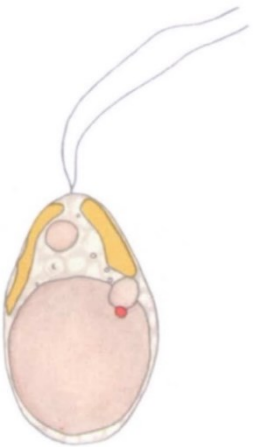
For this thesis, two Haptophytes belonging to Coccolithophyceae class were used: *Chrysotila elongata* and *Isochrisis galbana*. *C. elongata* is a unicellular algae. It was described first by Droop as *Syracosphaera elongata* (Figure 6) and to date it has never been used for ecotoxicological purposes.



Scientific classification	
Domain	Eukaryota
Kingdom	Chromista
Phylum	Haptophyta
Class	Coccolithophyceae
Order	Isochrysidales
Family	Isochrysidaceae
Genus	*Chrysotila*
Species	*C. elongata*

Figure 6: C. elongata *cell in culture (taken from Droop, 1955) and scientific classification.*

I. galbana Parke, 1949 (Figure 7) is a unicellular algae which, for its good nutritive characteristics (especially in relation to PUFAs composition), is of substantial interest in aquaculture, principally to feed mollusk larvae, as well as fish and crustaceans in the early stages of growth (Sánchez et al., 2000). This species is widely used in marine ecotoxicology to test a wide range of contaminants (i.e. metals, UV filters, plastics; Liu et al., 2011; Paredes et al., 2014, including plastic leachates; Meng et al., 2024).



Scientific classification	
Domain	Eukaryota
Kingdom	Chromista
Phylum	Haptophyta
Class	Coccolithophyceae
Order	Isochrysidales
Family	Isochrysidaceae
Genus	*Isochrysis*
Species	*I. galbana*

Figure 7: I. galbana *cell. Taken from Parke, 1949, and scientific classification.*

1.3.2. Echinoderms and their planktonic developmental stages

Echinoderms are keystone species in many marine ecosystems, particularly in coastal and benthic environments. Species such as sea urchins serve as herbivores and detritivores, influencing algal populations and nutrient cycling. *Paracentrotus lividus* and *Arbacia lixula* (Figure 8) are integral to the structure of benthic communities and the balance of the food web, inhabiting the intertidal and subtidal zone. *P. lividus* feeds more on flashy algae, while *A. lixula* with its superior Aristotle's lantern, that gives greater grazing ability, feeds on encrusting coralline algae as well as invertebrates (Agnetta et al., 2013). These two species are dominant in coastal areas of Canary Island and Ligurian Sea playing an important ecological role such as control of the benthic community. Due to their central role in ecosystem processes, any disruption to echinoderm populations can have cascading effects on the entire marine ecosystem, impacting biodiversity, species interactions, and habitat structure (Birkeland, 1989; Uthicke et al., 2009).

Scientific classification	
Domain	Eukaryota
Kingdom	Animalia
Phylum	Echinodermata
Class	Echinoidea
Order	Camarodonta
Family	Parechinidae
Genus	*Paracentrotus*
Species	*P. lividus*

Scientific classification	
Domain	Eukaryota
Kingdom	Animalia
Phylum	Echinodermata
Class	Echinoidea
Order	Arbacioida
Family	Arbaciidae
Genus	*Arbacia*
Species	*A. lixula*

Figure 8: Adults of P. lividus *(above) and* A. lixula *(below) with scientific classification. Pictures downloaded from WORMS website.*

Furthermore, echinoderms are considered bioindicators of environmental quality, particularly in the context of pollution (González-Delgado et al., 2024; Portocali Ph. et al., 1997). Due to their slow developmental cycles, long lifespans, relatively sedentary nature, and exposure to a wide range of environmental conditions, echinoderms are often used to monitor the health of marine ecosystems and test the effects of long-term exposure to low concentrations of pollutants. Their sensitivity to pollutants, such as heavy metals (Bonsignore et al., 2018), pesticides (Pesando et al., 2003), and emerging contaminants like plastic leachates (Rendell-Bhatti et al., 2021), provides valuable insight into the broader ecological impacts of marine pollution.

Sea urchin larvae have a well-defined life cycle (Figure 9), and their development is easily observable, particularly during the early stages, when they undergo critical processes like fertilization, cell division, and organogenesis. Disruptions at these critical stages can lead to abnormal development, reduced growth rates, and decreased survival, ultimately affecting the health of adult populations (Gambardella et al., 2024).

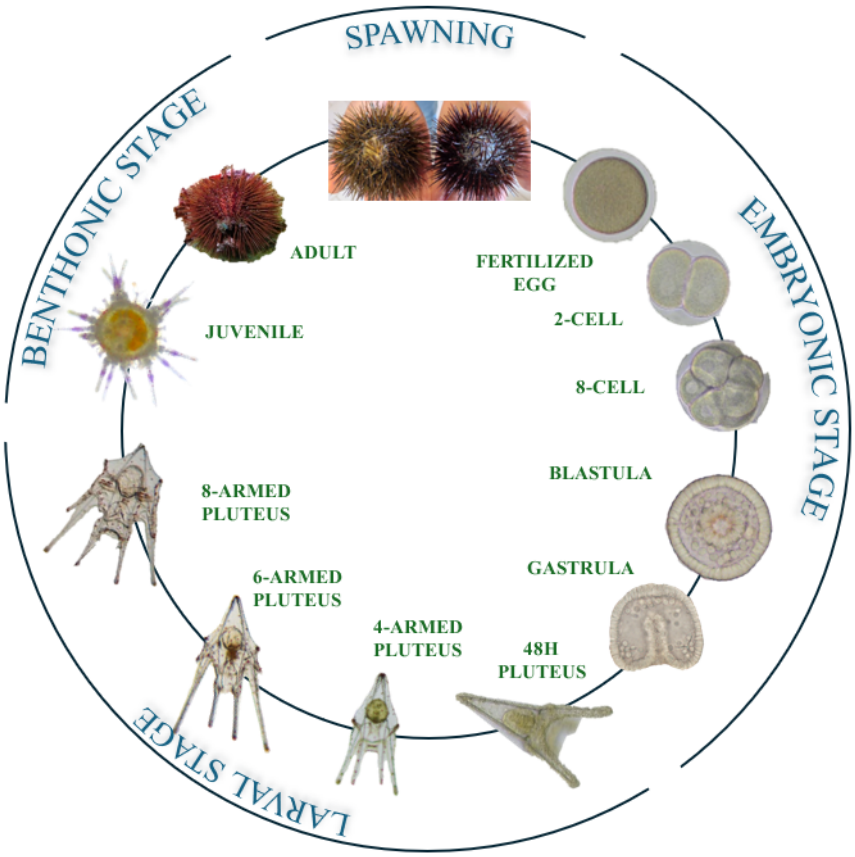


Figure 9: Sea urchin life cycle

Understanding toxicity effects of leachates during the larval stage and potential consequences on recruitment is essential for the maintenance of healthy populations of species that are ecologically and commercially important as *P. lividus* and *A. lixula* (Spalding et al., 2014).

1.3.3. Sensitivity of microalgae and echinoderms in ecotoxicology

Microalgae have different sensitivity to pollutants and can behave differently depending on the species and pollutant typology and concentration. For example Lv and colleagues (2024) have found different effects of TWP on the marine diatom *Phaeodactylum tricornutum*, reporting a low-dose growth stimulation and a high-dose growth inhibition.

Most of toxicity tests carried out with microalgae have been developed on *R. salina*, *I. galbana* (L. Li et al., 2024; López-Galindo et al., 2010) and *P. tricornutum*, which is also recognized by International Standardization Organization (ISO). *R. salina* and *I. galbana* are used in several studies as test organisms due to the importance in aquaculture and sensitivity (Hampel et al., 2001; Liu et al., 2011). Phytoplankton vary significantly in their size, morphology and growth rate, which might influence the resistance of the cells to harmful substances. For instance, the structure and composition of their cell wall differs. For example, the diatom *P. tricornutum* could be better protected against harmful particles and leachates due to their strong cell wall. Also, due to the uptake of chemicals through diffusion, cells with larger surface area could be more susceptible, as could cells that have a slow growth rate and therefore perhaps a longer retention time of harmful substances. With these assumptions the microalgae used in this experiment may be interesting for the high surface/volume ratio of *R. salina* and the lack of a cell wall in *I. galbana* and *C. elongata*.

The main endpoints used in ecotoxicology for microalgae are growth inhibition rate (IR), content of *Chl a*, photosynthetic activity and oxidative damage to cells (Nam et al., 2022).

R. salina is the only microalga used in this thesis that has been already tested to assess TWP leachate toxicity, showing to be highly sensitive to TWP leachates. Conversely, no studies are available on the effects of TWP leachate towards *I. galbana* and *C. elongata.* Despite, *I. galbana* sensitivity has been tested with different compounds, as metals (Trenfield et al., 2015) and plastic material (Garrido et al., 2019; López-Galindo et al., 2010; Trenfield et al., 2015; Venâncio et al., 2019).

No ecotoxicology studies are available in literature on *C. elongata*, so the relevance of this study to collect data for more species is underlined.

Echinoderm larvae, especially those of sea urchins, are particularly sensitive to pollutants, including plastic leachates, during early development (Beiras et al., 2019; Uribe-Echeverría & Beiras, 2022).

One of the primary reasons sea urchin larvae are used in ecotoxicological tests is their ability to serve as bioindicators of toxic effects at the population level and the sensitivity at this life stage to stress (Pandori & Sorte, 2019).

The sea urchin embryo-larval assay, in which fertilization success and embryo development are monitored after exposure to contaminants, is a widely accepted method in marine ecotoxicology. This test provides valuable information on the effects of pollutants on reproductive success and early development, which are key determinants of species survival and population health (Bielmyer et al., 2005; Marin et al., 2007). In this assay, the effects of chemical contaminants (including plastic leachates) on fertilization success, embryonic development, and larval morphology are closely monitored. For example, exposure to plastic leachates may lead to delayed development, abnormal skeletal formation, or failure to metamorphose into the adult form (Gambardella et al., 2021). These effects are particularly concerning given that echinoderm larvae are an important food source for many marine organisms, making their health and development crucial for maintaining marine biodiversity.

The impact of plastic leachates on sea urchins has been studied for several polymers (Cormier et al., 2021; Martínez-Gómez et al., 2017; Rendell-Bhatti et al., 2021), but there are only few tests with tyre particle addtives (Calle et al., 2025). Rist et al., 2023 investigated the effects of tyre particle leachates on early developmental stages of three keystone sea urchin species (*P. lividus*, *A. lixula* and *Diadema africanum*) and found clear concentration-dependent effects of tyre particle leachates on all species.

In Table 2 the main ecotoxicological endpoints for microalgae and sea urchins found in literature are summarized.

Table 2: Main endpoints for ecotoxicological tests with microalgae and sea urchins

Organism	Exposure time (h)	Endpoint
Microalgae	24 – 48 – 72 – 96	Growth inhibition
		Photosynthetic activity
		Chlorophyll content
		Oxidative stress
		DNA damages
Sea urchins	1	Spermiotoxicity
	24	Cholinesterase activity
	48	Size increase from eggs at day 0
	48	Total larval body length
	48	Percentage of fully developed pluteus
	24 – 48 – 72	Developmental anomalies percentage
	24 – 48 – 72	Swimming behaviour
	72	ETS and GST activity

Finally, it is important to keep in mind a general paradigm of ecotoxicology: the impact of a pollutant cascades through levels of biological organization such that biochemical changes at subcellular levels precede changes to cells and tissues, which in turn affect physiological functions and individual fitness (that is, populations) and ultimately ecosystems (Figure 10) (Galloway et al., 2017). Directly linking sub-organism-level impacts to the ecosystem level is hugely challenging for any environmental pollutant. An individual's behaviour forms an important link between physiological and ecological processes and is a sensitive measure of response to environmental stress or pollutants. Hence behavioural changes can serve as early warning signs for ecosystem level effects (Wong & Candolin, 2015). Understanding how microplastic itself and their leachates affect organisms is essential to understanding its ecological impact.

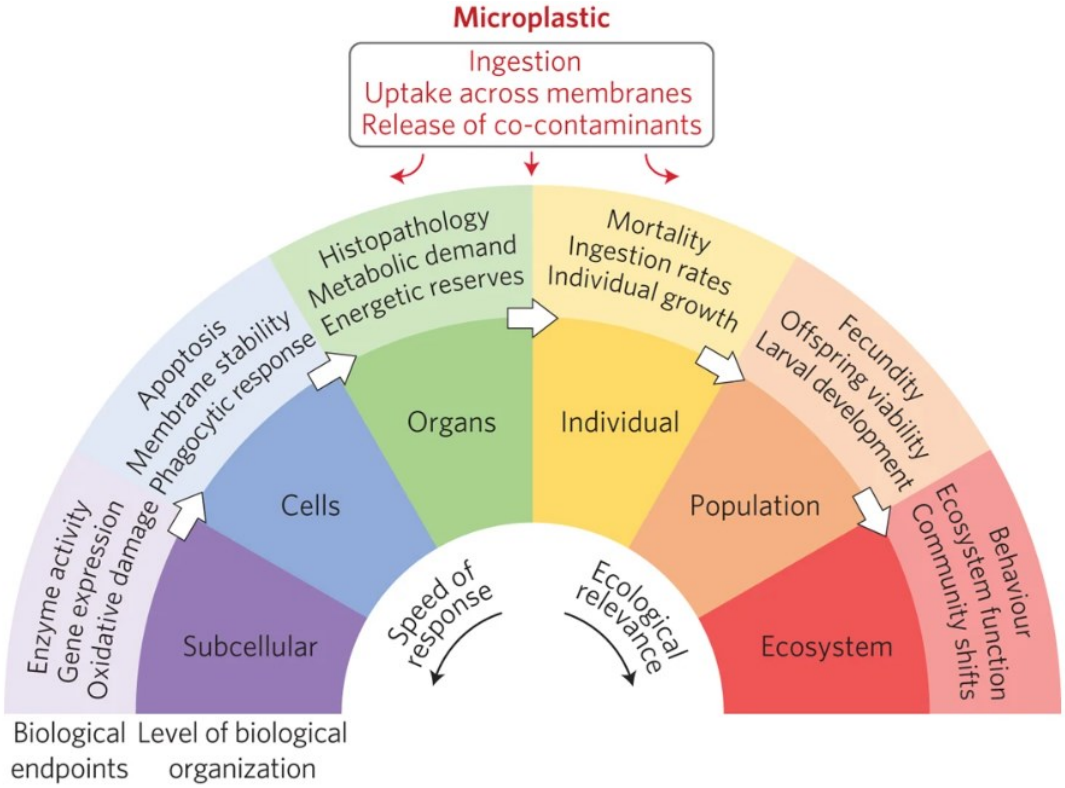


Figure 10: Simplified scheme illustrating impacts of exposure to microplastic across successive levels of biological organization (Galloway et al., 2017)

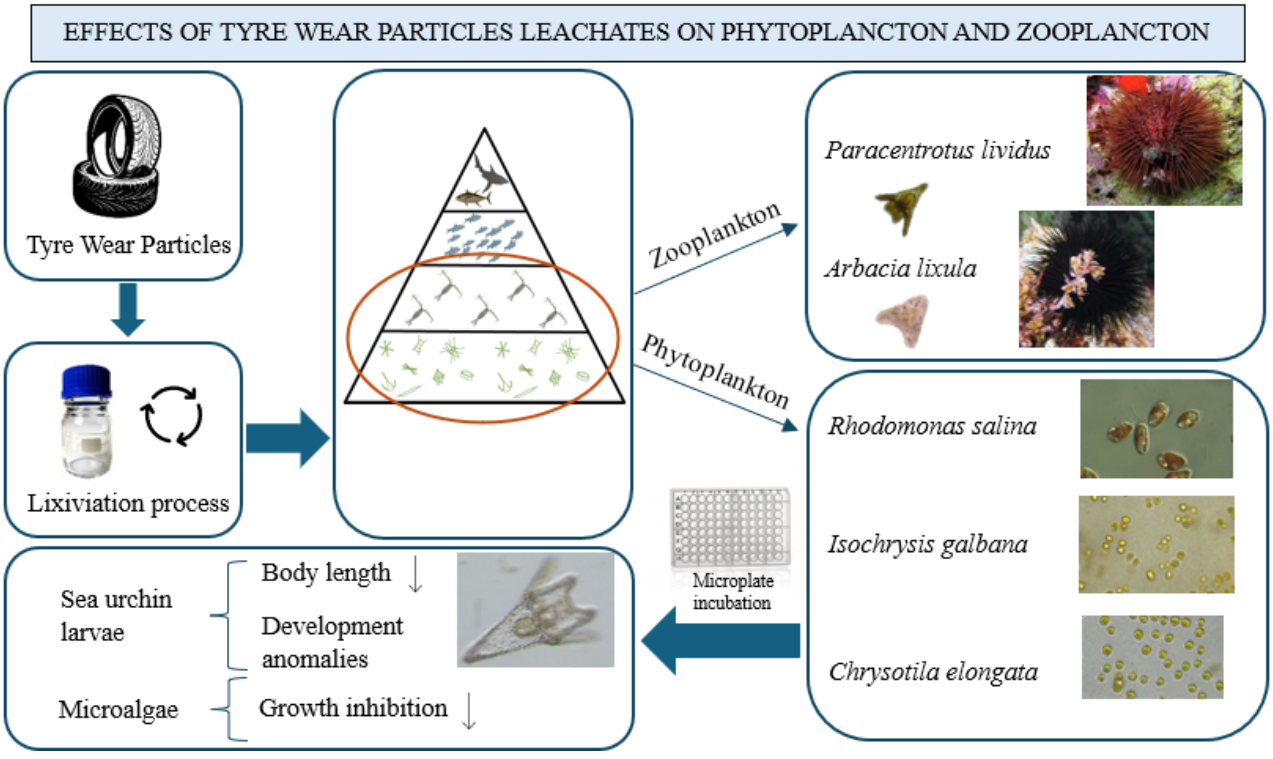
2. AIM AND OBJECTIVES OF THE STUDY

This thesis aims to investigate the acute effects of TWP leachates on key marine planktonic organisms. The specific objectives were to:

1) determine the effect of TWP leachates on the growth of several microalgae species.
2) estimate the survival and development of sea urchin embryo after acute exposure to TWP leachates
3) evaluate if microplate-based assays yield comparable results to glass bottle incubations.

To accomplish these objectives, bioassays were conducted on three species of microalgae (*Rhodomonas salina*, *Isochrysis galbana* and *Chrysotila elongata*) and the embryo/larvae of two keystone sea urchin species (*Paracentrotus lividus* and *Arbacia lixula*) using microplates. We hypothesize that TWP are toxic to planktonic organisms with toxicity differing among species/plankton groups and that microplate-based assays can yield comparable results to traditional glass bottle incubations.

By utilizing these planktonic organisms, we can evaluate the potential impacts of TWP pollution on marine food web, both on the primary producers and meroplanktonic larvae, which are the most vulnerable life stages. This study provides valuable insights into the ecological risks associated with leachate contamination from TWP, which can help for further environmental management and conservation efforts.



3. MATERIALS AND METHODS

Experiments were conducted in the faculty of Marine Sciences at the University of Las Palmas de Gran Canaria (Spain) and in the laboratories of CNR-IAS in Genoa (Italy). Tests and sampling sites are shown in Figure 11. In Las Palmas de Gran Canaria we analysed effects of TWP on microalga *R. salina* and larvae of *A. lixula*, while tests about effects of TWP on larvae of *P. lividus* and two other microalgae, *I. galbana* and *C. elongata*, were conducted in the IAS laboratory.

Figure 11: Sampling and tests sites. Red points are IAS and EOMAR laboratories (respectively above and below). Blue points are sampling sites (Ligurian Sea - Italy and Taliarte beach - Spain).

