The Effects of Plastic Debris on Bivalves

by

Sasha Holloway

A dissertation presented for the degree of Master of Science

Department of Biological Sciences

University of Cape Town

February 2025

Supervisor: Dr Lyle Vorsatz

Co-supervisor: Dr Maya Pfaff

Plagiarism declaration

I know the meaning of plagiarism and declare that all of the work in the dissertation, save for that which is properly acknowledged, is my own.

Signature: Signed by candidate Date: 11 February 2025

Acknowledgements

This dissertation would not have been possible without the assistance of a considerable number of people, and it is not possible to mention everyone personally.

To my supervisors, Dr Lyle Vorsatz and Dr Maya Pfaff, thank you for all the support and guidance you've given me. It has been a pleasure to work with you, and I truly appreciate everything you have done. Thank you also to Assoc Prof Coleen Moloney who was a superb supervisor through the first half of my thesis, I hope you are thoroughly enjoying retirement!

Thank you too to Andrea Plos and Calvan Hartnick for their help in setting up the experimental aquariums, and for keeping them running even during the worst of loadshedding! They also provided additional technical assistance in a multitude of ingenious ways, such as custom building me a mussel-puller for my byssal tenacity experiment. Thank you to Dr Mark Cyrus from the Marine Research Aquarium for growing and providing me with the *Rhodomonas salina* used in my experiments, your time and generosity were greatly appreciated.

A very special thank you goes to my friends and family. I could easily fill a novel with everything you have done for me, so I hope you will forgive the need for brevity. Not only did my family provide never-ending support, but went above and beyond by becoming lab assistants when my intended research partner could not join me due to COVID restrictions. Thank you, Mom, for your patience and willingness to drive me and my buckets of mussels all over Cape Town. Thank you especially to my sister, Kayla, who braved storms during field work and spent many long hours in the research aquarium working on 'yucky' mussels with me. Mom and Dad, I appreciate you both so much, thank you for everything you've done to help me achieve my dream.

A huge thank you to Dr Mark Lenz and to the whole GAME 2020 team. Despite the project working out *very* differently than we planned due to COVID-19, Mark managed to hold the group together and supported those of us who remained with the project. Thank you to all my GAME 2020 friends for our weekly Zoom-meetings where we motivated and assisted each other. Special thanks must go to Anni, who would have been my research partner if it weren't for COVID-19 restrictions. Thank you for taking me under your wing from the moment you picked me up from the train station at 2 am on my first day in Germany. I would have been so lost in Kiel without your friendship and kindness. I hope one day you get to come to South Africa and see the lions like we planned.

Thank you to the organisations that provided funding towards this project. The implementation of this project would not have been possible without the sponsoring of the GAME-partners, nor the Merit award provided by the University of Cape Town.

GAME programme 2020

Chapter 3 of this thesis was carried out within the framework established by the GAME 2020 research group. GAME (Globular Approach by Modular Experiments) is hosted by the GEOMAR Helmholtz Centre for Ocean Research Kiel and is funded by various institutions and companies. Initiated by the Benthic Ecology working group of GEOMAR in 2000, GAME offers students the opportunity to conduct their masters as part of a global study. This is done by pairing a German student with a student from a participating partner country, creating a bi-national team. The students then develop one experimental protocol to be replicated in each participating country. In 2020 the research focus of GAME was the effect of macroplastics on mussel aggregates. To create independent data for team members, one student focused on conducting experiments with film shaped macroplastics of different rigidities, and the other focused on filament shaped macroplastics of different rigidities.

Usually the programme starts in March with students meeting in Kiel to create a protocol to be followed by all teams. Teams start conducting their experiments at host institutes in April then return to Kiel in October to evaluate and present their results. Due to the COVID-19 pandemic, the usual schedule was interrupted in mid-March. Many students were no longer able to travel to their host institutes, forcing teams to split and in some cases re-shuffle. By August 2020 it was determined that the German student intended to travel to South Africa would not be able to do so due to COVID-19 travel restrictions, and the decision was made for the South African team to split and for the students to conduct their experiments separately. Several teams had to be split or withdraw entirely from the program due to disruptions from COVID-19. In 2020 GAME experiments took place both in teams and individually in: Germany, Denmark, Spain, South Africa, Cape Verde and Malaysia.

Due to the unusual circumstances created by the COVID-19 pandemic, some students were able to complete their experiments earlier than others. These studies have been referenced in Chapter 3 as: Baensch 2021, Berning 2021, Kumpitsch 2021. It should be noted that these are masters theses and are not peer-reviewed literature.

General abstract

Plastics have become increasingly pervasive in marine ecosystems, comprising 60-80% of all marine debris encountered. Numerous studies have investigated the effects of microplastics on mussel physiology, function and aggregate formation, however, the effects of macroplastics remain largely unknown. In this thesis, I firstly synthesised existing knowledge about the effects of plastic on bivalve physiology though a comprehensive literature review, identifying patterns, trends and knowledge gaps. Microplastics induce various physiological changes, act as vectors for other pollutants, and amplify physiological effects when co-exposed with chemical pollutants. Although studies often report no or limited effects, these tended to be omitted in other reviews. Experimental studies often fail to generate realistic environmental conditions, limiting their practical relevance. Macroplastics have been shown to smother bivalves, reducing their abundance and altering species compositions of their associated fouling communities by restricting the flow of oxygen and organic matter. Additionally, macroplastics serve as settlement substrate or rafting material in otherwise unsuitable habitats. However, bivalves that settled on macroplastic experienced increased mortality and altered sex ratios, and the species composition of associated communities differed with polymer type. Overall, the literature review revealed that effects of both micro and macroplastics on various aspects of bivalve physiology and associated communities are highly variable, and that macroplastics remain greatly understudied.

Secondly, I conducted an experiment to determine the effects of macroplastics on the physical structure, physiology and associated communities of mussel (*Mytilus galloprovincialis*) aggregates. Film (polyethylene plastic bags) and filament (plastic nylon fishing rope) shaped plastics were introduced into mussel clumps as they aggregated in the laboratory. The mussel aggregates were then suspended on platforms in a marina for 85-91 days before being retrieved and various attributes examined, including structural and physiological variables as well as associated infauna and epibionts. Results indicated that macroplastic exposure had no effect on particulate organic matter accumulation, respiration rate, spatial complexity, byssus strength, body condition index, or mortality of mussels in experimental aggregates. Infaunal abundance and species composition were also unaffected. Plastic shape and amount showed an interactive effect on the percentage cover of epibionts growing on the mussel aggregates. Epibiont cover increased with increasing amounts of filaments and decreased with increasing amounts of film shaped plastics. The presence, shape or amount of macroplastics in mussel beds had no clear and consistent effects on the physical structure of mussel beds, the physiological performance of mussels and the composition of epibionts and associated mobile fauna. This study thus suggests that macroplastics only have very limited effects on *M. galloprovincialis* and their epibiont communities.

In conclusion, this thesis highlights the inconsistency of plastic effects on bivalves and their associated communities within the literature and through primary experimental investigation. While isolated effects existed, the literature is likely underrepresenting studies that have not found any effects, as was the case for the experimental part of my study. While certain limitations should be addressed in future studies, such as the lack of standardised methods and units, the variability of results and trends may indicate that plastics only have very limited effects on the performance of bivalves, many of which are highly adaptive and resilient to environmental stressors.

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List of Abbreviations

ACHe - acetylcholinesterase

ACP - acid phosphatase

ACX - acyl-CoA oxidase

AKP - alkaline phosphatase

ATP - adenosine triphosphate

BaP - Benzo(a)pyrene

CAT - catalase

DCFH - dichlorofluroescein

DEGM - dietary exposure of the human gut microbiota

GABA - gamma-aminobutyric acid

GPx - glutathione peroxide

GR - glutathione reductase

GSH - total glutathione

GSSG - oxidised glutathione

GST - glutathione S-transferase

HDPE - high density polyethylene

Hg - mercury

LDPE - low density polyethylene

LPO - lipid peroxidation

MDA - malondialdehyde

MET/MDR - mean epithelial thickness/mean diverticular radius

ROS - reactive oxygen species

PA - polyamide (nylon)

PAH - polycyclic aromatic hydrocarbons

PE - polyethylene

PET - polyethylene terephthalate (polyester)

PFK - phosphofructokinase

PHB - polyhydroxybutyrate

PHC - petroleum hydrocarbon

PLA - polylactic acid

PMMA - polymethyl methacrylate (acrylic)

PP - polypropylene

PPA - polyphthalamide

PS - polystyrene

PVC - polyvinyl chloride

SOD - superoxide dismutase

T-AOC - total antioxidant capacity

TBARS - thiobarbituric acid reactive substances

THC - total haemocyte counts

TOSCA - total oxyradical scavenging capacity

VAC - velocity average current

VCL - velocity curvi linear

VvBAS - volume density of basophilic cells

1 Chapter 1 – General Introduction

Plastic, a synthetic polymer, was first produced in the 19th century (Law, 2017). Plastics are light, durable, and cheap to make. These qualities have made plastics a highly desirable material and their low cost has led to single-use plastics becoming staples in daily life, resulting in waste rapidly accumulating in landfills and leaking into the natural environment (Barnes *et al.*, 2009; Geyer, Jambeck and Law, 2017). In 2024, the global plastics industry was worth USD 651.55 billion (Towards Chem & Material, 2025) with 368 Mt of plastic being produced globally in 2019 (*Plastics Europe 2020*). At the present rate of growth, plastics production is estimated to double within the next 20 years (Lebreton and Andrady, 2019). It has been estimated that of the 6300 metric tonnes of plastic waste produced globally between 1950 and 2015, 12% was incinerated and only 9% recycled, leaving 79% to accumulate in land-fills or the natural environment (Geyer, Jambeck and Law, 2017).

Estimates of how long plastics take to degrade vary hugely and are often incorrectly cited (Ward and Reddy, 2020). This variation and uncertainty is due to degradation rate changes among polymer types and environmental conditions which differ across studies (Gu, 2003; Kale *et al.*, 2015; Quecholac-Piña *et al.*, 2020). Conventional plastics do not significantly biodegrade in anaerobic conditions that are typical for oxygen-depleted landfills (Gómez and Michel, 2013; Quecholac-Piña *et al.*, 2020). Plastics show minimal degradation in medium-term landfill experiments suggesting that physical degradation of plastics in landfills requires at least 20 years to occur, which is commonly the timescale for microplastics to appear in landfill leachates (He *et al.*, 2019; Quecholac-Piña *et al.*, 2020). Further research on the degradation of conventional (i.e. non-biodegradable) plastics is needed in controlled, terrestrial settings, as there is a clear lack of literature on the subject. Plastics are being produced at a rate far faster than they are managed or degraded, and are subsequently becoming a common pollutant worldwide (Borrelle *et al.*, 2020).

Plastic pollutants found in the environment are typically classed according to their size. Macroplastics are defined as plastic pieces equal or larger than 5 mm (Lechthaler *et al.*, 2020), microplastics are plastic particles ranging between 1 µm and 5 mm (Frias and Nash, 2019), and nanoplastics are a subset of microplastics smaller than 1 µm, although debate around the definitions for both macroplastics and microplastics ensues (Lechthaler *et al.*, 2020; Shi *et al.*, 2024). This debate is caused by a lack of standardisation of size classification (Hartmann *et al.*, 2019). Some studies also sort plastics larger than 5 cm into various sub-categories, such as meso-, macro-, or mega-debris (Barnes *et al.*, 2009), but these categories also suffer from a lack of consensus, and have no biological justification for their size boundaries (Hartmann *et al.*, 2019). Due to lack of consensus, this study therefore refers to all plastic larger or equal to 5 cm as macroplastic, with no

specific upper limit. Microplastics can be classified as primary or secondary. Primary microplastics are manufactured to be smaller than 5 mm, such as microbeads in cosmetics (Cole *et al.*, 2011). Secondary microplastics are formed when larger plastic debris breaks down into smaller fragments (Cole *et al.*, 2011). This fragmentation results from a culmination of physical, biological and chemical processes reducing the structural integrity of plastic debris over time, causing the plastic to break into smaller pieces (Zhang *et al.*, 2021). The abundance of plastic debris combined with fragmentation has resulted in microplastics becoming ubiquitous pollutants in both terrestrial and marine environments (Thompson *et al.*, 2004).

The qualities that make plastic so desirable to humans also make them a plague on natural environments. Their durable design allow their extended persistence in the natural environment facilitating their widespread dispersal (Bergmann, Tekman and Gutow, 2017; Brahney *et al.*, 2021). Plastic pollution is highest around landfills, urban areas, and beaches, although agricultural ecosystems have also been significantly affected (Ng *et al.*, 2018; Wan *et al.*, 2019). In terrestrial environments, microplastics can contaminate soils via several pathways, such as composts, greenhouse and irrigation tools, municipal waste, and atmospheric inputs (Dris *et al.*, 2016; Bläsing and Amelung, 2018; Bradney *et al.*, 2019; Galafassi, Nizzetto and Volta, 2019). It has been estimated that in Europe alone 63,000 to 430,000 tons of microplastics are released annually to farmlands (Nizzetto, Futter and Langaas, 2016). Despite being identified as an emerging global threat, little is known about the amounts or accumulation rates of macro- and microplastic in terrestrial environments (Barnes *et al.*, 2009; De Souza Machado *et al.*, 2018; Dissanayake *et al.*, 2022). The environmental fate of microplastics in marine systems is comparatively well studied even though microplastic contamination on land might be 4 to 23-fold larger than in the ocean (Horton *et al.*, 2017).

Plastics were first recorded in the ocean in 1972 (Carpenter and Smith, 1972; Carpenter *et al.*, 1972), but only recognised as a serious issue at the turn of the millennium (Stefatos *et al.*, 1999). Before this point, marine plastics were dismissed as a problem because the oceans were thought to be too vast to be affected by their accumulation (Laist, 1987). However, plastic debris is now a rapidly growing global challenge, with the amount of plastics recorded in marine environments increasing (Ryan and Moloney, 1990; Moore, 2008; Savoca, McInturf and Hazen, 2021). The majority of marine debris is plastic, making up 60 - 80% of total marine debris along shores, in coastal waters, on deep sea floors, mangroves, and estuaries (Galgani *et al.*, 1995; Derraik, 2002; Thiel *et al.*, 2013; Willis *et al.*, 2017). Even remote locations, such as the Arctic (Walker *et al.*, 1997; Bergmann *et al.*, 2022) and deep sea trenches (Peng *et al.*, 2020; Abel *et al.*, 2023) have recorded the presence of plastic debris.

Buoyant plastics can be transported offshore and enter oceanic gyres, where they can accumulate in huge abundances (Howell *et al.*, 2012; Eriksen, Thiel and Lebreton, 2016). One such example in the North Pacific Subtropical Gyre, commonly referred to as the 'Great Pacific Garbage Patch', a rapidly expanding area of over 1.6 million km^2 that holds at least 79 thousand tonnes of plastic (Lebreton *et al.*, 2018). Marine plastic debris will likely be a persistent problem because plastic in the deep sea is estimated to take 292 years to degrade (Zhang and Peng, 2022). However, another model suggests that plastic coating may take around 400 years to break down in shallow coastal waters, and 800 years on the sea floor (Oluwoye *et al.*, 2023).

Plastics enter the marine environment through several sources. Land-based sources contribute ~ 80% of marine plastics recorded (Li, Tse and Fok, 2016) with 19–23 million metric tonnes of mismanaged waste entering water from land based sources annually (Borrelle *et al.*, 2020). Marine sources make up the other ~ 20% (Li, Tse and Fok, 2016). Land-based plastic inputs are greatest around industrialised areas (Pruter, 1987; Gregory, 1991). Land-based plastics are often transported to the marine environment by rivers, municipal wastewater, and drainage systems (Pruter, 1987; Williams and Simmons, 1997; Sheavly and Register, 2007; Browne, Galloway and Thompson, 2010; Kanhai, Asmath and Gobin, 2022). Coastal plastic debris often stems from beach-goers (Storrier *et al.*, 2007; Jayasiri, Purushothaman and Vennila, 2013), comprising of food packaging or waste generated from recreational activities (Pruter, 1987; Kuo and Huang, 2014). Most of the plastics recorded on South Africa's beaches are from land-based sources (Ryan, 2020; Weideman *et al.*, 2020). Fishery-related debris accounts for less than 5% of beach debris by number (12% by mass) in South Africa (Ryan, 2020).

Marine sources of plastics include the shipping and fishing industries and offshore drilling rigs (Pruter, 1987; Sheavly and Register, 2007). Fishing gear can become an ocean pollutant either through accidental losses or from illegal dumping (Horsman, 1982; Pruter, 1987). Nylon nets and monofilament lines often contribute a large proportion to coastal plastic debris (Kuo and Huang, 2014). It is widely cited that 640 000 Mg of discarded fishing gear enter the ocean annually and contribute 10% to all marine plastics. However, these figures are likely an overestimate (Richardson *et al.*, 2021). An older report indicates that 134 628 Mg of fishing gear were lost globally in the 1980s (Merrell Jr., 1980). Newer studies estimate that industrial trawl, purse-seine and pelagic longline fisheries contributed 48 400 Mg of lost fishing gear (not including purposely abandoned gear) towards plastic debris recorded in 2018 (Kuczenski *et al.*, 2022). Additionally, over 25 million fishing traps and pots are estimated to be lost to the ocean annually (Richardson *et al.*, 2022).

Besides the commercial fishing industry, recreational fisheries are thought to contribute 52% of all rubbish dumped in US waters (UNESCO, 1994). Plastic items found around densely populated shorelines are more likely to be from recreational and land-based sources (Ross, Parker and Strickland, 1991) while plastics at more remote sites are more likely to be shipping and fishing debris (Benton, 1995; Walker *et al.*, 1997; Santos, Friedrich and Barretto, 2005; Lebreton *et al.*, 2018; Monteiro, Ivar Do Sul and Costa, 2018). Several studies have noted that estimates of plastic amounts in the ocean are far higher than estimates of how much plastic has entered the ocean (Cózar *et al.*, 2014; Ryan, 2020). This suggests a major sink for marine plastics exists that we have yet to discover, or we are overestimating the amount of plastic in our oceans.

Plastic debris has been recorded in many coastal habitats such as beaches, estuaries, mangroves, coastal lagoons, rocky shores, and fjords (Scott, 1972; Blašković *et al.*, 2018; Martin, Almahasheer and Duarte, 2019; Bissen and Chawchai, 2020; Velez *et al.*, 2020; Carlsson, Singdahl-Larsen and Lusher, 2021). Coastal areas tend to have high population densities and tourist activity, making them susceptible to pollution and other anthropogenic impacts (Baztan *et al.*, 2014; Corcoran, 2015). Globally, plastic bottles are the most common plastic debris item found in coastal environments, followed by cigarettes and fishing gear (de Deus *et al.*, 2024). However, in the intertidal, plastic bags are typically the most common item recorded (Willoughby, Sangkoyo and Lakaseru, 1997; Thiel *et al.*, 2013). Microplastics account for about 60% of the total number of plastics found in coastal zones (de Deus *et al.*, 2024).

Since the 2000s, research investigating the presence and effect of micro (and smaller) plastics on marine environments and organisms has exploded in number (Karbalaei *et al.*, 2019). Microplastics have been found in large proportions in sediments, primary consumers, and faeces of secondary consumers in intertidal habitats (Lourenço *et al.*, 2017). There is additional evidence that supports microplastic transfer along intertidal food webs (Lourenço *et al.*, 2017). Microplastics have an array of effects on marine life. Microplastic exposure can have toxic effects on feeding, fecundity and survival in copepods; can induce oxidative stress and increase mortality in crustaceans; and can suppress growth and cause malformations in molluscs, along with a myriad of other effects on many taxa (Rist *et al.*, 2019; Gonçalves and Bebianno, 2021; Cássio, Batista and Pradhan, 2022; Chouchene *et al.*, 2023). The effects of microplastics and plastic leachates on marine life are well documented, and have been summarized in several reviews (Koelmans, Besseling and Foekema, 2014; Paul-Pont *et al.*, 2018; Gunaalan, Fabbri and Capolupo, 2020; Sharifinia *et al.*, 2020; Gonçalves and Bebianno, 2021; Doyle, Sundh and Almroth, 2022; Chouchene *et al.*, 2023; Junaid *et al.*, 2023; Leistenschneider *et al.*, 2023; Liu *et al.*, 2023; Marmara *et al.*, 2023; Parolini *et al.*, 2023; Qu *et al.*, 2023). Despite an

abundance of research on microplastics, the effects of macroplastics on marine invertebrate life are still largely unknown.

There is both lack of consensus as well as research gaps in literature surrounding the effects of plastic debris on ecosystem engineering bivalves. In particular, very little is known about the effects of macroplastics on ecosystem engineering bivalves, both at an individual and assemblage level (Hammer, Kraak and Parsons, 2014; Rochman *et al.*, 2016; Barboza *et al.*, 2019). This thesis therefore aims to address these knowledge gaps. In this thesis, I synthesise the current body of literature on the effects of plastics on bivalves. I also present results from an experimental investigation of the effects of macroplastic on mussels, an aspect that constitutes as apparent knowledge gap. My thesis consists of four chapters. This first chapter is a general introduction of the topic and overview of the thesis. Chapter 2 consists of a systematic review of the effects of micro and macroplastics on the physiology of bivalves and attributes of their associated communities. In Chapter 3, I present the results of a field experiment on aggregates of the invasive mussel *Mytilus galloprovincialis* and their associated rocky-shore communities, with the aim to enhance the understanding on the effects of macroplastics at a population and community level. I addressed this by answering the research question: What are the effects of different shapes and quantities of macroplastics on mussel performance indicators and on the abundance and composition of their epibiont and mobile faunal communities? Finally, Chapter 4 draws general conclusions from the previous chapters. Each of the two main chapters (2 and 3) are written as stand-alone studies and in a few instances content is repeated for clarity.

2 Chapter 2 – A review of the effects of plastic on bivalves

2.1 Introduction

Plastic poses a number of threats to marine life in the forms of entanglement, ingestion, and smothering. These effects have been reviewed extensively (Laist, 1987, 1997; Derraik, 2002; Gregory, 2009; Li, Tse and Fok, 2016; Barboza *et al.*, 2019; Jepsen and De Bruyn, 2019). Animals can become entangled in plastic packaging and netting and at least 914 marine species have reportedly been affected by either entanglement or ingestion (Laist, 1987, 1997; Quayle, 1992; Kühn and Van Franeker, 2020). There have been numerous entanglement and ingestion records of turtles, seabirds, cetaceans, and pinnipeds (Gramentz, 1988; Moser and Lee, 1992; Blight and Burger, 1997; Baird and Hooker, 2000; Bugoni, Krause and Virginia Petry, 2001; Jepsen and De Bruyn, 2019). All sea turtle species, 55% of all seabird species and 69.9% of all marine mammal species have been affected by plastic entanglement or ingestion (Kühn and Van Franeker, 2020). Several studies have looked at the effects of plastic entanglement (including ghost fishing) on turtles (Duncan *et al.*, 2017; Naidoo, Rajkaran, and Sershen, 2020), seabirds (Ryan, 2018), sharks (Cliff *et al.*, 2002), and mammals (Shaughnessy, 1980; Meÿer *et al.*, 2011). In total, 280 South African vertebrate species have been affected by entanglement and 54 by ingestion of plastics (Naidoo, Rajkaran, and Sershen, 2020).

Ingestion of plastics can occur directly from the water column or through the food chain (Beaumont *et al.*, 2019). Sea turtle, seabirds and various bony and cartilaginous fish species selectively ingest plastic objects, mistaking them for prey items (Azzarello and Van Vleet, 1987; Gramentz, 1988; Moser and Lee, 1992; Shaw and Day, 1994). Once plastic is ingested, it can cause internal damage or block the digestive tract, leading to death in extreme cases (Ryan, 1988, 2016). If large amounts of plastic are ingested, buoyancy can be affected through the low density of the plastic itself, and gas can build up from impaired digestive functioning. In turtles, over-buoyancy can prevent them from diving to obtain food, leading to starvation (Ryan, 2016). Turtles ingest plastic at all life-stages and often die from gut impaction and perforation (Wilcox *et al.*, 2018). Birds ingesting plastic fill their stomachs with non-nutritive matter, reducing their ability to lay fat and thus their fitness (Ryan, 1988). In sharks, however, plastic entanglement is more of a danger than ingestion, with one study finding that between 1978 and 2000, only ~ 0.38% of 15 500 sharks had ingested plastic (Cliff *et al.*, 2002).

Plastic ingestion in vertebrates is well documented, but there is growing concern of the effects of plastics on invertebrate organisms, particularly filter-feeders (Nowack and Bucheli, 2007; Gregory, 2009; Ryan, 2016). Marine invertebrates such as lobsters, sea cucumbers, lugworms, and amphipods

also frequently ingest both macro- and microplastics (Thompson *et al.*, 2004; Graham and Thompson, 2009; Murray and Cowie, 2011). Predatory or scavenging invertebrates accumulate plastics through their diet, as was found when lobsters had plastic fibres in their guts after eating contaminated fish (Murray and Cowie, 2011; Farrell and Nelson, 2013). Mobile invertebrates can experience various physiological effects from microplastic exposure. For example, consumption of microplastics negatively affects crustacean, copepod, oyster, and sea urchin reproduction (Sharifinia *et al.*, 2020; Doyle, Sundh and Almroth, 2022). Microplastic exposure can impact cell functioning in planktonic organisms and corals, reducing photosynthetic efficiency (Reichert *et al.*, 2019; González-Fernández *et al.*, 2020) and negatively affect the immune functions of mussels, sea urchins, coral, rag worms, and crabs (Sharifinia *et al.*, 2020).

Benthic invertebrates can be smothered by plastics that have settled on the seabed, reefs, or beaches (Gregory, 2009). Plastics on the seafloor can damage flora and fauna and create anoxic environments through the inhibition of gas exchange between sediment and the water column (Lewis *et al.*, 2009; Green *et al.*, 2015). Sessile invertebrates are highly at risk to plastic exposure as they cannot move away from contaminants and are often filter-feeders. Corals can be smothered by macroplastics, and are 89% more prone to disease when in contact with them (Yoshikawa and Asoh, 2004; Lamb *et al.*, 2018). Bivalves and many other filter feeders indiscriminately collect suspended matter using their gills, putting them at risk of collecting microplastics from the water column. Microplastics can accumulate in bivalve tissue after they are taken in during filter feeding (Ward, Rosa and Shumway, 2019; Bom and Sá, 2021; Sendra *et al.*, 2021; Saraswati, 2023). Accumulation of microplastics in bivalves tissue can lead to decreased filtration, feeding, larval development and fertility, amongst other sub-lethal effects (Zhang *et al.*, 2020; Sendra *et al.*, 2021; Li *et al.*, 2022; Mkuye *et al.*, 2022; Khanjani, Sharifinia and Mohammadi, 2023; Liu *et al.*, 2023; Rios-Fuster, Alomar and Deudero, 2023; Xu *et al.*, 2024). In addition to physical damage, plastics can release harmful chemicals such as monomers, additives, and persistent organic pollutants (Gandara e Silva *et al.*, 2016; Capolupo *et al.*, 2021). These leachates also have sub-lethal effects on bivalves. Some bivalves reduce their filtration rates during exposure to microplastics, influencing their ability to mitigate coastal eutrophication and harmful algal blooms (Rist *et al.*, 2016; Christoforou *et al.*, 2020; Abidli *et al.*, 2021; Hamm and Lenz, 2021). Although there are records of macroplastics being entangled in mussel beds (Weideman *et al.*, 2020), to date, there are very few manipulative studies that have examined the effects of macroplastics on bivalves, and most of these are on general benthic systems, not focusing on a single species (Devakie and Ali, 2002; Green *et al.*, 2015; Jang *et al.*, 2016; Clemente, Paresque and Santos, 2018, 2022; Sorini *et al.*, 2021). Given the growing amounts of

macroplastics in the environment, this constitutes a gap in knowledge that needs to be addressed as a basis for effective management actions.

Not only is marine life affected by the plastic they ingest, the plastic is also affected by being ingested. When some organisms eat plastics, they fragment the plastic during chewing and ingestion, creating multiple smaller fragments (Rambacher *et al.*, 2023). Mechanisms of plastic fragmentation by macrofauna include biting, drilling, grazing and grinding though their mouthparts, gizzard or gastric mill (So *et al.*, 2022; Rambacher *et al.*, 2023). Both marine vertebrates (e.g. parrotfish, pufferfish, turtles, Northern Fulmar) and invertebrates (e.g. crabs, isopods, sea urchins, bivalves) exhibit these behaviours (Cadee, 2002; Jenner *et al.*, 2003; Eriksen, Thiel and Lebreton, 2016; Hodgson, Bréchon and Thompson, 2018; Markic *et al.*, 2018; Porter, Smith and Lewis, 2019; Zheng *et al.*, 2023). Amphipods, detrivores which naturally ingest and shred organic matter, have been found to shred plastic carrier bags through chewing, creating numerous microplastics in the process (Hodgson, Bréchon and Thompson, 2018). Antarctic krill can ingest microplastics, which, though digestive fragmentation, are transformed into smaller nanoplastics (Dawson *et al.*, 2018). Bivalves, *Martesia striata* clams, have also been recorded to fragment plastics though boring into plastic piping (Jenner *et al.*, 2003). Marine organisms are thus important agents in the transformation of macroplastics into microplastics.

With the aim to highlight knowledge gaps, I critically review the effects of macroplastics, microplastics, and the co-exposure of microplastics and other pollutants on bivalve species in this chapter. Specifically, I summarise the various physiological effects of microplastics on bivalves to evaluate trends among studies, suggest best practices going forward and points of interest for further study. Additionally, this review examines the extent to which studies used environmentally relevant microplastic exposures, and whether these confounded results. Despite extensive study, there are still significant gaps in research surrounding the effects of marine plastic debris on ecosystem engineering bivalves. Recently, the focus on microplastics has led to a lack of attention on the effects of macroplastics on bivalve individuals and assemblages (Devakie and Ali, 2002; Hammer, Kraak and Parsons, 2014; Jang *et al.*, 2016; Rochman *et al.*, 2016; Barboza *et al.*, 2019; McCoy *et al.*, 2020; Li *et al.*, 2021; Sorini *et al.*, 2021). This literature review thus also aims to draw attention to the effects of macroplastics on bivalves, highlighting this area of understudy.

2.2 Methods

The primary aim of this review was to summarise and synthesise existing knowledge and determine trends in the large body of literature on the physiological effects of nano-, micro- and macro-plastics on bivalves. Specifically, this review seeks to:

(I) Summarise and synthesise the physiological effects (focusing on filtration, feeding, excretion, respiration, reproduction, and oxidative stress) of micro/nanoplastics on bivalves, including studies which found no effects;

(II) Summarise and synthesise the physiological and community-level effects of macroplastics on bivalves, including studies which found no effects;

(III) Summarise and synthesise the degree to which co-exposing micro/nanoplastics and other pollutants have synergistic physiological effects on bivalves, including studies which found no effects;

(IV) Determine whether studies use environmentally relevant micro/nanoplastic doses, and unpack why this is not always easy to determine;

(V) Put forward some limitations of the current state of literature and suggestions for future research.

A literature search was conducted with cutoff publication date until 1 June 2024 on Google Scholar to find literature on the physiological effects of plastic exposure on bivalves, using key words and Boolean logic. The following terms were used: (plastic OR microplastic OR macroplastic OR “plastic debris”) AND (bivalve OR mussel OR oyster OR clam OR scallop OR cockle) AND (effect OR filtration OR ingestion OR respiration OR reproduction OR “oxidative stress”)

An initial search in the Google Scholar database yielded 655 potentially relevant articles. Next, the title, abstracts, and methodologies of the articles were read, removing articles not within the criteria.

Microplastic studies were retained or excluded based on the following criteria: 1) literature investigating the physiological effects caused by micro/nanoplastic exposure in bivalves, excluding papers that only recorded the amount of microplastics accumulated in laboratory or field settings, 2) dissertations, theses, technical reports, summaries of congresses and symposiums, reviews, book chapters, and papers which were not peer reviewed were excluded, 3) studies which did not provide details on plastic type or dosage level were excluded, 4) studies which did in-vitro exposures on tissues instead of whole living organisms were excluded, 5) in manipulative studies, papers which did not include a “no plastic” control were excluded. Some exclusions were specific to the subsection 2.5

which focused on co-exposure and microplastics acting as vectors for pollutants. As the aim was to compare the effect of microplastics alone versus combined with other pollutants, 6) studies were excluded if they did not have treatments with either microplastic or pollutants by themselves (i.e. *not* in co-exposure), 7) studies were also excluded from section 2.5 if they used plastic leachates or plastic additives as their co-exposure pollutants, as the aim was determine the effects of plastics acting as vectors for external pollutants, not the effects of pollutants originating from plastics.

Macroplastic studies were retained if they: 1) examined at least one effect of macroplastic exposure on bivalve physiology, or 2) examined at least one effect of macroplastic exposure on community structure or abundance on communities containing bivalves.

A lower time bound was not assigned, but the earliest paper recovered that met all inclusion criteria was published in 2008. A total of 94 relevant publications were identified published from 2000 to 2024. Of these, 78 related to the effects of microplastics, and 16 to the effects of macroplastics. Identified papers were classified in relation to the following considerations for ease of analysis and creating summary tables: if research was experimental or observational, if research was conducted in laboratory or in field conditions, species of study organisms, plastic polymer used, concentration of plastic used, shape of plastic used, size of plastic used, length of exposure to plastic, and whether study organisms were considered at an individual or population level. Subsection 2.5 also classified the type of pollutant used and the concentration of the pollutant used. Throughout the chapter, "microplastic" refers to any plastic smaller than 5 mm and thus includes nanoplastics (<1 µm), except when otherwise specified.

2.3 Physiological effects of microplastics on bivalves

2.3.1 Filtration

Filter-feeders filter the water around them indiscriminately, yet particle ingestion is selective in many species (Ward and Shumway, 2004). This, combined with increasing levels of microplastics in marine environments mean bivalves are almost certain to filter microplastics along with natural particles (Everaert *et al.*, 2018). It has long been established that bivalves are capable of filtering microplastics, with earlier studies using microplastics to investigate feeding processes (Ward and Targett, 1989; Solow and Gallager, 1990; Cranford *et al.*, 1998; Richoux and Thompson, 2001). Ward, Rosa and Shumway (2019), reviews these early studies as well as preingestive interactions such as microplastic particle capture and selection. A review by Khanjani, Sharifinia and Mohammadi (2023), focuses on how microplastic exposure decreases bivalves' filtration rates, but makes no mention of studies that found no effect. A recent review focused on how microplastics affect the feeding

behaviour of commercially important bivalves (Xu *et al.*, 2024). Despite several reviews on the topic, there is still no general agreement on the effects on microplastic exposure on the filtration rate of bivalves.

Trends emerging from studies which explore the effects of microplastics on the filtration rates of bivalves are highly dynamic and no consensus has been reached on the general effects (Table 1). All studies which explored the effects of microplastics on bivalve filtration rate took place in laboratory settings. Many studies that examined the effect of microplastics on bivalves' filtration found that exposure to microplastics had no effect (Table 1). An almost equal number found microplastic exposure decreased the filtration rate (Table 1). Two studies even found filtration rate to increase after microplastic exposure (Sussarellu *et al.*, 2016; Green *et al.*, 2017). Pedersen *et al.* (2020) reported a negative relationship between the filtration rate and the number of microplastic particles accumulated in (Quagga) mussel gills. They proposed several mechanisms underlying this pattern: 1) mussels reduced feeding upon recognising that the particles were non-edible, 2) physical filtration was slowed by either physiological or internal hinderance, or 3) consumption of microplastics resulted in faster satiation than just the algal food source. Rist *et al.* (2016) found that the mussel *Perna viridis* decreased both its filtration and respiration rates after exposure to microplastics (PVC, 1–50 µm) and thought the response was caused by particle-induced valve closure to prevent injury or blockage.

Some studies found that bivalves filtration rates were affected after both acute and chronic exposures with no deducible patterns linked to length of exposure. A study was considered chronic if exposure lasted at least four weeks as this is the time at which exposure studies on bivalves begin to identify themselves as long-term, presumably due to the exposure time relative to the life history of bivalves (Bour *et al.*, 2018; Choi *et al.*, 2022, p. 20; Jiang *et al.*, 2022). Several studies exposed bivalves to multiple concentrations of microplastics. Roughly half found concentration had no effect (Green, 2016; Green *et al.*, 2017; Harris and Carrington, 2020; Hamm *et al.*, 2022; Joyce and Falkenberg, 2022; Walkinshaw *et al.*, 2023). Of the studies that found concentration did have an effect, one found that filtration rate increased at higher concentrations of microplastic (Fernández Severini *et al.*, 2019) and the others found that filtration rate decreased with increasing microplastic concentration (Wegner *et al.*, 2012; Rist *et al.*, 2016; Abidli *et al.*, 2023), or that microplastic only had an affect at higher concentrations (Xu *et al.*, 2017; Hamm and Lenz, 2021; Abidli *et al.*, 2023). Distinguishing what might have caused these varying results is made difficult by the different units of microplastic concentration used between studies precluding direct comparisons.

Some studies have compared the effects of microplastics and natural particles of the same size on bivalve filtration rate. Harris and Carrington (2020) found that while the filtration rates of *Mytilus trossulus* mussels were not affected by acute exposure to silt of high (1876-2500 particles/ml) and high-medium (1251–1875 particles/ml) levels, they were negatively affected by microplastics of similar size (32–38 µm, PE) at the same concentration. However, a study which compared filtration rates when exposed to microplastic and similar sized natural particles (clay and diatom shells) found that filtration rate did not differ between the treatments (Hamm *et al.*, 2022). Similarly, Fernández and Albentosa (2019) found that there was no difference in the filtration rate when *M. galloprovincialis* filtered polyethylene (PE) particles (4-6 um) or algae (*Isochrysis galbana*) of similar size, and that mussels cleared both particle types faster at higher concentrations. Harris and Carrington (2020) proposed that microplastics reduced the filtration rates when silt did not due to their unique surface properties (Ward and Shumway, 2004; Rosa, Ward and Shumwy, 2018; Ward, Rosa and Shumway, 2019). Further comparative studies would need to be done to confirm this as bivalves otherwise seem to find microplastic as acceptable for ingestion as natural particles (Fernández and Albentosa, 2019; Hamm *et al.*, 2022).

Trends between, and even within, studies are highly dynamic, making it difficult to generalise or point to patterns caused by potentially important factors such as particle size, type and concentration, or exposure time. There is potentially a pattern linked to study species, as all investigated clam species' filtration rates decreased after microplastic exposure (Table 1). Additionally, Green *et al.* 2017), exposed *Mytilus edulis* mussels and *Ostrea edulis* oysters to identical microplastic conditions (Table 1) but found that after 50 days the exposed mussels were filtering 2.4 times *less* (algal cells/dry mussel mass/ hour) than unexposed mussels, and that the exposed oysters were filtering 7.5 times *more* than unexposed oysters. It is important to understand the mechanisms underlying reduced filtration in relation to microplastics, as a decreased filtration rate hinders bivalves' ability to gain sufficient food, potentially impacting their fitness if prolonged. Exposure to and ingestion of microplastics can negatively impact bivalves' ability to grow, function, and reproduce; these impacts are further explored in the following sections of this review (Sussarellu *et al.*, 2016; Webb *et al.*, 2020; Walkinshaw *et al.*, 2023).

Table 1: Studies exploring effects of microplastics on the filtration ability of bivalves. All effects are statistically significant and relative to controls without microplastic exposure unless otherwise stated.

Reference	Species	Type of plastic polymer	Methods	Highlights
Abidli *et al.*, 2021	*Mytilus galloprovincialis*	Polyethylene (ultra-high molecular weight), 40–48 µm. Shape unstated.	Individual adult mussels were exposed to varying concentrations (1 µg/l, 10 µg/l, 100 µg/l, and 1000 µg/l) of plastic for 14 days. Filtration rate and measures of oxidative stress were measured after 7 and 14 days.	Filtration rate decreased after 7 days for 10µg/l, 100 µg/l and 1000 µg/l and after 14 days for 100 µg/l and 1000 µg/l concentrations.
Abidli *et al.*, 2023	*Ruditapes decussatus*	Polyethylene (ultra-high molecular weight), 40–48 µm. Shape unstated.	Individual adult clams were exposed to varying concentrations of plastic (10 µg/l , 100 µg/l , and 1000 µg/l for 14 days. Filtration rate, growth rate and the integrity of immune cells were measured after 14 days.	Filtration rate decreased with increasing plastic concentration. However, only the filtration rate at 1000 µg/l (14.12 ± 2.31 ml/ animal/h) was lower than the control with no plastic (38.33 ± 7.59 ml/animal/h).
Barkhau *et al.*, 2022	*Semimytilus algosus*	Polyvinyl chloride (PVC) powder, 12.08 ± 13.65 µm; polymethyl methacrylate (PMMA) powder, 120.3 ± 69.92 µm. Red clay, 13.84 ± 14.55 µm; celite, 86.8 ± 90.37 µm.	Individual mussels were exposed to either PVC, PMMA, red clay, or celite at either 1.5, 15 or 150 mg/l for 68 days. After exposure the body condition index, respiration rate, clearance rate, byssus production, byssus strength and mortality were measured.	The clearance rate was highly variable and did not differ from the control for any treatment.
Baudrimont *et al.*, 2020	*Corbicula fluminea*	Polyethylene: Reference (PER) and collected from North Atlantic Gyre (PEN), reference was 350 µm, both were powders.	PEN plastics were created by collecting environmental plastics using manta nets then crushing them into a powder. Individual mussels were exposed to 1000 µg/l of either PEN or PER in the presence of *Scenedesmus subspicatus* algae (1×10^6) for 48 hours. After exposure their filtration rates and faeces production were measured.	Neither reference (PER) nor environmentally sourced (PEN) plastics had an effect on filtration rates.

Browne *et al.*, 2008	*Mytilus edulis*	Polystyrene (PS) spheres, either 3 µm or 9.6 µm.	Mussels were exposed to 42 857 particles/l of either 3 or 9.6 µm PS for 3 hours. Followed by a 48-day depuration period. After exposure, and on days 3, 6, 12, 24, and 48 of depuration, cell viability, oxidative stress, and clearance rate were measured. Sections of the midgut and circulatory system were examined for microplastics, as well as the haemolymph.	There was no effect on the clearance rate of the mussels.
Fernández and Albentosa, 2019	*Mytilus galloprovincialis*	Non-uniform HDPE, <22 µm, mean particle size of 4-6 µm.	Individual mussels were exposed to either plastic or *Isochrysis glabana* algae (spherical, 2-8 µm) at either 2 mm^3/l (low) or 4 mm^3/ (high) for 4 hours. They were then kept in clean water for a 144 hours (6 days) depuration period. The uptake, accumulation, and elimination of particles in the digestive gland were measured.	There was no difference in the clearance rate between plastic and algae. Clearance rate was higher for both the higher concentration of algae and plastic.
Goncalves *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 5, 6 and 10 µm diameter.	Individual mussels were exposed to 6 µm and 10 µm plastics (single and combined) at 1000 particles/ml for 90 min. Clearance rates were measured.	Mussels rapidly filtered both sized of plastic; after 20 minutes 40% and 60% of the 6 µm and 10 µm plastics were removed, respectively.
González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polyester (PS) spheres, 0.5 µm or 4.5 µm. Alone or sorbed with benzo(a)pyrene (BaP).	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experiments. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastic concentration was 0.058 mg/l (corresponding to 1000 particles/mL for 4.5 µm MPs and to 7.44 × 10^5 particles/mL for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	Clearance rate was highly variable and did not differ from the control for any treatment after either 7 or 26 days of exposure.

Green, 2016	*Ostrea edulis*	Polylactic acid (PLA, biodegradable) and conventional HDPE, 65.6 µm. Shape unstated.	Mesocosms were created by collecting intact cores containing sediment and algae from lower intertidal and shallow intertidal zones. Two adult oysters were placed in each mesocosm and exposed to either low (0.8 µg/l) or high (80 µg/l) plastic concentrations of either PLA or HDPE for 60 days. The filtration, respiration, and growth rates were measured as well as changes to the assemblage structures.	There was no difference in the filtration rate among treatments.
Green *et al.*, 2017	*Mytilus edulis* and *Ostrea edulis*	Polylactic acid (biodegradable), 65.6 µm; HDPE, 102.6 µm. Shape unstated.	Mesocosms were created by collecting intact muddy sediment cores. Each mesocosm contained either 7 mussels or 2 oysters to simulate natural densities. Bivalves were exposed to either low (2.5 µg/l) or high (25 µg/l) plastic concentrations of either PLA or HDPE for 50 days. The filtration rates were measured after exposure as well as ammonia, diatom biomass, and cyanobacteria biomass in the sediment. Changes to the assemblage structure were also measured.	Mussels filtered 2.4 times less microalgae per hour than unexposed mussels when exposed to either plastic type at 25 µg/l. Oysters filtered 7.5 times more microalgae per hour than unexposed oysters for both plastic types and concentrations.
Hamm and Lenz, 2021	*Mytilus edulis* juveniles	Polyvinylchloride (PVC) powder, 11-60 µm; PS beads, 40 µm.	Individual mussels were exposed to PVC at 15, 1500, 15 000, 150 000, or 1 500 000 particles/individual/week or to PS at 15, 1500, or 15 000 particles/individual/week for 42 weeks. Plastics were renewed once a week. Clearance rate, growth, and byssus production were measured ever sixth week during exposure, up to week 36. Condition index was measured after 16, 32, and 42 weeks. Measures of oxidative stress were taken after exposure.	After 36 weeks the clearance rates of mussels exposed to the highest concentration of PS were lowered.

Hamm *et al.*, 2022	*Brachidontes puniceus, Semimytilus algosus, Brachidontes pharaonis, M. trossulus, Mytilus galloprovincialis*	Polymethyl methacrylate (10-400 µm) and PVC (0.1-100 µm) VS red clay (0.1-100 µm) and diatom shells (4-400 µm). Shape unstated.	Individual mussels were exposed to one of four particle suspensions, each of which had three concentrations (1.5 mg/l, 15 mg/l and 150 mg/l) for 6 weeks. After exposure the clearance and respiration rates were measured as well as byssus production, condition index, and survival.	Clearance rate was not affected by any of the particle types nor particle concentrations.
Harris and Carrington, 2020	*Mytilus trossulus*	Polyethylene spheres, 32–38 µm. Algae *Dunaliella* sp., 10-20µm. Silt 30-37 µm.	Mussels were exposed to algae, algae + PE, or algae + silt. The treatment that had only algae had 4000–25,000 cells/ml, the other two treatments had 7000–12,000 cells/ml of algae. In addition to algae, two treatments added either silt or PE at concentrations of: low (1–625 particles/ml, low–med (626– 1250 particles/ml), high–med (1251–1875 particles/ml), and high (1876–2500 particles/ml). All exposure treatments lasted for 1 hour, during which water samples were taken every 15 minutes.	Clearance rate was affected by particle type but not concentration alone (there was an interaction between particle type and concentration). Clearance rate decreased by 50 % and 62% at med-high and high PE concentrations compared to algae alone. Silt had no effect at any concentration. Clearance rate was highly variable when exposed to just algae but was unaffected by concentration.
Joyce and Falkenberg, 2022	*Perna viridis*	Polyethylene terephthalate (PET) and biodegradable PLA, silver glitter, 200 µm, hexagon in shape.	Individual mussels were exposed to either normal or biodegradable plastic at either low (17-20 particles/l) or high (135-140 particles/l) amounts for 4 weeks. Plastics were delivered in 2-hour pulses daily. After exposure, the clearance and oxygen consumption rates, condition indexes and mortalities were measured.	Clearance rate was unaffected by plastic presence, type, and concentration. Low PET concentrations produced lower clearance rates than high PET concentrations, but neither differed from the control.
Moreschi *et al.*, 2020	*Anodontites trapesialis*	Polyethylene spheres, 55 - 110 µm.	Bivalves were exposed to 0.075 g/l of MPs for between 3 - 192 hours. Mussels were collected throughout the exposure period and assessed for the amounts of plastic they had filtered, assimilated, and eliminated.	There was a strong positive correlation between time and the weight of microplastics filtered by mussels. .

Pedersen *et al.*, 2020	*Dreissena bugensis*	Red HDPE powder, 10 - 45 µm.	Six mussels per tank were exposed to plastic at either 0.1 g/l, 0.4 g/l, and 0.8 g/l for 3 days. During and after exposure, filtration and oxygen consumption rates were measured. Survivorship and reproduction rates were also measured.	Microplastics impacted filtration rates in inconsistent ways. After day one, filtration rate was increased by the highest MP concentration. After day two, filtration decreased for all concentrations by 27%. Filtration rates were unaffected by any treatments by the end of the third day.
Revel *et al.*, 2019	*Mytilus edulis*	Polyethylene and polypropylene (PP) powder, <950 µm, mean sizes 287 µm (PE) and 204 µm (PP).	Mussels were exposed to a mixture of PE and PP at either 0.008 µg/l (low), 10 µg/l (medium), or 100 µg/l (high) for 10 days. Plastic doses were renewed daily. After exposure there was a 10 day depuration period. Physiological conditions of mussels were measured before exposure. Biodeposits were measured daily. After exposure, clearance rate, tissue structure, antioxidant defences, immune and digestive parameters, and DNA integrity were investigated, and plastic particles were identified in mussel tissues. The clearance rate and plastic content in tissues were also measured after depuration.	Clearance rate was unaffected by exposure and did not differ after the depuration period.
Rist *et al.*, 2016	*Perna viridis*	PVC submersed in fluoranthene-contaminated seawater for 24 days, 1-50 µm, shape unstated.	Mussels were exposed to either 21.6, 216, or 2160 mg/l (equivalent to 1.2 x 10^7, 1.2 x 10^8, and 1.2 x 10^9 particles/l, respectively) for 91 days. Plastic doses were renewed once a week. Response variables were measured at the start of the experiment and after 40-44 day. Filtration and oxygen consumption rates were measured as well as byssus production and survival.	Clearance rate decreased with increasing plastic load. At the highest and second highest concentrations, clearance rates were decreased by 79% and 41%

Santana *et al.*, 2018	*Perna perna*	Polyvinyl chloride powder, 0.1 µm – 1.0 µm.	Mussels were exposed to 0.125 g/l (equivalent to 1.115×10^{10} particles/l) PVC for 90 days. Plastics were replenished three times a week. After exposure, the clearance rate, absorption efficiency, growth rates, signals of cellular and molecular stress, and health condition (mortality, condition index, lysosomal integrity, LPO, and DNA damage) were measured.	Clearance rate was unaffected by MP exposure.
Sıkdokur *et al.*, 2020	*Ruditapes philippinarum*	Red fluorescent PE beads, 10-45 µm. Either virgin, co-exposed with mercury chloride, or pre-inoculated with mercury chloride.	Some plastics were pre-contaminated by mixing PE and Hg stock solution together for 96 hours. Clams were exposed for 7 days to: Hg alone at 10 µg/l, PE alone at 25 µg/l, co- exposure with 10 µg/l Hg and 25 µg /L PE, or 25 µg /L pre-contaminated PE. After exposure, the uptake and tissue distribution of PE and Hg were measured in addition to filtration rates, immunomodulation, oxidative stress, and histological alterations.	Filtration rates for all exposure treatments were lower than the control, however, exposure treatments did not differ from each other.
Sussarellu *et al.*, 2016	*Crassostrea gigas*	Yellow-green virgin PS spheres, 2 and 6 µm.	Adult oysters were exposed to 0.023 mg/l of plastics for 2 months during a reproductive cycle. During and after exposure, various ecophysiological parameters were measured; cellular, transcriptomic, and proteomic responses; fecundity; and offspring development. Plastic ingestion, algae consumption, and absorption efficiency were also measured.	Algal consumption (daily clearance) was increased in exposed oysters.
Walkinshaw *et al.*, 2023	Juvenile *Mytilus*	Red polyester (PE) fibres, mean length 293.5 µm; yellow cotton fibre, mean length 171.5 µm. Both 10–500 µm in length.	Juvenile mussels were exposed to either 8 fibres/l PE, 80 fibres/l PE, or 80 fibres/l cotton for 94 days. During exposure the clearance and respiration rates were measured, as well as shell length and mortality.	Clearance rate decreased over the experimental period for all treatments. Clearance rates varied greatly within exposure treatments. Both plastic and cotton groups had timepoints when they differed from the control, sometimes being higher and other times lower. After day 58 there was no difference in the clearance rate between treatments.

Wang, Hu, *et al.*, 2021	*Mytilus coruscus*	Polystyrene spheres of 0.07, 0.5, 5, 10 and 100 µm.	Mussels were exposed to one of five sizes of plastics at 0.2 mg/l for 3, 15 or 87 hours. This was followed by an 87 hour depuration period. Plastic intake and accumulation of particles in gill, digestive tract and mantle were measured after exposure and the depuration period.	Intake ratio (based on clearance rate) was higher after 12 hours of exposure than 3 hours. After 12 hours, 100 µm plastics were taken up less than the other sizes.
Wang, Zhong, *et al.*, 2021	*Mytilus coruscus*	Polystyrene, 70 nm and 10 µm. Shape unstated.	Mussels were exposed 0.20 mg/l of either 70 nm or 10 µm plastics for 2 weeks. Mussels were either exposed to plastics mixed with food (algae) or plastics alone (fed at an earlier time). Plastic intake was measured during and after exposure. After exposure the clearance rates, respiration rates, absorption efficiency, enzyme activity and oxidative responses were measured.	Clearance rate did not differ from the control for any treatment. However, clearance rate was higher when exposed to 10 µm plastic alone than when co-exposed with algae.
Webb *et al.*, 2020	*Perna canaliculus*	Polyethylene and triclosan spiked PE beads, 38–45 µm.	Adult mussels were exposed to either PE, triclosan, or triclosan spiked PE for 48 hours. Plastics were at a 0.5 g/l and triclosan at 0.36 mg/l. After exposure the clearance and oxygen consumption rates were measured as well as byssus production and oxidative stress.	No treatment affected the clearance rate.
Wegner *et al.*, 2012	*Mytilus edulis*	Polystyrene, 30 nm. Shape unstated.	Individual mussels were exposed to different combinations of algae and plastic for 8 hours. At plastic concentrations of 0.1 g/l the algae amounts were 0, 60,000, and 120,000 cells/ml. At plastic concentrations of 0.2 and 0.3 g/l, 60 000 cell/l of algae were used. Filtration rate was measured during exposure.	Filtering activity (number of mussels with open valves) decreased with increasing plastic concentration. Filtering activity did not differ with algal concentration.
Xu *et al.*, 2017	*Atactodea striata*	Polystyrene granules, 63 µm - 250 µm.	Mussels were exposed to plastics at either 10 particles/l or 1000 particles/l for two weeks, followed by a 7 day depuration period. Clearance and filtration rate were measured along with absorption efficiency and faeces production.	Clearance rate was decreased by the high concentration of PS.

2.3.2 Ingestion, absorption, assimilation, accumulation, and depuration

Bivalves often ingest microplastics from the water column, which increases their vulnerability to harm (Ward, Rosa and Shumway, 2019; Table 2). Many studies have recorded bivalves ingesting and accumulating microplastics in their tissues (Table 2). Several reviews have examined and summarised the literature from different angles: Bom and Sá, (2021) focused on the concentration of microplastics found in bivalves; Li *et al.* (2019) looked at the ability of mussels to ingest and accumulate microplastics and their suitability as bioindicators; Liu *et al.* (2023) focused on the nature and concentration of microplastics found in oysters, as well as their depuration time; Ward, Rosa and Shumway (2019) and Sendra *et al.* (2021) examined the mechanisms for ingestion and elimination of microplastics by bivalves; and Khanjani, Sharifinia and Mohammadi (2023) provides an extensive summary table of concentrations of microplastics found in mussels. Xu *et al.* (2024) also provides an extensive summary on microplastics found in commercially important bivalve species.

Whilst many publications have recorded bivalves ingesting microplastics, fewer have focused on whether microplastic exposure affects the feeding ability of bivalves or if microplastic type, size and concentration may cause these effect to vary (Cole and Galloway, 2015; Sussarellu *et al.*, 2016; Xu *et al.*, 2017; Cole *et al.*, 2020; Pedersen *et al.*, 2020; Li *et al.*, 2021; O'Brien *et al.*, 2021; Trestrail *et al.*, 2021; Wang, Hu, *et al.*, 2021). It is important to understand these impacts because reduced filtration and feeding rates can cause a decline in energy intake, potentially impacting bivalve growth and survival (Jiang *et al.*, 2022). The results of studies on the effects of bivalve feeding vary greatly and, once again, have no discernible trends. For example, one study found microplastic ingestion to increase with increasing microplastic concentration, however three others, including one that used a 10-fold higher microplastic concentration, found no difference (Cole and Galloway, 2015; Gardon *et al.*, 2018; Pedersen *et al.*, 2020; Weber *et al.*, 2021). Microplastic size has similar variability; one study found two sizes of microplastic (5 µm, 10 µm) to equally decrease ingestion rate, another found ingestion decreased with increasing microplastic size (0.07, 0.5, 5, 10 and 100 µm), and a third found bivalves to preferentially ingest 6 µm microplastics over 2 µm microplastics (Sussarellu *et al.*, 2016; Wang, Hu, *et al.*, 2021; Jiang *et al.*, 2022). Bivalve species, polymer type, and exposure time also provide no insight to differences among the results. It is unsurprising that the trends among ingestion are similarly dynamic as those among filtration, as the two processes are closely linked.

Changes to ingestion rate are not the only way microplastic exposure affects the feeding of bivalves. Other measures, such as digestive enzyme activity and food absorption efficiency, also have mixed results with no clear cause for the discrepancies. Changes in digestive enzyme activity can alter the

assimilation efficiency of bivalves, affecting the amount of energy they gain from food (Karasov and Douglas, 2013; Wang *et al.*, 2023). Several studies demonstrated that microplastics negatively affect the ability of bivalves to digest starch as indicated by decreased amylase activity after acute microplastic exposure (X. Wang *et al.*, 2020; O'Brien *et al.*, 2021; Trestrail *et al.*, 2021; Xu *et al.*, 2024). Notably, Trestrail *et al.* (2021) found amylase activity to decrease only after exposing *M. galloprovincialis* mussels to PS and found no decrease with PE exposure was detected. Similarly, Revel *et al.* (2019) found no effect on amylase activity after exposing *M. edulis* mussels to a mixture of PE and PP. Another study found amylase to increase at high microplastic levels (110 000 particles/l) when co-exposed to food, but to decrease when co-exposed with low levels of microplastic (55 000 particles/l) (O'Brien *et al.*, 2021).The effects of microplastics on other digestive enzymes such as cellulase, protease, lipase, pepsin, trypsin, laminarinase, and lipolytic esterases have also been investigated (X. Wang *et al.*, 2020; Trestrail *et al.*, 2021; Wang *et al.*, 2023; Lu *et al.*, 2024). These proteins were also affected depending on microplastic presence, polymer, size, and concentration (Table 2). There is some indication that polymer type may be important, as cellulase activity increased and amylase activity decreased when exposed to PS, but not PE (Revel *et al.*, 2019; Trestrail *et al.*, 2021; Wang *et al.*, 2023). Cellulose upregulation may be due to PS, or its chemical leachates, resembling the substrate (substance upon which an enzyme acts) at the molecular level, causing the epithelial cells in the digestive system to be stimulated and produce digestive enzymes (Mathers, 1973; Trestrail *et al.*, 2021). Amylase activity may be reduced due to epithelial cells being damaged chemically or physically or due to chemical interference with the substrate detection mechanism (Trestrail *et al.*, 2021).