3.1. Tyre particles and leachate preparation

A new car tire tread (Imperial 145/70-13 71T- Snowdragon HP-Vinterdæk) and a rapido trawling rubber were used to generate tire particles. They were composed of natural and synthetic rubber with a mixture of organic compounds and metals. Chemical analyses of the material used for this thesis were conducted during previous study (Table 3; Rist et al., 2023). To obtain tire particles, the tread of the tire was cut into strips and then micronized by grinding the strips with a stainless-steel pneumatic milling cutter (Page et al., 2022). Leachates were extracted following the standard protocol for plastic micronization and leaching published by Almeda et al., 2023 (Figure 12). A glass bottle with TWP at a concentration of 1g/L in FSW (Filtered Seawater) was placed in a laboratory roller at 20°C in darkness with a speed of 15 rpm, that assured gentle stirring, for 72.

Table 3: Compound identified in the undiluted tire particle leachates (1 g/L) and of the filtered seawater. All organic compounds and metals are shown for which concentrations in the leachate exceeded those of the blank. All values in µg/L. a, b indicate replicates. From Rist et al. (2023).

	Blank Seawater		Tire particle leachate
Compound/element	**a**	**b**	**mean ± sd**
Triethylphosphate	n.d.	n.d.	0.03 ± 0.00
Triisobutylphosphate	0.02	n.d.	0.03 ± 0.00
Tris (2-chloroethyl)phosphate	n.d.	n.d.	0.10 ± 0.01
Tris (2-chloroisopropyl)phosphate	0.02	n.d.	0.43 ± 0.02
Acenaphthene	n.d.	n.d.	0.13 ± 0.01
Naphthalene	0.04	n.d.	0.22 ± 0.02
Pyrene	n.d.	n.d.	0.48 ± 0.10
52 Cr	0.00	0.12	0.74 ± 0.16
55 Mn	0.00	0.00	4.10 ± 0.68
56 Fe	2.10	0.00	2.62 ± 1.33
60 Ni	0.00	0.00	8.27 ± 0.69
63 Cu	0.00	0.00	0.56 ± 0.06
66 Zn	11.20	4.71	43.98 ± 16.46
137 Ba	9.36	9.66	88.43 ± 10.41
208 Pb	0.00	0.08	0.72 ± 0.09

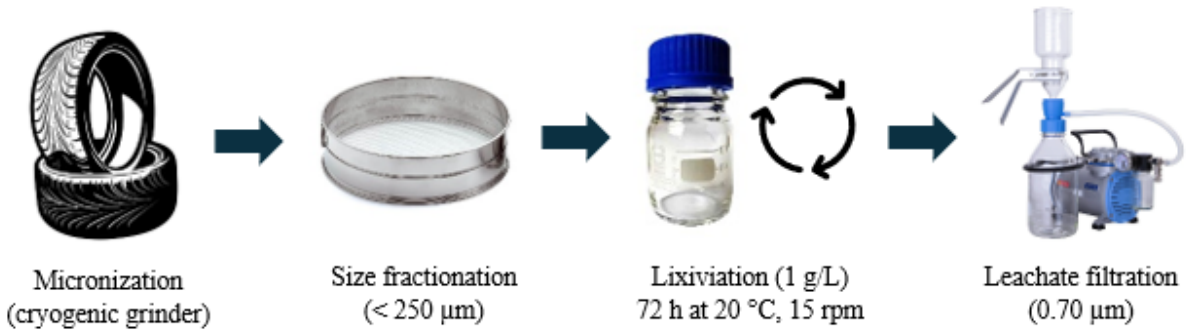


Figure 12: Lixiviation process

After lixiviation, microplastics were filtered with glass-fiber filters (Whatman GF/F filters 0.70 µm) on a vacuum filtration unit to obtain the final medium for the incubation (Figure 13). Following Almeda et al. (2023) recommendations, we used dilutions covering environmentally relevant predicted concentrations (Wik & Dave, 2009) and higher concentrations to enable precise estimation of toxic concentrations (EC_{50}) (Table 4).

The concentrations of the detected PAHs, flame retardants, and metals in the TWP leachates from the used materials can be found in Rist et al. (2023)

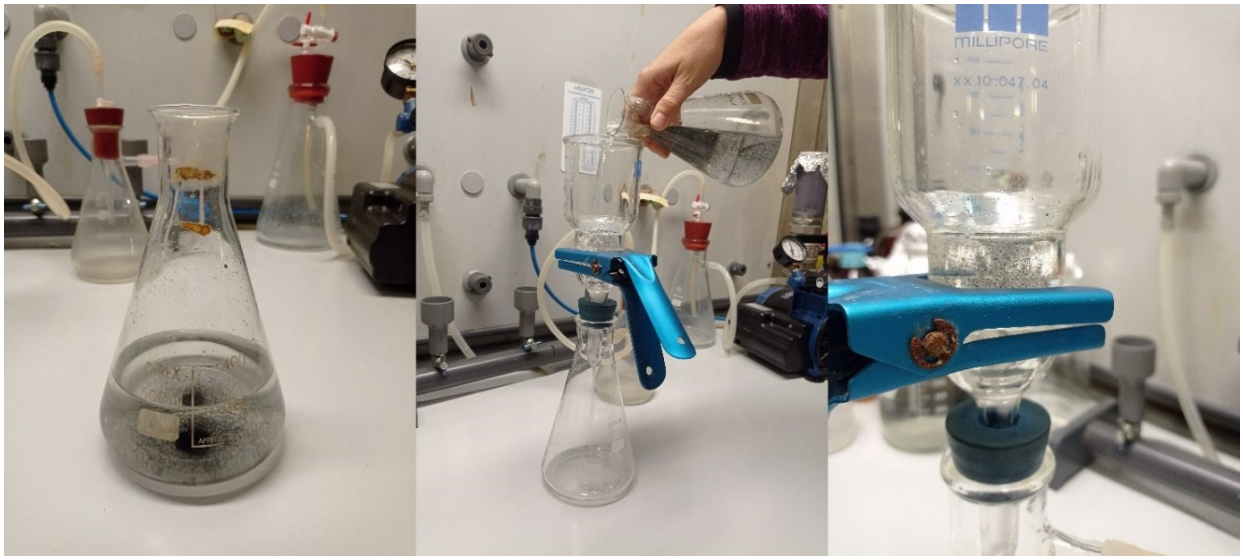


Figure 13: Filtration of TWP leachates using a vacuum filtration unit

3.2. Bioassay

Tests on *R. salina* and *A. lixula* were conducted in 96-well, flat-bottomed plates (capacity of 350 µl). Indeed, the effectiveness of these plates was to be tested to optimize future ecotoxicology tests (e.g Effect directed analyses, EDA) on microalgae and microzooplankton like sea urchin larvae.

Tests on *C. elongata*, *I. galbana* and *P. lividus* were conducted in 24-well, flat-bottomed plates (capacity of 3.4 ml) (Figure 14b).

We conducted serial dilutions with FSW to prepare the exposure solutions at various concentrations (Table 4; Figure 14a). Three replicates were used for each test concentration and control.

Table 4: Leachate testing conditions

Test organism	***Plastic material***	***Leachate plastic load***	***Dilutions tested***
R. salina	TWP	1 g/L	100%, 90%, 75%, 67%, 50%, 25%
C. elongata	TWP	1 g/L	100%, 75%, 50%, 25%
I. galbana			100%, 75%, 50%, 25%
A. lixula	TWP	1 g/L	100%, 75%, 50%, 25%, 10%, 5%
P. lividus			100%, 75%, 50%, 25%

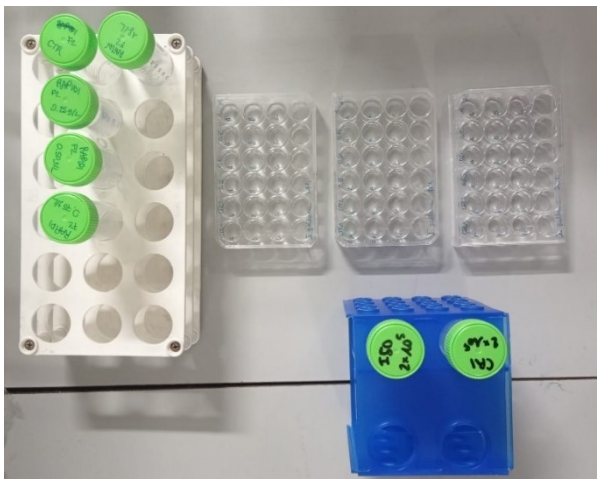


Figure 14a e b: Serial dilutions with F2 medium for tests with microalgae and test set up with C. elongata *e* I. galbana *in 24-wells microplates*

3.3. Experimental organisms

The experimental organisms, sources and measured variables for microalgae and sea urchin larvae are summarized in Table 5.

Table 5: Experimental setups

Test organism	***Sampling site/source***	***Exposure time (h)***	***Endpoint***
R. salina	EOMAR laboratory	24	Growth inhibition
C. elongata	CNR-IAS laboratory	24 - 48 - 72	Growth inhibition
I. galbana	CNR-IAS laboratory	24 - 48 - 72	Growth inhibition
A. lixula	Taliarte, Spain	48	Total body length
P. lividus	Genoa, Italy	48	Total body length Developmental anomalies percentage

For microalgae tests, growth was measured by counting cells with a Sedgewick-Rafter Counting Chamber (1 mL) for *R. salina* and a Burker Counting Chamber (20 µL) for *C. elongata* and *I. galbana*.

In the case of Sedgewick-Rafter Counting Chamber, three cells 1x1 µm for each replicate were counted and then mean was calculated. By multiplying the value by 1000, the concentration in cells/mL was obtained.

With the Burker Chamber, 12 cells were counted and the following formula applied to obtain final concentration: $C = \frac{(n*1736*12)}{6}$ [cells/mL]

C = concentration; n = number of cells

Rhodomonas salina

Cultures were grown in B1 medium and maintained at 20°C in aerated condition.

At the time of incubation cell concentration had to be maximum 20000 c/L to permit growth in wells. Concentration and size dimension were calculated with Beckman Coulter's Multisizer 4e before incubating.

Three replicates of initial culture were fixed with Lugol to know initial concentration C_0. The microplate was incubated on static at 20 °C with an irradiance of 2300 lux and a 16:8 h light:dark cycle.

After 24 hours of leachate exposure all the samples were fixated with Lugol solution to be counted with a Sedgewick-Rafter Counting Chamber as shown in Figure 15.

Since each treatment had a volume of 250 µl all the sample was added to the counting chamber. Three measurements per sample were conducted to determine the mean concentration of cells in each replicate for all the treatments.

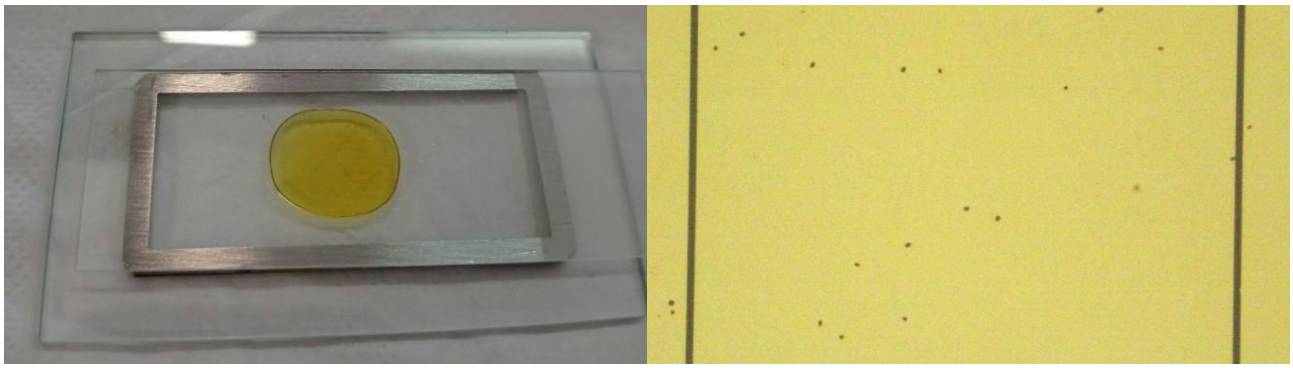


Figure 15: R. salina *in a Sedgewick-Rafter Counting Chamber*

Coccolithophyceae

Cultures (Figure 16) were grown in f/2 medium and maintained at 18 °C in aerated condition. About 2-4 days before the beginning of the test, an inoculum culture in the test medium was prepared as suggested by OECD (2011).

Before incubating, intermediate inoculum with concentration of 10^5 cell/ml was prepared.

Starting concentration was 20,000 c/ml and wells were filled with 1.8 mL of leachate at the right concentration and 0.2 mL of microalgae culture. Microplates were incubated on static at 20 °C with an irradiance of 10,000 lux and a 16:8 h light:dark cycle.

After 24, 48 and 72 hours of leachate exposure all the samples were fixated with Lugol to be counted with a Burker Counting Chamber.

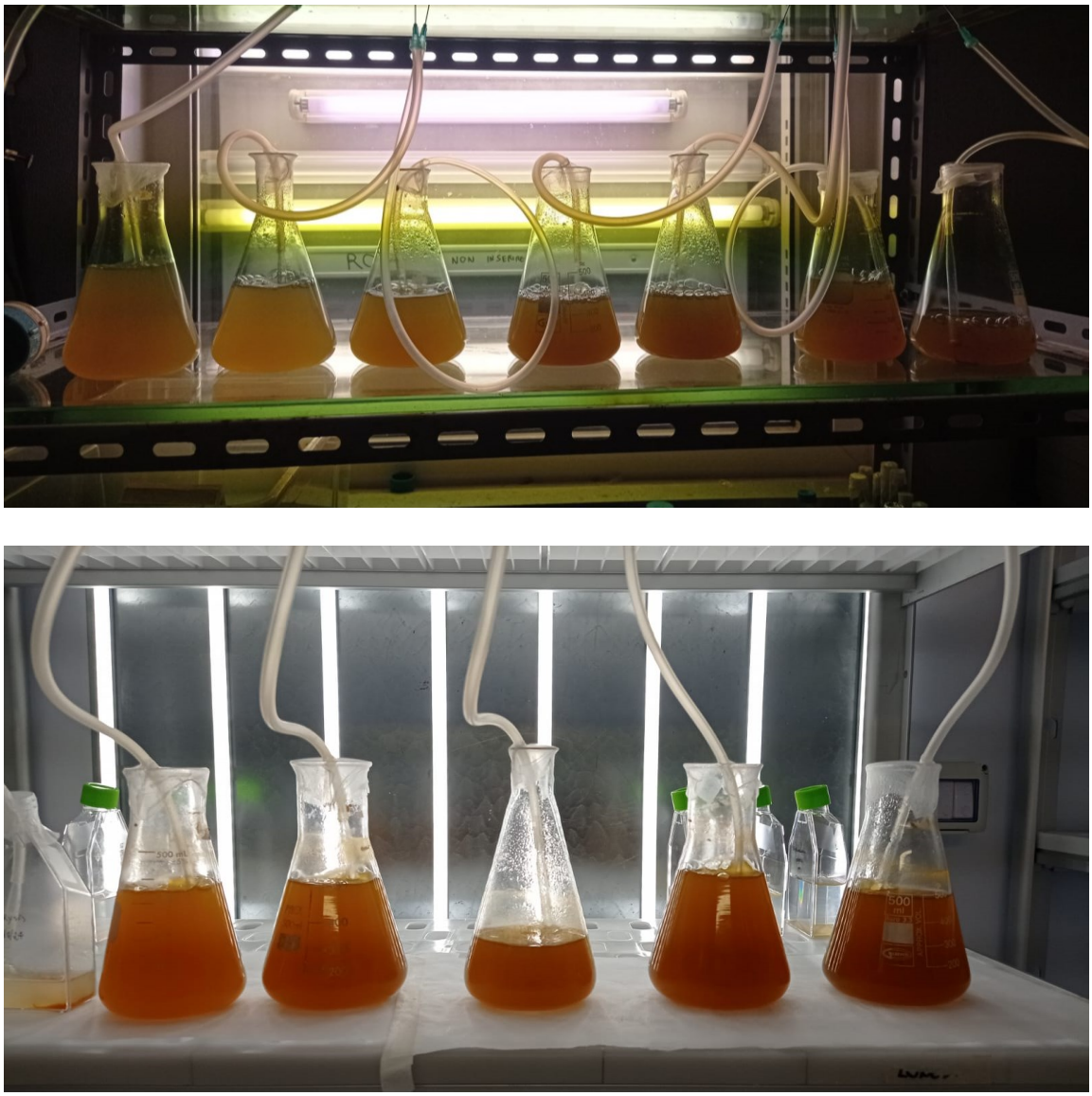


Figure 16: C. elongata *(above) and* I. galbana *(below) cultures.*

Arbacia lixula

Individuals of *A. lixula* were collected in Taliarte (Spain) on July 10th, 2024, and tests were conducted in EOMAR laboratory at the University of Las Palmas de Gran Canaria. Adults were maintained in two aquariums with sea water and air until fertilization was conducted the day after.

To induce spawning, 1 mL of 0.5 M KCl was injected through the peristomial membrane on the oral side. Oocytes and sperm were collected separately and transferred to glass beakers containing FSW (Figure 17). The beakers sperm were kept on a tray with ice until fertilization. Sperm quality (motility) and oocyte quality (uniformity of size and spherical shape) were confirmed with a stereomicroscope. For fertilization, oocytes from several females were mixed to create an oocyte suspension of approximately 2000 eggs/mL, and then diluted sperm (2-3 mL) from several males was added. After 15 minutes, fertilization success was verified by observing the fertilization envelope (> 90% of the examined oocytes; Figure 18). The fertilized oocyte suspension was filtered through a 20 µm mesh to remove sperm excess and then placed in a beaker with FSW. Finally, a subsample of the suspension was counted under the stereomicroscope to determine the embryo concentration before starting the experiment.