Microplastics can also affect bivalve absorption efficiency. Absorption efficiency is the efficacy of organic matter absorbed from ingested material. Microplastic exposure has been reported to have no effect, (Xu *et al.*, 2017; Santana *et al.*, 2018; Tallec *et al.*, 2021) decrease, (Gardon *et al.*, 2018; Wang, Zhong, *et al.*, 2021; Jiang *et al.*, 2022) or increase the absorption efficiency of bivalves (Sussarellu *et al.*, 2016; González-Soto *et al.*, 2019). Decreased absorption efficiency may result from reduced digestive enzyme activity or physical damage to the digestive gland due to microplastics (Sıkdokur *et al.*, 2020; X. Wang *et al.*, 2020; Jiang *et al.*, 2022). Increases in absorption efficiency were explained by a need to compensate for increased energy requirements due to stress caused from cellular and tissue damage from the microplastic exposure (Sussarellu *et al.*, 2016; González-Soto *et al.*, 2019). Trends on the effects of absorption efficiency are difficult to discern as there are limited studies to compare, all with highly varying results. It is possible that these differences are caused by the various model species, however, further studies would need to be done to confirm this. Among studied bivalves, both oyster species were affected; the two *Mytilus* mussels were

affected while the *Perna* mussels was unaffected; and one clam species was affected while the other was not (Table 2). The kind of microplastic polymer used may be playing a role in the variable results. All studies that used PS (except for one) found absorption efficiency to be affected, and the only study that used a polymer besides PS found no effect. This is supported by the fact that PS leaches the highest amount of chemical compounds compared to PP and PE (Biale *et al.*, 2022). However, more studies would need to be done to confirm whether polymer type influences the effects of microplastic exposure.

Co-exposing mussels to microplastic and food (algae) seems to mitigate the negative effects caused by the microplastic (Wang, Zhong, *et al.*, 2021; Weber *et al.*, 2021). *Dreissena polymorpha* mussels ingest fewer microplastics when they are fed algae at the same time as microplastic exposure than if they are exposed to microplastic alone (Weber *et al.*, 2021). Additionally, clearance rate was higher and absorption efficiency lower in *Mytilus coruscus* mussels when exposed to just microplastics, but mussels co-exposed to food were unaffected (Wang, Zhong, *et al.*, 2021). Co-exposure to food does not only affect feeding mechanisms, as respiration rates were significantly higher for *M. corusus* when exposed to just microplastic than when co-exposed with food (Wang, Zhong, *et al.*, 2021). Wang *et al.* (2021) proposed that the presence of food helped to reduce the physiological effects caused by the microplastics by interacting with the plastics and masking their characteristics. Scanning electron microscope images confirmed the alteration to the surface functional groups of the microplastic after mixing with algae in support of this. These surface alterations were suggested to be caused by a layer of biofilm, as biofilms are known to change the physical and chemical properties of microplastics (Jacquin *et al.*, 2019; Wang, Zhong, *et al.*, 2021).

Bivalves are capable of particle discrimination allowing them to bind unwanted particles in mucus and expel them as pseudofaeces without passing through the digestive tract (Ward *et al.*, 1994; Beninger, Dufour and Bourque, 1997; Ward and Shumway, 2004). Despite this ability, bivalves ingest and accumulate microplastics in their intestinal tracts, digestive cavity, and gills in natural and experimental conditions (Browne *et al.*, 2008; Goncalves *et al.*, 2019; Moreschi *et al.*, 2020; Pedersen *et al.*, 2020; Webb *et al.*, 2020; Jiang *et al.*, 2022). This accumulation has been extensively studied (Davidson and Dudas, 2016; Fernández and Albentosa, 2019; Bom and Sá, 2021; J. Li *et al.*, 2021). Bom and Sá (2021) found that 70 bivalve species had been recorded to accumulate microplastics, with studies primarily focusing on the mussels *Mytilus* spp. and the oysters *Crassostrea* spp. They noted that the lack of standardized methodologies in tissue digestion and microplastic identification limits the direct comparisons among studies. Accumulation can occur rapidly after bivalves are exposed to microplastics, highlighting their vulnerability, as shown for *M.*

edulis mussels, which accumulated PE microplastics (powder, > 0−80 µm) in the digestive system within hours of exposure (Von Moos, Burkhardt-Holm and Köhler, 2012). Beyond ingestion, microplastics are also taken up by the gills during respiration and adhere to bivalve soft tissue (Kolandhasamy *et al.*, 2018; Z. Li *et al.*, 2020).

Bivalves can expel most (90-100%) microplastics after a depuration period of several days, but some have been observed to remain in tissues for over six weeks (for a review see Xu *et al.* (2024)). Fernández and Albentosa (2019) found that after six days of depuration, mussels had expelled ~85% of microplastics accumulated but between 2 - 6% of the microplastic remained in the digestive gland. Xu *et al.* (2017) however, found no significant decrease in the amount of microplastics harboured by *Atactodea striata* clams after a 7-day depuration period. There is great variability in depuration efficiency with regards to study species, microplastic polymer, or microplastic shape. Any patterns are also obscured by the fact that both microplastic exposure and depuration time differ between studies, making comparisons difficult.

Mussels collected from the wild or open-sea aquaculture farms (which are only expelling microplastics they accumulated in the environment) have lower depuration efficiencies than those exposed to microplastics in laboratory settings (Van Cauwenberghe and Janssen, 2014; Birnstiel, Soares-Gomes and da Gama, 2019). Microplastics accumulated in farmed *M. edulis* and *Crassostrea gigas* decreased by 33% and 25% respectively after 3 days of depuration. Meanwhile, wild and farmed *P. perna* displayed 47% and 29% decreases respectively after 4 days of depuration (Van Cauwenberghe and Janssen, 2014; Birnstiel, Soares-Gomes and da Gama, 2019).

This possibly reflects that wild grown mussels are exposed to chronic low levels of microplastics compared to laboratory-exposed bivalves, which are given acute (but usually higher than environmental levels) exposures to microplastic. It may thus be worth investigating further if acute exposures are promoting an inaccurate image that bivalves can rapidly rid themselves of microplastic after exposure. Other studies showed that microplastic PS particles can rapidly move from the gut to the circulatory system of mussels, persisting there for over 48 days (Browne *et al.*, 2008). Larger microplastics are removed more readily than smaller microplastics, with smaller microplastics being more readily ingested and retained for longer (Von Moos, Burkhardt-Holm and Köhler, 2012; Van Cauwenberghe and Janssen, 2014; Al-Sid-Cheikh *et al.*, 2018; Fernández and Albentosa, 2019; Kinjo *et al.*, 2019; Wang, Hu, *et al.*, 2021; Jiang *et al.*, 2022). The longer it takes for microplastics to be expelled from an organism, the greater the potential of adhered contaminants to desorb and cause harm (Avio, Gorbi and Regoli, 2017; Fernández and Albentosa, 2019). Additionally, the longer microplastics are retained, the higher the likelihood for trophic transfer of the

microplastics to predatory species (Carbery, O'Connor and Palanisami, 2018; Fernández and Albentosa, 2019).

Table 2: Effects of microplastic on accumulation, feeding, and expulsion in bivalves. All effects are statistically significant and relative to controls without microplastic exposure unless otherwise stated.

References	Species	Type of plastic polymer	Methods	Highlights
Abidli *et al.*, 2021	*Mytilus galloprovincialis*	Polyethylene (ultra-high molecular weight), 40–48 µm. Shape unstated.	Individual adult mussels were exposed to varying concentrations (1 µg/l, 10 µg/l, 100 µg/l, and 1000 µg/l) of plastic for 14 days. Filtration rate and measures of oxidative stress were measured after 7 and 14 days.	There was no accumulation of MPs in soft tissues at any plastic concentration.
Baudrimont *et al.*, 2020	*Corbicula fluminea*	Polyethylene: Reference (PER) and collected from North Atlantic Gyre (PEN), reference was 350 µm, both were powders.	PEN plastics were created by collecting environmental plastics using manta nets then crushing them into a powder. Individual mussels were exposed to 1000 µg/l of either PEN or PER in the presence of *Scenedesmus subspicatus* algae (1×10^6) for 48 hours. After exposure their filtration rates and faeces production were measured.	There was an initially decrease in faeces production that normalized followed by an increase in faeces and pseudofaeces production towards the end of the exposure.
Browne *et al.*, 2008	*Mytilus edulis*	Experiment 1: Polystyrene spheres, either 2 µm or 4-16 µm. Experiment 2: PS spheres, either 3 µm or 9.6 µm.	Experiment 1: Mussels were exposed to 0.51 g/l of one of two sized plastics for 12 hours. Histological techniques were then used to determine the presence of microplastic in the gut. Experiment 2: Mussels were exposed to 42 857 particles/l of either 3 or 9.6 µm PS for 3 hours. After this was a 48 day depuration period. After exposure, and on days 3, 6, 12, 24, and 48 of depuration, cell viability, oxidative stress, and clearance rate were measured. Sections of the midgut and circulatory system were examined for microplastics, as well as the haemolymph.	Experiment 1: mussels exposed to both particle size treatments had accumulated PS microspheres in their gut cavity and digestive tubules. Experiment 2: Plastics were ingested and accumulated in digestive cavity and tubules. Particles translocated from the gut to the circulatory system within 3 days and persisted for over 48 days. Smaller particles were more abundant in tissues than larger particles.

Capolupo *et al.*, 2018	*Mytilus galloprovincialis* larvae	Polystyrene spheres, 3 µm.	Experiment 1: Larvae were collected at 48 h, 3, 6, or 9 days after fertilization and exposed to either 50, 100, 500, 1,000, 5000 and 10,000 particles/ml of PS for 24 hours. After exposure MP uptake was measured. Experiment 2: Larvae 6 days post-fertilization were exposed to 1000 particles/ml of PS for 24 hours. For the first 12 hours after exposure larvae were collected every 2 hours, after which they were collected every 12 hours. The number of larvae showing ingested MPs were counted at collection, and collection ended once there were 2 consecutive counts with no ingested MPs. Experiment 3: Larvae 6 days post-fertilization were exposed to either 2000 particles/ml of PS, 2000 particles/ml PS co-exposed with 2000 particles/ml algae, or 2000 particles/ml algae alone for 24 hours. Food and MP consumption was measured after exposure.	Experiment 1: MP concentration, time post fertilization, and their interaction affected MP uptake. MP uptake increased with increasing MP concentration. Experiment 2: Larvae needed 192 h (8 days) to achieve 100% gut clearance. Experiment 3: The consumption of MPs and algae did not differ between treatments. When plastic and algae were co-exposed, larvae consumed more algae (80%) than MPs (20%).

Cole and Galloway, 2015	*Crassostrea gigas* larvae	Experiment 1.1: Irregular PS beads, 70 nm - 20 µm (0.07, 0.16, 0.87, 1.84, 4.1, 7.3, 10.2, and 20.3 µm). Experiment 1.2: 0.87 µm fluorescent PS, 0.99 µm aminated PS (PSNH2), or 0.94 µm carboxylated PS (PS-COOH); all irregular bead shaped. Experiment 2: Irregular PS beads, 1 µm or 10 µm.	Experiment 1.1: Larvae (ages 3, 10, or 24 d.p.f) were exposed for 24 hours to a mixture of plastics ranging from 70nm - 20 µm at 1000 microplastics/ml at the same time as algae. After exposure larvae were assessed for the presence and number of ingested PS beads. Experiment 1.2: Larvae (age 8 d.p.f) were exposed for 24 hours to 1000 microplastics/ml of either 0.87 µm fluorescent PS, 0.99 µm aminated PS, or 0.94 µm carboxylated PS at the same time as algae. After exposure larvae were assessed for the presence and number of ingested PS beads. Experiment 2: Larvae (age 9 d.p.f) were exposed to algae and either 1 or 10 µm plastic, each at concentrations of either 1, 10, 100, or 1000 microplastics/ml for 24 hours. After exposure algal ingestion rate was calculated using carbon biomass.	Experiment 1.1: Larvae of all ages consumed plastics 0.07 - 7.3 µm, however, 20.3 µm plastics were only consumed by larger 24 d.p.f larvae. Larve aged 3 and 10 d.p.f. ingested decreasing amounts of plastics of increasing size. Experiment 1.2: Fewer larvae consumed carboxylated and nonfunctionalized PS (10-20% of larvae) than aminated PS (~ 50% of larvae). Experiment 2: Ingestion rate was affected by neither size nor concentration of plastic.
Cole *et al.*, 2020	*Mytilus* mussels	Polystyrene beads, 20 µm and 50 nm. Polyamide (PA) fibres, 10 x 30 µm.	Mussels were exposed to 500 ng/l of either 20 µm PS, 50 nm PS, or 10 x 30 µm PA for either 24 hours or 7 days. Plastics were renewed daily. After exposure MP uptake was measured. Biomarkers of immune response, oxidative stress, lysosomal destabilization and genetic damage were measured in the haemolymph, digestive gland, and gills.	MPs (20 µm and 10 x 30 µm) were observed in the digestive glands, with higher concentrations after 7 days than 24 hours. Quantifying 50 nm MPs was not possible as their signal did not differ from autofluorescence. Plastic shape (bead vs fibre) did not affect plastic accumulation or any other sub-lethal toxicity measurement.

Fernández and Albentosa, 2019	*Mytilus galloprovincialis*	Non-uniform HDPE, <22 µm, mean particle size of 4-6 µm.	Individual mussels were exposed to either plastic or *Isochrysis glabana* algae (spherical, 2-8 µm) at either 2 mm^3/l (low) or 4 mm^3/ (high) for 4 hours. They were then kept in clean water for a 144 hour (6 day) depuration period. The uptake, accumulation, and elimination of particles in the digestive gland were measured.	MP elimination rate was faster at the higher concentration of plastic. After depuration there was no difference in the amount of plastics in the digestive gland between high and low MP concentrations. After depuration all MPs still in the digestive gland were smaller than 8 µm.
Gardon *et al.*, 2018	*Pinctada margaritifera*	Virgin PS microbeads, 6 and 10 µm combined.	Adult oysters were exposed to plastics at either 0.25, 2.5, or 25 µg/l for 2 months. Respiration, growth and ingestion rates were measured, as well as assimilation efficiency and various measure of reproductive effort.	There was no effect on ingestion rate. Assimilation efficiency decreased with increasing MP concentration but was only lower than the control for the highest MP concentration.
Goncalves *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 5, 6, and 10 µm.	Experiment 2: Individual mussels were exposed to 6 µm and 10 µm MPs (single and combined) at 1000 particles/ml for 20 min with samples taken every 5 minutes. Bioassays of various mussel tissues were taken. Experiment 3: Individual mussels were exposed to 5 µm and 10µm plastics (single and combined) at 1000 particles/ml for 21 days followed by a 7 day depuration period. Various histologies of the digestive tract were performed.	Experiment 2: 10 µm MPs were digested faster than 6 µm MPS. Plastics were found in the lumen of the gut but not the gills or digestive gland. No severe histopathological alterations were found in the digestive tract. Experiment 3: MPs remained in the stomach after depuration, but not the gills or gonads. There were inflammatory loci in the digestive epithelia.

González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 0.5 µm or 4.5 µm. Alone or sorbed with BaP.	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experimentation. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastics concentration was 0.058 mg/l (corresponding to 1000 particles/mL for 4.5 µm MPs and to 7.44×10^5 particles/mL for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology, and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	Absorption efficiency was unaffected on day 7. After 26 days, absorption efficiency was raised in mussel exposed to 0.5 µm PS alone, 0.5 µm PS + BaP, and 4.5µm PS + BaP.
Jiang *et al.*, 2022	*Ruditapes philippinarum*	Polystyrene spheres, diameters 5 and 10 µm.	Mussels were exposed to 25 µg/l of either 5 or 10 µm plastics for 30 days. After exposure, the accumulation of plastics in various tissues were analysed and the ingestion rate and absorption efficiency were measured. Dynamic Energy Modelling (DEB) was also performed.	Plastics were ingested and accumulated in the gills, hepatopancreas and intestine (but not the digestive system). Smaller MPs accumulated more than larger MPs in the hepatopancreas and intestines. Ingestion rate and absorption efficiency decreased for both MP sizes.
Lu *et al.*, 2024	*Pinctada fucata martensii*	Polyvinyl chloride, 50 nm. Shape unstated.	Adult oysters were exposed to plastics at either 0.15, 1.5, or 15 mg/l for 15 days. Plastic concentration was renewed daily. Tissue samples were taken on days 1, 5, 10 and 15. These samples were used to measure effects on the: oxidation and reduction system; the immune system (acid phosphatase (ACP), alkaline phosphatase (AKP)); and the digestive system (amylase and protease).	Amylase and protease were initially raised by 0.15 mg/l PVC but were decreased by day 15. Both were raised by 1.5 mg/l PVC by day 15. Amylase was decreased by 15 mg/l PVC by day 15, but protease was raised.

Moreschi *et al.*, 2020	*Anodontites trapesialis*	Polyethylene spheres, 55 - 110 µm.	Mussels were exposed to 0.3 g/l of MPs for between 3 - 192 hours. Mussels were collected throughout the exposure period and assessed for the amounts of plastic they had filtered, assimilated, and eliminated.	The probability of finding plastics in the gills and gut increased with exposure time. There was a weak positive correlation between time and the number of eliminated particles.
O'Brien *et al.*, 2021	*Mytilus galloprovincialis*	Polystyrene spheres, 10 µm.	Mussels were exposed to MPs and food (particulate organic matter) in a fully crossed experiment for 7 days. Plastic exposure was 55 000 and 110 000 particles/l and food was 8 and 15 mg/l. After exposure the amylase activity and isoform Hsp70 expression in the gills were measured.	High MP levels increased amylase activity for both food levels. Surprisingly, amylase decreased under high food and low MPs. Plastic negatively affects the ability of mussels to digest starch under high food conditions but not low.
Pedersen *et al.*, 2020	*Dreissena bugensis*	Red HDPE powder, 10 - 45 µm.	Experiment 1: Mussels were exposed to MPs at either 0.1, 0.4, or 0.8 g/l for 24 hours, after which MP ingestion was calculated. After a 24 hour depuration period all expelled plastics were counted.	Experiment 1: MP ingestion increased with increasing MP concentration. MPs mostly accumulated in the intestines and gill tissue. MP excretion increased with increasing MP concentration.
Revel *et al.*, 2019	*Mytilus edulis*	Polyethylene and PP powder, <950 µm; mean sizes 287 µm (PE) and 204 µm (PP).	Mussels were exposed to a mixture of PE and PP at either 0.008 µg/l (low), 10 µg/l (medium), or 100 µg/l (high) for 10 days. Plastic doses were renewed daily. After exposure there was a 10 day depuration period. Biodeposits were measured daily. After exposure, clearance rate, tissue structure, antioxidant defenses, immune and digestive parameters, and DNA integrity were investigated and plastic particles were identified in mussel tissues. The clearance rate and plastic content in tissues were also measured after depuration.	After exposure, MPs were only found in the digestive glands of high MP mussels. MPs were in the biodeposits of all treatments after exposure. After depuration no plastics were found in any tissues. Amylase activity was unaffected.

Santana *et al.*, 2018	*Perna perna*	Polyvinyl chloride powder, 0.1 µm – 1.0 µm.	Mussels were exposed to 0.125 g/l (equivalent to 1.115×10^{10} particles/l) PVC for 90 days. Plastics were replenished three times a week. After exposure, the clearance rate, absorption efficiency, growth rates, signals of cellular and molecular stress, and health condition (mortality, condition index, lysosomal integrity, LPO, and DNA damage) were measured.	Absorption efficiency was unaffected by MP exposure. Faeces were always highly contaminated with PVC, confirming microplastic ingestion.
Sıkdokur *et al.*, 2020	*Ruditapes philippinarum*	Red fluorescent PE beads, 10-45 µm. Either virgin, co-exposed with mercury chloride, or pre-inoculated with mercury chloride.	Some plastics were pre-contaminated by mixing PE and Hg stock solution together for 96 hours. Clams were exposed for 7 days to: Hg alone at 10 µg/l, PE alone at 25 µg/l, co- exposure with 10 µg/l Hg and 25 µg /L PE, or 25 µg /L pre-contaminated PE. After exposure, the uptake and tissue distribution of PE and Hg were measured in addition to filtration rates, immunomodulation, oxidative stress, and histological alterations.	Microplastics were ingested, and translocated to all the examined tissues (gill, digestive gland, mantle and remaining tissues). Accumulation did not differ between the treatments.
Sussarellu *et al.*, 2016	*Crassostrea gigas*	Yellow-green virgin PS spheres, 2 or 6 µm in diameter.	Adult oysters were exposed to 0.023 mg/l of plastics for 2 months during a reproductive cycle. During and after exposure, various ecophysiological parameters were measured; cellular, transcriptomic, and proteomic responses; fecundity; and offspring development. Plastic ingestion, algae consumption, and absorption efficiency were also measured.	Absorption efficiency was raised by MP exposure. Average daily ingestion of MPs was 14 ± 2% of the 2 µm particles and 69 ± 6% of the 6 µm particles supplied. MPs were only detected in the stomach and intestine and did not cause cellular inflammatory features.
Tallec *et al.*, 2021	*Crossostrea gigas* embryos	Polystyrene beads, 50 nm, with amine functions.	Oyster embryos (Gen 1) were exposed to 0.1 µg/ml MPs for 24 hours. After exposure, the effects on larval performance were measured. Adult performance (growth, clearance rates, respiration rates, absorption efficiencies, reproductive outputs) was measured 10 months after exposure. Intergenerational effects were also measured.	Absorption efficiencies of Gen 1 adults were unaffected by MP exposure.

Trestrail *et al.*, 2021	*Mytilus galloprovincialis*	Polystyrene, 20 µm; PE, 20 and 75 µm. All sphere shaped.	Mussels were exposed to one of five treatments for 7 days; 20 µm PS at low concentration (10 000 MPs/l), 20 µm PE at low (10 000 MPs/l) or high (50 000 MPs/l) concentrations, and 75 µm PE at low (10 000 MPs/l) or high (50 000 MPs/l) concentrations. Digestive enzyme activities were measured after exposure.	PS reduced amylase and increased cellulase activity (PE caused no difference). Only high concentrations of MPs increased protease activity. Laminarinase, lipases and lipolytic esterases were unaffected.
Urban-Malinga, Jakubowska and Białowąs, 2021	*Cerastoderma glaucum* and *Limecola balthica*	Clear spherical PE, incubated in seawater for 5 weeks. 63–75 µm (small), 150–180 µm (medium) and 250–300 µm (large).	Microcosms contained either seven cockles or clams each with plastics mixed into the sediment. Clams were exposed to medium sized MPs at 0.5% sediment dry weight (low amount). Cockles were also exposed to all three sizes at 0.5 % sediment dry weight (low amount) as well as medium plastics as 0.1% sediment dry weight (high amount). After a 3 week exposure measurements were taken of; microplastic uptake, mortality, emergence and distribution in the sediment, energetic value, oxygen consumptions, body condition and sediment porosity.	After exposure to small, medium, and large plastics, at least 38%, 80%, and 44% of cockles had MPs in their tissue. After exposure to low and high amounts (medium size), at least 80 and 90% of cockles accumulated MPs. The highest number of MPs per individual were found in cockles exposed to the small MPs.
Von Moos, Burkhardt-Holm and Köhler, 2012	*Mytilus edulis*	High density polyethylene, 0−80 µm, nonuniformly shaped powder.	Mussels were exposed to 2.5 g/l of plastics for 96 hours. MP uptake was measured after 3, 6, 12, 48 and 96 hours. Histological assessments were also made.	Plastic particles were drawn into the gills as well as taken up into the stomach and transported into the digestive gland. Particle uptake didn't change with exposure time.
Wang *et al.*, 2020	*Mytilus coruscus*	Polystyrene spheres, 2 µm.	Mussels were co-exposed to water of either 8.1 or 7.7 pH, as well as plastics at either 0, 10, 10 000 or 1 000 000 particles/l in a fully crossed experiment for 14 days. Plastic concentration was maintained daily. They were then exposed to a recovery period of 7 days of 'normal' conditions (no plastics, 8.1 pH). Antioxidant enzyme, digestive enzyme, and lysozyme activities were measured on 1st, 7th, 14th, and 21st days of the experiment.	Pepsin (PES), trypsin (TRS), alpha-amylase (AMS) and lipase (LPS) were significantly inhibited by plastic exposure, and this inhibition was aggravated by acidification conditions. Only PES and AMS tended to recover during the recovery period.

Wang, Zhong, *et al.*, 2021	*Mytilus coruscus*	Polystyrene, 70 nm and 10 µm. Shape unstated.	Mussels were exposed to 0.20 mg/l of either 70 nm or 10 µm plastics for 2 weeks. Mussels were either exposed to plastics mixed with food (algae) or plastics alone (fed at an earlier time). Plastic intake was measured during and after exposure. After exposure the clearance rates, respiration rates, absorption efficiency, enzyme activity and oxidative responses were measured.	Lipase, trypsin, and amylase activities did not differ from the control for any treatment. Absorption efficiency was decreased by MPs alone but not when co-exposed with algae. Faecal organic dry weight and excretion rate were higher when exposed to MPs alone than co-exposure (70 nm MPs only). Excretion rate was raised by all treatments except for 70 nm co-exposure.
Wang, Hu, *et al.*, 2021	*Mytilus coruscus*	Polystyrene spheres; 0.07, 0.5, 5, 10 and 100 µm.	Individual mussels were exposed to one of five sizes of plastics at 0.2 mg/l for 3, 15 or 87 hours. Mussels were then moved to clean water for an 87 hour depuration period. Plastic intake and accumulation of particles in gill, digestive tract and mantle were measured after exposure and the depuration period.	MP accumulation was higher in the digestive tract than the gills or mantle. In the digestive tract, MP ingestion was negatively size dependent and positively related to exposure time. MPs in the digestive tract decreased after the depuration period. In the mantle, plastic accumulated during the depuration period after a delay, indicating the translocation of particles.
Wang *et al.*, 2023	*Mytilus galloprovincialis*	Polystyrene spheres, 100 nm.	Mussels were exposed to a combination of MP concentrations and dissolved oxygen patterns (DO) in a fully crossed design for 7 days. MP concentrations were: 0, 0.5, or 5 mg/l. Dissolved oxygen was either normoxia (6 mg/l DO, unchanging), constant hypoxia (2 mg/l DO, unchanging), or fluctuating hypoxia (changing between normoxia and hypoxia every 6 hours). Oxidative stress indicators, digestive capacity markers, and protein contents were measured after exposure.	α-Amylase activity was inhibited by MP exposure. Cellulase and trypsin activity were enhanced by MPs, and the increase was further stimulated by hypoxia. Lipase activity was not affected by MPs alone but decreased when co-exposed with fluctuating hypoxia.

Webb *et al.*, 2020	*Perna canaliculus*	Polyethylene and triclosan spiked PE beads, 38–45 µm.	Some MPs were pre-contaminated by mixing PE with a triclosan solution and then leaving the mixture to evaporate until dry. Adult mussels were exposed to either MPs, triclosan, or triclosan spiked MPs for 48 hours. MPs were at a 0.5 g/l concentration and triclosan at 0.36 mg/l. After exposure the clearance and oxygen consumption rates were measured as well as byssus production and oxidative stress.	All mussels in the two plastic treatments ingested plastics. Plastics accumulated equally in the digestive tracts of both plastic treatments.
Weber *et al.*, 2021	*Dreissena polymorpha*	Polystyrene spheres; 5 µm, 10 µm, 45 µm, 90 µm.	Experiment 1: Mussels were exposed to a mixture of three MP sizes (5 µm, 10 µm, 45 µm) at 3 particles/ml combined with 90 µm plastics at 0.1 particles/ml for 1, 3, 6, 13, 24, and 48 hours. Mussels were also exposed to the above MP combination but in co-exposure with 1 mg/l algae for 12 hours. There were 1, 3, 6, 12, 24, 72, and 168 hour depuration periods. Experiment 2: Mussels from three size classes were co-exposed to algae and MPs as in Experiment 1 for 12 hours. Experiment 3: Mussels were co-exposed to MPS for 12 hours as in Experiment 1 but algae was either 0.2, 1, or 5 mg/l. Experiment 4: Mussels were co-exposed to MPS for 12 hours as in Experiment 1, as well as MPS in the same combination but at a 10 times lower dose. After each experiment the number of ingested plastics per mussel was measured.	Experiment 1: MP body burden peaked after 1 hour of exposure then decreased, followed by another peak after 12 hours. MP body burden, decreased after 1 hour of depuration. The largest sized MPs were completely removed during depuration. Experiment 2: MP body burden did not differ between mussel size classes. However, the relative body burden was higher among the smallest individuals. Experiment 3: Mussels ingested less MPs when more food (algae) was available. EXP 4: The body burden of MPs did not differ with MP concentration.
Wegner *et al.*, 2012	*Mytilus edulis*	Polystyrene, 30 nm. Shape unstated.	Individual mussels were exposed to different combinations of algae and plastic for 8 hours. At plastic concentrations of 0.1 g/l the algae amounts were 0, 60,000, and 120,000 cells/ml. At plastic concentrations of 0.2 and 0.3 g/l, 60 000 cell/l of algae were used. Filtration rate was measured during exposure.	All mussels exposed to MPs produced pseudofaeces. Faeces and pseudofaeces production increased with increasing MP concentration. The presence and amount of algae did not affect pseudofaeces production.

Xu *et al.*, 2017	*Atactodea striata*	Polystyrene granules, 63 µm - 250 µm.	Mussels were exposed to plastics at either 10 particles/l or 1000 particles/l for two weeks, followed by a 7 day depuration period. Clearance and filtration rate were measured along with absorption efficiency and faeces production.	Absorption efficiency was unaffected. The number of MPs in the tissue did not differ with MP concentration. MPs in the tissue were the same after exposure and depuration. High concentrations of MPs produced more pseudofaeces than low concentrations.

2.3.3 Respiration

Bivalves use their gills for both feeding and respiration and as a result many species have evolved gills larger than needed for oxygen uptake to enable simultaneous feeding (Jorgensen, 1996; Riisgård, Larsen and Pleissner, 2014; Tang and Riisgard, 2018). Consequently, high concentrations of microplastics are trapped in the gill tissue, which - along with the digestive gland - are the organs most affected by microplastics (Von Moos, Burkhardt-Holm and Köhler, 2012; Avio *et al.*, 2015; Paul-Pont *et al.*, 2016; Sıkdokur *et al.*, 2020; Sendra *et al.*, 2021; Jiang *et al.*, 2022; Joshy, Krupesha Sharma and Mini, 2022). Exposure to microplastics can decrease the number of gill cilia and cause the epithelium to become thickened and disorganised (Bråte *et al.*, 2018).