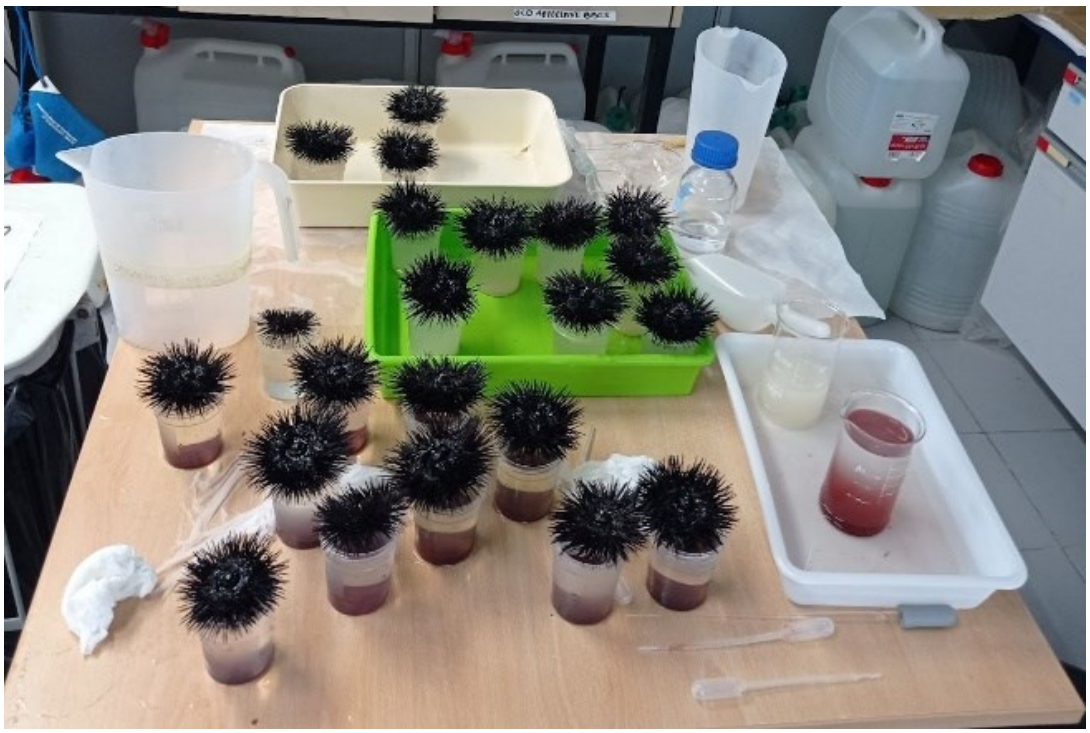


Figure 17: Collection of eggs and sperm in beakers with SW

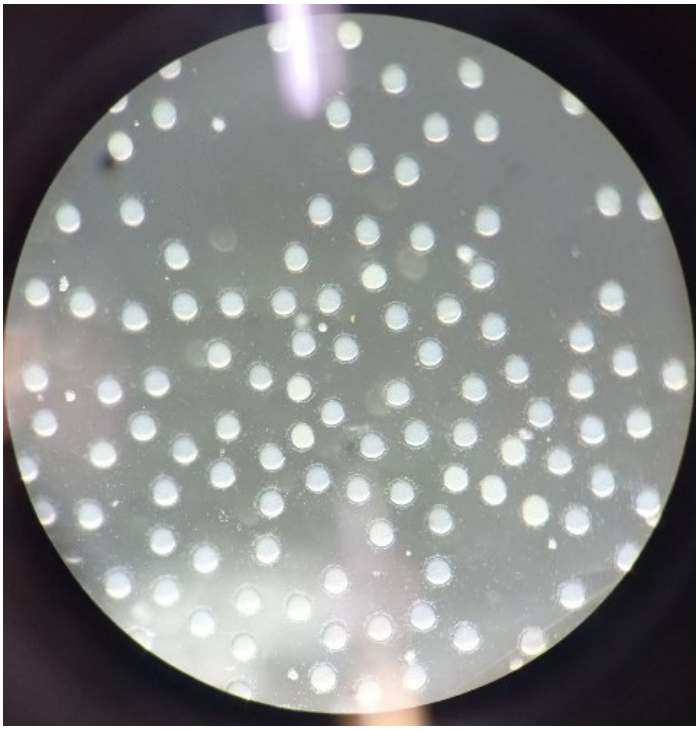
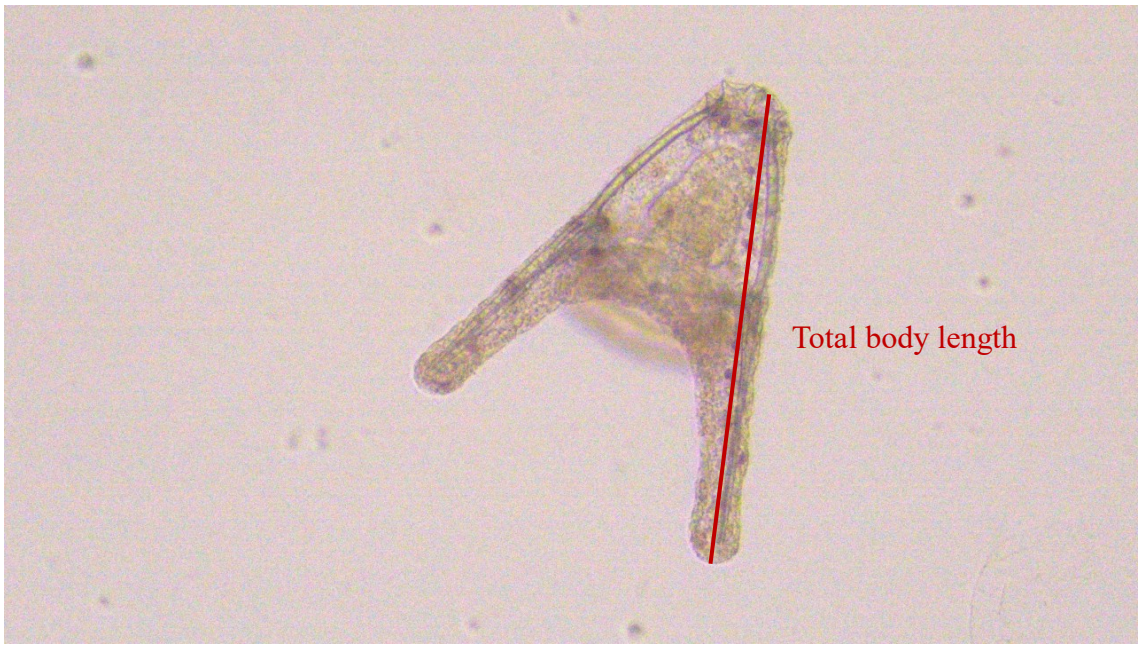


Figure 18: Fertilized eggs before test

Fertilized eggs were firstly diluted before putting 20 ± 5 eggs in each well containing undiluted and diluted leachates (100% / 75% / 50% / 25% / 10% / 5%). Three replicates were performed for each treatment, including negative control (SW).

After 48 hours of incubation in darkness at 18 °C, every sample with sea urchin larvae were fixated with 4% formalin (Fernández & Beiras, 2001; Laranjeiro et al., 2024). Photographs of samples were taken with a Leica inverted microscope (Figure 19), and finally larval development was investigated measuring the total body length (from the apex to the end of oral arms) with an Image J software.



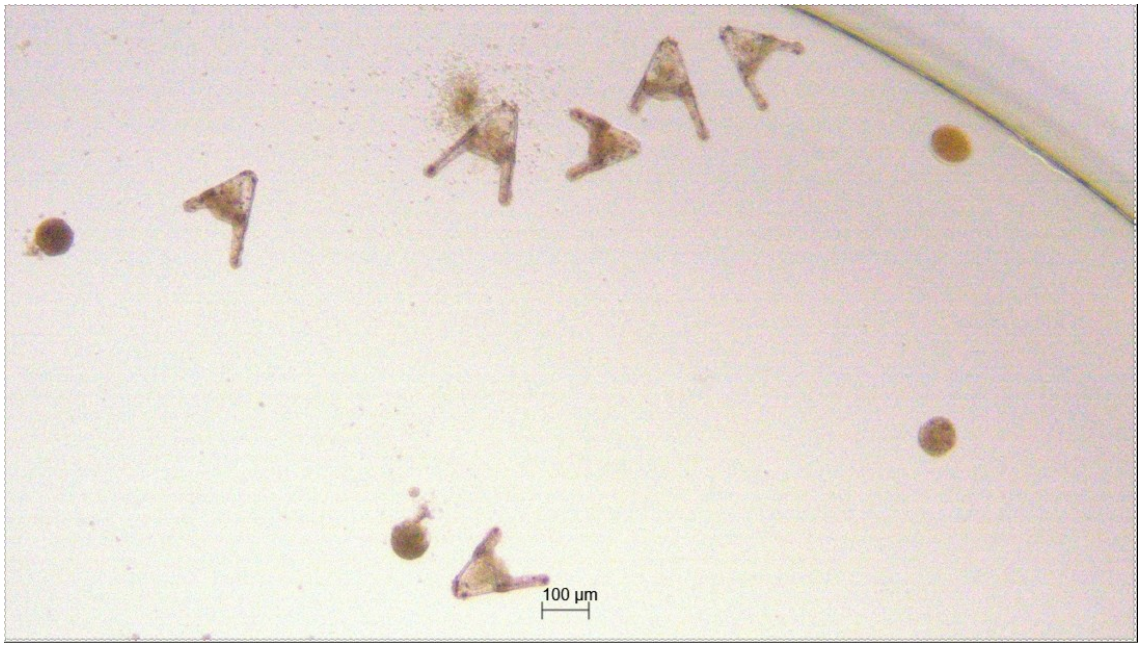


Figure 19: A. lixula *larvae after 48h of TWP exposure.*

Paracentrotus lividus

Adults of *P. lividus* were collected in the Ligurian Sea (Italy) and tests were conducted at CNR-IAS laboratories (Italy). To promote spawning, 0.5 M KCl was injected through the peristomial membrane on the oral side as previously reported for *A. lixula*. Sperm and eggs were collected separately in SW at 18 °C; eggs were diluted to a final concentration of 1000 eggs/mL. Fertilization was carried out by adding 10 L of pooled diluted sperm to egg suspension. After 15 minutes, four sub-samples were observed under a stereomicroscope to check fertilization success. About 1000 fertilized eggs/mL were added to each well containing undiluted (100%) and diluted leachates (75%, 50%, 25% and 0%). Three replicates were performed for each treatment, including negative control (SW).

Eggs were placed in darkness at 18 °C for 48 h to allow development (Fernández & Beiras, 2001; Laranjeiro et al., 2024). Then, larval development and developmental anomalies percentages were investigated. With this aim, larvae were fixed with 4% formalin and then photographs were taken with a Leica inverted microscope (Figure 20). Larval development was investigated by measuring the total body length with an Image J software; while observing images, it was possible to determine the developmental anomalies percentage.

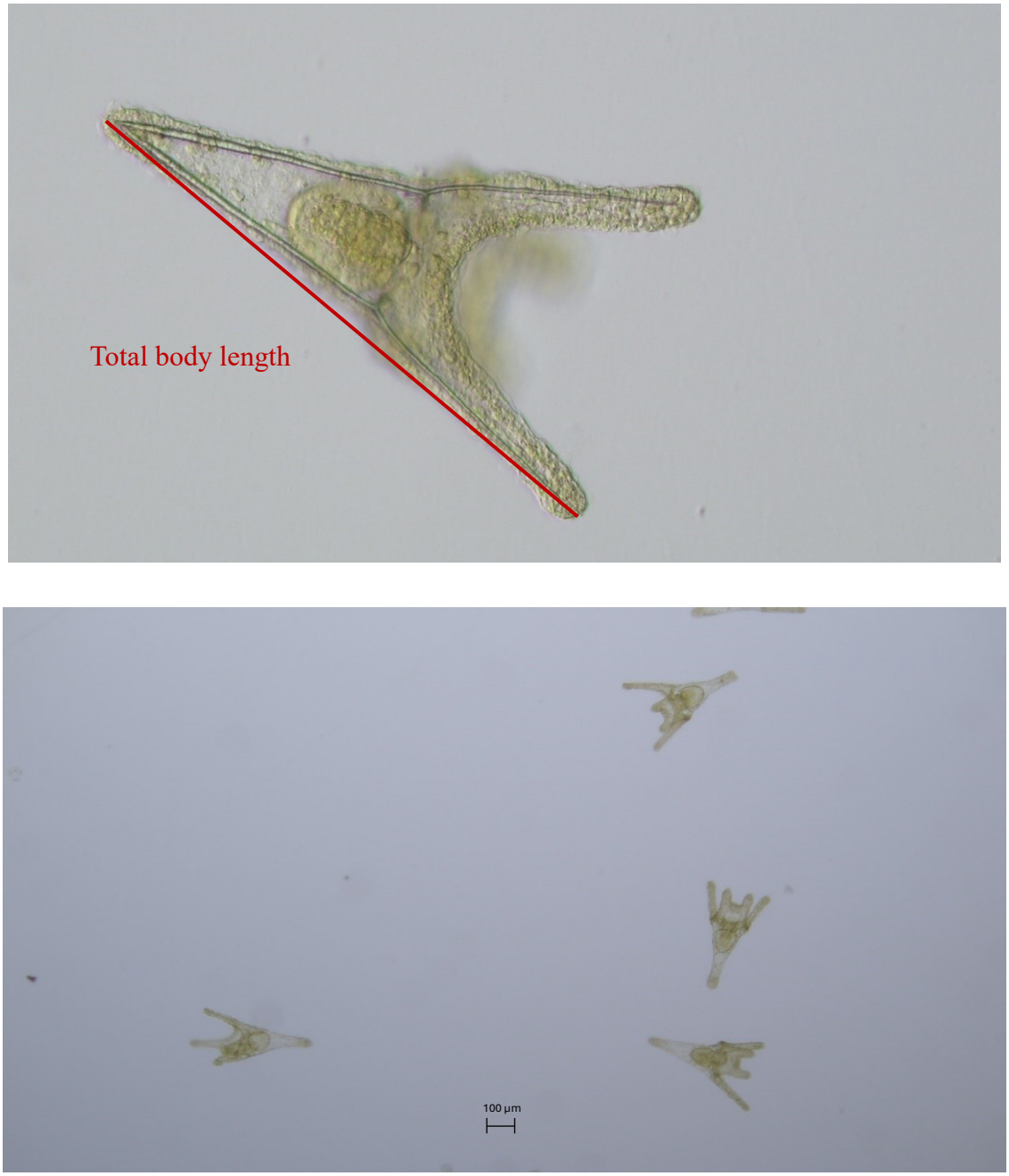


Figure 20: P. lividus *larvae after 48 h of TWP exposure. Magnitude of 20x (above) and 4x (down).*

3.4. Endpoints

Culture growth was used as endpoint for microalgae to assess any differences between controls and treated samples at different leachate concentrations. In particular, cell concentration was measured, and the growth inhibition rate (IR) was calculated according to the following formula:

$$IR\ (\%) = \left(1 - \frac{T}{C}\right) * 100\%$$

T and C were the cell concentrations of the experimental and control groups, respectively. IR > 0 indicates growth inhibition, and IR < 0 indicates growth promotion.

In sea urchins endpoints were Total Body Length of the larvae (TBL) and development anomalies at the pluteus larval stage following Gambardella et al. (2021). Specifically, for every TWP concentration, a minimum of 100 larvae were considered to count different types of anomalies based on images. For each concentration, larval anomalies were grouped according to anomalies frequency percentage. Once the frequency of each type of anomalies was determined to assess the degree of toxicity and the environmental impact of TWP, the Index of Contaminant Impact (ICI) was calculated by applying the following formula (Gambardella et al., 2021):

$$ICI = [0\ x\ \%\ level\ 0\ +\ 1\ x\ \%\ level\ 1\ +\ 2\ x\ \%\ level\ 2\ +\ 3\%\ level\ 3]/100$$

According to this formula, the ICI was calculated by considering sea urchin level of alteration, where:

- ✓ Level 0 was considered as normal development;
- ✓ Level 1 included light anomalies, easily reversible;
- ✓ Level 2 included moderate anomalies;
- ✓ Level 3 included severe anomalies, leading to arrested development or death.

The ICI was quantitatively evaluated by ranking the severity of anomalies in sea urchin larvae, as follows: 0 (no impact), 1 (slight impact), 2 (moderate impact), 3 (high impact). The ICI weighted the degree of developmental anomalies by the frequency (%) observed in all replicates from each concentration.

The TBL has been individuated as alternative endpoint for ecotoxicological test on sea urchin by Saco-Álvarez et al. (2010) to analyse the inhibition of development at larval stage.

3.5. Statistical Analysis

All statistical analyses were performed using the software R (version 4.3.1). Analysis of variance (ANOVA) was used to test differences between means of treatments in the size of larvae for *P. lividus* and *A. lixula* and in the microalgae growth inhibition. This analysis compares the means of the different factor level (TWP concentration) to verify whether the latter influences the response variable, that is the alteration of the endpoints. The test is based on two hypotheses: null hypothesis H_0 which assume that all the mean values of the levels of the factor are equal, so this has no effect on the response variable, and the alternative hypothesis H_1: all the mean values of the levels of the factor differ, therefore this has an effect on the response variable. The alternative hypothesis is accepted when the p-value < 0.05.

The assumptions of normality and homogeneity of variances were tested with the Shapiro-Wilk's and Levene's test, respectively. As a post hoc Dunnett's test was used. Data were considered significantly different when $p < 0.05$. The Lowest Observed Effect Concentrations (LOEC) were also determined.

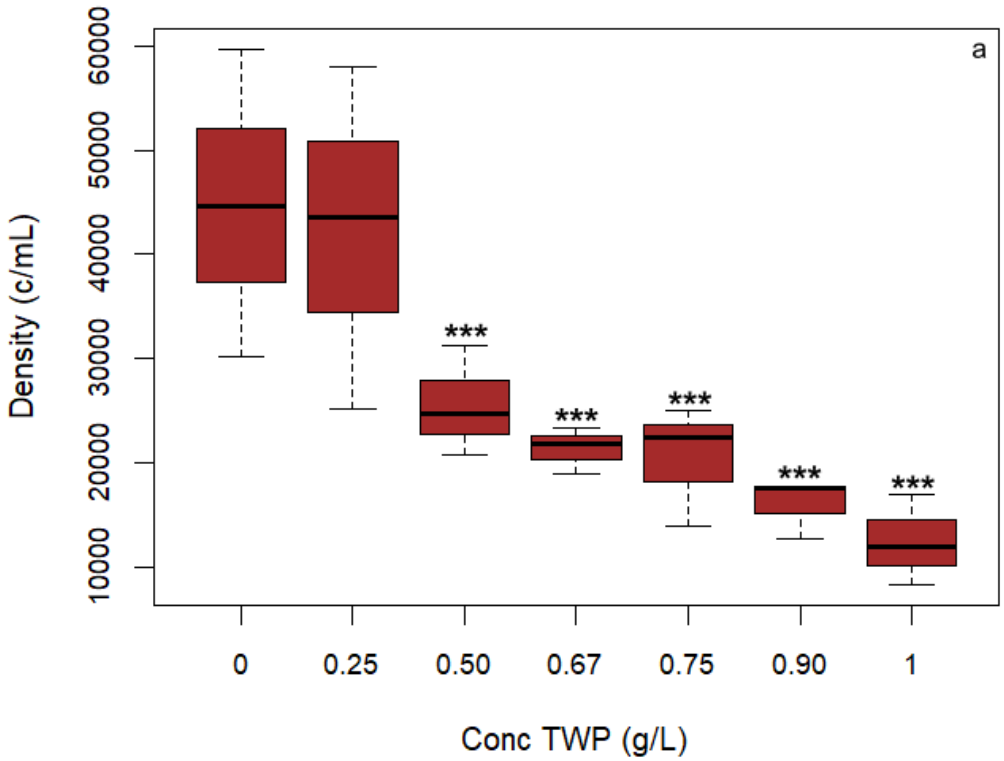
The median Effective Concentration (EC_{50}: median effective dilution of leachate resulting in 50% IR or length reduction for microalgae and sea urchins, respectively) was calculated only for *R. salina*. Indeed, for other species the maximum effect was inferior then 50%. The value and related 95% Confidence Limits (CL) were calculated using Trimmed Spearman–Karber analysis after 24 h exposure.

4. Results

4.1. Microalgae

4.1.1. Growth inhibition rate (24 h)

Figure 21 reports the density – measured as cells/ml - of the three species of microalgae exposed to TWP leachate for 24 hours. Overall, a decrease in algal density was observed for all species according to leachate increase, but significant differences were only found in *C. elongata* and *R. salina*, starting from 0.5 and 1 g/L, respectively.



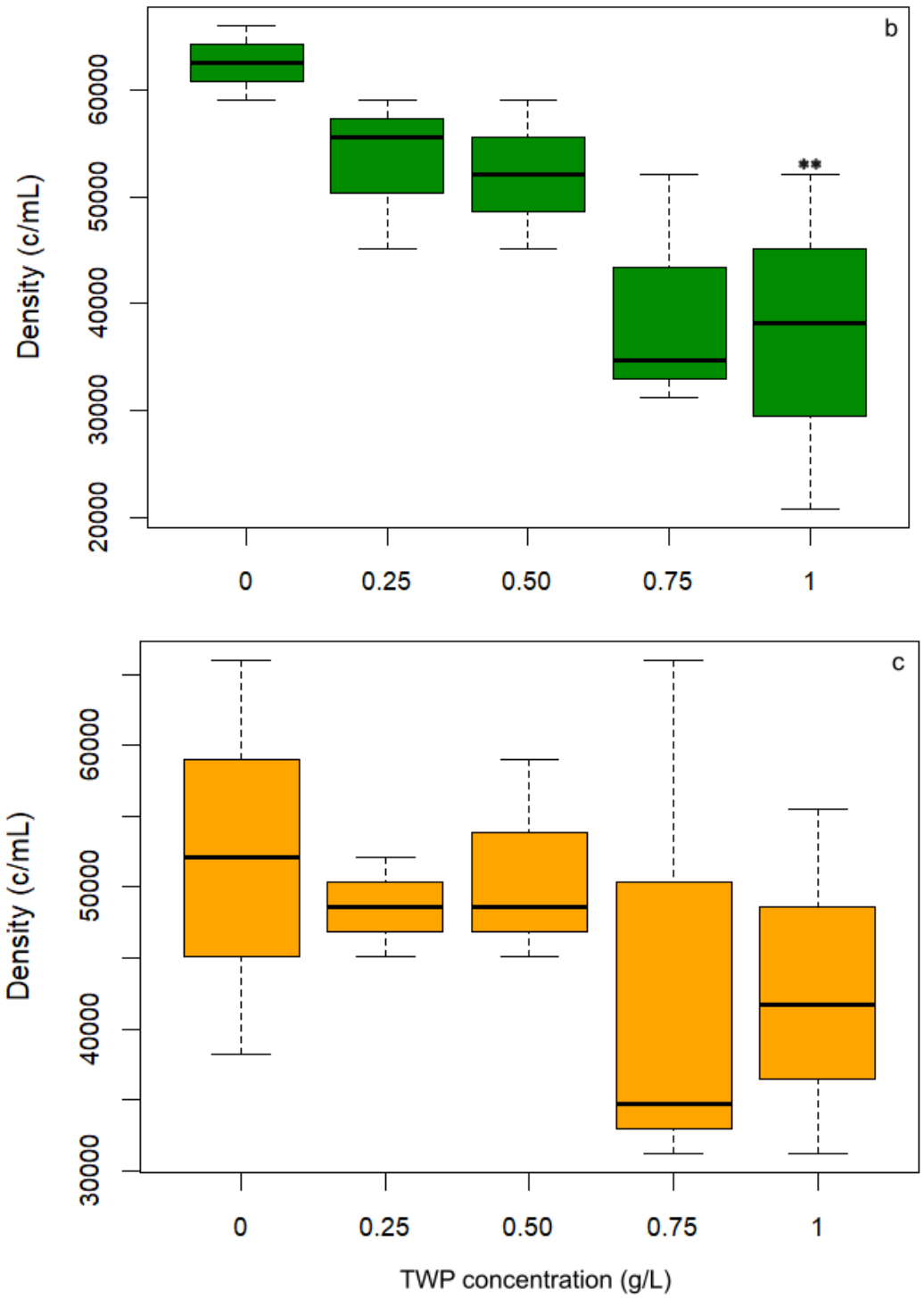


Figure 21: Boxplot of R. salina *(a),* C. elongata *(b) and* I. galbana *(c) representing density of cells at different leachate concentrations after 24 h exposure. ** = $p < 0.05$; *** = $p < 0.01$.*

The growth rate of the three microalgae species in the control after 24 hours was similar. Specifically, the rate was 2.24, 2.92, 2.78 d^{-1} for *R. salina*, *C. elongata* and *I. galbana,* respectively (Figure 22).

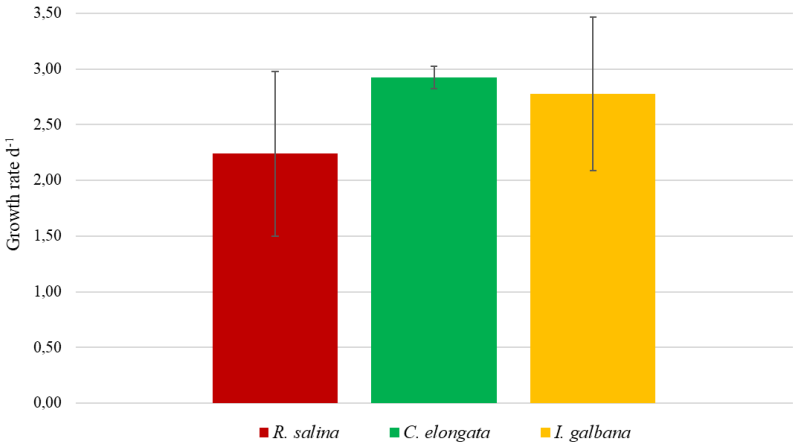


Figure 212: Growth rate of the three species of microalgae.

Conversely, the inhibition of the growth rate due to TWP leachate exposure differed among species. Thus, *R. salina* growth inhibition exponentially increased according to the leachate concentrations, showing a LOEC at 0.5g/L, corresponding to 50% leachates. The coccolithophyceae's growth inhibition showed a similar trend up to 0.5 g/L; after this threshold, *C. elongata* results to be more sensitive than *I. galbana* with increasing TWP concentration (Figure 23).

	Control	0.25	0.50	0.75	1
R. salina	0	6	43	54	72
C. elongata	0	12	15	36	40
I. galbana	0	11	16	17	18

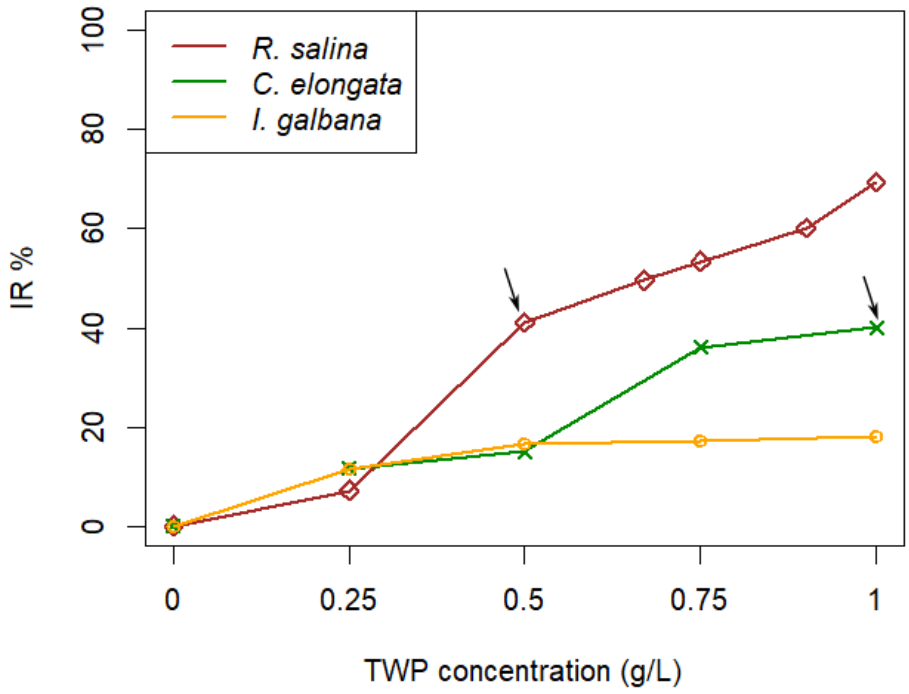


Figure 222: Percentage of growth inhibition rate of R. salina, C. elongata *and* I. galbana *after 24h exposure to different TWP concentrations. Values in the table are %. Data are mean of three replicates. Arrows represent LOEC with* $p < 0.05$ *and* $p < 0.01$ *for* C. elongata *and* R. salina, *respectively.*

Therefore, the sensitivity scale of microalgae after 24 h exposure to TWP was the following:

R. salina > *C. elongata* > *I. galbana*.

The cryptophyceae *R. salina* was more affected than coccolithophyceae not only in term of LOEC value, but also in term of EC_{50} (0.63 ± 0.7 g/L, corresponding to 63% leachate; Figure 24).

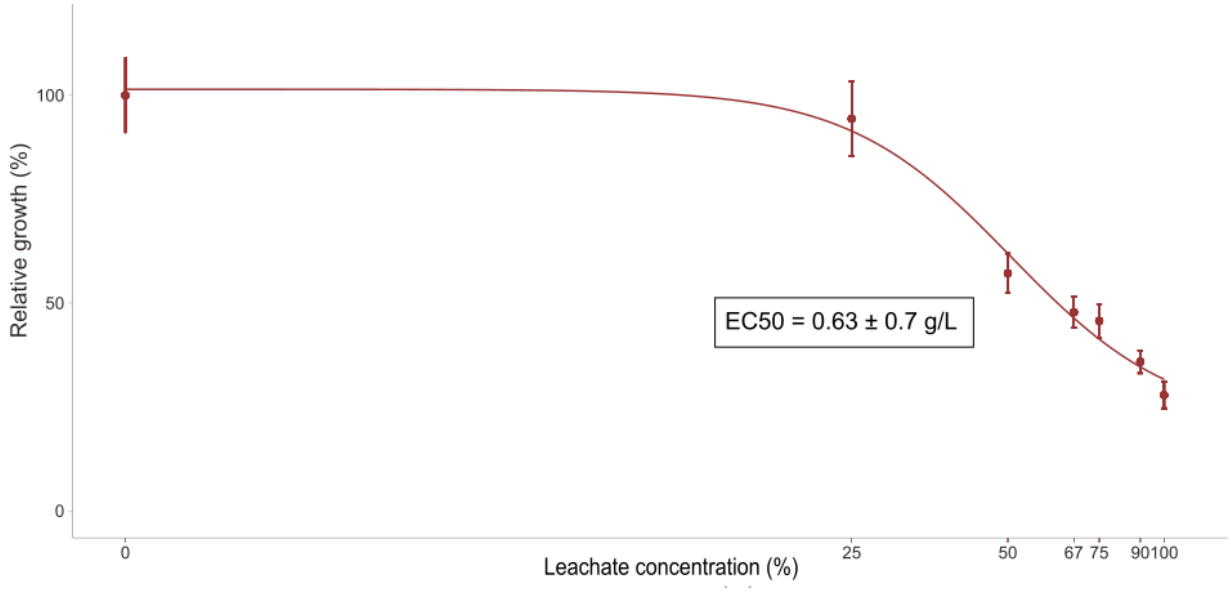
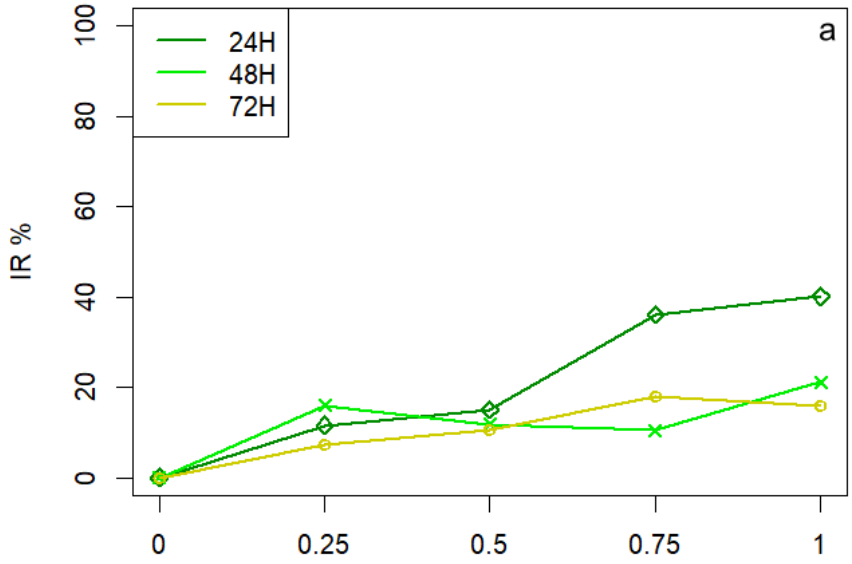


Figure 223: Dose-response curve for 24 h exposure of R. salina*. Relative growth refers to cells density compared to control in percentage.*

4.1.2. Growth inhibition rate (24 – 48 – 72 h)

Growth inhibition rate due to TWP was measured in *C. elongata* and *I. galbana* after 24, 48 and 72 h. The results (Figure 25) show a differences between the two species. Specifically, *C. elongata* was more sensitive after 24 h but not after longer exposures (48-72 h). *I. galbana* sensitivity increased along time, a time-dependent manner, showing the highest inhibition rate after 72 h (Figure 25).



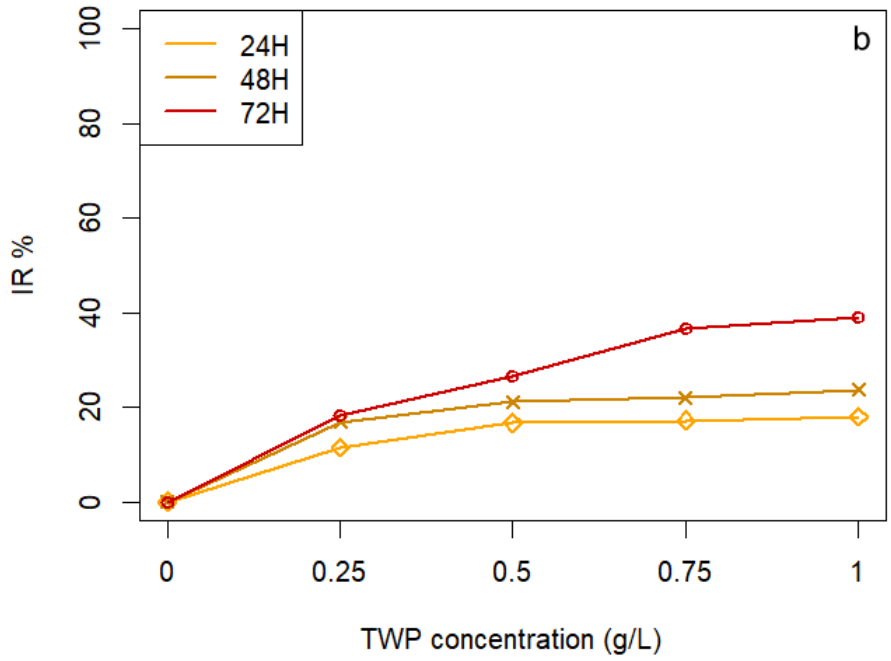


Figure 224: Growth Inhibition Rate after 24, 48 and 72 h exposure of C. elongata *(a) and* I. galbana *(b) at different TWP concentrations.*

4.2. Sea urchins

4.2.1. Total body length

The results on the body length measured in the two echinoderm species after 48 h exposure to TWP are reported in Figure 26; mean values with standard errors are shown in Table 6. The development of *P. lividus* showed longer larvae than *A. lixula*: the first species had maximum length (L_{max}) of 342.99 µm and a minimum (L_{min}) of 150.11µm while in the second one showed L_{max} = 159.33 µm and L_{min} = 76.75 µm.

Both species showed a decrease in body length according to increasing TWP concentration (Figure 26).

Table 6: Mean values and standard error (µm) of total body length of the larvae of P. lividus *and* A. lixula.

		Control	0.05	0.10	0.25	0.50	0.75	1
A. *lixula*	**Mean**	151.82	127.17	134.79	109.43	97.22	89.60	82.66
	Standard error	5.77	9.20	12.63	0.48	2.98	3.11	4.62
P. lividus	**Mean**	329.53	-	-	264.44	213.50	173.35	171.17
	Standard error	7.78	-	-	5.35	0.02	11.89	2.11

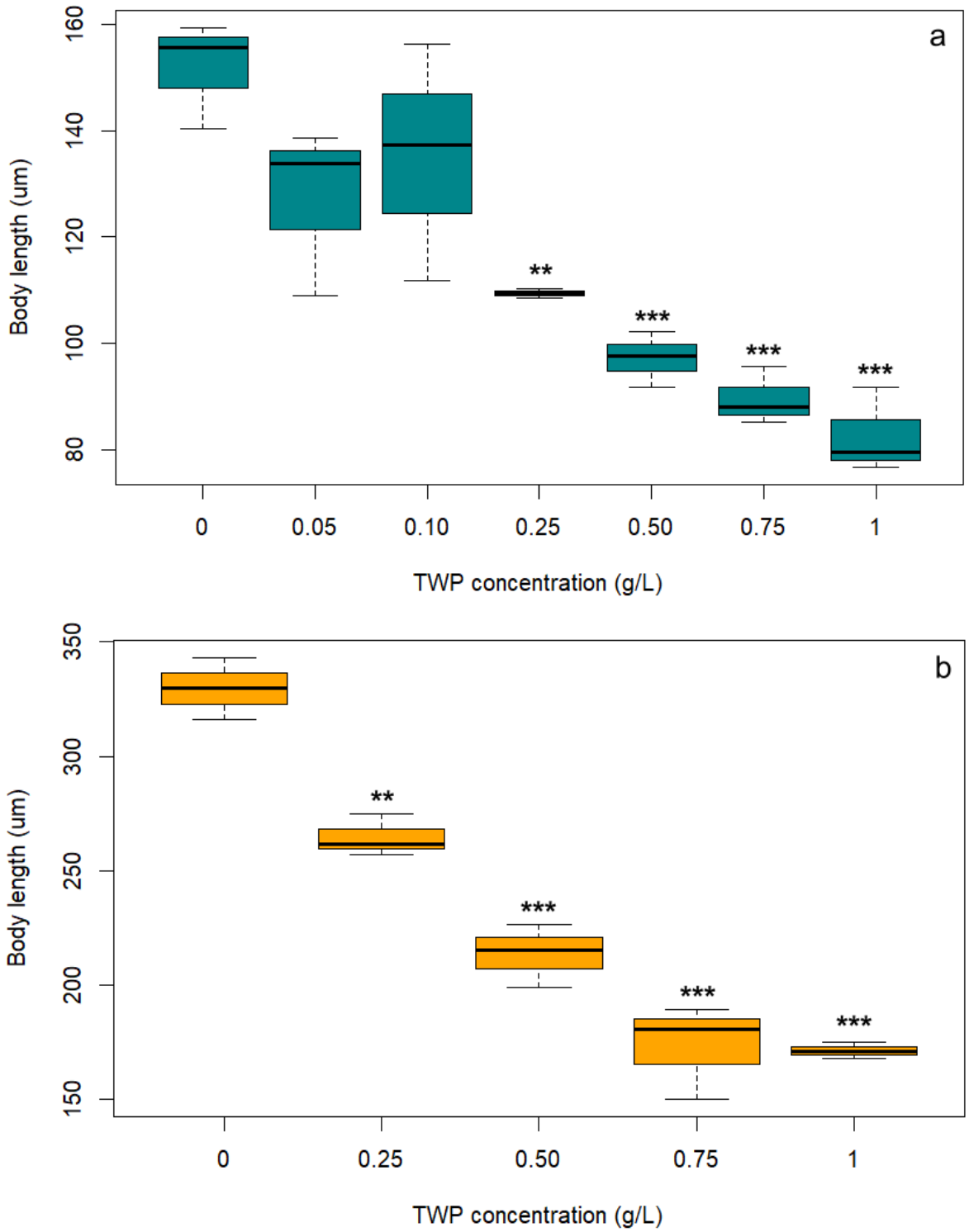
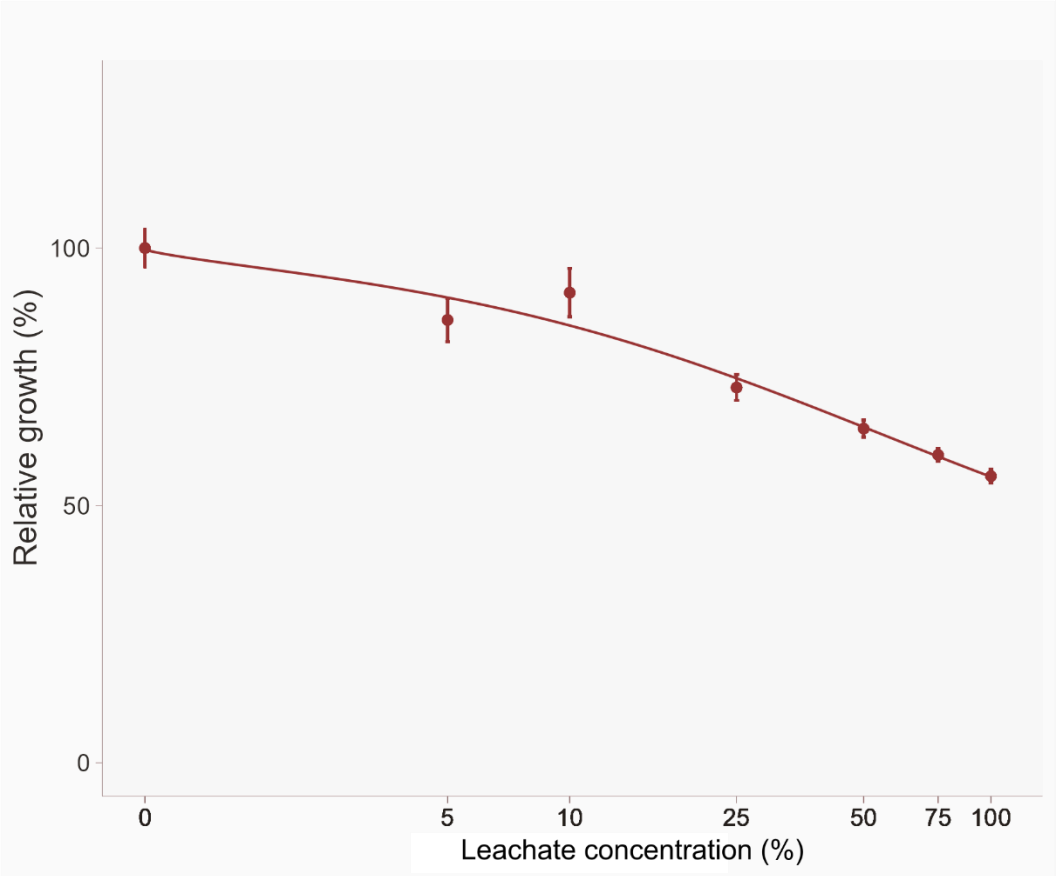


Figure 225: Boxplot of A. lixula *(a) and* P. lividus *(b) representing body length at different TWP concentrations after 48 h exposure. ** = $p < 0.05$; *** = $p < 0.01$.*

To assess the effects on both species due to TWP leachates and compare them, relative growth percentage was calculated (Figure 27). It showed a decrease in both species according to the increasing TWP leachate concentrations.