The presence of microplastics in the gills can cause physical harm and impede gaseous exchange, compromising the respiratory function of bivalves (Khanjani, Sharifinia and Mohammadi, 2023). Studies examining the effects of microplastics on bivalve respiration and oxygen consumption have varying results. Most found that there is either no effect on respiration (Xu *et al.*, 2017; Gardon *et al.*, 2018; González-Soto *et al.*, 2019; Pedersen *et al.*, 2020; Yap *et al.*, 2020; Tallec *et al.*, 2021; Barkhau *et al.*, 2022; Joyce and Falkenberg, 2022) or that respiration is increased (Urban-Malinga, Jakubowska and Białowąs, 2021; Wang, Zhong, *et al.*, 2021; Jiang *et al.*, 2022; Walkinshaw *et al.*, 2023). However, a study on *P. viridis* mussels found that respiration rate decreased upon exposing mussels to weathered PVC (1-50 µm, 216 mg/l and 2160 mg/l) for 91 days (Rist *et al.*, 2016). Additionally, two other studies on *Perna* found respiration to unaffected by both acute (*P. canaliculus*; 38-45 µm PE, 0.5 mg/l, 48 hours; Webb *et al.*, 2020) and chronic (*P. viridis*; 200 µm terephthalate, 20 and 140 particle/l, 4 weeks; Joyce and Falkenberg, 2022) exposures. One study found that while respiration did not differ from the control when exposed to microplastics (PVC and PMMA), it was lower than the respiration rate of mussels exposed to similar sized natural particles (red clay and diatom shells) (Hamm *et al.*, 2022). All studies looked at the effect on respiration of individual bivalves, except for Green (2016) who studied the effects on *Ostrea edulis* oyster assemblages. She found that oyster assemblages exposed to biodegradable PLA microplastic for 60 days had significantly higher respiration rates than oysters exposed to conventional HDPE microplastic, but their respiration rates did not differ from the control for either plastic type. Neither of the other two papers which used oysters (*Pinctada margaritifera* and *C. gigas*) found an effect on respiration (Gardon *et al.*, 2018; Tallec *et al.*, 2021). There is some indication that respiration responses may be species specific, as indicated by the responses of oysters and *Perna* mussels. Species specific responses may be due to differences in gill structure and the mechanism of particle selection (Webb *et al.*, 2020). Besides this observation, there are no patterns indicating that

respiration was affected by length of exposure, microplastic type, or microplastic size. Finding links was, however, obfuscated by studies varying largely in these experimental factors.

Some experiments where respiration rates increased upon microplastic exposure pointed to stress as the cause (Green, 2016; Wang, Zhong, *et al.*, 2021; Jiang *et al.*, 2022). Increased respiration may be linked to stress as the organisms tried to maintain physiological homeostasis (Smolders, Bervoets and Blust, 2002). Wang, Zhong, *et al.* (2021) found that respiration rates of *M. coruscus* mussels increased when exposed to PS particles, however, when co-exposed to microplastics and algae the effect was diminished. Wang, Zhong, *et al.* (2021) explained the increase in respiration as a stress response and proposed that the presence of food helped mitigate this response A solitary study reported that microplastic exposure resulted in a decrease in both respiration and clearance rates (Rist *et al.*, 2016). This was explained by mussel valve closure which is commonly observed in the presence of potentially harmful particles (Ward and MacDonald, 1996; Bacon, MacDonald and Ward, 1998; Madon *et al.*, 1998; Rist *et al.*, 2016). Authors who found no effects attributed this to the inherent resilience and ability of bivalves to tolerate, acclimate and quickly egest microplastic particles (Pedersen *et al.*, 2020; Yap *et al.*, 2020; Barkhau *et al.*, 2022; Joyce and Falkenberg, 2022). Others proposed that processes other than respiration were being impacted to compensate for changes in the energy balance caused by microplastic exposure (Xu *et al.*, 2017; Gardon *et al.*, 2018; Tallec *et al.*, 2021). Further multi-factorial experiments are needed to decipher under which interactive conditions bivalve respiration is affected by microplastics as well as to elucidate the mechanisms through which this is happening.

Table 3: Effects of microplastic exposure on bivalve respiration rate. All effects are statistically significant and relative to controls without microplastic exposure unless otherwise stated.

References	Species	Type of plastic polymer	Methods	Highlights
Barkhau *et al.*, 2022	*Semimytilus algosus*	Polyvinyl chloride powder, 12.08 ± 13.65 µm; PMMA powder, 120.3 ± 69.92 µm. Red clay, 13.84 ± 14.55 µm; celite, 86.8 ± 90.37 µm.	Mussels were exposed to either PVC, PMMA, red clay, or celite at either 1.5, 15 or 150 mg/l for 68 days. After exposure the body condition index, respiration rate, clearance rate, byssus production, byssus strength and mortality were measured.	Respiration rate did not differ from the control for any treatment.
Gardon *et al.*, 2018	*Pinctada margaritifera*	Virgin PS microbeads, 6 and 10 µm combined.	Adult oysters were exposed to plastics at either 0.25, 2.5, or 25 µg/l for 2 months. Respiration, growth and ingestion rates were measured, as well as assimilation efficiency and various measure of reproductive effort.	Respiration rate did not differ from the control for any treatment.
González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 0.5 µm or 4.5 µm. Alone or sorbed with BaP	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experimentation. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastics concentration was 0.058 mg/l (corresponding to 1000 particles/ml for 4.5 µm MPs and to 7.44×10^5 particles/ml for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology, and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	Respiration rate did not differ from the control for any treatment at either 7 or 26 days of exposure.

Green, 2016	*Ostrea edulis*	Polylactic acid (biodegradable) and conventional HDPE, 65.6 µm. Shape unstated.	Mesocosms were created by collecting intact cores containing sediment and algae from lower intertidal and shallow intertidal zones. Two adult oysters were placed in each mesocosm and exposed to either low (0.8 µg/l) or high (80 µg/l) plastic concentrations of either PLA or HDPE for 60 days. The filtration, respiration, and growth rates were measured as well as changes to the assemblage structures.	Oysters exposed to high concentrations of PLA had ~2.6 times higher respiration rates than oysters exposed to high concentrations of HDPE, but neither differed from the control.
Hamm *et al.*, 2022	*Brachidontes puniceus, Semimytilus algosus, Brachidontes pharaonis, M. trossulus, Mytilus galloprovincialis*	Polymethyl methacrylate, 10-400 µm; PVC, 0.1-100 µm, red clay, 0.1-100 µm; diatom shells, 4-400 µm. Shape unstated.	Mussels were exposed to one of four particle suspensions, each of which had three concentrations (1.5 mg/l, 15 mg/l and 150 mg/l) for 6 weeks. After exposure the clearance and respiration rates were measured as well as byssus production, condition index, and survival.	Respiration rate did not differ from the control for any treatment. Respiration rates differed between particle types but not concentration levels. Mussels exposed to MPs had 9% lower respiration rates than those exposed to natural particles.
Jiang *et al.*, 2022	*Ruditapes philippinarum*	Polystyrene spheres, 5 and 10 µm.	Mussels were exposed to 25 µg/l of either 5 or 10 µm plastics for 30 days. After exposure, the accumulation of plastics in various tissues were analysed and the ingestion rate and absorption efficiency were measured. Dynamic Energy Modelling (DEB) was also performed.	Oxygen consumption rate was increased by both MP sizes.
Joyce and Falkenberg, 2022	*Perna viridis*	Polyethylene terephthalate, PLA (biodegradable), and silver glitter, 200 µm, hexagon in shape.	Mussels were exposed to either normal or biodegradable MPs at either low (17-20 particles/l) or high (135-140 particles/l) amounts for 4 weeks. MPs were delivered in 2 hour pulses daily. After exposure, the clearance and oxygen consumption rates, condition indexes, and mortalities were measured.	Respiration rate did not differ from the control for any treatment.
Pedersen *et al.*, 2020	*Dreissena bugensis*	Red HDPE powder, 10 - 45 µm.	Mussels were exposed to MPs at either 0.1 g/l, 0.4 g/l, or 0.8 g/l for 3 days. During and after exposure, filtration and oxygen consumption rates, survivorship, and reproduction rates were measured.	Respiration rate did not differ from the control for any treatment.

Rist *et al.*, 2016	*Perna viridis*	PVC submersed in fluoranthene-contaminated seawater for 24 days, 1-50 µm. Shape unstated.	Mussels were exposed to either 21.6, 216, or 2160 mg/l (equivalent to 1.2 x 10^7, 1.2 x 10^8, and 1.2 x 10^9 particles/l, respectively) for 91 days. Plastic doses were renewed once a week. Response variables were measured at the start of the experiment and after 40-44 day. Filtration and oxygen consumption rates, byssus production, and survival were measured.	Respiration rate was decreased by the two higher MP concentrations and decreased with increasing particle concentration. At the highest MP concentration, respiration decreased by 64%.
Tallec *et al.*, 2021	*Crossostrea gigas* embryos	Polystyrene beads, 50 nm, with amine functions.	Oyster embryos (Gen 1) were exposed to 0.1 µg/ml MPs for 24 hours. After exposure, the effects on larval performance were measured. Adult performance (growth, clearance rates, respiration rates, absorption efficiencies, reproductive outputs) was measured 10 months after exposure. Intergenerational effects were also measured.	Respiration rates of Gen 1 adults were unaffected by MP exposure.
Urban-Malinga, Jakubowska and Białowąs, 2021	*Cerastoderma glaucum* and *Limecola balthica*	Clear spherical PE, incubated in seawater for 5 weeks. 63–75 µm (small), 150–180 µm (medium) and 250–300 µm (large).	Microcosms contained either seven cockles or clams each with plastics mixed into the sediment. Clams were exposed to medium sized MPs at 0.5% sediment dry weight (low amount). Cockles were also exposed to all three sizes at 0.5 % sediment dry weight (low amount) as well as medium plastics as 0.1% sediment dry weight (high amount). After a 3 week exposure, measurements were taken of microplastic uptake, mortality, emergence and distribution in the sediment, energetic value, oxygen consumptions, body condition and sediment porosity.	*C. glaucum* respiration rates were raised by exposure to large MPs.
Walkinshaw *et al.*, 2023	*Mytilus* juveniles	Red polyester fibres (PE), mean length 293.5 µm; yellow cotton fibre, mean length 171.5 µm. All fibres 10–500 µm in length.	Juvenile mussels were exposed to either 8 fibres/l PE, 80 fibres/l PE, or 80 fibres/l cotton for 94 days. During exposure the clearance and respiration rates were measured, as well as shell length and mortality.	Respiration rate was raised by 8 fibres/l PE for the first three experimental time points (days 22, 37, 51), but after this point (days 65, 79, 93) respiration did not differ. Respiration rates of 80 fibres/l PE and cotton did not differ from the control or each other.

Wang, Zhong *et al.*, 2021	*Mytilus coruscus*	Polystyrene, 70 nm and 10 µm, shape unstated.	Mussels were exposed 0.20 mg/l of either 70 nm or 10 µm plastics for 2 weeks. Mussels were either exposed to plastics mixed with food (algae) or plastics alone (fed at an earlier time). Plastic intake was measured during and after exposure. After exposure the clearance rates, respiration rates, absorption efficiency, enzyme activity and oxidative responses were measured.	Respiration rate was raised by 70 nm MPs alone, but co-exposure with algae had no effect. Respiration rate was raised by 10 µm MP alone and co-exposed with algae.
Webb *et al.*, 2020	*Perna canaliculus*	Polyethylene and triclosan spiked PE beads, 38–45 µm.	Some MPs were pre-contaminated by mixing PE with a triclosan solution and then leaving the mixture to evaporate until dry. Adult mussels were exposed to either MPs, triclosan, or triclosan spiked MPs for 48 hours. MPs were at a 0.5 g/l concentration and triclosan at 0.36 mg/l. After exposure the clearance and oxygen consumption rates were measured as well as byssus production and oxidative stress	Respiration did not differ from the control for any treatment
Xu *et al.*, 2017	*Atactodea striata*	Polystyrene granules, 63 µm - 250 µm.	Mussels were exposed to plastics at either 10 particles/l or 1000 particles/l for two weeks, followed by a 7 day depuration period. Clearance and filtration rate were measured along with absorption efficiency and faeces production.	Respiration rate did not differ from the control for any treatment.
Yap *et al.*, 2020	*Mytilus galloprovincialis*	Polyvinyl chloride, 12-14 µm; red clay. Shape unstated.	Mussels were exposed to either PVC or red clay at 1.5, 15, or 150 mg/l for 35 days. After exposure, respiration rates, byssus production, dry weight (soft tissue) and the body condition index (BCI) were measured.	Respiration rate did not differ from the control for any treatment.

2.3.4 Reproduction

Bivalves are broadcast spawners, putting their gametes, embryos, and young larvae in contact with microplastics in the surrounding water when they reproduce. Microplastics have been shown to negatively affect the reproductive success of bivalves in a number of ways, and several reviews briefly cover the topic (Sendra *et al.*, 2021; Khanjani, Sharifinia and Mohammadi, 2023; Liu *et al.*, 2023; Xu *et al.*, 2024). Studies have found that bivalve gametes exposed to microplastics suffer decreases in fertilization success as well as negative embryo-larval developmental effects (Tallec *et al.*, 2018, 2020; Shi *et al.*, 2022; Lu *et al.*, 2023). The mechanisms by which reproductive success is reduced are still uncertain but microplastics have been found to affect multiple stages in the bivalve reproductive cycle by reducing gamete quality (Bringer *et al.*, 2022; Shi *et al.*, 2022; Romdhani *et al.*, 2024), changing gene expression and damaging DNA (Sussarellu *et al.*, 2016; Capolupo *et al.*, 2018; Shi *et al.*, 2022; Contino *et al.*, 2023; Romdhani *et al.*, 2024), changing in spermatozoa swimming speeds and trajectories (Sussarellu *et al.*, 2016; Tallec *et al.*, 2020; Shi *et al.*, 2022; Contino *et al.*, 2023; Lu *et al.*, 2023; Romdhani *et al.*, 2024), decreasing oocyte numbers and sizes (Sussarellu *et al.*, 2016), reducing larval settlement (Bringer *et al.*, 2021), and stunting larval growth (Sussarellu *et al.*, 2016; Tallec *et al.*, 2021; Bringer *et al.*, 2022).

Gametes exposed to microplastic show a variety of deleterious effects. González-Fernández *et al.* (2018) found that *C. gigas* gametes exposed to PS nanoplastics had increased spermatozoa aggregates. Additionally, flow cytometry revealed increased spermatozoa size and cellular complexity, suggesting nanoplastic adhesion to spermatozoa surfaces, which was confirmed by microscopy observations. González-Fernández *et al.* (2018) noted that oocytes exposed to the same conditions were unaffected, and that microplastic exposure affected spermatozoa more than oocytes, possibly due to their membrane characteristics (Kline, 1991; González-Fernández *et al.*, 2018). Spermatozoa are sensitive to water quality, as once sperm cells are in contact with water, they are activated through a combination of chemical signals such as pH, ions, and cyclic nucleotides (Liu, Innes and Thompson, 2011; Boulais *et al.*, 2019). Usually, once activated, they swim in a curving trajectory interspersed by small linear segments (Van Der Horst, Bennett and Bishop, 2018). However, sperm motility is affected when experimentally exposed to microplastics (either PS or environmental mixes, for up to an hour) (Table 4). Either the number of actively swimming sperm declined (Tallec *et al.*, 2020; Contino *et al.*, 2023; Romdhani *et al.*, 2024), their swimming speed declined (Shi *et al.*, 2022; Lu *et al.*, 2023), or their trajectories changed and became more linear (Shi *et al.*, 2022). It has been found that carboxylic PS nanoplastic beads attach to the acrosome of sperm heads' (González-Fernández *et al.*, 2018; Tallec *et al.*, 2020), possibly causing a decrease in sperm motility similar to the effect of colloidal matter on coral sperm (Humanes *et al.*, 2017; Tallec *et al.*,

2020). Microplastics have also been found to decrease adenosine triphosphate (ATP) contents of sperm and eggs and inhibit ATP synthases (PK and PFK) in sperm (Shi *et al.*, 2022; Lu *et al.*, 2023). As sperm swimming behaviour is directly related to sperm ATP levels, decreased sperm velocity may therefore be caused by lack of energy provisions to sustain sperm flagellar beating and motility (Mita and Nakamura, 1998; Tani and Kamimura, 1998; Shi *et al.*, 2022). In addition, sperm DNA fragmentation has been observed in bivalves exposed to microplastics. Sperm DNA fragmentation is negatively correlated with sperm motility (Huang *et al.*, 2005; Shi *et al.*, 2022; Contino *et al.*, 2023; Romdhani *et al.*, 2024). External fertilisation relies on sperm and eggs successfully colliding (Vogel *et al.*, 1982), but changes in sperm movements can reduce sperm-egg collision probabilities (Vogel *et al.*, 1982; Shi *et al.*, 2022; Lu *et al.*, 2023). The sperm-egg collision frequency is one of the most important factors determining fertilization success in broadcast-spawning marine invertebrates, and impairments caused by microplastics may have a significant impact on successful bivalve reproduction (Styan and Butler, 2000; Shi *et al.*, 2022).

Exposure to microplastics also affects embryo and larval development. Exposure to plastics can reduce larval growth, quality, and cause malformations (Balbi *et al.*, 2017; Tallec *et al.*, 2018, 2021; Bringer *et al.*, 2022; Romdhani *et al.*, 2024). *Mytilus galloprovincialis* mussel larvae exposed to amino modified PS (50 nm) for 48 hours after fertilisation had stunted larval development, with higher malformations, decreased shell length, and downregulation of chitin synthetase, carbonic anhydrase, ABC transporter p-glycoprotein, and lysozyme mRNS levels (Balbi *et al.*, 2017). A similar study on *Meretrix meretrix* clams exposed three stages of larvae to either 200 nm PS-COOH or 100nm PS-NH2 for between 24 hrs – 7 days (Luan *et al.*, 2019). They found that microplastics were more toxic at the hatching and metamorphic stages than the D-veliger larvae stage, which was only affected by the highest two exposure concentrations.

Some studies exposed adult bivalves to microplastics and then measured effects on their reproductive outputs (Sussarellu *et al.*, 2016; Gardon *et al.*, 2018; González-Soto *et al.*, 2019; Pedersen *et al.*, 2020; Bringer *et al.*, 2022; Jiang *et al.*, 2022). Effects included fewer gametes of good quality, smaller oocytes, smaller, slower growing larvae with slowing swimming speeds, increased malformation, and increased gametogenic regression. The gametes of mature *C. gigas* oysters exposed to virgin PS (2 and 6 µm) for two months showed a 38% reduction in oocyte number, 5% decrease in diameter (-5%), and a 21% decline in sperm velocity (Sussarellu *et al.*, 2016). Adult *C. gigas* exposed to a mixture of crushed marine plastics (138.6 um) had fewer gametes of good quality compared to unexposed oysters as well as slower swimming larvae with different swimming trajectories (circular) compared to unexposed oysters (rectilinear) (Bringer *et al.*, 2022). One study

used Dynamic Energy Modelling after exposing *Ruditapes philippinarum* clams to PS (5 and 10 µm) for 30 days and predicted that after 200 days of exposure growth and reproduction would be impacted and that their reproductive energy would also decrease by 12.4-102.2 % (Jiang *et al.*, 2022).

Some authors have proposed that in reaction to stress caused by microplastics, bivalves are sacrificing reproductive efforts in favour of maintaining metabolism and vital functions (Sussarellu *et al.*, 2016; Gardon *et al.*, 2018). Gardon *et al.* (2018) propose that gonads provide missing energy to maintain bivalves' metabolism through the production of metabolites derived from germ cells phagocytosis. Sussarellu *et al.* (2016) exposed mature oysters, *C. gigas*, to 2 and 6 µm PS beads for two months and found there to be signs of disturbed homeostasis coupled with strong negative effects on reproductive indices. Dynamic energy budget (DEB) modelling of bivalves exposed to microplastics has also shown a shift in energy allocation from reproduction to structural growth and high maintenance costs, to the detriment of reproductive output (Sussarellu *et al.*, 2016; Jiang *et al.*, 2022).

Some studies found no effects on the reproduction of bivalves exposed to microplastics, all of which used either *M. galloprovincialis* or *D. burgensis* mussels (Capolupo *et al.*, 2018; González-Soto *et al.*, 2019; Pedersen *et al.*, 2020). Other studies found that effects on reproduction had some experimental measures that were unaffected. For example, González-Fernández *et al.* (2018) found that despite microplastic aggregates attaching to the head of sperm, and higher sperm complexity and size, sperm velocity and movement was unaffected. Studies that used multiple sizes found smaller sized microplastic often produced an effect when larger sized microplastics did not or at least produced stronger effects than larger microplastics (Tallec *et al.*, 2018; Luan *et al.*, 2019; Shi *et al.*, 2022; Contino *et al.*, 2023). Smaller microplastic particles are thought to be more harmful than larger microplastics as they are more readily internalised by marine organisms and can cross biological barriers such as those in the gut and spread to other tissue (Lu *et al.*, 2016; Jeong *et al.*, 2017; Mitrano, Wick and Nowack, 2021; Wang, Hu, *et al.*, 2021; Jiang *et al.*, 2022; Li *et al.*, 2023; Liu *et al.*, 2024). This increased harm is relative to larger microplastics, there is no threshold size point at which harm is increased. In addition, studies which exposed bivalves to different sized microplastics found that smaller microplastics were retained longer than larger microplastics (Browne *et al.*, 2008; Fernández and Albentosa, 2019; Heo *et al.*, 2022). However, slower depuration could only contribute to the increased effects of small microplastics in some of the mentioned reproductive studies, as exposures were often highly acute with no depuration time (Table 4). Smaller microplastics also create greater toxic effects in comparison to larger microplastics, such as increased DNA damage in

M. galloprovincialis larvae and a slew of increased deleterious effects on other bivalves (González-Soto *et al.*, 2019; Cole *et al.*, 2020; Contino *et al.*, 2023; Liu *et al.*, 2024).

Generally, effects on the reproduction of bivalves increased with increasing concentration or only occurred at the higher concentrations of microplastics exposure (Balbi *et al.*, 2017; González-Fernández *et al.*, 2018; Tallec *et al.*, 2018, 2020; Luan *et al.*, 2019; Bringer *et al.*, 2021; Shi *et al.*, 2022). When *C. gigas* oyster gametes were exposed to different functional groups of PS, PS-COOH produced increased sperm aggregates and decreased sperm relative size and complexity (FSG and SSC) when PS-NH2 did not (González-Fernández *et al.*, 2018). In *C. gigas,* fertilization yields were unaffected by plain PS but reduced by PS-COOH and PS-NH2 (Tallec *et al.*, 2018). Furthermore, exposure to PS-NH2 decreased reproductive success in *C. gigas* and was more toxic to three life stages of *M. meretrix* than PS-COOH (Luan *et al.*, 2019; Tallec *et al.*, 2020). This may be because PS-NH2 particles are positively charged and are more likely to adsorb onto the negatively charged lipid bilayer of embryos than PS-COOH which is negatively charged (Lunov *et al.*, 2011; Bergami *et al.*, 2016; Galloway, Cole and Lewis, 2017; Luan *et al.*, 2019). This suggests that the interactive effects of study species, microplastic size, microplastic concentration, and microplastic type should all be considered in future studies.

Table 4: Effects of microplastic exposure on bivalve reproduction. All effects are statistically significant and relative to controls without microplastic exposure unless otherwise stated.

Reference	Species	Type of plastic polymer	Methods	Highlights
Balbi *et al.*, 2017	*Mytilus galloprovincialis* larvae	Amino-modified PS, 50 nm. Shape unstated.	Experiment 1: Newly fertilised embryos were exposed to either 0.001, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 or 20 mg/l MPs for 48 hours. After exposure, larval morphology (shell size, degree of mineralisation) and development were measured. Experiment 2: Newly fertilised embryos and embryos 24 hours post fertilisation (hpf) were exposed to 0.150 mg/l MPs for 48 hours, after which embryotoxicity tests were carried out. Experiment 3: Embryos at 30 min pf were exposed to 0.150 mg/l MPs for 24 and 48 hours. After exposure, gene expressions relating to neuroendocrine signalling antioxidant defence, biotransformation, biomineralization, shell growth, autophagy, growth and metabolism, apoptosis, and immune response were evaluated.	Experiment 1: There was a dose-dependent decrease in normal larval development for all MP concentrations (except the lowest). The EC50 for embryogenesis success was 0.142 mg/l. MP exposure caused D-larvae malformations and delayed development. Shell length was decreased (20-30 %) by all treatments. Experiment 2: Age at exposure did not affect development. Experiment 3: After 24 hours there was an upregulation in genes relating to biotransformation, biomineralization, and shell growth, but after 48 hours these were downregulation. Lysozyme mRNS levels were decreased at both time points. No effects were observed for the other genes at either time point.
Bringer *et al.*, 2021	*Crassostrea gigas* larvae	Polyethylene (28%), PP (40%), PVC (32%), 138.6 ± 2.3 µm, shapes uneven (from crushed marine macroplastics).	Pediveliger larvae were exposed to MPs at either 0.1 or 10 mg/l for 7 days. After exposure the larvae were moved to a nursery for 190 days, then to experimental beds until 338 days (11 months) after exposure. After exposure and until the end of the experiment, measures of growth and development were taken.	After 7 days, there were fewer fixed larvae in the 10mg/l group than in the 1mg/L group or control. In the first 28 days, MP exposure caused decreased growth rates. After 338 days, MP exposure increased growth rates. No malformations or metamorphosis abnormalities were observed.

Bringer *et al.*, 2022	*Crassostrea gigas*	Polyethylene (28%), PP (40%), PVC (32%), 138.6 ± 2.3 µm, shapes uneven (from crushed marine macroplastics).	Adult oysters were exposed MPs at either 0.1 or 10 mg/l for 2 months. During exposure, oysters were measured for MP quantity and toxicity biomarkers. After exposure, oysters were mated and their embryos were grown and measured at 24, 48, and 72 hours after fertilisation. Measurement of D-larvae included swimming behaviour, development and growth.	Exposed adult oysters had reduced gametes of good quality. Larvae of exposed oysters had reduced swimming speeds. Swimming trajectories differed between the larvae of exposed oysters (circular) and control (rectilinear). Exposed parents had an increased number of larvae with malformations and arrested development, and larvae grew slower and were smaller.
Capolupo *et al.*, 2018	*Mytilus galloprovincialis* larvae	Polystyrene spheres, 3 µm.	During fertilisation, embryos were exposed to either 50, 100, 500, 1,000, 5000, or 10,000 MP/ml for 48 hours. Embryotoxicity was measured after exposure.	Larvae morphological development was unaffected by MP exposure.
Contino *et al.*, 2023	*Mytilus galloprovincialis* gametes	Amino-modified PS spheres, 50 and 100 nm.	Mussel spermatozoa were exposed to either 1, 10, 20, 50, or 100 µg/l MPs of either 50 or 100 nm for 30 minutes. After exposure, measures of sperm motility, plasma membrane integrity, DNA fragmentation, and oxidative stress were taken. After exposure, sperm were combined with eggs, and the fertilisation success was recorded.	Exposure caused the proportion of motile spermatozoa to decline, more so with the smaller MPs. Spatial pathways differed between exposed and unexposed sperm. Plasma membrane integrity decreased with increasing MP concentration. Small MPs caused increased DNA fragmentation and decreased fertilisation rates, but small MPs had no effect.
Gardon *et al.*, 2018	*Pinctada margaritifera*	Virgin PS microbeads, 6 and 10 µm combined.	Adult oysters were exposed to plastics at either 0.25, 2.5, or 25 µg/l for 2 months. Respiration, growth and ingestion rates were measured, as well as assimilation efficiency and various measure of reproductive effort.	Gonad development index was unaffected by MP exposure. Exposed oysters had an increased number of individuals with gametogenesis regression.

González-Fernández *et al.*, 2018	*Crassostrea gigas* gametes	Amino (PS-NH2) and carboxylic (PS-COOH) PS beads, 100 nm.	Spermatozoa and oocytes were exposed to either 0.1, 1, 10, or 100 mg/l of PS-NH2 or PS-COOH for 1, 3, or 5 hours. Gamete motility, MP-gamete interactions, gamete health, gamete mortality, and oxidative stress were measured after exposure.	MP aggregates were found attached to the spermatozoa acrosome. Spermatozoa aggregates increased after exposure to PS-COOH (no effect by PS-NH2, oocyte aggregation unaffected). Exposed spermatozoa had higher cellular relative complexity and relative size (oocytes unaffected). There was no effect on sperm cell mortality, the percentage of motile sperm, movement linearity, or velocity.
González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 0.5 µm or 4.5 µm. Alone or sorbed with BaP	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experimentation. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastics concentration was 0.058 mg/l (corresponding to 1000 particles/ml for 4.5 µm MPs and to 7.44×10^5 particles/ml for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology, and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	Percentage of gametogenic stages was unaffected. Gonads showed no histopathological alterations.
Jiang *et al.*, 2022	*Ruditapes philippinarum*	Polystyrene spheres, 5 and 10 µm.	Mussels were exposed to 25 µg/l of either 5 or 10 µm plastics for 30 days. After exposure, the accumulation of plastics in various tissues were analysed and the ingestion rate and absorption efficiency were measured. Dynamic Energy Modelling (DEB) was also performed.	DEB modelling showed the specific growth rates and reproductive energy of mussels exposed to 5 and 10 µm MPs for 200 days would decrease by 12.4%– 102.2%, with significant impacts on reproduction.

Lu *et al.*, 2023	*Tegilarca granosa* gametes	Polystyrene beads, 5 µm and 500 nm.	Gametes were exposed to either 0.69 µg/l of MPs, 0.20 µg/l triclosan, or 0.69 µg/l MPs and 0.20 µg/L triclosan co-exposed for 1 hour. After exposure, sperm and eggs were mixed to determine fertilisation success. Measures of oxidative stress, ATP content, ion-transport, and caspase activity were measured.	All treatments reduced fertilisation success, sperm ATP synthase, and sperm ATP content *. MPs and co-exposure reduced sperm swimming speed* and sperm-egg collision rates. All treatments reduced gamete fusion efficiency, sperm viability, capase activity, and Ca_{2+}-ATPase. Sperm Na_{+}/K_{+}-ATPase activity was reduced by triclosan and MP. *effect enhanced by co-exposure
Luan *et al.*, 2019	*Meretrix meretrix* larvae	Polystyrene beads; orange carboxylated (PS-COOH), 200 nm; and red aminated (PS-NH2), 100 nm.	Clam larvae were allowed to develop into three stages: fertilised egg, D-veliger larvae, and umbo larvae. Embryos/larvae at each stage were exposed to either COOH-PS or NH2-PS at either 0.02, 0.2, 0.5, 1, or 2 mg/l for either 24 hours, 24h - 4 days, or 4 - 7 days. After exposure, the development and malformations of larvae were measured.	PS-NH2 was more toxic than PS-COOH at all life stages. Exposure to MPs at the fertilised egg stage reduced the hatching rate, shell height, and shell length. The malformation rate was greatly increased. At the D-veliger larvae stage, only higher MP concentrations reduced developmental rates and increased malformation rates. Shell length and height were unaffected. At the umbo larvae stage, MP exposure decreased metamorphosis rates and shell height.
Pedersen *et al.*, 2020	*Dreissena bugensis*	Red HDPE powder, 10 - 45 µm.	Mussels were exposed to MPs at either 0.1 g/l, 0.4 g/l, or 0.8 g/l for 3 days. During and after exposure, filtration and oxygen consumption rates, survivorship, and reproduction rates were measured.	Reproduction rates did not differ between treatments and the control.