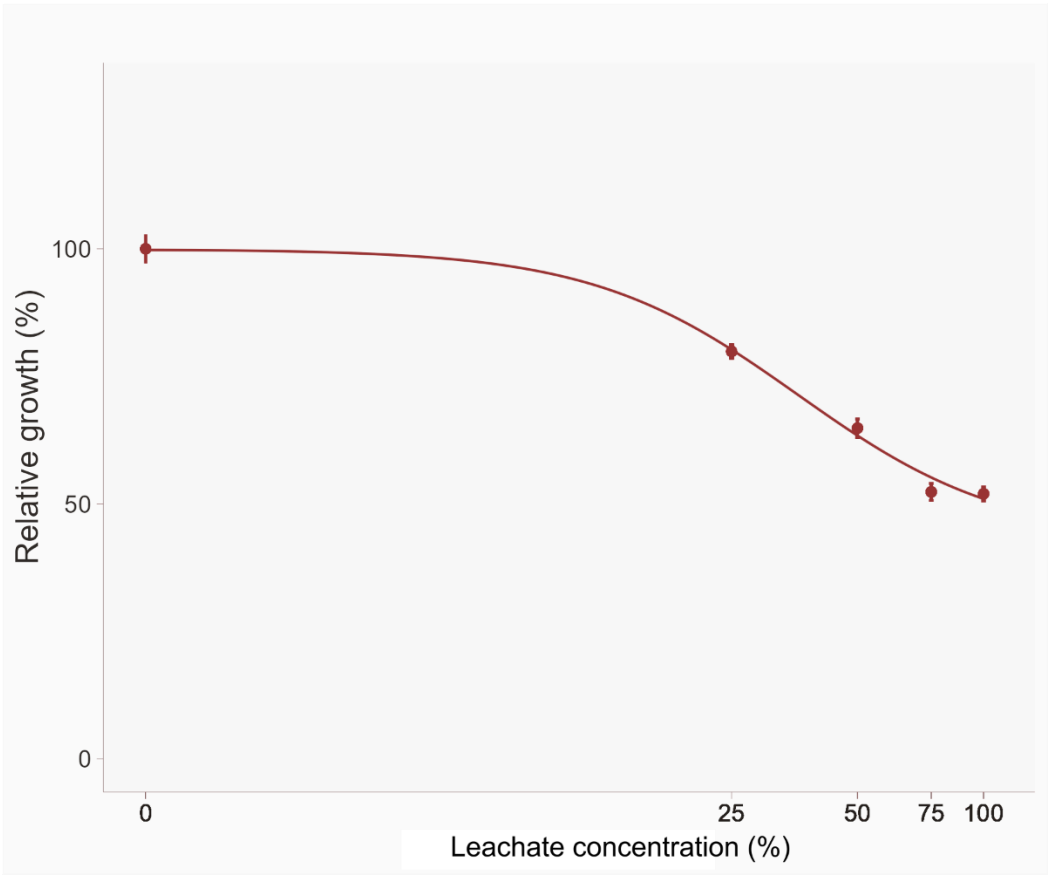


Figure 226: Dose-response curve for 48 h exposure of A. lixula *(above) and* P. lividus *(below) larvae. Relative growth referes to larval body length compared to control in percentage.*

Statistical analysis shows that sea urchins larval development was affected significantly by TWP concentration, resulting in a similar reduction of the total body length (TBL; Figure 28), characterized by a LOEC equals to 0.25 g/L.

	Control	0.05	0.10	0.25	0.50	0.75	1
A. lixula	0	15.89	10.78	27.72	35.63	40.66	45.16
P. lividus	0	-	-	19.61	35.07	47.30	48.03

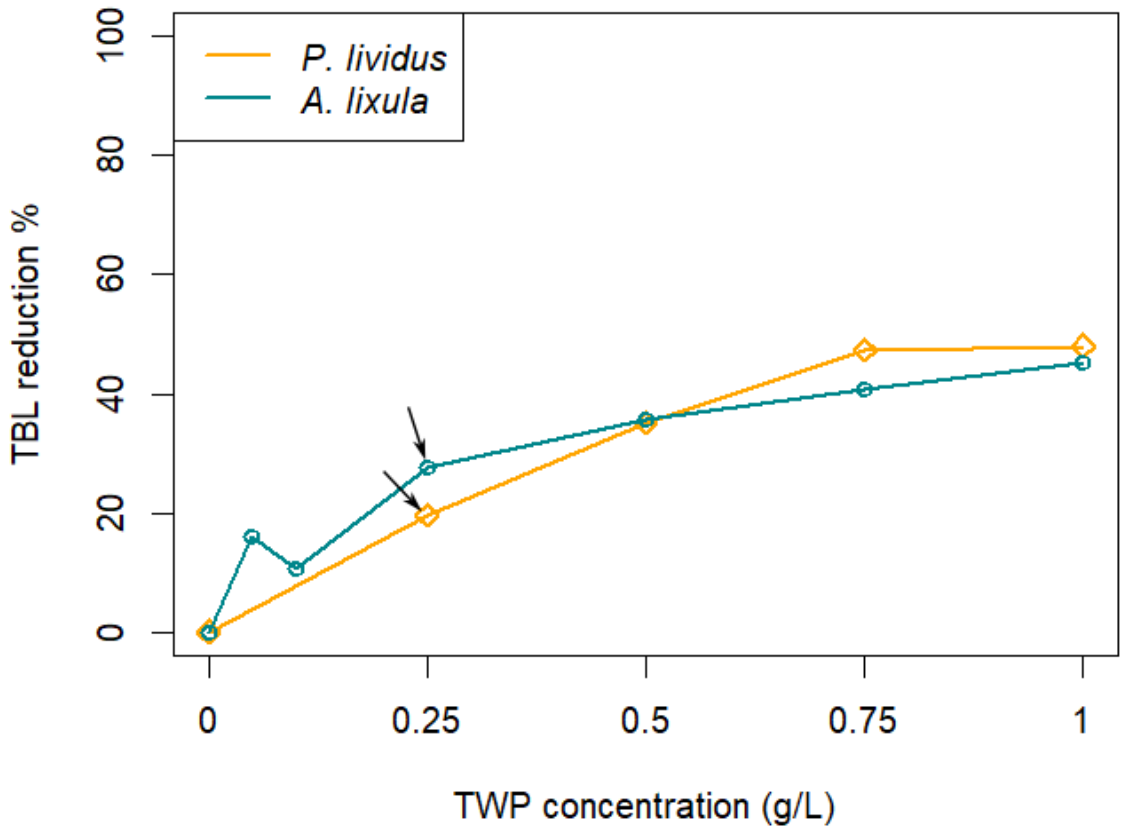


Figure 227: Percentage of TBL reduction of P. lividus *and* A. lixula *larvae after 48h exposure to different TWP concentrations. Values in the table are %. Data are mean of three replicates. Arrows represent LOEC with $p<0.05$.*

4.2.2. Development anomalies

Development anomalies were found in both sea urchins' species exposed to TWP. They were classified according to type described by Gambardella and colleagues (2021; Table 7) with a different severity level.

Table 7: Type of anomalies encountered in this test with severity level of alteration (Gambardella et al. 2021).

Type of anomalies	Anomalies	Level of alteration
5	Exposure during the cleavage and blastula stage may cause the loss of adhesion among blastomeres, followed by embryonic disaggregation.	3
6	Anomalous of PMCs, which are unable to enter the coelom cavity and are extruded, forming exogastrulae.	3
8	Altered aspects of PMCs migration, causing gastrulae lacking a coelom. The effect of exposure on PMC migration is also responsible for defective skeletogenesis at further stages (Type 9–12).	2
9	Small larvae, with swollen aspect, short and thin skeletal rods.	1
10	Short plutei, with little or no developed hind spines, so that the larva has a truncated appearance.	1
11	Larvae slightly smaller or equal to controls, with skeletal rods of the anterior arms fused, or with crossed tips. Sometimes both the aspects are present in the same larva.	1
12	Asymmetrical or bent larval body.	1
13	Presence of spines and supernumerary rods or entirely doubled skeleton.	2
14	Severe skeletal regression usually in the arms, or in both the parts of larval body.	3
17	Larvae with swollen and dilated intestine and anus.	2
18	Dead larvae or with lethal anomalies due to acute exposure to toxicants. These larvae may present a disrupted aspect, with vestigial skeleton and degenerating tissues.	3

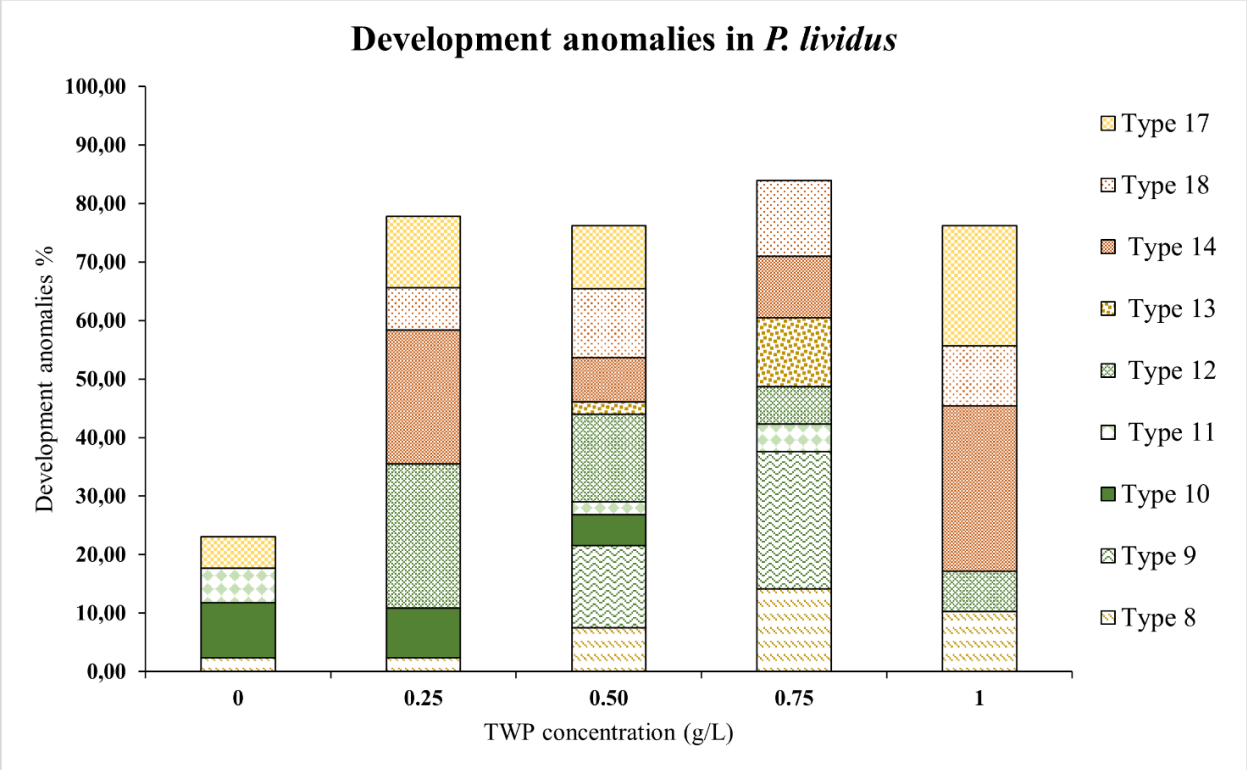
Regarding controls, a percentage of developmental anomalies ranging between 23.05% and 48.13% was found in *P. lividus* and *A. lixula*, respectively (Figure 29). However, it is worth noticing that the reference to evaluate anomaly types and to calculate ICI is based on *P. lividus* (Gambardella et al. 2021) and not on *A. lixula*.

Overall, the percentage of leachate anomalies differs from the control in each species (Figure 29). Thus, such percentages varied from 76.19% to 83.98% in *P. lividus* (Table 8a) and from 63.16% to 95.37% (Table 8b) in *A. lixula*. No dose-dependent effect due to TWP leachate was observed in any species.

Larvae of *P. lividus* were affected by 9 different developmental anomalies. Figure 29 shows that the anomalies with the highest severity (alteration level 3) were type 14 and 18 (Figure 30), responsible for skeletal regression in the arms/body and a disrupted aspect, with degenerating tissue respectively. Moreover, high percentages of anomaly Type 8, 13 and 17 - corresponding to delayed embryos, supernumerary rods and swollen and dilated intestine - were also observed in samples exposed to TWP.

According to the data, about 15% of type 1 anomalies were observed in the control while in the treated this percentage increased up to two times, reaching a maximum percentage of 36.44% at 0.5 g/L.

A. lixula larvae were affected with 7 anomaly types; the main anomalies only present in treated samples were type 5 (i.e. adhesion loss among blastomeres) and 6 (exogastrulae formation) shown in Figure 31, both with high severity level (alteration level 3), which involved early stage of development, before gastrula formation.



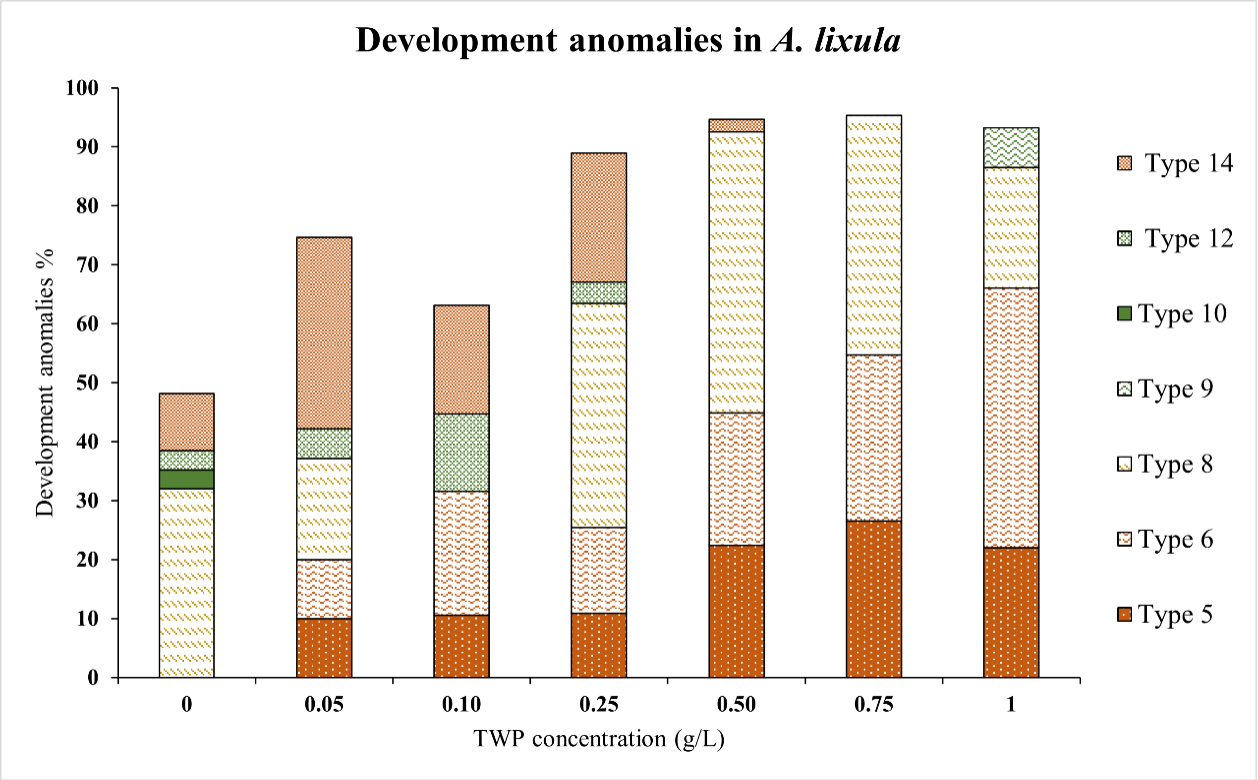


Figure 228: Development anomalies divided by type and severity level in P. lividus *(above) and* A. lixula *(below). Red tones = high severity; yellow tones = medium severity; green tones = low severity.*

Table 8a (above) and 8b (below): Percentage of different types of anomalies measured at different leachate concentrations.

P. lividus		**Leachate concentration (g/L)**					
		0	**0.25**	**0.50**	**0.75**	**1**	**Alteration level**
Anomalies type	Type 8	2,35	2,41	7,53	14,12	10,26	2
	Type 9	0	0	13,98	23,53	0	1
	Type 10	9,41	8,43	5,38	0	0	1
	Type 11	5,88	0	2,15	4,71	0	1
	Type 12	0	24,67	14,94	6,33	6,94	1
	Type 13	0	0	2,15	11,76	0	2
	Type 14	0	22,89	7,53	10,59	28,21	3
	Type 17	5,40	12,16	10,71	0	20,59	2
	Type 18	0	7,23	11,83	12,94	10,26	3
	Total	**23,05**	**77,79**	**76,19**	**83,98**	**76,25**	

A. lixula		Leachate concentration (g/L)							
		0	**0.05**	**0.10**	**0.25**	**0.50**	**0.75**	**1**	**Alteration level**
Anomalies type	Type 5	0	10,00	10,53	10,91	22,45	26,56	22,03	3
	Type 6	0	10,00	21,05	14,55	22,45	28,13	44,07	3
	Type 8	32,00	17,14	0	38,00	47,70	40,68	20,37	2
	Type 9	0	0	0	0	0	0	6,78	1
	Type 10	3,23	0	0	0	0	0	0	1
	Type 12	3,23	5,00	13,16	3,64	0	0	0	1
	Type 14	9,68	32,50	18,42	21,82	2,04	0	0	3
	Total	**48,13**	**74,64**	**63,16**	**88,91**	**94,64**	**95,37**	**93,25**	

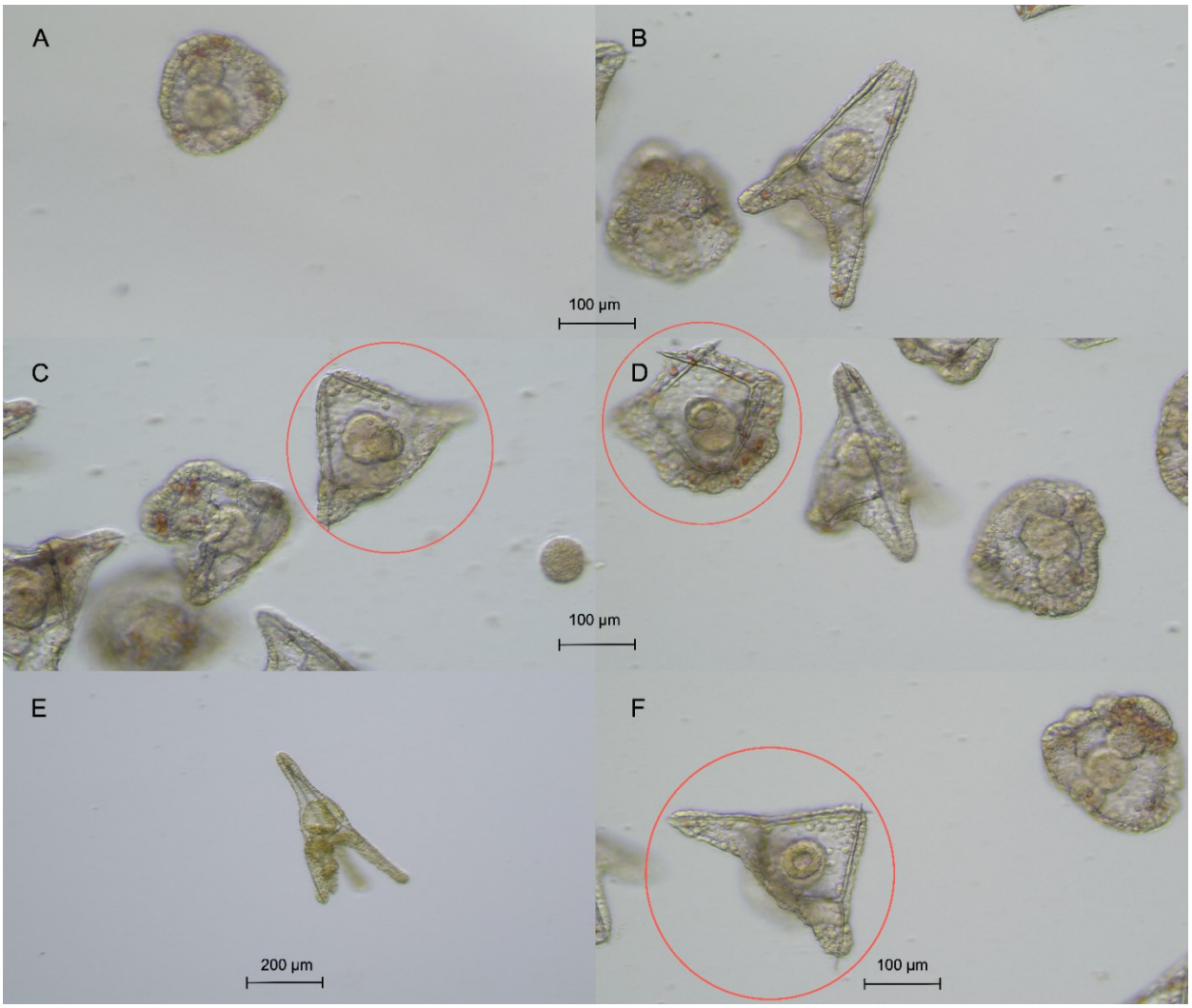


Figure 29: Main anomalies encountered during P. lividus *development. A: type 8; B: type 10 and 12; C: type 14; D: type 13; E: type 17; F: type 18.*

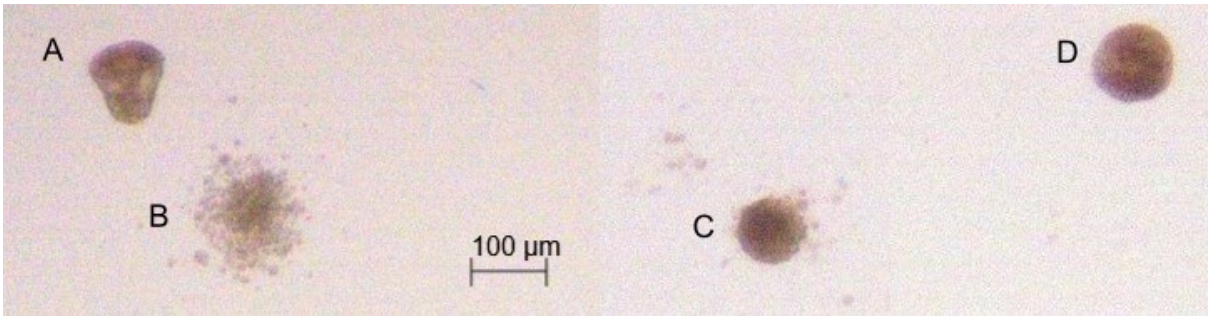


Figure 30: Main anomalies encountered during A. lixula *development. A: type 14; B: type 5; C: type 6; D: type 8.*

According to the ICI (Index of Contaminant Impact) values, *P. lividus* larvae exhibit a moderate level due to TWP concentrations from 0.25 g/L onwards, with only slight variations observed across ICI values ranging from 1.66 to 2.04 (Table 9). On the other hand, *A. lixula* exhibits a moderate level of impact from the lowest concentration, increasing to a high impact at concentrations higher than 0.50 g/L.

Table 9: ICI values with the corresponding impact levels (0 = no impact; 1 = slight impact; 2 = moderate impact; 3 = high impact). Values are rounded up after .50.

	P. lividus		***A. lixula***	
Treatment	**ICI**	**Impact Level**	**ICI**	**Impact Level**
0	0.34	0	0.81	1
0.05	-	-	2.20	2
0.10	-	-	1.88	2
0.25	1.66	2	2.36	2
0.50	1.46	1	2.52	3
0.75	1.68	2	2.59	3
1	2.04	2	2.65	3

5. DISCUSSION

The results reported in this thesis demonstrate that acute exposure to TWP leachate affects negatively microalgae growth and larval development of echinoderms. Our initial hypotheses were validated, as we found differences in sensitivity to this pollutant between plankton groups (phytoplankton vs larvae). Within a plankton group, these differences were more notable among phytoplankton species. By comparing our results with other studies, we also confirmed that plastic microplate-based assays can yield comparable results to traditional glass bottle incubations for this type of pollutants.

5.1. Microalgae growth inhibition

Microalgal species tested in this thesis showed a different sensitivity to TWP leachate exposure in terms of growth rates. Specifically, *R. salina* was more affected by TWP leachates if compared to the other two microalgae, either in terms of LOEC and EC_{50} (0.63±0.7) values. This result is in the same range as the one reported by Page and colleagues (2022) for a similar exposure time, finding a 0.39 g/L in *R. salina* exposed to TWP leachates. The toxicity value reported in the present thesis differs from that measured in the marine diatom *Skeletonema costatum* exposed to car tire rubber (Capolupo et al., 2020). They calculated an EC_{50} equal to 15.2 g/L (19% of 80 g/L, the highest concentration), a higher value in comparison with the one found for *R. salina*. Taken together these results demonstrate the higher sensitivity of Cryptophyte than diatoms. The major sensitivity of *R. salina,* compared to the other algae, could be due to its surface/volume ratio; actually, species with larger surface area could be more susceptible because of the major uptake through the cell wall (Weiner et al., 2004). In addition, Haptophytes such as *I. galbana* and *C. elongata* can evolve specific adaptive mechanisms to enhance their tolerance to stress conditions (Wang et al., 2024). This may explain the lower toxicity measured in these species than *R. salina*, belonging to Cryptophyte.

No ecotoxicity studies are available on TWP for *C. elongata* and only one for *I. galbana* (Li et al., 2024); however Haptophytes growth may vary among species after exposure to pollutants (metals; Faucher et al., 2017). According to these findings, we observed a different sensitivity in algal growth in the two microalgae belonging to Haptophytes. In particular, *C. elongata* was more sensitive than *I. galbana* but to date this is the only study comparing the two microalgae. It would be interesting to increase knowledge on these two species exposed to other contaminants and their leachates to verify a species-specific sensitivity. In addition, it would be worth investigating whether *I. galbana* shows time-dependent growth inhibition even with other contaminants, and if *C. elongata* short exposure (24 h) is the most suitable to detect an effect than long exposure (48-72 h; Figure 25). The latter is an important issue to suggest future studies based on different exposure times to investigate short and long-term effects of contaminants.

5.2. Effects on sea urchins' larvae

The length of both sea urchin species in not exposed samples (controls) after 48 hours from fertilization is consistent with previous literature on *P. lividus* (329.53 ± 7.78 and 218 μm; Saco-Álvarez et al. 2010) and *A. lixula* (151.82 ± 5.57 and 160 μm; Visconti et al., 2017).

The exposure of both sea urchin species to TWP significantly affected the development, since about 50% of the population showed a body length reduction at 1 g/L exposure (Figure 28).

These results differ from those found by Rist et al. (2023) who found an EC_{50} equal to 0.16 g/L and 0.35 g/L for *P. lividus* and *A. lixula,* respectively. This difference is likely due to the different time exposure (48 h versus 72 h); overall, the toxicity increases in a time-dependent way, which may explain the lower values found after a longer exposure period if compared to a short one.

We found a reduction in the body length of both sea urchin species exposed to TWP, according to Rist et al. (2023). Body length has been demonstrated to be a useful parameter to be investigated in marine invertebrates' larvae exposed to TWP. For instance, Moreira et al. (2024) reported a significant decrease (10–22%) in the body length of the nauplii and copepodites of *Acartia tonsa* after exposure to 0.25 and 0.5 g/L of TWP leachates, according to the LOEC values calculated for sea urchins.

The highest concentration of TWP (1 g/L) caused 76.25 % and 93.25 % of developmental anomalies in *P. lividus* and *A. lixula*, respectively. Similar percentages (70%, 100%) were reported in the same species exposed to the same TWP (Gambardella et al. 2024; Rist et al. 2023), confirming the replicability of the data. In addition, in the present study the anomalies were classified according to the criteria established for *P. lividus* (Gambardella et al. 2021). Thus, we found developmental anomalies causing high and moderate alteration levels in both species, such as anomaly type 5, 6, 8, 13, 14, 17 and 18. Moderate alterations, represented by type 8, 13 and 17, are mainly found after exposure of fertilized eggs to several contaminant categories, including neurotoxic pesticides, pharmaceuticals and personal care products (PPCPs) or sunscreens (Corinaldesi et al., 2017). Surfactants cause an altered PMC migration resulting in type 8 anomaly only at high concentration and nanoparticles causes type 13 anomaly through an enhancement of acetylcholine expression (Gambardella et al., 2013, 2021).

High developmental alterations can be caused by the same contaminants as moderate ones, but at higher concentrations or after exposure at different stages of development (Corinaldesi et al., 2017; Morale et al., 1998). Other contaminants causing anomaly types 5, 6, 14 and 18, which have a high alteration level, are typical of sea urchin exposure to persistent organic pollutants (POPs) like PAHs, endocrine disrupters like Bisphenol-A (BPA) that act as deregulator of calcium and MPs, including

their leachates (Hose et al., 1983; Oliviero et al., 2019; Roepke et al., 2005). Specifically, PAHs as acenaphthene, naphthalene and pyrene, were found also in TWP used for the experiment. Anomalies involving skeletal rods are mostly caused by altered calcium processes.

Overall, *A. lixula* initially shows higher impact values compared to *P. lividus* and exhibits a continuous and more pronounced increase (Figure 32). Specifically, developmental anomalies in this species allowed to estimate a moderate impact (ICI equals to 2; Table 9) already at the lowest tested TWP concentration (0.05 mg/L), while the same effect was estimated in *P. lividus* from 0.25 mg/L upwards. Generally the two species display similar sensitivity when exposed to other contaminants (Carballeira et al., 2012); however Buric et al. (2016) found a major sensitivity of *A. lixula* to silver nanoparticle of large size (> 60 nm) respect to *P. lividus*. Taking these results into account, we can assume that this difference may be due to the specificity of the anomaly classification (Gambardella et al., 2021), which is based on *P. lividus* but further research is needed to confirm this hypothesis.

The trend of ICI of *A. lixula* is similar to that of the body length (Figure 28 and 32) representing a potential new promising criterion for the detection of contaminants or chemical mixtures (Carballeira et al., 2012).

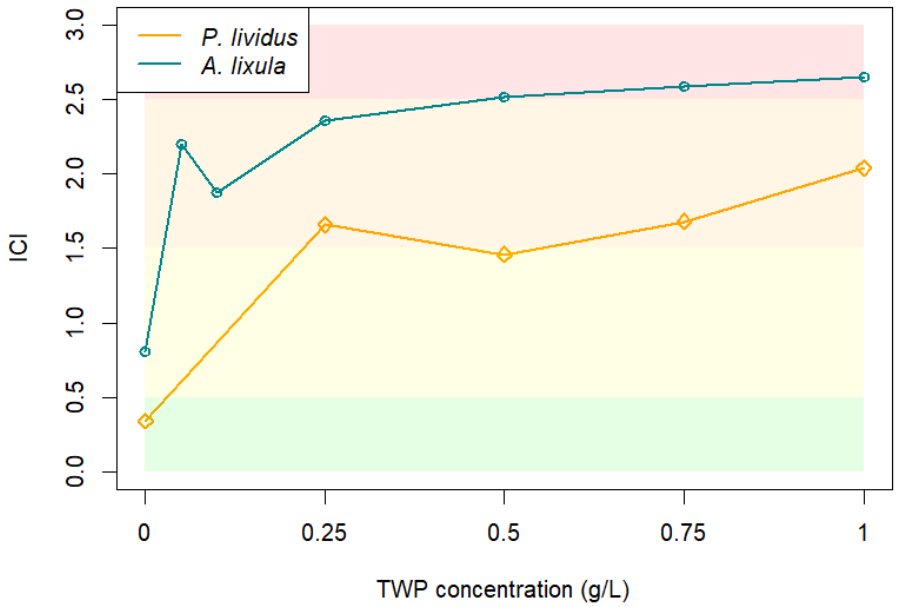


Figure 31: Index of Contaminant Impact trend for P. lividus *and* A. lixula. *Green = no impact; Yellow = slight impact; Orange = moderate impact; Red = high impact*

The solid to liquid ratio (S:L) influences the toxicity of plastic leachates, with toxicity to zooplankton increasing as the S:L ratio rises. However, the increase in toxicity was not linear with the increase in leachate concentration. A 4-fold increase in the TWP concentration (from 0.25 to 1 g/L) produced a 2.5-fold and a 1.6-fold increase in length reduction for *P. lividus* and *A. lixula*, respectively (Table 10).

5.3. General considerations

Plastic additives could result toxic when assimilated by the biota (Nobre et al., 2015) and usually the adverse effects are directly related to them and not to the polymer (Oliviero et al. 2019) . Chemical analyses of the material used for this study (Table 3) revealed elevated concentration of barium and zinc (88.43 and 43.98 µg/L, respectively). High zinc e concentration is in accordance with other studies that found zinc as the most prominent metal in TWP leachates, since it is a common additive serving as a vulcanization activator (Capolupo et al., 2020; Halsband et al., 2020). Specifically, high zinc concentrations in TWP have been shown to be directly correlate with toxicity towards aquatic planktonic organisms such as *I. galbana* (Yap et al., 2004), the freshwater microalgae *Pseudokirchneriella subcapitata* (Gualtieri et al., 2005) and the copepod *Tigriopus japonicus* (Yang et al., 2022). In addition, high zinc content can lead to development anomalies due to a disruption of calcium homeostasis explaining the presence of skeletal abnormalities in the Mediterranean sea urchin (Carballeira et al., 2012; Tellis et al., 2014), according to the findings showed in the present thesis.

As the TWP leachates are a complex mixture of chemicals, potentially unknown effect mechanisms can be involved in growth inhibition for microalgae or anomalous development of sea urchins larvae. Indeed, the leaching of plastic additives into the environment can create co-exposure scenarios in which organisms are simultaneously exposed to multiple chemicals (Ding et al., 2017; Nguyen et al., 2022). As a next step, the use of a method coupling chemical fractionation associated with bio-tests, such as Effect-Directed Analysis, to identify chemicals that individually or associated with others are responsible for the observed toxicity is suggested (Hong et al., 2023).

Overall, there are still many uncertainties regarding how compounds may leach from TWP (Halsband et al., 2020), especially considering variables such as initial concentration, particle size, incubation time, temperature, and salinity. The application of standard protocols for leachate extraction from TWP would allow for more reliable comparisons of effect concentrations between studies (Almeda et al., 2023).

5.4. Ecological consequences

Phytoplankton are primary producers and their absence or decrease can cause cascading effects on upper levels, starting with meroplanktonic larvae like sea urchins' pluteus. An interesting effect observed by Kaposi et al. (2014) is the increase in microplastic ingestion by the sea urchin *Tripneustes gratilla* larvae due to the absence of a food source such as microalgae.

Due to their role as grazers in benthic coastal rocky ecosystems, the two species of sea urchins studied in the present thesis are important. Negative impacts on the development of their larvae can modify the structure and the dynamics of their habitat directly influencing organisms on upper positions in the food web. Both species, *P. lividus* and *A. lixula*, were affected even at low concentration (0.25 g/L). A reduction of these species could result in fewer barren patches within algal forests, decreasing heterogeneity and the habitat for associated encrusting and cryptic organisms (Agnetta et al., 2013). No significant differences in larval length reduction percentage were found between sea urchin species. This can be connected to no relevant differences in egg size but only in colour, differently to other sea urchin species like *D. africanum*, which is probably more sensitive due to the lower energy content in eggs, as demonstrated by the smaller size (Rist et al., 2023).

Tian et al. (2021) have shown that leached plastic additives from TWP can have ecological impacts at environmental concentrations, specifically caused by quinone transformation product 6PPD. When TWP concentrations are measured in open waters, there is a considerable lack of data (Mennekes & Nowack, 2022). Moreover, there is still a lack of data concerning both the concentration and composition of TWP leachates, and the identification of compounds responsible for toxicity towards marine planktonic species. Therefore, more environmental chemical data is needed to evaluate the potential ecological impacts of TWP on plankton food webs.

6. CONCLUSIONS

Acute exposure to TWP leachates affects negatively both microalgal growth and echinoderm larval development, suggesting that tire particle pollution can have a harmful impact on the base of the marine food web and coastal benthic recruitment.

The sensitivity towards the leachates differed among microalgae species but not significatively among sea urchins' species. Overall, *R. salina* was the most sensitive species with EC_{50} = 0.63 g/L and both sea urchin species registered a percentage near to 50% reduction at the highest concentration. Considering the impact level determined by the encountered anomalies, it is crucial not to exceed TWP concentration levels of 0.05 for *A. lixula* – already representing an environmentally relevant concentration - and 0.25 for *P. lividus*.

The use of planktonic organisms, including phytoplankton and meroplanktonic larvae, to evaluate the chemical toxicity of microplastics is important to help in the understanding of the impacts on critical biological processes such as photosynthesis and larval development, which could change the structure of populations and communities. Furthermore, these findings raise concerns about the long-term

ecological impacts of TWP accumulation in marine environments, especially considering the increasing volume of tyre wear entering coastal ecosystems worldwide.

Regarding methodology, the use of microplate with sea urchins' larvae has been successfully tested, and it could be further employed in future investigations, such as Effect-directed analysis (EDA).

Overall, this study emphasizes the potential risk of TWP pollution to key marine organisms. With rising vehicle usage and population growth, implementing effective emission reduction strategies and developing ecologically safer tire additives are crucial for mitigating the aquatic toxicity of TWP pollution and safeguarding marine biodiversity and ecosystem services.