Romdhani *et al.*, 2024	*Mytilus galloprovincialis* gametes	Environmentally sourced mix (PE and PET most prevalent), <100 µm, shapes uneven (from crushed marine macroplastics)	Sperm were exposed to either 1, 10, 50, or 100 µg/l of MPs for 1 hour. After exposure, viability, mitochondrial membrane potential, oxidative stress, and motility were measured.	Sperm viability was decreased by the two higher MP concentrations. Mitochondrial membrane potential, Bax/Bcl-s ratio (apoptosis indicator), and AO-positive cells (DNA integrity) were increased, and the percentage of mobile sperm and PTMA protein levels decreased after exposure to all MP concentrations except the lowest.
Shi *et al.*, 2022	*Tegilarca granosa* gametes	Polystyrene beads, 0.5 and 5 µm.	Gametes were exposed to either 0.26 mg/l or 0.69 mg/l MPs for 1 hour. After exposure, measures of sperm health and oxidative stress were taken. After exposure, sperm were mixed with eggs to determine fertilisation success.	Fertilisation success was decreased by MP exposure, with smaller MPs and higher concentrations having greater effects. Gamete collision rate, gamete fusion efficiency, sperm-egg fusion probabilities, and sperm viability decreased from MP exposure. MP exposure increased sperm caspase activities and DNA damage. Exposure reduced sperm velocity (VCL and VAC), linearity, and ATP contents.
Sussarellu *et al.*, 2016	*Crassostrea gigas*	Yellow-green virgin PS spheres, 2 and 6 µm in diameter.	Adult oysters were exposed to 0.023 mg/l of plastics for 2 months during a reproductive cycle. During and after exposure, various ecophysiological parameters were measured; cellular, transcriptomic, and proteomic responses; fecundity; and offspring development. Plastic ingestion, algae consumption, and absorption efficiency were also measured.	Oocyte number, diameter, and D-larval yield were decreased by MPs. Exposed males had decreased sperm velocity. Progeny of exposed genitors had slower larval growth, shorter shell lengths, and delayed metamorphosis.
Tallec *et al.*, 2018	*Crassostrea gigas* gametes and larvae	Polystyrene spheres, 50 nm, 500 nm and 2 µm without functionalisation (plain); 50 nm coated with carboxyl groups (COOH); 50 nm coated with amine groups (NH2).	Oyster gametes, embryos, and larvae were exposed to one of five types of PS at either 0.1, 1, 10, or 25 µg/ml for 1.5 hours. Measures of fertilisation success and embryo-larval development were taken after exposure.	Fertilisation yield decreased with increasing concentration for all 50 nm MPs. D-larval yield was reduced by the two highest concentrations of 50 nm MPs, with total inhibition at 1, 10 and 25 mg/L NH2-PS. Metamorphosis success was unaffected.

Tallec *et al.*, 2020	*Crassostrea gigas* gametes	Polystyrene beads, 50 nm, coated with carboxyl groups (COOH) or amine groups (NH2).	Sperm were exposed to COOH-PS or NHS-PS at either 0.1, 1, 10, or 25 µg/ml for 1 hour. After exposure, sperm motility, morphology, lipid composition, and interactions with plastic were measured. After exposure, sperm were mixed with eggs to measure reproductive success 48 hours after fertilisation.	Sperm motility was decreased after exposure to 25 µg/ml MPs. The relative size and complexity of sperm were decreased by 10 and 25 µg/l of COOH-PS. The functional characteristics of the sperm were unaffected, and lipid composition was generally unaffected. MPs adhered to the external membrane of the sperm head. D-larval yield was decreased by 10 and 25 µg/ml NH2-PS.
Tallec *et al.*, 2021	*Crassostrea gigas* embryos	Polystyrene beads, 50 nm, with amine functions.	Oyster embryos (Gen 1) were exposed to 0.1 µg/ml MPs for 24 hours. After exposure, the effects on larval performance were measured (larval yield, morphology, lipid composition). Adult performance was also measured at 4 weeks (growth, lipid index, settlement yield) and 10 months (growth, clearance rates, respiration rates, absorption efficiencies, reproductive outputs) after exposure. In some treatments, the embryos of Gen 1 oysters (Gen 2) were then exposed to the same plastic treatment. Gen 2 larval performance (larval yield, growth, lipid index) was measured after 24 hours as well as adult performance (growth, lipid index) after 4 weeks. Treatments included: exposing neither generation to plastic, exposing both generation to plastic, not exposing Gen 1 but exposing Gen 2, exposing Gen 1 but not Gen 2.	Gen 1 larvae exposed to MPs had decreased larval growth and lipid storage. They also had increased cardiolipin content, suggesting a modification of mitochondrial functioning. Adult Gen 1 oyster reproductive outputs (gonadic development, gamete quality) were unaffected. Gen 2 larvae which were exposed to MPs but had unexposed Gen 1 progenitors had reduced growth.

2.3.5 Oxidative Stress

When organisms are faced with stressful contaminants, they undergo processes which generate reactive oxygen species (ROS) that increase the natural oxidation of cells. This oxidation can lead to DNA damage and cell death, negatively affecting the health of bivalves (Khanjani, Sharifinia and Mohammadi, 2023; Liu *et al.*, 2023). To counteract this, cells produce various antioxidant enzymes which can be measured to determine if oxidative stress is occurring in an organism (Matés, 2000; Furtado-Filho *et al.*, 2007; Chahouri *et al.*, 2023). Many studies have explored the effects of microplastic exposure on the oxidative stress of bivalves and these have been summarised to various degrees in review papers, often included in impacts on immunity (Sendra *et al.*, 2021; Li *et al.*, 2022; Mkuye *et al.*, 2022; Chahouri *et al.*, 2023; Khanjani, Sharifinia and Mohammadi, 2023; Liu *et al.*, 2023; Xu *et al.*, 2024). Oxidative stress is often associated with or is the cause of other effects such as immune responses and DNA damage (Avio *et al.*, 2015; Ribeiro *et al.*, 2017; Sharifinia *et al.*, 2020; Chahouri *et al.*, 2023; Khanjani, Sharifinia and Mohammadi, 2023; Xu *et al.*, 2024).

Several biomarkers have been used to measure oxidative stress, with some of the most common being superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx), glutathione reductase (GR), total glutathione (GSH), oxidised glutathione (GSSG), glutathione S-transferase (GST), and malondialdehyde (MDA; as an indicator of LPO). It is well established that individual oxidative biomarkers are prone to variation, and even within treatments single parameter responses might be heterogeneous (Regoli *et al.*, 2002; Magara *et al.*, 2018). It is therefore unsurprising that oxidative stress biomarkers in bivalves have shown highly variable responses upon exposure to microplastics. Despite this, some patterns have emerged. Superoxide dismutase was generally found to increase after microplastic exposure. However, one study found it to decrease after chronic exposure to both PVC and PS (11-60 µm), and that regardless of dose, mussels exposed to PS had less than half the SOD of those exposed to PVC (Hamm and Lenz, 2021). Another study found that while SOD in *Mytilus* mussels increased after 24 hours of exposure to 50 nm PS, there was no effect after the full exposure of 7 days. Several other papers found no effect at all (Cole *et al.*, 2020; Revel *et al.*, 2020; X. Wang *et al.*, 2020; Bringer *et al.*, 2022; Parolini *et al.*, 2023). Catalase (CAT) has been shown to both increase (Ribeiro *et al.*, 2017; Magara *et al.*, 2018; Revel *et al.*, 2019; Abidli *et al.*, 2021; Wang, Zhong, *et al.*, 2021; Parolini *et al.*, 2023) decrease (Paul-Pont *et al.*, 2016; González-Soto *et al.*, 2019; Wang *et al.*, 2024) and be unaffected (Avio *et al.*, 2015; Magara *et al.*, 2018; Revel *et al.*, 2020; Sıkdokur *et al.*, 2020; Martyniuk *et al.*, 2022; von Hellfeld *et al.*, 2022) after both acute and chronic exposures. Acute microplastic exposure has resulted in increases, decreases and no changes in GPx (Table 5). The only chronic study reported that GPx increased in *M. coruscus* after the first 72 hours of PS exposure but decreased after 30 days. Both effects were only seen in the smaller microplastic

sizes (Wang *et al.*, 2024). Oxidative effects on GST are similar to those of CAT (Table 5). Neither study on oysters (*C. gigas*) that measured GST found it to be affected, and other studies which found no effect usually used microplastics larger than 50 µm (Avio *et al.*, 2015; Goncalves *et al.*, 2019; Revel *et al.*, 2020; Webb *et al.*, 2020; Bringer *et al.*, 2022; Parolini *et al.*, 2023). Lipid peroxidation (LPO, measured by MDA/TBARS) increased after microplastic exposure in over half (12 of 21) the studies in which it used as a biomarker to detect oxidative stress (Table 5). Only two studies reported a decrease in LPO after exposure (Paul-Pont *et al.*, 2016; Abidli *et al.*, 2021), the rest found no effect.

Reactive oxygen species (ROS) usually increased after acute microplastic exposure (Paul-Pont *et al.*, 2016; González-Fernández *et al.*, 2018; Z. Li *et al.*, 2020; Lu *et al.*, 2023; Romdhani *et al.*, 2024), but has also been reported as unaffected (Revel *et al.*, 2020; Contino *et al.*, 2023; Parolini *et al.*, 2023). A minority (8 of 30) of studies found microplastic to have no effects on oxidative stress (Table 5). It is not possible to make comparisons between these studies based on microplastic concentration as they are reported in different units between studies. Some studies report microplastic concentration in both particles/volume and weight/volume, future studies should continue to report both units together to allow for direct comparisons (Paul-Pont *et al.*, 2016; Santana *et al.*, 2018; González-Soto *et al.*, 2019; Revel *et al.*, 2020; Wang *et al.*, 2024). Generally, results varied greatly among and within studies, with no clear patterns according to model species, microplastic size, microplastic shape, or microplastic type. This is most clearly displayed in a study by O'Donovan *et al.* (2018) who found several biomarkers (SOD, CAT, GPx, GST, AChE, and LPO) to change with tissue type (gill or digestive gland), time, and microplastic type (virgin or contaminated). This wide array of results in the literature may be due to the large variation in species, plastics and experimental protocols used.

There is some evidence that the effects of microplastics on oxidative stress are time-dependent, a finding supported by the meta-analysis of Li *et al.* (2022). Chronic (4 weeks or longer) studies generally found biomarkers of oxidative stress (CAT, SOD, H2O2, GPx) to have decreased by the end of exposure, even if responses increased initially (González-Soto *et al.*, 2019; Hamm and Lenz, 2021; Wang *et al.*, 2024). For example, one study on the effects of PS (0.5 and 4.5 um; sorbed with BaP) on *M. galloprovincialis* mussels found that CAT was raised after 7 days, but that after the full exposure of 26 days CAT had decreased compared to the control (González-Soto *et al.*, 2019). Another study found SOD to decrease and MDA to increase with increasing exposure time, but this difference was only statistically significant between time points of exposed mussels, and did not differ from the control at each time point (Bringer *et al.*, 2022). Not all chronic studies found biomarkers to have decreased by the end of exposure. Lipid peroxidation (MDA, TBARS), GST, and SOD have also been reported to increase or remain unaffected after long-term exposure (Santana *et al.*, 2018; Hamm

and Lenz, 2021; Bringer *et al.*, 2022; Wang *et al.*, 2024). Acute studies (less than 4 weeks) often observed SOD to initially increase within the first couple of days, but that this effect sometimes subsided or was reversed within two weeks of exposure (Paul-Pont *et al.*, 2016; Cole *et al.*, 2020; Webb *et al.*, 2020; Z. Li *et al.*, 2020; Martyniuk *et al.*, 2022; Parolini *et al.*, 2023). For example, a study on *Mytilus* mussels noted SOD in the gills to increase after expose to both PS and PA for 24 hours, but after 7 days, differences between microplastics and the control were not evident (Paul-Pont *et al.*, 2016). These studies show that while there may be time-related patterns for some oxidative stress biomarkers, some are also incongruent. In addition, some biomarkers appear to be more strongly affected by other factors, such as tissue selection or inadequate replication to compare and determine driving factors.

Li *et al.* (2022) proposed that contradictory findings relating to oxidative stress may be due to differences in microplastics type, microplastic concentration, exposure time, and the use of different oxidative stress indicators. The type of oxidative stress indicator used is important as CAT and GSH are generally more sensitive to microplastics than SOD, GPx or GST (Li *et al.*, 2022). The mix of results may also be due to different tissues being examined for oxidative stress. Two studies have found CAT to be affected differently depending on the tissues examined, with one finding CAT to increase in the gills but not the digestive gland and the other study finding the reverse (Ribeiro *et al.*, 2017; Parolini *et al.*, 2020). Catalase is not the only biomarker to respond differently between tissues: GST was lower than the control in the digestive gland of *M. edulis* but higher in the gills after exposure to a mixture of PE and PP powder for 10 days (Revel et al., 2019). Parolini et al. (2020) found no changes in the oxidative stress of the digestive glands of *Ruditapes philippinarum* clams after seven days of exposure to PET (220 µm), but did find that their gill tissue was affected, causing GPx to be inhibited and CAT activity and LPO to increase. This was not the only case of LPO responding with high variability between and within different tissues of clams. One study found LPO increased in the visceral mass of *Corbicula fluminea* after PS (80 nm, 96 hours) exposure, but decreased in the gills and mantle (Z. Li *et al.*, 2020). The authors proposed that the gills either had a stronger antioxidant capacity than the visceral mass or that visceral mass was the main target/detoxification organ when the clams were under oxidative stress (Z. Li *et al.*, 2020). Additionally, LPO decreased in the gills of *Scrobicularia plana* after 20 µm PS exposure after 3 day and increased in the digestive gland from day 7 onwards (Ribeiro *et al.*, 2017). Further research is required to determine if the unpredictability of LPO levels is specific to clams or is a wider phenomenon in bivalves. There is also a general need for future studies to consider appropriate biomarkers before measuring oxidative stress, as some are more erratic than others to microplastic exposure.

Table 5: Effects of microplastic exposure on bivalve oxidative stress. All effects are statistically significant and relative to controls without microplastic exposure unless otherwise stated.

References	Species	Type of plastic polymer	Methods	Highlights
Abidli *et al.*, 2021	*Mytilus galloprovincialis*	Polyethylene (ultra-high molecular weight), 40–48 µm. Shape unstated.	Individual adult mussels were exposed to varying concentrations (1 µg/l, 10 µg/l, 100 µg/l, and 1000 µg/l) of plastic for 14 days. Filtration rate and measures of oxidative stress were measured after 7 and 14 days.	CAT was raised and GST was reduced by the two higher MP concentrations in the digestive glands of female mussels. GST was reduced in males at the highest concentration. LPO was reduced in females at the highest concentration and was unaffected in males.
Avio *et al.*, 2015	*Mytilus galloprovincialis*	Polyethylene and (PS) powder, <100 µm.	Prior to experimentation, some plastics were sorbed with pyrene by mixing MPs with pyrene for 6 days. Mussels were exposed to either PE, PS, pyrene-treated PE (PE-PYR), or pyrene-treated PS(PS-PYR) at 20 g/l for 7 days. MPs were nominally re-dosed daily at 1.5 g/l. After exposure, tissue samples were taken and immune responses, antioxidant systems changes, neurotoxic effects and gene expressions were measured.	CAT, MDA, GSH, GR, GST, GPx, and oxyradical scavenging capacity were unaffected. Se-dependent GPx was reduced by all treatments except for PE alone.
Brandts *et al.*, 2018	*Mytilus galloprovincialis*	Polystyrene spheres, 110 nm. Prepared by miniemulsion polymerization of styrene.	Mussels were exposed to either: 0.005, 0.05, 0.5, 5, or 50 mg/l plastic alone (PS), 6.3 µg/l carbamazepine alone (Cbz), or to 0.05 mg/l plastics combined with 6.3 µg/l Cbz (PS+Cbz) for 96 hours. After exposure, samples of the haemolymph, digestive gland and gills were taken to measure gene expression, DNA damage, cell-tissue repair, immune system response, oxidative status, neurotoxicity, carbohydrate metabolism and metabolic enzymes.	In the digestive gland (but not gills), total oxidant status was increased by 0.5 mg/l PS, and total antioxidant capacity (T-AOC) was increased by 50mg/l PS. LPO was increased by 0.05 mg/l PS.

Bringer *et al.*, 2022	*Crassostrea gigas*	Mixture of PE (28%), PP (40%), PVC (32%), 138.6 ± 2.3 µm, shapes uneven (from crushed marine macroplastics).	Adult oysters were exposed MPs at either 0.1 or 10 mg/l for 2 months. During exposure, oysters were measured for MP quantity and toxicity biomarkers. After exposure, oysters were mated and their embryos were grown and measured at 24, 48, and 72 hours after fertilisation. Measurement of D-larvae included swimming behaviour, development and growth.	In the digestive glands of adult oysters, SOD was unaffected. Other measures (GST, MDA, laccase) differed with time but not from the control.
Browne *et al.*, 2008	*Mytilus edulis*	Polystyrene spheres, either 3 or 9.6 µm.	Mussels were exposed to 42 857 particles/l of either 3 or 9.6 µm PS for 3 hours. After this was a 48 day depuration period. After exposure, and on days 3, 6, 12, 24, and 48 of depuration, cell viability, oxidative stress, and clearance rate were measured. Sections of the midgut and circulatory system were examined for microplastics, as well as the haemolymph.	The oxidative status of the haemolymph was unaffected.
Cole *et al.*, 2020	*Mytilus* spp.	Polystyrene beads, 20 µm and 50 nm. PA fibres, 10 x 30 µm.	Mussels were exposed to 500 ng/l of either 20 µm PS, 50 nm PS, or 10 x 30 µm PA for either 24 hours or 7 days. Plastics were renewed daily. After exposure MP uptake was measured. Biomarkers of immune response, oxidative stress, lysosomal destabilization and genetic damage were measured in the haemolymph, digestive gland, and gills.	After 24 hours, SOD activity in the gills was increased 50 nm PS, and in the digestive gland by 20 µm PS and PA. After 7 days, SOD was unaffected. Thiobarbituric acid reactive substances (TBARS) was lowered in the gills after 24 h exposure to 50 nm PS.
Contino *et al.*, 2023	*Mytilus galloprovincialis* gametes	Amino-modified PS spheres, 50 and 100 nm.	Mussel spermatozoa were exposed to either 1, 10, 20, 50, or 100 µg/l MPs of either 50 or 100 nm for 30 minutes. After exposure, measures of sperm motility, plasma membrane integrity, DNA fragmentation, and oxidative stress were taken. After exposure, sperm were combined with eggs, and the fertilisation success was recorded.	Dichlorofluroescein (DCFH, used to measure ROS) levels were unaffected.

Goncalves *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 5 and 10 µm.	Individual mussels were exposed to 5 µm and 10µm plastics (single and combined) at 1000 particles/ml for 21 days followed by a 7 day depuration period. Various histologies of the digestive tract were performed.	Neither LPO nor GST differed from the control for any treatment or timepoint.
González-Fernández *et al.*, 2018	*Crassostrea gigas* gametes	Amino (PS-NH2) and carboxylic (PS-COOH) PS beads, 100 nm.	Spermatozoa and oocytes were exposed to either 0.1, 1, 10, or 100 mg/l of PS-NH2 or PS-COOH for 1, 3, or 5 hours. Gamete motility, MP-gamete interactions, gamete health, gamete mortality, and oxidative stress were measured after exposure.	ROS production was raised in sperm by 10 and 100 mg/L PS-COOH, and the increase was dose-dependent (PS-NH2 caused no effect). ROS production in oocytes was unaffected.
González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 0.5 µm or 4.5 µm. Alone or sorbed with BaP.	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experimentation. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastics concentration was 0.058 mg/l (corresponding to 1000 particles/ml for 4.5 µm MPs and to 7.44 × 105 particles/ml for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology, and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	CAT was increased by both PS sizes sorbed with BaP after 7 days, however after 26 days CAT was decreased compared to both the control and day 7 levels.

Hamm and Lenz, 2021	*Mytilus edulis* juveniles	Polyvinylchloride (PVC) powder, 11-60 µm; PS beads, 40 µm.	Individual mussels were exposed to PVC at 15, 1500, 15 000, 150 000, or 1 500 000 particles/individual/week or to PS at 15, 1500, or 15 000 particles/individual/week for 42 weeks. Plastics were renewed once a week. Clearance rate, growth, and byssus production were measured ever sixth week during exposure, up to week 36. Condition index was measured after 16, 32, and 42 weeks. Measures of oxidative stress were taken after exposure.	SOD was decreased by all treatments. Regardless of dose, mussels exposed to PS had lower SOD activity than those exposed to PVC. MDA was unaffected.
Li *et al.*, 2020	*Corbicula fluminea*	Fluorescent labelled PS, 80 nm. Shape unstated.	Clams were exposed to MPS at either 0.1, 1, or 5 mg/l for 96 hours. After exposure, tissue was extracted and antioxidant enzyme activity, histological analyses, and integrated biomarkers responses were measured. Oxidative stress (SOD, CAT, GSH, GPx, GR, GST, MDA) was measured in the visceral mass, gill and mantle. Liver damage, intestinal inflammation, and the levels of AChE were also measured.	SOD was increased in all tissues, and the increase was dose dependent. CAT, GPx, GSH, and GST were also increased in all tissues. GR decreased after exposure to 5mg/l MPs. MDA in the visceral mass was induced by 0.1 mg/L exposure but inhibited in the gill and mantle.
Lu *et al.*, 2023	*Tegilarca granosa* gametes	Polystyrene beads, 5 µm and 500 nm.	Gametes were exposed to either 0.69 µg/l of MPs, 0.20 µg/l triclosan, or 0.69 µg/l MPs and 0.20 µg/L triclosan co-exposed for 1 hour. After exposure, sperm and eggs were mixed to determine fertilisation success. Measures of oxidative stress, ATP content, ion-transport, and caspase activity were measured.	ROS production was increased in oocytes by 500 nm PS alone and co-exposed with triclosan (5 µm had no effect). Intracellular MDA levels in oocytes and sperm increased when co-exposed to MPs and triclosan, but not when exposed to MPs alone.
Lu *et al.*, 2024	*Pinctada fucata martensii*	Polyvinyl chloride (PVC), 50 nm. Shape unstated.	Adult oysters were exposed to plastics at either 0.15, 1.5, or 15 mg/l for 15 days. Plastic concentration was renewed daily. Tissue samples were taken on days 1, 5, 10 and 15. These samples were used to measure effects on the: oxidation and reduction system (SOD, MDA, GSH, T-AOC); the immune system (ACP, AKP); and the digestive system (amylase and protease).	SOD was raised by 15 mg/l MPs on day 15. MDA activity was always raised, except by 0.15 mg/L on day 1 and 15 mg/L on day 10. GSH was always raised, except by 0.15 mg/l MPs on day 5. All treatments lowered T-AOC at all time points.

Magara *et al.*, 2018	*Mytilus edulis*	Polyethylene powder, 10-90 µm; virgin or fluoranthene (FLU) contaminated.	Some plastics were pre-mixed (incubated) with FLU of 50 or 100 µg/l and left overnight until dry. Mussels were exposed for 96 hours to either: FLU only, PE only, FLU and PE co-exposed, or FLU incubated PE. Treatments were conducted at a low and high concentrations (50 µg/l and 100 µg/l FLU; 100 and 1000 PE particles/mL) that were not crossed i.e. low co-exposure used only low amounts of both FLU and PE. After exposure, FLU uptake and antioxidant response (SOD, CAT, SeGPx, GPx, GSH) were measured in the gills and digestive gland.	SOD in the gills was decreased by both concentrations of FLU-incubated MPs (unaffected in digestive gland). CAT was raised in the gills and digestive gland by PE alone and FLU-incubated PE. SeGPx activity was raised in the gills by most plastic exposures and was lowered in the digestive gland by high PE-only. GPx in the gills was raised by all treatments, in the digestive gland only by high FLU-incubated PE. GSH was decreased by all treatments in the gills and most in the digestive gland.
Martyniuk *et al.*, 2022	*Unio tumidus*	Polyethylene terephthalate, 0.1-0.5 mm. Shape unstated.	Mussels collected from either reference (Pr) or contaminated (Ct) areas were exposed to either 1 mg/l PET, 0.8 µg/l Ibuprofen, or their combination for 14 days. Controls consisted of unexposed mussels from both collection sites. After exposure, measures of antioxidant activities (Mn-SOD, Cu,Zn-SOD, CAT) and oxidative injury (TBARS, protein carbonyl concentrations) were measured.	Mn-SOD was higher for Ct mussels co-exposed to PET and ibuprofen; PET alone had no effect and PR mussels were unaffected. Cu,Zn-SOD activity was supressed and TBARS was increased in Pr mussels exposed to PET. Protein carbonyl levels were increased in Pr mussel co-exposed to PET and Ibuprofen but decreased in Ct mussels; PET alone had no effect on either mussel group. Protein carbonyl concentrations were higher in Pr mussel after co-exposure compared to plastic alone.
O'Donovan *et al.*, 2018	*Scrobicularia plana*	Low density polyethylene powder (LDPE), 11-13 µm; virgin, pre-contaminated with BaP, or pre-contaminated with perfluorooctane sulfonic acid (PFOS).	Prior to experimentation, some plastics were with adsorbed with BaP or PFOS. Clams were exposed to either 1 mg/l of virgin LDPE, LDPE pre-contaminated with BaP, or LDPE pre-contaminated with PFOS for 14 days. Plastics were re-supplied every 72 hours and no food was supplied during exposure. Samples were taken on days 0, 3, 7, and 14 to measure BaP accumulation, condition index, oxidative stress (SOD, CAT, GTPx, GST, LPO), genotoxicity and neurotoxicity.	All oxidative stress biomarkers were affected, but these effects were highly variable between time points, tissue types (gill vs digestive gland), and biomarkers. SOD in the gills was higher after co-exposure to BaP than plastic alone. GPx and LPO in the gills were higher after co-exposure to PFOS than plastic alone. In the digestive gland, both co-exposures had higher LPO than plastic alone.

Parolini *et al.*, 2020	*Ruditapes philippinarum*	Polyethylene terephthalate, 8 - 1054 µm (mean length 220 µm), irregular shapes (from grinding bottle-grade PET).	Clams were exposed to either 0.125 or 12.5 µg/ml PET for 7 days. After exposure, histological analyses were performed and measures of oxidative stress, (SOD, CAT, GPx) and detoxifying (GST) enzymes, as well as levels of LPO, were determined in gills and digestive gland.	In the digestive gland, oxidative stress measures (ROS, SOD, CAT, GST, LPO) were unaffected by plastic exposure, except GPx which was inhibited at the higher PET concentration. In the gills, CAT activity and TBARS (LPO) increased and GPx was inhibited after exposure to the higher concentration of plastic.
Paul-Pont *et al.*, 2016	*Mytilus* spp.	Polystyrene beads, 2 and 6 µm mixed.	Plastics were a mixture of 1800 particles/ml of 2 µm PS combined with 200 particles/ml of 6 µm PS to create a final concentration of 2000 particles/day (32 µg/l). Mussels were exposed to either: fluoranthene (FLU) at 30 µg/l, PS at 32 µg/l, or PS and fluoranthene co-exposed for 7 days followed by a 7 day depuration period. Doses were renewed daily at the same time as algal feeding. Mussels were sampled after exposure and depuration to measure haemolymph, fluoranthene quantification, gene expressions, oxidative stress (SOD, CAT, H2O2, GR, GST, LPO), and enzyme activity.	After exposure, ROS and GR were increased and CAT and LPO were reduced by PS alone. GST and SOD activity were unaffected by plastic treatments. After depuration, GR was decreased by co-exposure. LPO was reduced by all treatments and GST and SOD were increased by PS alone. Generally, co-exposures did not cause greater effects than single exposures in oxidative stress measures.
Pittura *et al.*, 2018	*Mytilus galloprovincialis*	Low density polyethylene powder, 20-25 µm; virgin and pre-contaminated with BaP.	Prior to experimentation, some plastics were adsorbed with BaP by mixing LDPE and BaP for 2 days. Mussels were exposed to either 10 mg/l ($2.34x10^7$ particles/l) virgin LDPE, 10 mg/l LDPE pre-contaminated with BaP, or 150 ng/l BaP for 4 weeks. Treatments were re-dosed daily 12 hours after feeding. Samples were taken on days 7, 14, and 28 to measure LDPE tissue accumulation, BaP accumulation, immune system alteration, oxidative stress (CAT, GST, GPx, GR, GSH, MDA), genotoxicity, and neurotoxicity.	Measures of antioxidant defence did not differ from the control for any treatment at any timepoint.