7. REFERENCES

A typology of microplastics released from textiles and tyres. (2021, October 20). OECD. https://www.oecd.org/en/publications/policies-to-reduce-microplastics-pollution-in-water_7ec7e5ef-en/full-report/component-6.html

Agnetta, D., Bonaviri, C., Badalamenti, F., Scianna, C., Vizzini, S., & Gianguzza, P. (2013). Functional traits of two co-occurring sea urchins across a barren/forest patch system. Journal of Sea Research, 76, 170–177. https://doi.org/10.1016/j.seares.2012.08.009

Almeda, R., Gunaalan, K., Alonso-López, O., Vilas, A., Clérandeau, C., Loisel, T., Nielsen, T. G., Cachot, J., & Beiras, R. (2023). A protocol for lixiviation of micronized plastics for aquatic toxicity testing. Chemosphere, 333, 138894. https://doi.org/10.1016/j.chemosphere.2023.138894

Ayata, S.-D., Stolba, R., Comtet, T., & Thiébaut, É. (2011). Meroplankton distribution and its relationship to coastal mesoscale hydrological structure in the northern Bay of Biscay (NE Atlantic). Journal of Plankton Research, 33(8), 1193–1211. https://doi.org/10.1093/plankt/fbr030

Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. (2020). Tyre and road wear particles (TRWP)—A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. Science of The Total Environment, 733, 137823. https://doi.org/10.1016/j.scitotenv.2020.137823

Balestrieri, A., Winkler, A., Scribano, G., Gazzola, A., Lastrico, G., Grioni, A., Pellitteri-Rosa, D., & Tremolada, P. (2022). Differential effects of microplastic exposure on anuran tadpoles: A still underrated threat to amphibian conservation? Environmental Pollution, 303, 119137. https://doi.org/10.1016/j.envpol.2022.119137

Barbier, E. B. (2017). Marine ecosystem services. Current Biology, 27(11), R507–R510. https://doi.org/10.1016/j.cub.2017.03.020

Beiras, R., Tato, T., & López-Ibáñez, S. (2019). A 2-Tier standard method to test the toxicity of microplastics in marine water using Paracentrotus lividus and Acartia clausi larvae. Environmental Toxicology and Chemistry, 38(3), 630–637. https://doi.org/10.1002/etc.4326

Bejgarn, S., MacLeod, M., Bogdal, C., & Breitholtz, M. (2015). Toxicity of leachate from weathering plastics: An exploratory screening study with Nitocra spinipes. Chemosphere, 132, 114–119. https://doi.org/10.1016/j.chemosphere.2015.03.010

Bielmyer, G. K., Brix, K. V., Capo, T. R., & Grosell, M. (2005). The effects of metals on embryo-larval and adult life stages of the sea urchin, Diadema antillarum. Aquatic Toxicology, 74(3), 254–263. https://doi.org/10.1016/j.aquatox.2005.05.016

Birkeland, C. (1989). The influence of echinoderms on coral-reef communities. Echinoderm Studies, 3, 1–79.

Bonsignore, M., Salvagio Manta, D., Mirto, S., Quinci, E. M., Ape, F., Montalto, V., Gristina, M., Traina, A., & Sprovieri, M. (2018). Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. Ecotoxicology and Environmental Safety, 162, 554–562. https://doi.org/10.1016/j.ecoenv.2018.07.044

Boucher, J., & Friot, D. (2017). Primary microplastics in the oceans: A global evaluation of sources. IUCN International Union for Conservation of Nature. https://doi.org/10.2305/IUCN.CH.2017.01.en

Brierley, A. S. (2017). Plankton. Current Biology, 27(11), R478–R483. https://doi.org/10.1016/j.cub.2017.02.045

Calle, L., Le Du-Carrée, J., Martínez, I., Sarih, S., Montero, D., Gómez, M., & Almeda, R. (2025). Toxicity of tire rubber-derived pollutants 6PPD-quinone and 4-tert-octylphenol on marine plankton. Journal of Hazardous Materials, 484, 136694. https://doi.org/10.1016/j.jhazmat.2024.136694

Campanale, C., Stock, F., Massarelli, C., Kochleus, C., Bagnuolo, G., Reifferscheid, G., & Uricchio, V. F. (2020). Microplastics and their possible sources: The example of Ofanto river in southeast Italy. Environmental Pollution, 258, 113284. https://doi.org/10.1016/j.envpol.2019.113284

Capolupo, M., Sørensen, L., Jayasena, K. D. R., Booth, A. M., & Fabbri, E. (2020). Chemical composition and ecotoxicity of plastic and car tire rubber leachates to aquatic organisms. Water Research, 169, 115270. https://doi.org/10.1016/j.watres.2019.115270

Carballeira, C., Ramos-Gómez, J., Martín-Díaz, L., & DelValls, T. A. (2012). Identification of specific malformations of sea urchin larvae for toxicity assessment: Application to marine pisciculture effluents. Marine Environmental Research, 77, 12–22. https://doi.org/10.1016/j.marenvres.2012.01.001

Carpenter, E. J., & Smith, K. L. (1972). Plastics on the Sargasso Sea Surface. Science, 175(4027), 1240–1241. https://doi.org/10.1126/science.175.4027.1240

Chen, G., Feng, Q., & Wang, J. (2020). Mini-review of microplastics in the atmosphere and their risks to humans. Science of The Total Environment, 703, 135504. https://doi.org/10.1016/j.scitotenv.2019.135504

Chung, M. K., Hu, R., Wong, M. H., & Cheung, K. C. (2007). Comparative toxicity of hydrophobic contaminants to microalgae and higher plants. Ecotoxicology, 16(5), 393–402. https://doi.org/10.1007/s10646-007-0134-4

Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F., & Danovaro, R. (2017). Sunscreen products impair the early developmental stages of the sea urchin Paracentrotus lividus. Scientific Reports, 7(1), 7815. https://doi.org/10.1038/s41598-017-08013-x

Cormier, B., Gambardella, C., Tato, T., Perdriat, Q., Costa, E., Veclin, C., Le Bihanic, F., Grassl, B., Dubocq, F., Kärrman, A., Van Arkel, K., Lemoine, S., Lagarde, F., Morin, B., Garaventa, F., Faimali, M., Cousin, X., Bégout, M.-L., Beiras, R., & Cachot, J. (2021). Chemicals sorbed to environmental microplastics are toxic to early life stages of aquatic organisms. Ecotoxicology and Environmental Safety, 208, 111665. https://doi.org/10.1016/j.ecoenv.2020.111665

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., & Geissen, V. (2019). Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Science of The Total Environment, 671, 411–420. https://doi.org/10.1016/j.scitotenv.2019.03.368

Daly, K. L., & Smith, W. O. (1993). Physical-Biological Interactions Influencing Marine Plankton Production. Annual Review of Ecology and Systematics, 24, 555–585. JSTOR.

Dang, V. D. H., Cheung, P.-Y., Fong, C.-L., Mulla, A. J., Shiu, J.-H., Lin, C.-H., & Nozawa, Y. (2020). Sea Urchins Play an Increasingly Important Role for Coral Resilience Across Reefs in Taiwan. Frontiers in Marine Science, 7. https://www.frontiersin.org/journals/marine-science/articles/10.3389/fmars.2020.581945

Dayras, P., Bialais, C., Lee, J.-S., & Souissi, S. (2020). Effects of microalgal diet on the population growth and fecundity of the cyclopoid copepod Paracyclopina nana. Journal of the World Aquaculture Society, 51(6), 1386–1401. https://doi.org/10.1111/jwas.12685

Dekiff, J. H., Remy, D., Klasmeier, J., & Fries, E. (2014). Occurrence and spatial distribution of microplastics in sediments from Norderney. Environmental Pollution, 186, 248–256. https://doi.org/10.1016/j.envpol.2013.11.019

Delaeter, C., Spilmont, N., Bouchet, V. M. P., & Seuront, L. (2022). Plastic leachates: Bridging the gap between a conspicuous pollution and its pernicious effects on marine life. Science of The Total Environment, 826, 154091. https://doi.org/10.1016/j.scitotenv.2022.154091

Ding, K., Lu, L., Wang, J., Wang, J., Zhou, M., Zheng, C., Liu, J., Zhang, C., & Zhuang, S. (2017). In vitro and in silico investigations of the binary-mixture toxicity of phthalate esters and cadmium (II) to Vibrio qinghaiensis sp.-Q67. Science of The Total Environment, 580, 1078–1084. https://doi.org/10.1016/j.scitotenv.2016.12.062

Droop, M. R. (1955). Some new supra-littoral Protista. Journal of the Marine Biological Association of the United Kingdom, 34(2), 233–245. Cambridge Core. https://doi.org/10.1017/S0025315400027612

Dunstan, Graeme. A., Brown, M. R., & Volkman, John. K. (2005). Cryptophyceae and rhodophyceae; chemotaxonomy, phylogeny, and application. Phytochemistry, 66(21), 2557–2570. https://doi.org/10.1016/j.phytochem.2005.08.015

Egbeocha CO, Malek S, Emenike CU, & Milow P. (2018). Feasting on microplastics: Ingestion by and effects on marine organisms. Aquatic Biology, 27, 93–106.

Environment, U. N. (2021, October 21). Drowning in Plastics – Marine Litter and Plastic Waste Vital Graphics | UNEP - UN Environment Programme. https://www.unep.org/resources/report/drowning-plastics-marine-litter-and-plastic-waste-vital-graphics

Falkowski, P. G. (1994). The role of phytoplankton photosynthesis in global biogeochemical cycles. Photosynthesis Research, 39(3), 235–258. https://doi.org/10.1007/BF00014586

Faucher, G., Hoffmann, L., Bach, L. T., Bottini, C., Erba, E., & Riebesell, U. (2017). Impact of trace metal concentrations on coccolithophore growth and morphology: Species-specific responses in past and present ocean. 15537. EGU General Assembly Conference Abstracts.

Fenchel, T. (1988). Marine Plankton Food Chains. Annual Review of Ecology and Systematics, 19, 19–38. JSTOR.

Fernández, N., & Beiras, R. (2001). Combined Toxicity of Dissolved Mercury With Copper, Lead and Cadmium on Embryogenesis and Early Larval Growth of the Paracentrotus Lividus Sea-Urchin. Ecotoxicology, 10(5), 263–271. https://doi.org/10.1023/A:1016703116830

Fernández-Reiriz, M. J., Irisarri, J., & Labarta, U. (2015). Feeding behaviour and differential absorption of nutrients in mussel Mytilus galloprovincialis: Responses to three microalgae diets. Aquaculture, 446, 42–47. https://doi.org/10.1016/j.aquaculture.2015.04.025

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science, 281(5374), 237–240. https://doi.org/10.1126/science.281.5374.237

Fontúrbel, F. E., & Castaño-Villa, G. J. (2011). Relationships between nutrient enrichment and the phytoplankton community at an andean oligotrophic lake: A multivariate assessment. Ecología Aplicada, 10(2), 75–81.

Frias, J. P. G. L., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. Marine Pollution Bulletin, 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

Gallitelli, L., Cera, A., Cesarini, G., Pietrelli, L., & Scalici, M. (2021). Preliminary indoor evidences of microplastic effects on freshwater benthic macroinvertebrates. Scientific Reports, 11(1), 720. https://doi.org/10.1038/s41598-020-80606-5

Galloway, T. S., Cole, M., & Lewis, C. (2017). Interactions of microplastic debris throughout the marine ecosystem. Nature Ecology & Evolution, 1(5), 0116. https://doi.org/10.1038/s41559-017-0116

Gambardella, C., Aluigi, M. G., Ferrando, S., Gallus, L., Ramoino, P., Gatti, A. M., Rottigni, M., & Falugi, C. (2013). Developmental abnormalities and changes in cholinesterase activity in sea urchin embryos and larvae from sperm exposed to engineered nanoparticles. Aquatic Toxicology, 130–131, 77–85. https://doi.org/10.1016/j.aquatox.2012.12.025

Gambardella, C., Marcellini, F., Falugi, C., Varrella, S., & Corinaldesi, C. (2021). Early-stage anomalies in the sea urchin (Paracentrotus lividus) as bioindicators of multiple stressors in the marine environment: Overview and future perspectives. Environmental Pollution, 287, 117608. https://doi.org/10.1016/j.envpol.2021.117608

Gambardella, C., Miroglio, R., Costa, E., Cachot, J., Morin, B., Clérandeau, C., Rotander, A., Rocco, K., d'Errico, G., Almeda, R., Alonso, O., Grau, E., Piazza, V., Pittura, L., Benedetti, M., Regoli, F., Faimali, M., & Garaventa, F. (2024). New insights into the impact of leachates from in-field collected plastics on aquatic invertebrates and vertebrates. Environmental Pollution, 355, 124233. https://doi.org/10.1016/j.envpol.2024.124233

Garrido, S., Linares, M., Campillo, J. A., & Albentosa, M. (2019). Effect of microplastics on the toxicity of chlorpyrifos to the microalgae Isochrysis galbana, clone t-ISO. Ecotoxicology and Environmental Safety, 173, 103–109. https://doi.org/10.1016/j.ecoenv.2019.02.020

Geyer, R. (2020). A Brief History of Plastics. In M. Streit-Bianchi, M. Cimadevila, & W. Trettnak (Eds.), Mare Plasticum—The Plastic Sea: Combatting Plastic Pollution Through Science and Art (pp. 31–47). Springer International Publishing. https://doi.org/10.1007/978-3-030-38945-1_2

González-Araya, R., Lebrun, L., Quéré, C., & Robert, R. (2012). The selection of an ideal diet for Ostrea edulis (L.) broodstock conditioning (part B). Aquaculture, 362–363, 55–66. https://doi.org/10.1016/j.aquaculture.2012.06.029

González-Delgado, S., Lozano-Bilbao, E., Hardisson, A., Paz, S., Gonález-Weller, D., Rubio, C., & Gutiérrez, Á. J. (2024). Metal concentrations in echinoderms: Assessing bioindicator potential and ecological implications. Marine Pollution Bulletin, 205, 116619. https://doi.org/10.1016/j.marpolbul.2024.116619

Gualtieri, M., Andrioletti, M., Vismara, C., Milani, M., & Camatini, M. (2005). Toxicity of tire debris leachates. Environment International, 31(5), 723–730. https://doi.org/10.1016/j.envint.2005.02.001

Guzzetti, E., Sureda, A., Tejada, S., & Faggio, C. (2018). Microplastic in marine organism: Environmental and toxicological effects. Environmental Toxicology and Pharmacology, 64, 164–171. https://doi.org/10.1016/j.etap.2018.10.009

Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., & Purnell, P. (2018). An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. Journal of Hazardous Materials, 344, 179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014

Halsband, C., Sørensen, L., Booth, A. M., & Herzke, D. (2020). Car Tire Crumb Rubber: Does Leaching Produce a Toxic Chemical Cocktail in Coastal Marine Systems? Frontiers in Environmental Science, 8. https://doi.org/10.3389/fenvs.2020.00125

Hampel, M., Moreno-Garrido, I., Sobrino, C., Lubián, L. M., & Blasco, J. (2001). Acute Toxicity of LAS Homologues in Marine Microalgae: Esterase Activity and Inhibition Growth as Endpoints of Toxicity. Ecotoxicology and Environmental Safety, 48(3), 287–292. https://doi.org/10.1006/eesa.2000.2028

Hong, S., Lee, J., Cha, J., Gwak, J., & Khim, J. S. (2023). Effect-Directed Analysis Combined with Nontarget Screening to Identify Unmonitored Toxic Substances in the Environment. Environmental Science & Technology, 57(48), 19148–19155. https://doi.org/10.1021/acs.est.3c05035

Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. WIREs Water, 5(2), e1268. https://doi.org/10.1002/wat2.1268

Hose, J. E., Puffer, H. W., Oshida, P. S., & Bay, S. M. (1983). Developmental and cytogenetic abnormalities induced in the purple sea urchin by environmental levels of benzo(a)pyrene. Archives of Environmental Contamination and Toxicology, 12(3), 319–325. https://doi.org/10.1007/BF01059409

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, 347(6223), 768–771. https://doi.org/10.1126/science.1260352

Kaposi, K. L., Mos, B., Kelaher, B. P., & Dworjanyn, S. A. (2014). Ingestion of Microplastic Has Limited Impact on a Marine Larva. Environmental Science & Technology, 48(3), 1638–1645. https://doi.org/10.1021/es404295e

Kedzierski, M., D'Almeida, M., Magueresse, A., Le Grand, A., Duval, H., César, G., Sire, O., Bruzaud, S., & Le Tilly, V. (2018). Threat of plastic ageing in marine environment. Adsorption/desorption of micropollutants. Marine Pollution Bulletin, 127, 684–694. https://doi.org/10.1016/j.marpolbul.2017.12.059

Kole, P. J., Löhr, A. J., Van Belleghem, F. G. A. J., & Ragas, A. M. J. (2017). Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment. International Journal of Environmental Research and Public Health, 14(10), Article 10. https://doi.org/10.3390/ijerph14101265

Koski, M., Søndergaard, J., Christensen, A. M., & Nielsen, T. G. (2021). Effect of environmentally relevant concentrations of potentially toxic microplastic on coastal copepods. Aquatic Toxicology, 230, 105713. https://doi.org/10.1016/j.aquatox.2020.105713

Laranjeiro, F., Rotander, A., López-Ibáñez, S., Vilas, A., Södergren Seilitz, F., Clérandeau, C., Sampalo, M., Rial, D., Bellas, J., Cachot, J., Almeda, R., & Beiras, R. (2024). Comparative assessment of the acute toxicity of commercial bio-based polymer leachates on marine plankton. Science of The Total Environment, 946, 174403. https://doi.org/10.1016/j.scitotenv.2024.174403

Larsen, S. h., & Beardall, J. (2021). The effect of CO2 concentration on DMSP production in Gephyrocapsa oceanica (Isochrysidales, Coccolithophyceae). Phycologia, 60(5), 439–448. https://doi.org/10.1080/00318884.2021.1944016

Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., & Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. Environment International, 163, 107199. https://doi.org/10.1016/j.envint.2022.107199

Li, J., Xu, J., & Jiang, X. (2023). Urban runoff mortality syndrome in zooplankton caused by tire wear particles. Environmental Pollution, 329, 121721. https://doi.org/10.1016/j.envpol.2023.121721

Li, L., Huang, W., Qiao, D., Zhong, Z., Shang, Y., Khan, F. U., Wei, S., & Wang, Y. (2024). Marine Heatwaves Exacerbate the Toxic Effects of Tire Particle Leachate on Microalgae. Environmental Science & Technology. https://doi.org/10.1021/acs.est.4c08986

Liu, G., Chai, X., Shao, Y., Hu, L., Xie, Q., & Wu, H. (2011). Toxicity of copper, lead, and cadmium on the motility of two marine microalgae Isochrysis galbana and Tetraselmis chui. Journal of Environmental Sciences, 23(2), 330–335. https://doi.org/10.1016/S1001-0742(10)60410-X

López-Galindo, C., Garrido, M. C., Casanueva, J. F., & Nebot, E. (2010). Degradation models and ecotoxicity in marine waters of two antifouling compounds: Sodium hypochlorite and an alkylamine surfactant. Science of The Total Environment, 408(8), 1779–1785. https://doi.org/10.1016/j.scitotenv.2010.01.029

Lu, T., Zhang, Q., Zhang, Z., Hu, B., Chen, J., Chen, J., & Qian, H. (2021). Pollutant toxicology with respect to microalgae and cyanobacteria. Journal of Environmental Sciences, 99, 175–186. https://doi.org/10.1016/j.jes.2020.06.033

Lv, M., Meng, F., Man, M., Lu, S., Ren, S., Yang, X., Wang, Q., Chen, L., & Ding, J. (2024). Aging increases the particulate- and leachate-induced toxicity of tire wear particles to microalgae. Water Research, 256, 121653. https://doi.org/10.1016/j.watres.2024.121653

Marin, A., Montoya, S., Vita, R., Marín-Guirao, L., Lloret, J., & Aguado, F. (2007). Utility of sea urchin embryo–larval bioassays for assessing the environmental impact of marine fishcage farming. Aquaculture, 271(1), 286–297. https://doi.org/10.1016/j.aquaculture.2007.05.030

Martínez-Gómez, C., León, V. M., Calles, S., Gomáriz-Olcina, M., & Vethaak, A. D. (2017). The adverse effects of virgin microplastics on the fertilization and larval development of sea urchins. Marine Environmental Research, 130, 69–76. https://doi.org/10.1016/j.marenvres.2017.06.016

Meng, F., Ni, Z., Tan, L., Cai, P., & Wang, J. (2024). Oxidative stress and energy metabolic response of Isochrysis galbana induced by different types of pristine and aging microplastics and their leachates. Chemosphere, 348, 140755. https://doi.org/10.1016/j.chemosphere.2023.140755

Mennekes, D., & Nowack, B. (2022). Tire wear particle emissions: Measurement data where are you? Science of The Total Environment, 830, 154655. https://doi.org/10.1016/j.scitotenv.2022.154655

Morale, A., Coniglio, L., Angelini, G., Cimoli, G., Bolla, A., Alleteo, D., Russo, P., & Falugi, C. (1998). Biological effects of a neurotoxic pesticide at low concentrations on sea urchin early development. A terathogenic assay. Chemosphere, 37(14), 3001–3010. https://doi.org/10.1016/S0045-6535(98)00341-5

Moreira, W., Alonso, O., Paule, A., Martínez, I., Le Du-Carreé, J., & Almeda, R. (2024). Life stage-specific effects of tire particle leachates on the cosmopolitan planktonic copepod Acartia tonsa. Environmental Pollution, 343, 123256. https://doi.org/10.1016/j.envpol.2023.123256

Morgan, S. G. (2020). Life and death in the plankton: Larval mortality and adaptation. Ecology of Marine Invertebrate Larvae, 279–321.