Revel *et al.*, 2019	*Mytilus edulis*	Polyethylene and PP powder, <950 µm, mean sizes 287 µm (PE) and 204 µm (PP).	Mussels were exposed to a mixture of PE and PP at either 0.008 µg/l (low), 10 µg/l (medium), or 100 µg/l (high) for 10 days. Plastic doses were renewed daily. After exposure, clearance rate, tissue structure, antioxidant defences, immune and digestive parameters, and DNA integrity were investigated and plastic particles were identified in mussel tissues.	SOD and CAT activities were increased in the digestive gland after exposure to low and medium doses and increased in the gills after exposure to the highest dose of MPs. Digestive gland GST activity was decreased in mussels exposed to the highest dose of plastic but was increased in the gills.
Revel *et al.*, 2020	*Crassostrea gigas*	Polyethylene and PP powder mixture, <500 µm, mean sizes 300 µm (PE) and 200 µm (PP). Cryo-milled from commercial plastics.	Oysters were exposed to 0.008, 10, or 100 µg/l of an MP mixture (corresponding to about 9, 11200 and 112000 particles/l) for 10 days followed by 10 days of depuration. Plastics were renewed daily at a separate time from feeding. Samples were taken on days 0, 10, and 20. Histopathological exams, AcP activity, oxidative stress, genotoxicity, condition index, clearance rate and MP content of tissue were measured.	CAT, SOD and GST activities in the gills and digestive gland were unaffected by plastic exposure. Gene expression of Gpx, GST, SOD, and CAT, as well as ROS content were unaffected by plastic exposure.
Ribeiro *et al.*, 2017	*Scrobicularia plana*	Polystyrene, 20 µm. Shape unstated.	Clams were exposed to 1 mg/l (~4 particles/ml) MPs for 14 days, followed by 7 days of depuration. MPs were renewed daily. Clams were sampled after 0, 3, 7 and 14 days of exposure and after 7 days of depuration (day 21). Haemolymphs, gill and digestive glands were sampled to measure microplastic accumulations, condition index, antioxidant enzymes (SOD, CAT, GPx, GST), oxidative damage (LPO/MDA), genotoxicity, and AChE activity.	CAT and LPO were higher in the digestive gland than the gills. In both tissues, SOD was raised on days 14 and 21. CAT and GPx in the gills were raised on day 3 and 21. GST in the gills was increased after 14 days of exposure, in the digestive gland it was increased on days 3 and 21. LPO in the gills was lowered on day 3, and on day 21 in the digestive gland.
Romdhani *et al.*, 2024	*Mytilus galloprovincialis* gametes	Environmentally sourced mix (PE and PET most prevalent), <100 µm, shapes uneven (from crushed marine plastics)	Sperm were exposed to either 1, 10, 50, or 100 µg/l of MPs for 1 hour. After exposure, viability, mitochondrial membrane potential, oxidative stress, and motility were measured.	ROS increased after exposure to the three highest concentrations.

Santana *et al.*, 2018	*Perna perna*	Polyvinyl chloride powder, 0.1 µm – 1.0 µm.	Mussels were exposed to 0.125 g/l (equivalent to 1.115×10^{10} particles/l) PVC for 90 days. Plastics were replenished three times a week. After exposure, the clearance rate, absorption efficiency, growth rates, signals of cellular and molecular stress, and health condition (mortality, condition index, lysosomal integrity, LPO, and DNA damage) were measured.	Lipid peroxidation was unaffected.
Shi *et al.*, 2022	*Tegilarca granosa* gametes	Polystyrene beads, 0.5 and 5 µm.	Gametes were exposed to either 0.26 mg/l or 0.69 mg/l MPs for 1 hour. After exposure, measures of sperm health and oxidative stress were taken. After exposure, sperm were mixed with eggs to determine fertilisation success.	MDA content of oocytes was increased for all treatments except for 5 µm MPs at 0.26 mg/l.
Sıkdokur *et al.*, 2020	*Ruditapes philippinarum*	Red fluorescent PE beads, 10-45 µm. Either virgin, co-exposed with mercury chloride, or pre-inoculated with mercury chloride.	Some plastics were pre-contaminated by mixing PE and Hg stock solution together for 96 hours. Clams were exposed for 7 days to: Hg alone at 10 µg/l, PE alone at 25 µg/l, co- exposure with 10 µg/l Hg and 25 µg /L PE, or 25 µg /L pre-contaminated PE. After exposure, the uptake and tissue distribution of PE and Hg were measured in addition to filtration rates, immunomodulation, oxidative stress, and histological alterations.	Measures of oxidative stress (CAT, GSH, LPO) in the gills and digestive gland were unaffected.
von Hellfeld *et al.*, 2022	*Mytilus galloprovincialis*	Polystyrene spheres, 4.5 µm: pristine, sorbed with cadmium (Cd), or sorbed with BaP.	Prior to experimentation, some PS was combined with BaP or Cd for 1 day. Mussels were exposed to either: 1000 particles/ml virigin PS, 1000 particles/ml PS sorbed with Cd, 1000 particles/ml PS sorbed with BaP, 1 µM (113.4 µg/l) Cd, or 1 uM BaP (252.3 µg/l) for 3 days. Doses were renewed every 24 hours. After exposure, antioxidant and peroxisomal enzymes activity (CAT, SOD, ACX) in the digestive gland and gills, lysomal membrane stability, and PS and metal localisation were measured.	Neither pristine nor contaminated MP exposure affected oxidative stress.

Wang *et al.*, 2020	*Mytilus coruscus*	Polystyrene spheres, 2 µm.	Mussels were co-exposed to water of either 8.1 or 7.7 pH, as well as MPs at either 0, 10, 10 000 or 1 000 000 particles/l in a fully crossed experiment for 14 days. Plastic concentration was maintained daily. They were then exposed to a recovery period of 7 days of 'normal' conditions (no plastics, 8.1 pH). Antioxidant enzyme, digestive enzyme, and lysozyme activities were measured on 1st, 7th, 14th, and 21st days of the experiment.	SOD and GPx were unaffected. CAT activity was increased after co-exposure to the highest MP dose and pH 7.7. GSH was raised at all time points by the highest MP dose.
Wang, Zhang *et al.*, 2021	*Mytilus coruscus*	Polystyrene, 70 nm and 10 µm. Shape unstated.	Mussels were exposed 0.20 mg/l of either 70 nm or 10 µm plastics for 2 weeks. Mussels were either exposed to plastics mixed with food (algae) or plastics alone (fed at an earlier time). Plastic intake was measured during and after exposure. After exposure the clearance rates, respiration rates, absorption efficiency, enzyme activity and oxidative responses were measured.	SOD was unaffected. CAT activity was raised by 10 µm MPs alone but not the other treatments. MDA was increased by both MP sizes alone (70 nm was higher than 10 µm), but not when co-exposed with algae.
Wang *et al.*, 2023	*Mytilus galloprovincialis*	Polystyrene spheres, 100 nm.	Mussels were exposed to a combination of MP concentrations and dissolved oxygen patterns (DO) in a fully crossed design for 7 days. MP concentrations were: 0, 0.5, or 5 mg/l. Dissolved oxygen was either normoxia (6 mg/l DO, unchanging), constant hypoxia (2 mg/l DO, unchanging), or fluctuating hypoxia (changing between normoxia and hypoxia every 6 hours). Oxidative stress indicators (SOD, GST, MDA), digestive capacity markers, and protein contents were measured after exposure.	SOD increased when exposed to either MP concentration under normoxia. GST increased after exposure to 5mg/l MPs under normoxia, as well as 0.5 mg/l MPs under fluctuating hypoxia. MDA increased after exposure to 5 mg/l MPs under normoxia and fluctuating hypoxia.

Wang *et al.*, 2024	*Mytilus coruscus*	Polystyrene spheres: 70 nm, 500 nm, 5 µm, 10 µm, 100 µm.	Mussels were exposed to 0.2 mg of either 70 nm, 500 nm, 5 µm, 10 µm or 100 µm PS (2.65 × 109, 7.28 × 106, 7.28 × 103, 3.64 × 102, 0.9 particles/l , respectively) for 3 h, 72 h, and 30 days, followed by 7 days of depuration. MPs were renewed every 12 h, within half an hour of feeding. Digestive glands were sampled after each time point, as well as after depuration. After exposure, oxidative stress (hydrogen peroxide, CAT, GPx, MDA) was measured in the digestive gland and haemolymph.	Hydrogen peroxide was decreased by the three smallest sized MPs after 30 days but was restored to initial values after depuration. CAT activity was decreased after 30 days by all MP sizes and was restored after depuration. GPx was decreased after 30 days by the two smallest sized MPs. MDA was increased by all MP sizes after 30 days.
Webb *et al.*, 2020	*Perna canaliculus*	Polyethylene and triclosan spiked PE beads, 38–45 µm.	Some MPs were pre-contaminated by mixing PE with a triclosan solution and then leaving the mixture to evaporate until dry. Adult mussels were exposed to either MPs, triclosan, or triclosan spiked Mps for 48 hours. MPs were at a 0.5 g/l concentration and triclosan at 0.36 mg/l. After exposure the clearance and oxygen consumption rates were measured as well as byssus production and oxidative stress	LPO in gill tissues was raised by triclosan and triclosan spiked MPs. SOD was only increased after exposure to triclosan spiked MPs. GST activity was unaffected.

2.3.6 Other physiological and behavioural effects

Many different physiological measurements have been used to determine the effects of microplastics on bivalves. These include measures of mortality, growth and condition, byssus growth and strength, and immunity. Few reviews haves specifically covered the effects on these measures (Sendra *et al.*, 2021; Xu *et al.*, 2024), with no reviews highlighting effects on bivalve growth or condition. However, these measure (mortality, growth and condition, byssus growth and strength, and immunity) are sometimes included in non-specific summaries in review studies on the effects of microplastics on bivalves (Chahouri *et al.*, 2023; Khanjani, Sharifinia and Mohammadi, 2023; Liu *et al.*, 2023). Xu et al. (2024) provides detail on the impacts of MPs on the immunity, survival, byssus production, and burrowing behaviour of commercially important bivalves. Like effects on filtration, respiration, reproduction, and oxidative stress, other physiological measures vary greatly both within and between studies after exposure to microplastics.

Microplastic exposure is generally non-lethal, with most studies finding no effect on mortality (Rist *et al.*, 2016; Santana *et al.*, 2018; Pedersen *et al.*, 2020; Yap *et al.*, 2020; Abidli *et al.*, 2021; Urban-Malinga, Jakubowska and Białowąs, 2021; Barkhau *et al.*, 2022; Hamm *et al.*, 2022; Joyce and Falkenberg, 2022; Walkinshaw *et al.*, 2023). There have, however, been cases where microplastic exposure caused mortality to increase (Rahim, Yaqin and Rukminasari, 2019; Thomas *et al.*, 2020; Phothakwanpracha, Lirdwitayaprasit and Pairohakul, 2021; Bringer *et al.*, 2022). Microplastic exposure can still induce a wide range of sub-lethal effects. Microplastics can negatively affect bivalves growth rates, condition index and scope for growth (Gardon *et al.*, 2018; Santana *et al.*, 2018; Thomas *et al.*, 2020; Bringer *et al.*, 2021; Hamm and Lenz, 2021; Wang, Zhong, *et al.*, 2021; Barkhau *et al.*, 2022; Hamm *et al.*, 2022; Jiang *et al.*, 2022; Joyce and Falkenberg, 2022; Abidli *et al.*, 2023; Walkinshaw *et al.*, 2023). An isolated study showed condition index and scope for growth to increase after microplastic exposure (González-Soto *et al.*, 2019), while others reported no effect on growth or condition (Thomas *et al.*, 2020). Bivalve growth can be rapidly affected by microplastic exposure, exemplified by a study that observed total weight to decrease after just two weeks of exposure to PE (Abidli *et al.*, 2023). In addition to impacting general growth, exposure to microplastics can reduce byssus thread growth/production and tenacity/attachment strength, possibly due to additional stress compromising the energy budget of the animals (Rist *et al.*, 2016; Green *et al.*, 2019; Webb *et al.*, 2020; Hamm and Lenz, 2021; Hamm *et al.*, 2022). One study on *M. galloprovincialis* found that byssus thread production increased under high microplastic particle loads, possibly as a strategy to survive in areas of high wave action (Yap *et al.*, 2020).

Stress responses to microplastic also occur at the cellular level. Some studies have included measures of immunity and genotoxicity after microplastic exposure (Pittura *et al.*, 2018; Cole *et al.*, 2020; Sıkdokur *et al.*, 2020; Lu *et al.*, 2024). Effects on bivalve immunity are often measured by the number of haemocyte cells, haemocyte viability, and lysomal membrane stability. Haemocyte viability and lysomal membrane stability often decline after microplastic exposure (Von Moos, Burkhardt-Holm and Köhler, 2012; Avio *et al.*, 2015; Pittura *et al.*, 2018; Abidli *et al.*, 2023). Immunity is also affected through immunomodulation, involving the up and down regulation of various genes (Brandts *et al.*, 2018; Capolupo *et al.*, 2018; Green *et al.*, 2019). Effects on immunity are complex. Some studies reported bivalve immunity to be unaffected after both acute and chronic microplastic exposure (Browne *et al.*, 2008; Santana *et al.*, 2018; Revel *et al.*, 2020; Thomas *et al.*, 2020; von Hellfeld *et al.*, 2022; Lu *et al.*, 2024). Genotoxicity is most commonly assessed through changes to DNA fragmentation/damage, which has been observed to increase after microplastic exposure (Avio *et al.*, 2015; González-Soto *et al.*, 2019; Zhang *et al.*, 2022; Contino *et al.*, 2023). Impacts on genotoxicity may be caused by the production of ROS not being sufficiently handled by antioxidant defence mechanisms (Ribeiro *et al.*, 2017). Microplastic exposure triggers a wide range of stress responses in bivalves, affecting nearly every aspect of their physiology. However, the specific ways in which different types and amounts of microplastic influence these effects remain unclear.

2.4 The effects of macroplastics on bivalves

Despite the growing evidence of the negative effects of macroplastics on biota, there have been considerably few studies on the effects of macroplastics on bivalves (Li, Tse and Fok, 2016; Bucci, Tulio and Rochman, 2020; Naidoo, Rajkaran, and Sershen, 2020; Table 6, Table 7, Table 8). Only five studies could be found where bivalves were the primary study species (Devakie and Ali, 2002; Jang *et al.*, 2016; Ke *et al.*, 2019; McCoy *et al.*, 2020; Sorini *et al.*, 2021). The remaining 11 studies examined benthic or fouling communities which included at least one bivalve species (Table 6, Table 7, Table 8). Numerous studies examined the effects of marine debris on bivalves (or bivalve containing communities) and in all these macroplastic was the most abundant type of debris (Goldstein, Carson and Eriksen, 2014; Shabani, Nasrolahi and Thiel, 2019; Rumbold, García and Seco Pon, 2020; De-la-Torre *et al.*, 2021; Rizzo, Musco and Crocetta, 2021). These publications focused on the effects macroplastics have on bivalves and their communities through smothering organisms in benthic systems, acting as hard substrate in soft-bottom habitats, providing artificial substrata or rafts for fouling communities, or serving as a source of leachates.

The impact of macroplastics smothering on bivalve densities and their associated communities have shown mixed results in the literature and thus remain inconclusive (Table 6). All smothering studies focused on soft-bottom benthic assemblages with various bivalve species, except one that solely investigated *C. fluminea* clams (McCoy *et al.*, 2020). Some studies recorded decreases in bivalve and macrofauna density when beneath or near macroplastic litter (McCoy *et al.*, 2020; Clemente, Paresque and Santos, 2022). For example, Green *et al.* (2015) reported the complete absence of *M. edulis* in plots containing plastic bags. Others observed no changes in macrofaunal density in the presence of macroplastic litter items (Uneputty and Evans, 1997; Green *et al.*, 2015; Clemente, Paresque and Santos, 2018). Impacts on community composition are also mixed. Some studies noted that macroplastic alters benthic assemblages (Uneputty and Evans, 1997; Green *et al.*, 2015; Clemente, Paresque and Santos, 2018), however, another identified no difference in the community structure of estuarine benthic macrofauna when comparing areas with or without macroplastic litter (Clemente, Paresque and Santos, 2022). These mixed results could be due to the variability of organic matter and oxygen available to deposit feeders, both with and without macroplastic-covered sediment (Sara, 2006). Some studies have found that both organic matter and organism abundance are lower beneath macroplastic litter compared to uncovered sediment (Green *et al.*, 2015; Clemente, Paresque and Santos, 2022). This is caused by the plastic acting as a barrier, preventing the deposition of organic matter from the water column and reducing the availability of organic matter to deposit feeders (Green *et al.*, 2015; Clemente, Paresque and Santos, 2022). Macroplastic

debris also act as a barrier to oxygen diffusion, creating anoxic sediment conditions with negative redox potentials and increased ammonia pools below plastic bags (Green *et al.*, 2015).

Several studies have recorded macroplastics acting as settlement substrata for bivalves and assemblages containing bivalves (Table 7). Two of these examined the effects of using macroplastics as settlement material for *Crassostrea* oysters in laboratory settings (Devakie and Ali, 2002; Sorini *et al.*, 2021). Others collected macroplastic and other debris in soft-bottom benthic environments and recorded which species had colonised them (Gündoğdu, Çevik and Karaca, 2017; Rumbold, García and Seco Pon, 2020; De-la-Torre *et al.*, 2021; Subías-Baratau *et al.*, 2022). In one manipulative experiment, *C. virginica* oyster spat were settled on bags containing either oyster shells or macroplastic shaped similarly to oyster shells (Sorini *et al.*, 2021). After three weeks, fewer oysters had settled onto the plastic. After three months mortality in oysters settled on the plastic was higher and those that did survive were ~50% of the size of those growing on natural shells. After a further ten months of growing the oyster in metal cages, the proportion of oysters initially grown on macroplastic skewed significantly more female (44% female and 56% male) than those grown on shell (18% female and 82% male). Sex ratio shifts towards female have also been observed in other bivalves exposed to common aquatic anthropogenic pollutants (Blaise *et al.*, 2003; Nice *et al.*, 2003; Andrew *et al.*, 2010). Species recorded to have sex ratio shifts in this way include freshwater mussels, *Elliptio complanate,* exposed to municipal effluent (Blaise *et al.*, 2003), and oysters, *C. gigas*, exposed to the plastic leachates nonylphenol and xenoestrogen (Nice *et al.*, 2003). If exposure to macroplastics cause oyster populations to have altered sex ratios there may also be impacts on reproductive output. Devakie and Ali (2002) investigated how macroplastics affect oyster larval settlement. They focused on whether plastics with different features, such as texture and presence of biofilm, would affect settlement. They reported both features and their interactions impacted settlement. The highest settlement rate observed was for rough plastics without biofilm, and the lowest settlement rate was for smooth plastics without biofilm. These examples indicated that oysters have poor settlement rates on plastic, particularly smooth, un-weathered plastic. In addition, once settlement has occurred, oysters experience higher mortality rates and skewed sex ratios, which could have potential ramifications for a populations persistence. Due to the limited laboratory studies for comparison, it is difficult to assess the generality of these effects.

A few field studies recorded bivalves using macroplastic as attachment in otherwise unsuitable habitats, e.g. by providing hard settlement substrata in soft-bottom habitats (Gündoğdu, Çevik and Karaca, 2017; Rumbold, García and Seco Pon, 2020) or rafting material at the ocean surface (Table 7). Results are, however, mixed as to whether different debris types support different fouling

communities. One study quantified fouled debris items in one-hour collection efforts from a sandy beach in the Persian Gulf and immediately transferred samples to the lab (Shabani, Nasrolahi and Thiel, 2019). They recorded 21 different species, five of which were bivalves. They found that while the total coverage of rafting species on beached debris did not change with marine debris type (macroplastic, wood, glass, metal cans), species richness and species assemblage composition did change, with macroplastic debris having fewer species than either wood or metal cans (Shabani, Nasrolahi and Thiel, 2019). Another study collected samples from a lagoon in Argentina using the efforts of the public, who placed debris items in designated bins that were collected weekly before being transferred to the lab (Rumbold, García and Seco Pon, 2020). Only five fouling species were recorded, with barnacles dominating samples and only two bivalve groups present. They found no difference in species richness of macrofauna with different debris types or buoyancy (Rumbold, García and Seco Pon, 2020). As the research is highly limited, it is difficult to determine whether or why different debris types support different bivalve fouling communities.

Several field studies have identified the polymer types of macroplastic debris fouled by bivalves and other macrofauna (Shabani, Nasrolahi and Thiel, 2019; De-la-Torre *et al.*, 2021; Subías-Baratau *et al.*, 2022). One noted that species diversity and abundance differed based on plastic polymer type, and another that fouling community compositions differed with polymer type (Gündoğdu, Çevik and Karaca, 2017; Subías-Baratau *et al.*, 2022). This indicates that plastic type is an important consideration when accounting for differences in biofouling benthic diversity, especially when bivalves are the target taxon. Colonised benthic macroplastic collected with a bottom trawl net revealed that PE and PET litter had higher species diversity and abundance than PP litter (Gündoğdu, Çevik and Karaca, 2017). *Corbula gibba* clams and *Anomia ephippium* oysters were found solely on PE and PET respectively, while *Neopycnodonte cochlear* and *Musculus subpictus* were both found on a variety of plastic polymers (Gündoğdu, Çevik and Karaca, 2017). Another study noted that PET and PE had the highest diversity of biofouling organisms (out of eight polymer types) but did not attempt to statistically differentiate between polymer types (Subías-Baratau *et al.*, 2022). Similarly, other research concluded that PP was the most abundant type of fouled macroplastic but did not confirm this with statistical testing (De-la-Torre *et al.*, 2021). While it is possible that fouling organisms are selecting settlement substrata that correspond with different plastic types, this would need to be studied further to ascertain the generality of patterns (Gündoğdu, Çevik and Karaca, 2017). Future studies into this topic should also collect and identify all macroplastic within a study area to be able to quantify the percentage of fouled plastic per polymer type to validate whether invertebrates are indeed choosing or avoiding certain polymers or if substrate choice is indiscriminate. Additionally, differences found between the effects of debris and macroplastic types may be due to the size of

debris. Several studies found correlations between debris size and the number of colonising organisms, and that the larger the item the more species were found on the item (Goldstein, Carson and Eriksen, 2014; Gündoğdu, Çevik and Karaca, 2017; Shabani, Nasrolahi and Thiel, 2019). If common plastic items are usually the same size and material, e.g. water bottles made from PET, shopping bags made from HDPE, piping made from PVC, then differences in fouling assemblages that appear to be due to plastic type may be the result of the size of an item rather than its synthetic polymer.

One study examined if macroplastic debris acted as a source of chemical additives and whether mussels using the macroplastic as substrate were being influenced by microplastic production through fragmentation and leaching additives (Jang *et al.*, 2016). *Mytilus galloprovincialis* mussels were collected from a variety of buoys made from either styrofoam, HDPE, metal, or naturally occurring rock. The mussels' tissues were analysed for the presence and concentration of hexabromocyclododecane (HBCD), a plastic additive that has been detected in plastic marine buoys and in sediment near plastic marine buoys (Rani *et al.*, 2013; Al-Odaini *et al.*, 2015), Mussels growing on styrofoam had far higher concentrations of HBCD (mean 523 ± 11 ng/g lipid weight) than mussels growing on other substrates (mean 60.0 ± 17.3 ng/g lipid weight). It was found that the levels of HBCD in the substrate were reflected in the mussels inhabiting them, with mussels having high HBCD levels when attached to buoys with high HBCD contents. Styrofoam particles were also found in all mussels' tissue which had used styrofoam buoys as substrate. Jang *et al.* (2016) proposed that the HBCDs in the styrofoam substrate were being transferred to the mussels through indirect uptake via the water column or styrofoam particle ingestion. The HBCDs could leach from the styrofoam into the water column, adsorb onto particulate organic matter or dissolve in the water, and then be taken up indirectly by the mussels through ingestion or across the gills. After ingestion in either of these scenarios the HBCDs leach out while inside the digestive tract and are absorbed. This study highlights the important link between macroplastics, microplastics and plastic leachates. Even if macroplastics are not causing physical effects (e.g. smothering), they are acting as a source of leachates and microplastics, both of which have been repeatedly shown to negatively impact bivalves (Ke *et al.*, 2019; Gunaalan, Fabbri and Capolupo, 2020; Capolupo *et al.*, 2021; Delaeter *et al.*, 2022).

In addition to the effects of macroplastics on bivalves being understudied, research on marine debris has overwhelmingly focused on sandy shores, leaving the impacts on intertidal systems such as rocky shore habitats understudied (Browne *et al.*, 2015; GESAMP, 2019). We therefore know little about intertidal rocky shore plastic debris (Moore *et al.*, 2001; Thiel *et al.*, 2013; Weideman *et al.*, 2020), or

its effects on rocky shore organisms. No studies have investigated how macroplastic debris affects rocky shore bivalves. One study examined the different fouling communities (including bivalves) found on macroplastic debris on both sandy and rocky intertidal beaches, but found no difference in the abundance or diversity of organisms between the two habitats (De-la-Torre *et al.*, 2021).

Table 6: Effects of macroplastics smothering on bivalves

Reference	Species	Plastic	Methods	Highlight
Clemente, Paresque and Santos, 2018	Tropical benthic estuary community including bivalves. Bivalve species unspecified.	Environmental plastic bags.	Cylindrical samplers were used to take samples of biogeochemical measures and macrofauna at various distances from naturally weathered and deposited plastic bags in an estuary. Samples were taken from under, border and distant from (50 cm) the bags.	Biogeochemical measurements (e.g. sediment redox potential, silt-clay percentage, organic matter) were unaffected by plastic bags. Macrofauna density and diversity were unaffected by the bags. Taxonomic richness was slightly higher beneath bags and community structure differed between treatments (under, border or distant from bags).
Clemente, Paresque and Santos, 2022	Tropical benthic estuary community including bivalves. Bivalve species unspecified.	Plastic bags.	In an estuary, plastic bags were firmly attached to sediment using pins. Macrofauna and sediment samples were taken before the bags were placed and after 8 weeks of exposure. Both sediment and macrofauna samples were collected using core samplers.	Both organic matter and silt-clay percentage of sediment were lower beneath bags after exposure. Macrofauna abundance decreased (97%) beneath bags, as well as species richness and Shannon-Wiener diversity. Community structure was unaffected.
Green *et al.*, 2015	Benthic macro and meio-faunal assemblages including *Mytilus edulis*.	Bags, HDPE and biodegradable plastic made from corn starch.	Conventional and biodegradable bags were randomly interspersed on mud flats (at least 2 m apart) and secured using pins. Pins were also used to mark control plots. After 9 weeks a corer was inserted 5cm deep into the centre of the plots to collect infauna. Biogeochemical measurements of the sediment were also taken. Chlorophyll-a, -b, and -c were also measured from the surfaces of the bags.	Conventional and biodegradable bags had similar effects. Infauna abundance was six times lower beneath bags, but species richness and Shannon-Wiener diversity were unaffected. Infaunal assemblage structure differed under bags. Mytilus edulis mussels were present on control plots but absent beneath plastic bags. The top sediment layer was affected by the plastic bags: redox potential was negative (was positive on control plots), organic matter was lower, ammonium was higher, and biogenic silicate was higher under bags.
Uneputty and Evans, 1997	Tidal flat assemblages including bivalves. *Dosinia* sp, Pectinidae, *Tellina* sp.	Environmental plastic from beach litter.	Paired quadrats were chosen within the same tidal level on a tidal flat. In each quadrat pair, one was litter free and the other covered in plastic debris. In each quadrat, meiofauna and diatoms were collected from the surface sediment and macrofauna were collected from the top 25 cm of sediment. Fauna and diatoms were identified and counted.	Meiofauna was higher in littered quadrats and assemblage composition differed between litter and litter-free quadrats. Diatoms were less abundant under litter, but their assemblage structure did not differ.

McCoy *et al.*, 2020	*Corbicula fluminea*	Wet wipes.	A transect was taken along the foreshore with quadrats every 2 m. The density of clams and wet wipes was measured per quadrat.	There was negative correlation between the number of wet wipes and the number of clams present per quadrat.

Table 7: Macroplastics can act as substrate and rafting material for bivalves

Reference	Species	Plastic	Methods	Highlight
Devakie and Ali, 2002	*Crassostrea iredalei*	Sheets of colourless HDPE 1 mm thickness.	Plastic of different textures (rough vs soft) and conditions (with or without biofilm were used for larval settlement. Success of settlement was measured.	Both condition and texture of plastic affected settlement percentage. Rough surfaced plastics had higher settlement rates than smooth plastic. Best settlement rates were obtained for rough plastic without biofilm (37.6%).
Sorini *et al.*, 2021	*Crassostrea virginica*	Oyster shell-shaped PET (cut from bottles).	Experiment 1: Oyster larvae were settled onto either oyster shells or bags filled with oyster-shaped plastic pieces. Settled spat were reared for three weeks in the laboratory. Number of settled spat per fake or real shell was measured as well as well as shell length. Experiment 2: Spat were reared for a further 10 months in metal cages on natural reefs. Survival, growth, sex ratio, and gene expression were measured.	Experiment 1: Juvenile oysters grown on PET showed significantly higher mortality rates and reduced growth than those grown on shell. Experiment 2: The proportion of oysters grown on plastic skewed significantly more female (44% female and 56% male) than those grown on shell (18% female and 82% male). However, substrate had no impact the level of expression in any of the spermatogenesis or oogenesis genes measured.
Gündoğdu, Çevik and Karaca, 2017	Fouling assemblages including bivalve species. *Neopycnodonte cochlear, Musculus subpictus, Anomia ephippium, Corbula gibba.*	Environmental plastic from coastal benthic. Mean weight 0.97 kg.	A trawling vessel collected benthic plastic using a bottom trawl net for 20 min. All macroplastic debris was collected and any attached fouling species were identified. Plastic type was identified using ATIR-FTIR spectroscopy.	Plastic debris as a substrate can contain a high diversity of life. Species diversity and abundance differed based on plastic debris type; PE and PET had higher species diversity and abundance than PP. There was a positive correlation between plastic size and organism abundance, as well as plastic size and species richness.

Rumbold, García and Seco Pon, 2020	Fouling assemblages including bivalves. *Ostrea* sp., *Brachidontes rodriguezii.*	Environmental plastic from lagoon litter. Rubber litter (15%) included.	Beach debris was collected by the public and placed in designated bins which were collected by authors weekly. Macrobenthic fouling organisms were recorded and identified per piece of debris. The volume of debris was calculated using water displacement and plastic was classified as buoyant if it floated in a bucket of water. Debris type was classified as styrofoam plastic, undetermined plastic, undetermined plastic/metal, and rubber.	There was a positive correlation between debris mass and species richness but not between debris size and species richness. Species richness was not affected by debris type or debris buoyancy.
De-la-Torre *et al.*, 2021	Fouling assemblage, Bivalvia the most abundant class at 53.5%. E.g. *Semimytilus algosus.*	Environmental plastic from beach litter. Cloth and wood (22%) debris included.	Colonized litter items were collected from the entire vertical shore area per site. The number of fouling or entangled macroinvertebrates was recorded for each taxon after identification. A piece of each fouled item was sampled for FTIR spectroscopy analysis in order to identify the plastic polymer.	All fouled items were found in intertidal rocky zones. Bivalvia was the most abundant class (53.5%). Abundance and diversity were not affected by source (land vs sea). Polypropylene was the most frequently fouled polymer type (7 items), followed by LDPE and polyester (4 items each). Mesh bags and plastic bags were the most common colonised items.
Rizzo, Musco and Crocetta, 2021	Benthic assemblages including bivalves. *Ostrea edulis, Pinna nobilis.*	Environmental plastic from 50m deep. Metal and textile debris included.	A Remotely Operated Vehicle (ROV) was used to measure coastal benthic litter in three subtidal habitats using camera observations. The habitats were: fine sands well sorted, coastal detritic bottoms, and scattered rocky bottoms. Four transects of 100m^2 of each habitat were sampled from subtidal waters to 50 m deep. Macro biota were identified and reported as individuals/100 m^2. Litter were categorised by size, source, % of colonisation, and composition. Composition was classified as either concrete, fiberglass, metal, plastic, rubber, textile, and other.	Most items were not colonised, and the number of colonised items did not differ with habitat. There were no cases of entangled animals in marine litter. The litter composition did not explain variations in benthic assemblages.

Goldstein, Carson and Eriksen, 2014	Rafting species including bivalves. Arcidae, *Teredo* spp., *Zirfaea* spp., *M. galloprovincialis, C. gigas, Chlamys* spp., *Pinctada* spp., Unknown oyster species.	Floating plastic. Rope and styrofoam debris included.	Floating debris items were opportunistically collected by dip net (mesh 1 mm). Pieces of debris with attached fauna was preserved and fauna identified. A subset of plastics collected using a standard manta net (mesh 333-µm) were also included in this study.	Provides evidence that bivalves will foul floating plastic. There was a significant positive correlation between the size of the debris object and the number of taxa found on that object.
Shabani, Nasrolahi and Thiel, 2019	Biofouling organisms including bivalves. *Saccostrea cucullata, Isognomon legumen*, two unknown bivalve species.	Environmental plastic from beach litter. Other debris included.	Fouled debris items on sandy beaches or inside ports were collected for 1 hour. The identity and surface area covered by fouling organisms was recorded. Debris was classified as plastic, wood, glass, or metal can.	Provides evidence that bivalves will foul floating plastic. The total coverage of debris by organisms was not affected by debris type. Species richness and species assemblages differed with debris type. There was a weak positive correlation between size of debris and the number of taxa fouling it.
Subías-Baratau *et al.*, 2022	Biofouling organisms including bivalves. *Barbatia barbata, Neopycnodonte cochlear.*	Floating and benthic plastic.	Floating plastic debris was collected along transects between 100 and 200 m from the shoreline using a manta trawl parallel to the shoreline. Benthic plastic debris was collected using a trawling vessel at ranges 100-366 m by transects parallel to the shoreline. Additionally, beached plastic debris was collected after storms in the wrack line. Fouling organisms on debris were identified.	Provides evidence that bivalves will foul floating, benthic, and beached plastic. Plastic composed of PET, PE and PS showed the highest diversity of biofouling organisms. Fouling communities differed with polymer substrate type.

Table 8: Macroplastics can act as a source of microplastics and leachates to bivalves

Reference	Species	Plastic	Methods	Highlight
Ke *et al.*, 2019	*Meretrix meretrix* clam embryos and larvae.	Polyethylene bags.	Leachates were collected by soaking 10g/l of plastic bag pieces in seawater for 48 h in dark and with a shaking speed of 90 r min−1 at 25 °C. Serial dilutions of each stock solution (10 g L−1) were made with filtered seawater at concentrations of 0.05, 0.10, 0.50, 1, and 10 g L−1. Experiment 1: Fertilisation rates of clams were measured under the different treatment concentrations after 1 hr of leachate exposure. Experiment 2: Fertilized eggs were exposed to leachates for 24 hours at each concentration and the number of hatching embryos were counted. Number of deformed larvae and shell height were also measured. Experiment 3**:** Larvae were exposed to leachates for three days at each concentration and the mortalities were counted.	Experiment 1: Fertilisation of embryos was unaffected by leachate. Experiment 2: There were dose-dependent effects on the development of D-veliger larvae. Higher concentrations caused higher deformation rates of larvae, smaller larvae sizes, and more abnormal embryos. Experiment 3: Leachates caused higher mortality and caused all larvae to die at the highest leachate concentrations.
Jang *et al.*, 2016	*Mytilus galloprovincialis*	Styrofoam and HDPE buoys.	Experiment 1: Mussels were collected from buoys made from either styrofoam, HDPE, metal, or rocks. After mussels were collected their biometric data were recorded. Sections of the styrofoam buoys were collected and analysed for HBCD. Experiment 2: Mussels were collected from 2 styrofoam buoys, one with a high level of HBCD (4001 ug/g) and one with a low level (13 ug/g). Some mussels were frozen immediately while other had a 2 day depuration period. All mussel tissue was analysed for HBCD. Mussel faeces were collected after depuration and styrofoam particles were identified.	The mussels inhabiting the styrofoam substrate accumulated more Hexabromocyclododecanes (HBCDs) than the mussels from the other substrates. Hexabromocyclododecanes levels in styrofoam buoys were reflected in the mussels inhabiting them, i.e. HBCD concentrations were high in mussels from styrofoam buoys with high HBCD contents. Styrofoam particles were found in all mussel tissues of mussels using styrofoam as substrate.

2.5 Plastics as vectors for other contaminants in the marine environment

Both macroplastics and microplastics can adsorb and transport various organic and inorganic contaminants on their surfaces, as well as release pollutants into the marine environment and the digestive tracts of organisms through desorption (Teuten *et al.*, 2009; Ziccardi *et al.*, 2016; Avio, Gorbi and Regoli, 2017; Amelia *et al.*, 2021; Gao *et al.*, 2021; Santos, Rodríguez-Mozaz and Barceló, 2021; Sendra *et al.*, 2021; Tumwesigye *et al.*, 2023; Xia, Niu and Yu, 2023). Microplastics accumulate large amounts of toxic pollutants due to their hydrophobic properties and large surface area to volume ratios (Teuten *et al.*, 2009; Avio, Gorbi and Regoli, 2017; Amelia *et al.*, 2021; Gao *et al.*, 2021; Santos, Rodríguez-Mozaz and Barceló, 2021; Tumwesigye *et al.*, 2023; Xia, Niu and Yu, 2023). These pollutants are known to desorb in the digestive tracts of marine organisms, sometimes at a faster rate than in seawater (Liu *et al.*, 2020; Santos, Rodríguez-Mozaz and Barceló, 2021). Therefore, ingested microplastics may act as vectors for contaminants entering the marine food web, increasing their bioavailability (Horton *et al.*, 2017). Contaminants such as heavy metals, pharmaceuticals, parabens, cyanotoxins, pesticides, and pathogenic microbes have all been recorded to adsorb onto the surface of microplastics (Zettler, Mincer and Amaral-Zettler, 2013; Brennecke *et al.*, 2016; X. Wu *et al.*, 2019; T. Wang *et al.*, 2020; Gao *et al.*, 2021; Mo *et al.*, 2021; Santos, Rodríguez-Mozaz and Barceló, 2021; Klavins *et al.*, 2022; Moura *et al.*, 2022; Mejías *et al.*, 2023). This knowledge has led researchers to investigate what effects contaminated plastics may have on marine organisms.

Researchers have investigated the combined effects of microplastics and various co-occurring contaminants on bivalves, including pesticides, persistent organic pollutants (POPs), antibiotics and other medicines, petroleum hydrocarbons (PHCs), and metals (Avio *et al.*, 2015; Brandts *et al.*, 2018; O'Donovan *et al.*, 2018; González-Soto *et al.*, 2019; J. Li *et al.*, 2020; Y. Sun *et al.*, 2020; Bringer *et al.*, 2021; Han *et al.*, 2021; Lebordais *et al.*, 2021; Martyniuk *et al.*, 2022). Rios-Fuster, Alomar and Deudero (2023) extensively review the subject, and include studies which use plastic leachates, plastic additives, and unknown contaminants (from weathering) as co-exposure contaminants with microplastics. My review focuses on co-exposure to pollutants which are *not* sourced from plastics but that can co-occur in the marine environment. Whether microplastics and co-occurring pollutants have a synergistic effect on bivalves varies greatly between studies. I found that among 23 studies co-exposing microplastics with other pollutants, eight found co-exposure to majorly aggravate physiological effects, ten found it to minorly aggravate effects, and five found no difference between single and co-exposures (Table 9). Effects were considered major if at least half of the response variables measured experienced a statistically significant effect when co-exposed to microplastics

and contaminants than to either alone. Most studies which found minor effects only found 1 – 3 measures to be synergistically affected by co-exposure treatment, whilst most studies showing major impact had all measures synergistically affected. All studies which found that bioaccumulation of pollutants was not enhanced by co-exposure with microplastics also found that no physiological effects were enhanced after co-exposure. Several studies did find that co-exposure increased the bioaccumulation of pollutants compared to when pollutants were exposed on their own (Avio *et al.*, 2015; O'Donovan *et al.*, 2018; Webb *et al.*, 2020; Zhou *et al.*, 2020; Han *et al.*, 2021). In addition to increased pollutant load, physiological effects which are synergistically enhanced by co-exposure to microplastics include a decrease in shell regeneration, increases in histopathological events (e.g. tissue alterations, haemocyte infiltration, vacuolation), oxidative stress, genotoxicity and immunotoxicity, and gene expressions being increasingly up and down regulated (Table 9). Exposure time did not seem to impact whether co-exposure aggravated effects. Plastic polymer may play a role in in the degree of aggravation caused by co-exposure, as all PE studies either found co-exposure only minorly enhanced effects, or did not enhance them at all. Additionally, all PS studies found co-exposure to enhance effects. Polymer type playing a role is supported by the fact that the physical and chemical properties of microplastics affect their pollutant sorption capacity, with different plastic polymers having differing affinities for different environmental pollutants (Fries and Zarfl, 2012; Hüffer and Hofmann, 2016; Fisner *et al.*, 2017; Amelia *et al.*, 2021). There were no obvious trends between studies which used mussels (13), clams (8), and oysters (2) as their study organism. However, within studies which used clams, all *T. granosa* (6 of 8 clam studies) were majorly affected after co-exposure, and within studied mussels*, M. coruscus* (2 of 13 mussel studies) were the only species which were majorly affected. Thus, some bivalve species would appear to be more at risk and less resilient than others. Microplastic size may also be impacting the degree to which co-exposure aggravates effects, as all studies which used microplastics smaller than 20 µm (range 0.11 -13 µm) found co-exposure to have aggravating effects. Additionally, all studies which found no enhanced effect used PE larger than 20 µm. This is unsurprising as smaller plastics have relatively larger surface areas capable of adsorbing more pollutants. All medicines and antibiotics found co-exposure to have an aggravating effect, however most of these used PS so it is difficult to tell if the plastic polymers or the contaminants were the influencing factors.

Not all co-exposures had aggravating effects. Some authors found co-exposure to mitigate the effects of pollutants (Pittura *et al.*, 2018; Tang *et al.*, 2020; Zhou *et al.*, 2020). Pittura *et al.* (2018) found that after 4 weeks, co-exposure with LDPE (20-25 µm, 10 mg/l) mitigated the decrease in the phagocytic capacity and the granulocyte/hyalinocyte ration caused by benzo(a)pyrene (BaP, 150 ng/l) exposure in *M. galloprovincialis* mussels. Another study found that co-exposure to

fluoranthene (FLO, 42 ng/l) and PS (<500 nm, 0.26 mg/l) for 4 weeks mitigated the effects on UGT and MRP2 gene expression compared to PS alone (both still lower than control) in *T. granosa* clams (Zhou *et al.*, 2020). This mitigation was thought to be caused by the microplastics decreasing the bioavailability of the pollutants when in combination (Brandts *et al.*, 2018; Tang *et al.*, 2020; Zhou *et al.*, 2020). Tang *et al.* (2020) found size-dependent effects when co-exposing *T. granosa* clams to PS (1 mg/l, 500 nm or 30 μm) and BaP (5 or 50 μg/l) or 17B-estradiol (E2, 0.1 or 1 μg/l) for four days. Co-exposure to smaller PS particles exacerbated the effects caused by PS or the pollutants alone, while co-exposure to larger PS particles mitigated the effects. For example, both BaP concentrations co-exposed with small PS microplastics caused ROS (measure of oxidative stress) to be higher than BaP alone, and co-exposure with large PS microplastics caused ROS to be lower than BaP alone; this pattern was the same for E2

It is possible that exposure to the larger microplastics caused the mussels to increase their clearance rates, shortening the in vivo time of microplastic particles and contaminants (Tang *et al.*, 2020). This would give the BaP and E2 absorbed on the microplastics less time to discharge into the intestine and cause damage, thus mitigating their effects (Tang *et al.*, 2020). The effects of microplastic co-exposure with pollutants therefore have similarly variable effects as those when microplastics are exposed alone. This is unsurprising, as in addition to studies using microplastics of varying types at differing doses, there is the further variability caused by different types of pollutants being used at different doses. Multiple factors effect bioaccumulation of pollutants, including desorption, organism internal environment, and retention time (Bakir *et al.*, 2016; Barboza *et al.*, 2019; Menéndez-Pedriza and Jaumot, 2020). Any of these factors could be impacting how much of an effect co-exposure would have and would differ with experimental protocol and study species. The fact that these studies use different plastic polymers, microplastic sizes and amounts, contaminant type and amounts, and study species, and all in different combinations makes comparisons difficult and may explain the range of results.

Despite these findings, several studies have suggested that due to the current concentration of microplastics in the ocean, bioaccumulation of pollutants from microplastics is negligible or minor in bivalves when compared to uptake from natural pathways such as contaminated food and surrounding water (Gouin *et al.*, 2011; Koelmans, Besseling and Foekema, 2014; Bakir *et al.*, 2016; Paul-Pont *et al.*, 2016; Ziccardi *et al.*, 2016; Sıkdokur *et al.*, 2020).

Table 9: Effects of co-exposing microplastics with other contaminants on bivalve physiology. All effects are statistically significant and relative to controls without microplastic or contaminant exposure unless otherwise stated.

Reference	Bivalve species	Type of plastic polymer and contaminant	Methods	Highlights and co-exposure effects
Avio *et al.*, 2015	*Mytilus galloprovincialis*	Polyethylene and PS powder, <100 µm. Pyrene (persistent organic pollutant).	Prior to experimentation, some plastics were sorbed with pyrene by mixing PE with pyrene for 6 days. Mussels were exposed to either PE, PS, pyrene-treated PE (PE-PYR), or pyrene-treated PS (PS-PYR) at 20 g/l for 7 days. Plastics were nominally re-dosed daily at 1.5 g/l. After exposure, tissue samples were taken and immune responses, antioxidant systems changes, neurotoxic effects and gene expressions were measured.	Catalase, MDA, GSH, GR, GST, GPx, total oxyradical scavenging capacity (TOSCA), lipofuscin, neutral lipids, and labialization period were unaffected. Se-dependent GPx was reduced by all treatments except for PE alone. Co-exposure increased pyrene concentrations in the gills and digestive gland compared to pyrene exposure alone. Se-dependent GPx was reduced by PE-PYR but not virgin PE.
Brandts *et al.*, 2018	*Mytilus galloprovincialis*	Polystyrene spheres, 110 nm. Prepared by miniemulsion polymerization of styrene. Carbamazepine (medicine).	Mussels were exposed to either: 0.005, 0.05, 0.5, 5, or 50 mg/l plastic alone (PS), 6.3 µg/l carbamazepine alone (Cbz), or to 0.05 mg/l plastics combined with 6.3 µg/l Cbz (PS+Cbz) for 96 hours. After exposure, samples of the haemolymph, digestive gland and gills were taken to measure gene expression, DNA damage, cell-tissue repair, immune system response, oxidative status, neurotoxicity, carbohydrate metabolism and metabolic enzymes.	In the gills, some genes relating to biotransformation and detoxification processes were affected by PS and PS+Cbz (digestive gland unaffected). In the digestive gland (but not gills), measures of oxidative stress were increased by PS exposure at varying concentrations. DNA integrity decreased after exposure to PS, Cbz and their mixture. Co-exposure had a greater effect on the downregulation of gene expression (e.g. hsp70) than individual exposure.
(Bringer *et al.*, 2021)	*Crassostrea gigas* juveniles	High density polyethylene powder, 20-25 µm. Chlortoluron (pesticide).	Oysters were exposed to either: 112 particles/l HDPE, 85 µg/l of chlortoluron, or 108 particles/l HDPE co-exposed with 97 µg/l chlortoluron for 24 days. During exposure, valve opening activity and shell growth were measured.	Plastic exposure resulted in increased valve micro-closures (VMC), and decreased valve opening duration (VOD) and growth. Chlortoluron exposure caused increased valve opening amplitude (VOA) and decreased VMC. Co-exposure had no additional effect.

González-Soto *et al.*, 2019	*Mytilus galloprovincialis*	Polystyrene spheres, 0.5 µm or 4.5 µm. Benzo(a)pyrene (persistent organic pollutant)	Some plastics were sorbed with BaP (PS + BaP) for 24 hours prior to experimentation. Mussels were exposed to either 0.5 µm PS alone, 4.5 µm PS alone, 0.5 PS + BaP, or 4.5 PS + BaP for 26 days. Plastics concentration was 0.058 mg/l (corresponding to 1000 particles/mL for 4.5 µm MPs and to 7.44×10^5 particles/mL for 0.5 µm MPs) and was renewed daily mixed with algae. Mussels were sampled after 7 and 26 days. Effects were determined on early cellular biomarkers in haemocytes, structure and cell type composition of digestive tubules (DTs), histopathology, and whole organism responses (condition index, clearance rate, food absorption efficiency, respiration rate, and scope for growth).	Catalase (CAT) initially increased for both PS sizes sorbed with BaP but was decreased by day 26 compared to both the control and day 7 levels. The percentage of gametogenic stages was unaffected and gonads showed no histopathological alterations. Respiration rate and clearance rate were highly variable but unaffected. After 26 days absorption efficiency was raised by all treatments except for 4.5 µm PS alone. Catalase, structural integrity of digestive tubules, and cell viability were decreased after exposure to contaminated plastics but not virgin plastics.
Han *et al.*, 2021	*Mytilus coruscus*	Polystyrene beads, 500 nm. Oxytetracycline (OTC), florfenicol (FLO), sulfamethoxazole (SMX) (veterinary antibiotics).	Mussels were exposed to either: PS alone, each contaminant alone, or PS mixed with each contaminant for 4 weeks. Plastic concentration was 0.26 mg/l PS, and contaminant concentrations were 270 ng/l OTC, 42 ng/l FLO, or 140 ng/l SMX. After exposure, measures of contaminant accumulation, immune toxicity (phagocyte activity, haemocyte counts, haemocyte cell viability, distribution of F-actin cytoskeleton), oxidative stress (ROS, GST, LPO), and gene expression were taken.	Phagocyte activity, total haemocyte count (THC), haemocyte cell viability, F-actin cytoskeleton distribution, and GST activity were decreased by all treatments. ROS and LPO were increased by all treatments. All antibiotic accumulation was greater when co-exposed with PS than to antibiotics alone. Generally, gene expressions relating to immunity were lower in co-exposure treatments than single exposure. Effects on immune toxicity and oxidative stress were aggravated by co-exposure compared to single exposures.

Lebordais *et al.*, 2021	*Crassostrea virginica*	Polystyrene: carboxylated additive free latex spheres (PSL), 390 nm; crushed pristine powder (PSC) 692 nm; crushed environmental (from beach debris) powder (NPG), 1071 nm. Arsenic (metal).	Microalgae were pre-exposed to one of each kind of PS (PSL, PSC, NPG) at either 10 or 100 µg/l for 48 hours. Oysters were exposed for 1 week to three single treatments (NPG, PSC, PSL) at both 10 and 100 µg/l as well as three combined treatments (also at 10 and 100 µg/l) which co-exposed algae with 1 mg/l arsenic. Every two days oysters were fed with contaminated algae, and arsenic levels were adjusted to maintain constant concentration. There was an additional control of 1 mg/l arsenic. After exposure, measures of shell length, weight, arsenic bioaccumulation (gills, visceral mass), and gene expressions were taken. The investigated gene functions were endocytosis, oxidative stress, mitochondrial metabolism, cell cycle regulation, apoptosis, detoxification, and energy storage.	Mortality, tissue weight, and condition index were unaffected. Effects on genes were highly variable between plastic concentrations, plastic types, tissue (gills and visceral mass) and genes. All plastic types affected oxidative stress genes, cell cycle genes were repressed by PSC and PSL in both tissues, endocytosis genes were affected by NPG and PSC in the gills, and mitochondrial metabolism genes were repressed by PSC alone but raised by PSC and NPG co-exposure. Co-exposure to plastics did not increase arsenic accumulation compared to arsenic alone. Co-exposure increased expression of 12S (mitochondrial metabolism) and bax (apoptosis) genes compared to single exposures.
Li *et al.*, 2020	*Mytilus edulis*	Ground virgin PVC powder, dyed red, 1-75 µm. Cadmium (Cd) (metal).	Mussels were exposed for 7 days to either: 20 particles/mL PVC alone, 200 µg/l cadmium alone, or a co-expose of 20 particles/mL PVC and 200 µg/l cadmium together which had been pre-mixed and kept in suspension for 3 days prior to exposure. Treatments were renewed after 4 days. After exposure, measures of mortality, shell length, Cd tissue accumulation, haemocyte lysomal membrane stability, transcriptional responses for metal-related stress (Mt-20), antioxidant defence (CAT), and metabolic impact (PK) were taken.	Shell length was unaffected by all treatments. Gene expressions were unaffected by PVC, either alone or co-exposed, except for MT-20 (metal-related stress) which was increased after Cd alone and co-exposure. Co-exposure did not aggravate cadmium uptake. Lysomal membrane stability was not affected by PVC alone but was decreased by co-exposure.

Magara *et al.*, 2018	*Mytilus edulis*	Polyethylene powder, 10-90 µm; virgin or pre-contaminated Fluoranthene (FLU) (persistent organic pollutant).	Some plastics were pre-mixed (incubated) with FLU of 50 or 100 µg/l and left overnight until dry. Mussels were exposed for 96 hours to either: FLU only, PE only, FLU and PE co-exposed, or FLU incubated PE. Treatments were conducted at a low and high concentrations (50 µg/l and 100 µg/l FLU; 100 and 1000 PE particles/mL) that were not crossed i.e. low co-exposure used only low amounts of both FLU and PE. After exposure, FLU uptake and antioxidant response (SOD, CAT, SeGPx, GPx, GSH) were measured in the gills and digestive gland.	SOD was decreased in the gills but unaffected in the digestive gland. CAT and GPx were raised in both tissues. GSH was decreased in both tissues. SeGPx activity was raised in the gills but decreased in the digestive gland. FLU accumulation was higher from FLU-incubated plastics (not co-exposure) than FLU alone. Antioxidant responses were similar for single exposure, co-exposure, and incubation.
Magara *et al.*, 2019	*Mytilus edulis*	White PE powder), 10-90 µm, virgin and contaminated. White polyhydroxybutyrate (PHB) beads, 10-90 µm, virgin and contaminated. Fluoranthene (FLU) (persistent organic pollutant).	Some plastics were pre-mixed (incubated) with FLU of 100 µg/l and left overnight until dry. Individual mussels were exposed to either: FLU only, microplastics only (PE or PHB), FLU and microplastic (PE and PHB) co-exposure, and FLU incubated microplastic (PE and PHB) for 96 hours. The FLU concentrations were 100 µg/l and plastic concentrations were 1000 particles/mL. After exposure, FLU uptake and antioxidant response (SOD, CAT, SeGPx, GPx, GSH) were measured.	In the gills: SOD and GR were unaffected, and CAT, SeGPx, and GST were reduced by most treatments. In the digestive gland: SOD, CAT, SeGPx were reduced by most treatments, GR was raised by some treatments, and GST was unaffected. Co-exposure did not increase FLU uptake compared to FLU alone. Antioxidant responses were similar for single exposure, co-exposure, and incubation.
Martyniuk *et al.*, 2022	*Unio tumidus*	Polyethylene terephthalate, 0.1-0.5 mm. Shape unstated. Ibuprofen (medicine).	Mussels collected from either reference (Pr) or contaminated (Ct) areas were exposed to either 1 mg/l PET, 0.8 µg/l Ibuprofen, or their combination for 14 days. Controls consisted of unexposed mussels from both collection sites. After exposure, measures of antioxidant activities (Mn-SOD, Cu,Zn-SOD, CAT) and oxidative injury (TBARS, protein carbonyl concentrations) were measured.	Mn-SOD was higher for Ct mussels co-exposed to PET and ibuprofen; PET alone had no effect and PR mussels were unaffected. Cu,Zn-SOD activity was supressed and TBARS was increased in Pr mussels exposed to PET. Protein carbonyl levels were increased in Pr mussel co-exposed to PET and Ibuprofen but decreased in Ct mussels; PET alone had no effect on either mussel group. Protein carbonyl concentration and AChE activity were higher in Pr mussel after co-exposure compared to plastic alone.

O'Donovan *et al.*, 2018	*Scrobicularia plana*	Low density polyethylene powder, 11-13 µm; either virgin, pre-contaminated with BaP, or pre-contaminated with perfluorooctane sulfonic acid (PFOS). Benzo(a)pyrene and perfluorooctane sulfonic acid (PFOS) (persistent organic pollutants).	Prior to experimentation, some plastics were with adsorbed with BaP or PFOS. Clams were exposed to either 1 mg/l of virgin LDPE, LDPE pre-contaminated with BaP, or LDPE pre-contaminated with PFOS for 14 days. Plastics were re-supplied every 72 hours and no food was supplied during exposure. Samples were taken on days 0, 3, 7, and 14 to measure BaP accumulation, condition index, oxidative stress (SOD, CAT, GTPx, GST, LPO), genotoxicity and neurotoxicity.	Condition index and DNA damage were unaffected. All oxidative stress biomarkers were affected, but these effects were highly variable between time points, tissue types (gill vs digestive gland), and biomarkers. The digestive gland was less affected than the gills by mechanical damage caused by virgin LDPE. Co-exposure increased pollutant concentrations in clam tissues compared to pollutant exposure alone**.** SOD and acetylcholinesterase (ACHe) in the gills were higher after co-exposure to BaP than plastic alone. GPx and LPO in the gills were higher after co-exposure to PFOS than plastic alone. In the digestive gland, both co-exposures had higher LPO than plastic alone.
Paul-Pont *et al.*, 2016	*Mytilus* spp.	Polystyrene beads, 2 and 6 µm mixed. Fluoranthene (FLU) (persistent organic pollutant).	Plastics were a mixture of 1800 particles/ml of 2 µm PS combined with 200 particles/ml of 6 µm PS to create a final concentration of 2000 particles/day (32 µg/l). Mussels were exposed to either: fluoranthene (FLU) at 30 µg/l, PS at 32 µg/l, or PS and fluoranthene co-exposed for 7 days followed by a 7 day depuration period. Doses were renewed daily at the same time as algal feeding. Mussels were sampled after exposure and depuration to measure haemolymph, fluoranthene quantification, gene expressions, oxidative stress (SOD, CAT, H_2O_2, GR, GST, LPO), and enzyme activity.	**After exposure,** ROS and GR were increased and CAT and LPO were reduced by PS alone. GST and SOD activity were unaffected by plastic treatments. The number of histopathological observations was increased by all treatments. **After depuration**, GR was decreased by co-exposure. LPO was reduced by all treatments and GST and SOD were increased by PS alone. The number of histopathological observations were increased by co-exposure but not plastic alone. Co-exposure increased FLU levels in the digestive gland compared to FLU alone. After both exposure and depuration, co-exposure with FLU caused more histopathological events than plastic alone. Generally, co-exposures did not cause greater effects than single exposures in oxidative stress measures.

Pittura *et al.*, 2018	*Mytilus galloprovincialis*	Low density polyethylene powder, 20-25 µm; virgin and contaminated. Benzo(a)pyrene (persistent organic pollutant).	Prior to experimentation, some plastics were adsorped with BaP by mixing LDPE and BaP for 2 days. Mussels were exposed to either 10 mg/l ($2.34x10^7$ particles/l) virgin LDPE, 10 mg/l LDPE pre-contaminated with BaP, or 150 ng/l BaP for 4 weeks. Treatments were re-dosed daily 12 hours after feeding. Samples were taken on days 7, 14, and 28 to measure LDPE tissue accumulation, BaP accumulation, immune system alteration, oxidative stress (CAT, GST, GPx, GR, GSH, MDA), genotoxicity, and neurotoxicity.	Immune effects were observed for all treatments. Oxidative stress and DNA fragmentation were unaffected. BaP accumulation in the gills and digestive gland did not differ between BaP alone and BaP contaminated plastics. BaP decreased phagocytic capacity and granulocyte/ hyalinocyte ratio to a greater degree than either virgin or contaminated plastics.
Shi *et al.*, 2020	*Telligarca granosa*	Polystyrene beads, 500 nm and 30 µm. Sertraline (medicine).	Clams were exposed to either: 500 nm or 30 µm of 0.29 mg/l PS alone, 100 ng/l sertraline (Ser) alone, or co-exposure to 0.29 mg/l PS (500 nm or 30 µm) and 100 ng/l Ser for 14 days. After exposure measures of immunity (THC), phagocytosis, haemocyte viability, caspase-3 gene activity (apoptosis) and oxidative stress (ROS, LPO), ATP content, plasma cortisol (PK), and gene expressions were taken.	Phagocytosis and THC were reduced by all treatments. All treatments (except for 30 µm PS alone) reduced haemocyte activity. All treatments increased capase-3 activity, ROS, and LPO. ATP was reduced by 500 nm PS alone and co-exposed. All effects caused by single exposures were aggravated by co-exposure. Plastic alone did not affect PK, ACh, or gamma-aminobutyric acid (GABA), but co-exposure increased them.
Sıkdokur *et al.*, 2020	*Ruditapes philippinarum*	Red fluorescent PE beads, 10-45 µm. Either virgin, co-exposed with mercury chloride, or pre-inoculated with mercury chloride. Mercury (metal).	Some plastics were pre-contaminated by mixing PE and Hg stock solution together for 96 hours. Clams were exposed for 7 days to: Hg alone at 10 µg/l, PE alone at 25 µg/l, co- exposure with 10 µg/l Hg and 25 µg /L PE, or 25 µg /L pre-contaminated PE. After exposure, the uptake and tissue distribution of PE and Hg were measured in addition to filtration rates, immunomodulation, oxidative stress, and histological alterations.	Oxidative stress (CAT, GSH, LPO) was unaffected. Filtration rates were reduced by all exposures. After ingestion, microplastics translocated to all the examined tissues: gill, digestive gland, mantle, and remaining tissues. Mercury accumulation did not differ between treatments and neither co-exposure nor pre-contamination aggravated any effects.