Nam, S.-H., Lee, J., & An, Y.-J. (2022). Towards understanding the impact of plastics on freshwater and marine microalgae: A review of the mechanisms and toxicity endpoints. Journal of Hazardous Materials, 423, 127174. https://doi.org/10.1016/j.jhazmat.2021.127174

Naselli-Flores, L., & Padisák, J. (2023). Ecosystem services provided by marine and freshwater phytoplankton. Hydrobiologia, 850(12), 2691–2706. https://doi.org/10.1007/s10750-022-04795-y

Nguyen, V. T., Huynh, A. T., & Dao, T. S. (2022). Acute and chronic toxicity of mixtures of bisphenol A and trace metals (Cd and Pb) to micro-crustacean, Daphnia magna. IOP Conference Series: Earth and Environmental Science, 964(1), 012012. https://doi.org/10.1088/1755-1315/964/1/012012

Nobre, C. R., Santana, M. F. M., Maluf, A., Cortez, F. S., Cesar, A., Pereira, C. D. S., & Turra, A. (2015). Assessment of microplastic toxicity to embryonic development of the sea urchin Lytechinus variegatus (Echinodermata: Echinoidea). Marine Pollution Bulletin, 92(1), 99–104. https://doi.org/10.1016/j.marpolbul.2014.12.050

Nowicki, M., DeVries, T., & Siegel, D. A. (2022). Quantifying the Carbon Export and Sequestration Pathways of the Ocean's Biological Carbon Pump. Global Biogeochemical Cycles, 36(3), e2021GB007083. https://doi.org/10.1029/2021GB007083

Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S., & Beiras, R. (2019). Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus lividus. Environmental Pollution, 247, 706–715. https://doi.org/10.1016/j.envpol.2019.01.098

Omidoyin, K. C., & Jho, E. H. (2024). Environmental occurrence and ecotoxicological risks of plastic leachates in aquatic and terrestrial environments. Science of The Total Environment, 954, 176728. https://doi.org/10.1016/j.scitotenv.2024.176728

Page, T. S., Almeda, R., Koski, M., Bournaka, E., & Nielsen, T. G. (2022). Toxicity of tyre wear particle leachates to marine phytoplankton. Aquatic Toxicology, 252, 106299. https://doi.org/10.1016/j.aquatox.2022.106299

Pandori, L. L. M., & Sorte, C. J. B. (2019). The weakest link: Sensitivity to climate extremes across life stages of marine invertebrates. Oikos, 128(5), 621–629. https://doi.org/10.1111/oik.05886

Paredes, E., Perez, S., Rodil, R., Quintana, J. B., & Beiras, R. (2014). Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. Chemosphere, 104, 44–50. https://doi.org/10.1016/j.chemosphere.2013.10.053

Parker-Jurd, F. N. F., Napper, I. E., Abbott, G. D., Hann, S., & Thompson, R. C. (2021). Quantifying the release of tyre wear particles to the marine environment via multiple pathways. Marine Pollution Bulletin, 172, 112897. https://doi.org/10.1016/j.marpolbul.2021.112897

Passarelli, M. C., Cesar, A., Riba, I., & DelValls, T. A. (2017). Comparative evaluation of sea-urchin larval stage sensitivity to ocean acidification. Chemosphere, 184, 224–234. https://doi.org/10.1016/j.chemosphere.2017.06.001

Pearse, J. S. (2006). Ecological Role of Purple Sea Urchins. Science, 314(5801), 940–941. https://doi.org/10.1126/science.1131888

Pennington, J. T., Rumrill, S. S., & Chia, F.-S. (1986). Stage-specific predation upon embryos and larvae of the Pacific sand dollar, Dendraster excentricus, by 11 species of common zooplanktonic predators. Bulletin of Marine Science, 39(2), 234–240.

Pesando, D., Huitorel, P., Dolcini, V., Angelini, C., Guidetti, P., & Falugi, C. (2003). Biological targets of neurotoxic pesticides analysed by alteration of developmental events in the Mediterranean sea urchin, Paracentrotus lividus. Marine Environmental Research, 55(1), 39–57. https://doi.org/10.1016/S0141-1136(02)00215-5

Pfaendner, R. (2006). How will additives shape the future of plastics? Polymer Degradation and Stability, 91(9), 2249–2256. https://doi.org/10.1016/j.polymdegradstab.2005.10.017

Piscitello, A., Bianco, C., Casasso, A., & Sethi, R. (2021). Non-exhaust traffic emissions: Sources, characterization, and mitigation measures. Science of The Total Environment, 766, 144440. https://doi.org/10.1016/j.scitotenv.2020.144440

Portocali Ph., Iliopoulou-Georgudaki, J., Catsiki, V. A., & Papapetropoulou, M. (1997). The role of echinoderms as bioindicators of seawater pollution: A case study from patraicos and corinthiacos Gulf, N. Peloponnesus, Greece. Toxicological & Environmental Chemistry, 59(1–4), 293–303. https://doi.org/10.1080/02772249709358443

Prowe, A. E. F., Pahlow, M., Dutkiewicz, S., Follows, M., & Oschlies, A. (2012). Top-down control of marine phytoplankton diversity in a global ecosystem model. Progress in Oceanography, 101(1), 1–13. https://doi.org/10.1016/j.pocean.2011.11.016

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M. C. A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., & Giorgini, E. (2021). Plasticenta: First evidence of microplastics in human placenta. Environment International, 146, 106274. https://doi.org/10.1016/j.envint.2020.106274

Rech, S., Borrell, Y., & García-Vazquez, E. (2016). Marine litter as a vector for non-native species: What we need to know. Marine Pollution Bulletin, 113(1), 40–43. https://doi.org/10.1016/j.marpolbul.2016.08.032

Redondo-Hasselerharm, P. E., de Ruijter, V. N., Mintenig, S. M., Verschoor, A., & Koelmans, A. A. (2018). Ingestion and Chronic Effects of Car Tire Tread Particles on Freshwater Benthic Macroinvertebrates. Environmental Science & Technology, 52(23), 13986–13994. https://doi.org/10.1021/acs.est.8b05035

Rendell-Bhatti, F., Paganos, P., Pouch, A., Mitchell, C., D'Aniello, S., Godley, B. J., Pazdro, K., Arnone, M. I., & Jimenez-Guri, E. (2021a). Developmental toxicity of plastic leachates on the sea urchin Paracentrotus lividus. Environmental Pollution, 269, 115744. https://doi.org/10.1016/j.envpol.2020.115744

Rendell-Bhatti, F., Paganos, P., Pouch, A., Mitchell, C., D'Aniello, S., Godley, B. J., Pazdro, K., Arnone, M. I., & Jimenez-Guri, E. (2021b). Developmental toxicity of plastic leachates on the sea urchin Paracentrotus lividus. Environmental Pollution, 269, 115744. https://doi.org/10.1016/j.envpol.2020.115744

Rillig, M. C., Lehmann, A., de Souza Machado, A. A., & Yang, G. (2019). Microplastic effects on plants. New Phytologist, 223(3), 1066–1070. https://doi.org/10.1111/nph.15794

Rist, S., Le Du-Carrée, J., Ugwu, K., Intermite, C., Acosta-Dacal, A., Pérez-Luzardo, O., Zumbado, M., Gómez, M., & Almeda, R. (2023). Toxicity of tire particle leachates on early life stages of keystone sea urchin species. Environmental Pollution, 336, 122453. https://doi.org/10.1016/j.envpol.2023.122453

Roepke, T. A., Snyder, M. J., & Cherr, G. N. (2005). Estradiol and endocrine disrupting compounds adversely affect development of sea urchin embryos at environmentally relevant concentrations. Aquatic Toxicology, 71(2), 155–173. https://doi.org/10.1016/j.aquatox.2004.11.003

Saco-Álvarez, L., Durán, I., Ignacio Lorenzo, J., & Beiras, R. (2010). Methodological basis for the optimization of a marine sea-urchin embryo test (SET) for the ecological assessment of coastal water quality. Ecotoxicology and Environmental Safety, 73(4), 491–499. https://doi.org/10.1016/j.ecoenv.2010.01.018

Sánchez, S., Martínez, Me., & Espinola, F. (2000). Biomass production and biochemical variability of the marine microalga Isochrysis galbana in relation to culture medium. Biochemical Engineering Journal, 6(1), 13–18. https://doi.org/10.1016/S1369-703X(00)00071-1

Sartori, D., Scatena, G., Vrinceanu, C. A., & Gaion, A. (2023). Increased sensitivity of sea urchin larvae to metal toxicity as a consequence of the past two decades of Climate Change and Ocean Acidification in the Mediterranean Sea. Marine Pollution Bulletin, 194, 115274. https://doi.org/10.1016/j.marpolbul.2023.115274

Shin, H., Sukumaran, V., Yeo, I.-C., Shim, K.-Y., Lee, S., Choi, H.-K., Ha, S. Y., Kim, M., Jung, J.-H., Lee, J.-S., & Jeong, C.-B. (2022). Phenotypic toxicity, oxidative response, and transcriptomic deregulation of the rotifer Brachionus plicatilis exposed to a toxic cocktail of tire-wear particle leachate. Journal of Hazardous Materials, 438, 129417. https://doi.org/10.1016/j.jhazmat.2022.129417

Silberberger MJ, Renaud PE, Espinasse B, & Reiss H. (2016). Spatial and temporal structure of the meroplankton community in a sub-Arctic shelf system. Marine Ecology Progress Series, 555, 79–93.

Spalding, M. D., Ruffo, S., Lacambra, C., Meliane, I., Hale, L. Z., Shepard, C. C., & Beck, M. W. (2014). The role of ecosystems in coastal protection: Adapting to climate change and coastal hazards. Ocean & Coastal Management, 90, 50–57. https://doi.org/10.1016/j.ocecoaman.2013.09.007

Stefatos, A., Charalampakis, M., Papatheodorou, G., & Ferentinos, G. (1999). Marine Debris on the Seafloor of the Mediterranean Sea: Examples from Two Enclosed Gulfs in Western Greece. Marine Pollution Bulletin, 38(5), 389–393. https://doi.org/10.1016/S0025-326X(98)00141-6

Tellis, M. S., Lauer, M. M., Nadella, S., Bianchini, A., & Wood, C. M. (2014). Sublethal mechanisms of Pb and Zn toxicity to the purple sea urchin (Strongylocentrotus purpuratus) during early development. Aquatic Toxicology, 146, 220–229. https://doi.org/10.1016/j.aquatox.2013.11.004

Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. (2011, July 27). OECD. https://www.oecd.org/en/publications/test-no-201-alga-growth-inhibition-test_9789264069923-en.html

Thomsen, E. S., Almeda, R., & Nielsen, T. G. (2024). Tire particles and their leachates reduce the filtration rate of the mussel Mytilus edulis. Marine Environmental Research, 195, 106348. https://doi.org/10.1016/j.marenvres.2024.106348

Thorson, G. (1950). Reproductibe and larval ecology of marine bottom invertebrates. Biological Reviews, 25(1), 1–45. https://doi.org/10.1111/j.1469-185X.1950.tb00585.x

Tian, Z., Zhao, H., Peter, K. T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A. E., Biswas, R. G., Kock, F. V. C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., … Kolodziej, E. P. (2021). A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science, 371(6525), 185–189. https://doi.org/10.1126/science.abd6951

Tremblay, R., Cartier, S., Miner, P., Pernet, F., Quéré, C., Moal, J., Muzellec, M.-L., Mazuret, M., & Samain, J.-F. (2007). Effect of Rhodomonas salina addition to a standard hatchery diet during the early ontogeny of the scallop Pecten maximus. Aquaculture, 262(2), 410–418. https://doi.org/10.1016/j.aquaculture.2006.10.009

Trenfield, M. A., van Dam, J. W., Harford, A. J., Parry, D., Streten, C., Gibb, K., & van Dam, R. A. (2015). Aluminium, gallium, and molybdenum toxicity to the tropical marine microalga Isochrysis galbana. Environmental Toxicology and Chemistry, 34(8), 1833–1840. https://doi.org/10.1002/etc.2996

Turner, A. (2016). Heavy metals, metalloids and other hazardous elements in marine plastic litter. Marine Pollution Bulletin, 111(1), 136–142. https://doi.org/10.1016/j.marpolbul.2016.07.020

Uribe-Echeverría, T., & Beiras, R. (2022). Acute toxicity of bioplastic leachates to Paracentrotus lividus sea urchin larvae. Marine Environmental Research, 176, 105605. https://doi.org/10.1016/j.marenvres.2022.105605

Uthicke, S., Schaffelke, B., & Byrne, M. (2009). A boom–bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. Ecological Monographs, 79(1), 3–24. https://doi.org/10.1890/07-2136.1

Venâncio, C., Ferreira, I., Martins, M. A., Soares, A. M. V. M., Lopes, I., & Oliveira, M. (2019). The effects of nanoplastics on marine plankton: A case study with polymethylmethacrylate. Ecotoxicology and Environmental Safety, 184, 109632. https://doi.org/10.1016/j.ecoenv.2019.109632

Visconti, G., Gianguzza, F., Butera, E., Costa, V., Vizzini, S., Byrne, M., & Gianguzza, P. (2017). Morphological response of the larvae of Arbacia lixula to near-future ocean warming and acidification. ICES Journal of Marine Science, 74(4), 1180–1190. https://doi.org/10.1093/icesjms/fsx037

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T., & Reemtsma, T. (2018). Tire wear particles in the aquatic environment—A review on generation, analysis, occurrence, fate and effects. Water Research, 139, 83–100. https://doi.org/10.1016/j.watres.2018.03.051

Wang, J., Zeng, C., & Feng, Y. (2024). Meta-analysis reveals responses of coccolithophores and diatoms to warming. Marine Environmental Research, 193, 106275. https://doi.org/10.1016/j.marenvres.2023.106275

Wangensteen, O. S., Dupont, S., Casties, I., Turon, X., & Palacín, C. (2013). Some like it hot: Temperature and pH modulate larval development and settlement of the sea urchin Arbacia lixula. Journal of Experimental Marine Biology and Ecology, 449, 304–311. https://doi.org/10.1016/j.jembe.2013.10.007

Wayman, C., & Niemann, H. (2021). The fate of plastic in the ocean environment – a minireview. Environmental Science: Processes & Impacts, 23(2), 198–212. https://doi.org/10.1039/D0EM00446D

Weiner, J. A., DeLorenzo, M. E., & Fulton, M. H. (2004). Relationship between uptake capacity and differential toxicity of the herbicide atrazine in selected microalgal species. Aquatic Toxicology, 68(2), 121–128. https://doi.org/10.1016/j.aquatox.2004.03.004

Wik, A., & Dave, G. (2009). Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. Environmental Pollution, 157(1), 1–11. https://doi.org/10.1016/j.envpol.2008.09.028

Wik, A., Nilsson, E., Källqvist, T., Tobiesen, A., & Dave, G. (2009). Toxicity assessment of sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. Chemosphere, 77(7), 922–927. https://doi.org/10.1016/j.chemosphere.2009.08.034

Wong, B. B. M., & Candolin, U. (2015). Behavioral responses to changing environments. Behavioral Ecology, 26(3), 665–673. https://doi.org/10.1093/beheco/aru183

Yang, K., Jing, S., Liu, Y., Zhou, H., Liu, Y., Yan, M., Yi, X., & Liu, R. (2022). Acute toxicity of tire wear particles, leachates and toxicity identification evaluation of leachates to the marine copepod, Tigriopus japonicus. Chemosphere, 297, 134099. https://doi.org/10.1016/j.chemosphere.2022.134099

Yap, C. K., Ismail, A., Omar, H., & Tan, S. G. (2004). Toxicities and tolerances of Cd, Cu, Pb and Zn in a primary producer (Isochrysis galbana) and in a primary consumer (Perna viridis). Environment International, 29(8), 1097–1104. https://doi.org/10.1016/S0160-4120(03)00141-7

Young, C. M. (1987). Abundance and distribution of pelagic larvae as influenced by predation, behaviour, and hydrographic factors. In Reproduction of Marine Invertebrates, 385–463.

Zhang, M., Yin, H., Tan, J., Wang, X., Yang, Z., Hao, L., Du, T., Niu, Z., & Ge, Y. (2023). A comprehensive review of tyre wear particles: Formation, measurements, properties, and influencing factors. Atmospheric Environment, 297, 119597. https://doi.org/10.1016/j.atmosenv.2023.119597

8. ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to all those who have supported me during this research and the preparation of my thesis.

First, I am grateful to my external supervisor Rodrigo Almeda who accepted and supported me throughout the research. It has been a unique and rewarding experience to have the opportunity to work on a study in a foreign country. I would also like to thank Jessy for the continuous guidance, valuable feedback, and support throughout this study. Your expertise and encouragement have been instrumental in the completion of this work.

I am also deeply grateful to my Italian supervisors Marco Faimali and Chiara Gambardella. Specifically, Chiara has been an exceptional guide, and her invaluable advice has greatly enriched this work.

I would also like to thank the IAS laboratory team for their warm welcome and guidance, particularly to Roberta, Pietro, and Francesco for their support and assistance.

Now, I would like to switch to Italian to express my gratitude to those closest to me, as it is my native language and allows me to more accurately convey my feelings.

Un grazie speciale va alla mia famiglia, in particolare ai miei genitori, per il vostro supporto costante e per permettermi ogni giorno di avere fiducia in me stessa e nel percorso di vita che mi sto costruendo. Grazie anche ai miei bro, Adri, Rebe e Mary, perché grazie alle nostre differenze rendete più ricca e divertente la vita.

Come non ringraziare poi la famiglia genovese, i Sapori Kiara, Clara, Giulio ed Emilia; senza di voi questi due anni sarebbero stati più noiosi e meno piccanti. Grazie per il confronto, per le risate, per la pazzia. Mi sento a casa con voi. Grazie anche alle amiche di una vita, Otty, Ceci, Zazu, Sofi, Vale e Carococchi. Legami così sono rari e da coltivare.

Infine, vorrei ringraziare l'incontro più inaspettato che Genova mi ha regalato, Antony, perché trovare una persona con cui mettersi in discussione e crescere sentendosi sé stessi è una fortuna. Grazie per la dolcezza, per il supporto, per le esperienze vissute e per i sogni futuri.