Sun *et al.*, 2020	*Telligarca granosa*	Polystyrene beads, 30 µm. Petroleum hydrocarbons, standard mix (persistent organic pollutant).	Clams were exposed to: PS alone, PHCs alone (low and high concentration), or PS co-exposed with PHCs (PHCs at low and high concentrations) for 14 days. Plastics were always at 0.26 mg/l, and PHCs were at 50 µg/l (low) or 100 µg/l (high). After exposure, measures of immunity (THC, haemocyte cell composition, phagocytosis), intracellular ROS content, cell viability, degree of DNA damage, and expression levels of genes from immune-, apoptosis-, and immunotoxicity-related pathways were analysed.	THC, red granulocytes, and phagocytic activity were reduced, and basophil granulocytes activity was increased by all treatments. Intracellular ROS was increased, and cell viability was decreased by all treatments. DNA damage was increased, and immune related genes were suppressed by almost all treatments. Immunotoxicity and apoptosis related genes were increased by 50 µg/l PHCs co-exposed and were decreased by 100 µg/l co-exposed. Generally, 100 µg/l doses had greater effects than 50 µg/l doses, both alone and when co-exposed with plastic. The immunotoxicity and genotoxicity of PHCs was aggravated by co-exposure with PS, but this increased effect was sometimes only seen in high (100 µg/l) PHC co-exposures.
Sun *et al.*, 2021	*Telligarca granosa*	Polystyrene beads, 30 µm. Polycyclic aromatic hydrocarbons (PAH), standard mix (persistent organic pollutant).	Adult clams were exposed to either 0.26 mg/l ($1.76x10^4$ particle/L) PS, 64 µg/l PAH mixture (4 µg/l for each PAH), or a co-exposure of the two with 0.26 mg/l PS and 64 µg/l PAH mix for 2 weeks. Plastics and PAH mixture were renewed daily. After exposure, THC, haemocyte composition and phagocytic activity, oxidative stress (ROS, LPO), DNA damage, and gene expressions were measured.	All treatments decreased THC and haemocyte cell viability, changed haematic composition, and inhibited phagocytosis of haemocytes. All treatments elevated ROS, but LPO and DNA damage were only raised by co-exposure. All gene expressions were affected by PAH alone, and some by PS alone. All effects were aggravated by co-exposure compared to single exposures.

Tang *et al.*, 2020	*Telligarca granosa*	Polystyrene, 30 µm and 500 nm. Shape unstated. Benzo(a)pyrene and 17B-estradiol (E2) (persistent organic pollutants).	Adult clams were exposed to both single and co-exposure treatments for 4 days: Single exposure were: 1 mg/l PS at size 30 µm or 500 nm, BaP at either 5 or 50 µg/l, E2 at either 0.1 or 1 µg/l. Co-exposure treatments were crossed in a way that both sizes of PS were co-exposed with both concentrations of each contaminant. Doses were renewed every day 2 hours after feeding. After exposure, haemocyte counts (THC), cell type and phagocytic activity, intracellular ROS and Ca^{2+} concentration, LZM activity, and gene expressions relating to immunity, Ca2+, and apoptosis were measured.	All single treatments reduced THC, red granulocytes, phagocytic activity, and LZM activity. All single treatments increased ROS content of haemocytes. All single treatments (except for 30 µm PS) reduced Ca^{2+}. Almost all genes measuring immunity and apoptosis were affected by single treatments. Co-exposure to smaller plastics (500nm) aggravated most effects, and co-exposure to larger plastics (30 µm) mitigated most effects in comparison to single treatments.
von Hellfeld *et al.*, 2022	*Mytilus galloprovincialis*	Polystyrene spheres, 4.5 µm: virgin or contaminated. Cadmium (Cd) and BaP (metal and persistent organic pollutant).	Prior to experimentation, some PS was combined with BaP or Cd for 1 day. Mussels were exposed to either: 1000 particles/mL virgin PS, 1000 particles/mL PS sorbed with Cd, 1000 particles/mL PS sorbed with BaP, 1 µM (113.4 µg/l) Cd, or 1 µM BaP (252.3 µg/l) for 3 days. Doses were renewed every 24 hours. After exposure, antioxidant and peroxisomal enzymes activity (CAT, SOD, acyl-CoA oxidase (ACX)) in the digestive gland and gills, lysomal membrane stability, and PS and metal localisation were measured.	Neither virgin nor contaminated plastic affected oxidative stress enzymes, peroxisomal enzymes, or lysomal membrane stability. Both Cd and BaP alone and PS-BaP co-exposure caused histological alterations in the digestive gland. Some histological effects (VvBAS increase and MET/MDR decrease) were aggravated by BaP-PS co-exposure compared to PS alone.
Webb *et al.*, 2020	*Perna canaliculus*	Polyethylene beads, 38–45 µm; virgin or pre-contaminated. Triclosan (medicine: antibacterial).	Some MPs were pre-contaminated by mixing PE with a triclosan solution and then leaving the mixture to evaporate until dry. Adult mussels were exposed to either MPs, triclosan, or triclosan spiked MPs for 48 hours. MPs were at a 0.5 g/l concentration and triclosan at 0.36 mg/l. After exposure the clearance and oxygen consumption rates were measured as well as byssus production and oxidative stress.	LPO and SOD were unaffected by virgin PE but were increased by contaminated PE. Byssus production was reduced by virgin PE. GST activity, filtration rate, and respiration rate were unaffected. Plastics accumulated in the digestive tract equally for the two plastic treatments. Triclosan tissue levels were higher after exposure to triclosan spiked plastics than triclosan alone.

Yu *et al.*, 2022	*Mytilus coruscus*	White PS spheres, 5 µm. Carbamazepine (CBZ) (medicine).	Adult mussels were exposed to either: 0.26 mg/l PS, 10 µg/l CBZ, or co-exposed with 0.26 mg/l PS and 10 µg/l CBZ for 4 weeks. Treatments were renewed daily, one hour after feeding. Two weeks into exposure, 1.42 mm holes were drilled in the middle axis of shells. After exposure, shell regeneration was measured as well as ATP content, phosphofructokinase (PFK) activity, $Ca2^{+}$-ATPase activity, Ca^{2+} content, CA and BMPR2 gene expression, and expression of genes encoding organic shell matrix formation.	Shell regeneration and the expression of shell-formation genes was inhibited by all treatments. All treatments caused ATP, PFK, Ca^{2+}ATPase, Ca^{2+}, CA, and BMPR2 to decrease. Co-exposure greatly aggravated the effects on shell regeneration, shell-formation gene expression, ATP, Ca^{2+}ATPase, Ca^{2+} , and CA in comparison to single treatments.
Zhou *et al.*, 2020	*Telligarca granosa*	Polystyrene powder, <500 nm. Oxytetracycline (OTC), florfenicol (FLO) (veterinary antibiotics).	Clams were exposed to either: 0.26 mg/l PS, 270 ng/l OTC, or 42 ng/l FLO, as well as PS co-exposed to each antibiotic at the same doses for 4 weeks. Doses were renewed daily. After exposure, antibiotic accumulation and oxidative stress (GST activity and 5 gene expressions) were measured, and a food safety risk assessment was done.	GST activity was decreased by all treatments. All treatments reduced the expressions of detoxification genes, except UGT was not reduced by FLO alone. In cooked clams, no treatments posed a risk to antibiotic resistance after human consumption, however raw clams exposed to OTC alone and OTC co-exposed with PS had DEGM (dietary exposure of the human gut microbiota) values within the risk-range. Antibiotic accumulation in clam tissue was higher for PS co-exposure than either antibiotic alone. Co-exposure aggravated effects compared to single treatments for GST activity, most detoxification genes, and DEGM for both raw and cooked clams.

Zhou *et al.*, 2021	*Telligarca granosa*	Polystyrene powder, <500 nm. Oxytetracycline (OTC), florfenicol (FLO) (veterinary antibiotics).	Clams were exposed to either: 0.26 mg/l PS, 270 ng/l OTC, or 42 ng/l FLO, as well as PS co-exposed to each antibiotic at the same doses for 2 weeks. Treatments were renewed daily. After exposure, haemocyte count (THC) and cell type, haemocyte phagocytosis, oxidative stress (ROS and LPO), cell viability and DNA damage, lectin content of serum, and gene expressions were measured.	THC was reduced and LPO was increased by all treatments except by FLO alone. ROS was increased and the number of red granulocytes, phagocytosis, cell viability of haemocytes, and lectin content of serum were reduced by all treatments. DNA damage and Capase-3 were increased by plastic alone and co-exposed to both antibiotics. All immune- and detoxification-related genes were downregulated by all treatments (except IKKa in FLO alone). All effects were aggravated by co-exposure in comparison to single exposures (except for IKKa co-exposed to FLO and PS).

2.6 Properties of plastics in experimental studies vs. environmentally relevant conditions

Microplastic studies vary greatly in the shapes, sizes, materials, and concentrations of microplastics used. Determining whether the plastics used reflect realistic aquatic conditions is key to ensuring experimental insights are applicable and relevant. Using realistic doses is complicated by the fact that different freshwater, estuarine and marine systems accumulate different microplastics loads, and that local-scale microplastics concentrations often vary in both space and time (Skalska *et al.*, 2020; Wu *et al.*, 2020; Mutuku *et al.*, 2024). Additionally, sampling methods and units of measurements differ vastly between studies examining environmental microplastic loads, rendering results incomparable (Burns and Boxall, 2018; Cunningham and Sigwart, 2019). It should be noted that due to common sampling biases in mesh sizes used for collection, there are few data available on marine plastics smaller than 333 µm and almost none on plastics smaller than 50 µm (P. Wu *et al.*, 2019). Nanoplastics are particularly underrepresented due to limitations of sampling apparatus and have only been successfully sampled in recent years (Ter Halle *et al.*, 2017). Additionally, to assess the effects of environmentally relevant microplastic concentrations and characteristics, it is necessary to compare experimental exposures to environmental microplastics found in the water column and not inside wild bivalves, as microplastics ingested by bivalves are not indicative of the plastics in their surroundings (Dahms, van Rensburg and Greenfield, 2020). There is a growing awareness over using microplastic doses and exposure conditions that closely mimic natural systems, with the term “environmentally relevant” having increased 10-fold in studies over the last 20 years (Qu *et al.*, 2023). Consequently, many papers now cite relevant literature after validating that their methodologies and microplastic doses are environmentally relevant (e.g. Walkinshaw *et al.*, 2023).

2.6.1.1 *Relevance of plastic polymer type*

Globally, PP and PE microplastics are numerically dominant in aquatic systems, however, other polymers can dominate locally and are thus also important for understanding the impacts of microplastics on bivalves (Paul-Pont *et al.*, 2018; Baroja *et al.*, 2021). For example, PE (79.9% relative frequency) and PP (77.2% relative frequency) are the most common polymer types in the marine environment, yet, PS dominates the Mediterranean and nylon the North-Western Pacific (Kannankai *et al.*, 2022). Polymer frequency also differs among marine habitats, however, this may vary among locations. For example, PE is the most frequent polymer in the water column, while, PP is most frequently recorded in beach and bottom sediments (Kannankai *et al.*, 2022). Within intertidal and subtidal areas, PP, PE, PA (nylon) and PMMA (acrylic) are the respective dominant polymers (Erni-

Cassola *et al.*, 2017). When considering only marine surface waters (top 10 m), PS (28% of MPs), PP (24%), and polyphthalamide (PPA, 22%) are the most dominant polymer types, presumably due to their low densities (Li*et al.*, 2021; Mutuku *et al.*, 2024).

There is evidence that plastic polymer compositions differ at different size ranges. A study in the North Atlantic Subtropical Gyre found that large microplastics (>300 µm) were solely PE and PP, while smaller microplastics (<20 µm) and nanoplastics (<1 µm) had more diverse compositions. Nanoplastics were primarily PVC (70%) and PET (17%), differing from the larger microplastics (Ter Halle *et al.*, 2017).

The most commonly used micro/nanoplastic in laboratory exposure experiments on bivalves is PS at 55.1% (43 of 78 studies), followed by PE at 29.5% (23 of 78), PVC (10.3%, 8 of 78), and plastic mixes collected from the environment (5.1%, 4 of 78) (Figure 1; this review). All other plastic types (PLA, PET, PP, PHB, PMMA, PA) were each used in 3 or less studies. Most studies reviewed here used environmentally relevant polymers (87.2%), based on the criteria that they used environmentally sourced plastic mixes from their specific study system, or one of the polymers most commonly found in aquatic environments aside from PP (PS, PE). As regional microplastic compositions are often unknown, global data on microplastics had to be used to determine the likelihood that organisms would interact with a particular polymer, i.e. the plastic's environmental relevance, when non-environmentally sourced microplastics were used. Environmentally sourced mixes consisted mainly of PP, PVC, and PE (Bringer *et al.*, 2021, 2022), PE and PET (Romdhani *et al.*, 2024) or were not identified (Lebordais *et al.*, 2021). Some studies (11.5 %; 9/78) compared the effects between two polymer types, and others compared surface properties of the same polymer type, such as using carboxylated vs. aminated microplastics, or virgin vs. environmentally sourced microplastics (9.0%, 7/78). Only two studies which did not use environmentally sourced mixes combined plastic polymers together during exposure (e.g. Revel *et al.*, 2019, 2020). This alone makes most exposure studies different from actual environmental conditions, as plastic polymers of different kinds co-exist within aquatic environments. Considering that PP is one of the most common polymer types found both environmentally and within bivalves (Baroja *et al.*, 2021; Bom and Sá, 2021), it is underrepresented in the current literature, being used in only two studies reviewed here (e.g. Revel *et al.*, 2019, 2020). Future studies should either use environmentally sourced microplastics that are identified after collection or use different polymers of manufactured microplastics mixed in an approximation of local ratios. It is advisable to use local ratios from the same environment that the study species are found, if possible, as polymer ratios differ greatly with location and environment. If local surveys are unavailable, consideration should be given to the habitat of the study species, as low-density plastics

such as PE and PP are buoyant, whilst high density plastics are more likely to sink to the sediment and affect benthic systems (Ivar Do Sul and Costa, 2014; Amelia *et al.*, 2021). Studies that aim to investigate whether microplastics of different polymers have different effects should include PP, as this polymer is common yet currently underrepresented in exposure studies.

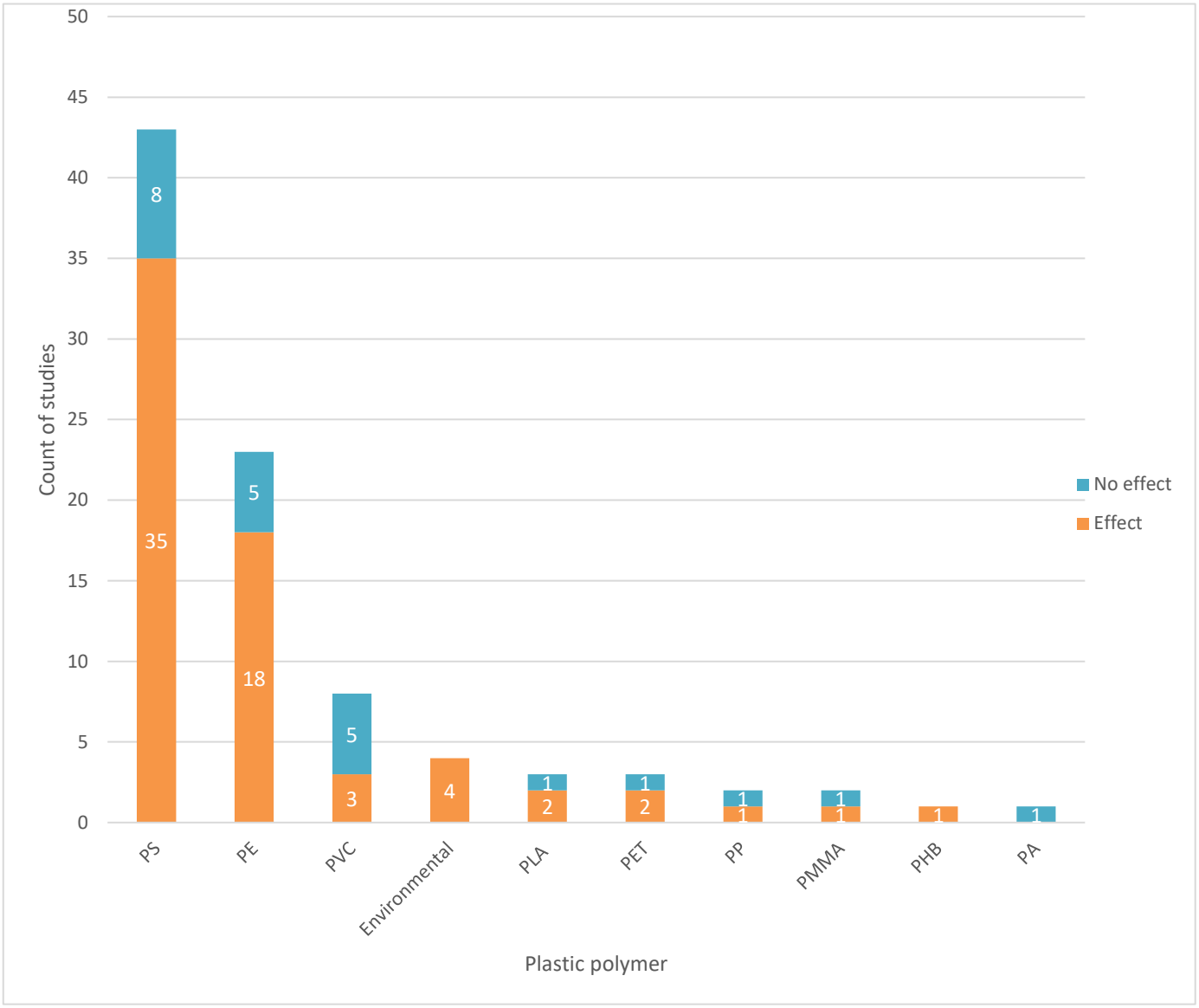

Figure 1: Different plastic polymers used in 78 reviewed studies on the physiological effects of micro/nanoplastics on bivalves: some found effects (orange bars) and others did not (blue bars). Abbreviations: polystyrene (PS), polyethylene (PE), polyvinyl chloride (PVC), polylactic acid (PLA), polyethylene terephthalate (PET), polypropylene (PP), polyhydroxybutyrate (PHB), polymethyl methacrylate (PMMA), polyamide (PA). 'Environmental' refers to plastics that are a mixture of polymers that were collected from aquatic environments.

2.6.1.2 *Relevance of plastic shape*

Microplastics are commonly separated into five main shapes: pellets, fragments, granules, fibres, and films (Van Cauwenberghe *et al.*, 2015). Although a range of shapes can be found in marine ecosystems, fibres are the most common, accounting for up to 91% of all microplastics particles (Desforges *et al.*, 2014; Amélineau *et al.*, 2016; Barrows, Cathey and Petersen, 2018; Railo *et al.*, 2018; Harris, 2020; Baroja *et al.*, 2021; Mutuku *et al.*, 2024). The proportion of fibres do, however, differ between marine systems. They account for 90% of microplastic particles in sandy beach environments, 61% in shallow coastal environments, and 49% in tide-dominated estuarine systems (Harris, 2020). In surface waters (top 10 m), beads and spheres are the least common shape of microplastic (Mutuku *et al.*, 2024). Fragments dominate the surface waters of all oceans except for the Arctic, where fibres are the most common shape (Mutuku *et al.*, 2024).

Beads (50%) and powders (33.3%) are the most commonly used microplastic shapes in experimental studies on bivalves (Figure 2). Fibres were only used in 2.6 % of reviewed studies and a single paper used hexagon shaped glitter. Several papers (17.9%) did not include the shape of the microplastic they used. One paper compared the effects of beads vs. fibres (Cole *et al.*, 2020), and several others compared the effects of beads vs. powders (Magara *et al.*, 2019; Hamm and Lenz, 2021; Weber *et al.*, 2021). Powder-shaped microplastics were either purchased from manufacturers or created through crushing or cryo-milling both environmentally and commercially sourced plastics. There is a clear difference between the shapes of micro/nanoplastics used in exposure experiments and those recorded in environmental surveys.

Using relevant microplastic shapes is important, as some may be more detrimental to bivalves than others due to differing retention time in the gut and gills (De Witte *et al.*, 2014; Gray and Weinstein, 2017). For example, fibres are removed from the digestive track of bivalves more slowly than other shapes of microplastic (De Witte *et al.*, 2014). Despite being the most common shape found in aquatic environments, fibres are rarely used in experimental settings. Only two studies in this review used fibre shaped microplastics (Cole *et al.*, 2020; Walkinshaw *et al.*, 2023), possibly because microfibres are not readily commercially available and are difficult and time-consuming to produce in a uniform size (Cole, 2016; Baroja *et al.*, 2021). Conversely, beads are commonly used because they are convenient. Beads are commercially available and can be embedded with fluorescent labelling or dyes for easier detection (Paul-Pont *et al.*, 2018; Ward, Rosa and Shumway, 2019). Despite the labour required for production, future studies should use microfibres for several reasons. Firstly, they are the most abundant microplastic shape in the aquatic environment (Barrows, Cathey and Petersen, 2018; Baroja *et al.*, 2021). Secondly, they are the most abundant microplastic shape found within both wild and commercially grown bivalves (Li *et al.*, 2015; Baroja *et*

al., 2021; Bom and Sá, 2021; Sendra *et al.*, 2021; Khanjani, Sharifinia and Mohammadi, 2023). Thirdly, they cause the most damage to bivalves due to their elongated shape (Beecham, 2008); Lastly, they are currently severely understudied in controlled exposures. If it is not feasible to use microfibres, virgin beads should be avoided as they over-represented in exposure studies compared to environmental levels.

Secondary microplastics are created through fragmentation, so it is highly possible that the abundance of fragment shaped microplastics will increase in future. In cases where microfibres cannot be sourced, fragments should be used instead (e.g. created by cryo-milling plastic) or microplastics should be collected from the environment (e.g. filtered or milled for size control) (Paul-Pont *et al.*, 2018).

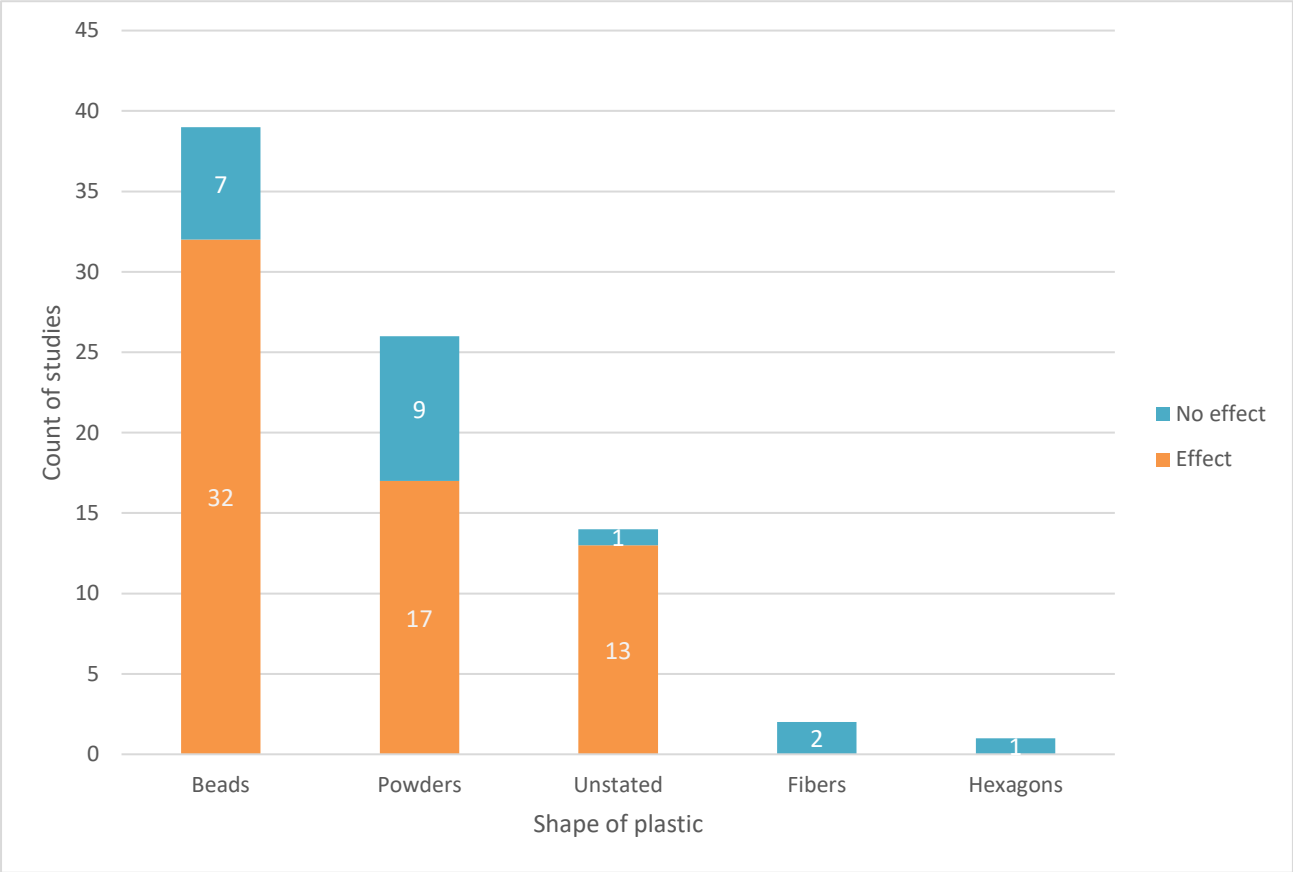

Figure 2: Different plastic shapes used in 78 reviewed studies on the physiological effects of micro/nanoplastics on bivalves: some found effects (orange bars) and others did not (blue bars).

2.6.1.3 *Relevance of plastic size*

A sampling bias towards microplastics >333 µm heavily influences the current understanding on the impacts of microplastic in marine environments (Paul-Pont *et al.*, 2018; P. Wu *et al.*, 2019; Alimi, Fadare and Okoffo, 2021). Studies that have successfully collected smaller microplastics found that they are far more abundant than larger microplastics, and that small microplastics are increasingly

abundant with decreasing particle size (Lusher *et al.*, 2015; Di Mauro, Kupchik and Benfield, 2017; Erni-Cassola *et al.*, 2017; Ter Halle *et al.*, 2017; Paul-Pont *et al.*, 2018). For example, micro/nanoplastics smaller than 300 µm are thought to make up 92% of all microplastics in the South China Sea (Cai *et al.*, 2018).

Even when smaller microplastics or nanoplastics are successfully collected, detection methods like dynamic light scattering (DLS) have limitations in accuracy and resolution, depending on particle size and concentration. For example, 100 nm particles can only be detected within a concentration range of 0.00002 – 0.2 g/l. If plastic concentrations are too low then accurate size analyses are not possible (e.g. Ter Halle *et al.*, 2017; Shi *et al.*, 2024). Additionally, there are few non-destructive particle-based methods for analysing plastics smaller than 100 nm (Shi *et al.*, 2024). There is therefore no current consensus on the size distributions of micro/nanoplastics in aquatic habitats (Baroja *et al.*, 2021).

As there is no consensus on the size composition and distribution of environmental microplastics, it is unclear whether exposure studies use microplastic sizes that reflect real-world conditions. Studies in this review were instead split into biologically relevant size brackets, taking bivalve particle selection and ingestion into account. Bivalves can ingest plastic microspheres ranging from 5 – 300 µm, but as particle size increases above 100 µm, anatomical constraints of the gills, labial palps, and mouth begin to reduce the likelihood of ingestion (Ward and Shumway, 2004; Ward *et al.*, 2019; Ward, Rosa and Shumway, 2019). At ~ 20 µm, more plastic microspheres are ingested than rejected, for particles larger than this the reverse is true (Ward, Rosa and Shumway, 2019). Generally, particle retention/ingestion is efficient down to 1 µm, but for small particles (1-6 µm) there is species-specific variability depending on gill and cilia structure (Møhlenberg and Riisgård, 1978; Rosa, Ward and Shumwy, 2018; Ward *et al.*, 2019; Ward, Rosa and Shumway, 2019). Based on these considerations, size brackets of <1 µm, 1 - 6 µm, >6 - <20 µm, 20 - <100 µm, 100 - <300 µm, and 300 - 500 µm were chosen to classify the biologically-relevant sizes of microplastic used in experimental studies. Studies were classified as “mixed” if they used plastics belonging to more than one size bracket.

Many papers (39.7%) used plastics of mixed sizes, mostly containing both nano (<1 µm) and microplastics in their ranges (Figure 3). In studies that only used plastics from one size class, nanoplastics (<1 µm) were most often used (21.8%), followed by plastics between 20 to <100 µm (15.4 %). None of the reviewed studies used plastics with a mean size larger than 300 µm. As there is no consensus on the actual size distribution of aquatic micro/nanoplastics other than the fact that smaller micro/nanoplastics are more abundant, it is not possible to determine whether a study was

using environmentally relevant sizes of plastic. No studies used microplastics that are larger than those captured by bivalves, and most used plastics that are easily ingestible (<100 µm). Despite reported species specific capture efficiency for particles between 1 to 6 µm and above 100 µm, studies examined here showed no consistent species-specific differences at these size ranges. This suggests that capture efficiency may not be relevant to the effects caused by microplastic exposure in bivalves. Similar numbers of studies found or did not find effects using microplastics < or >20 µm, suggesting effects occur without ingestion or bivalves ingest all sizes indiscriminately.

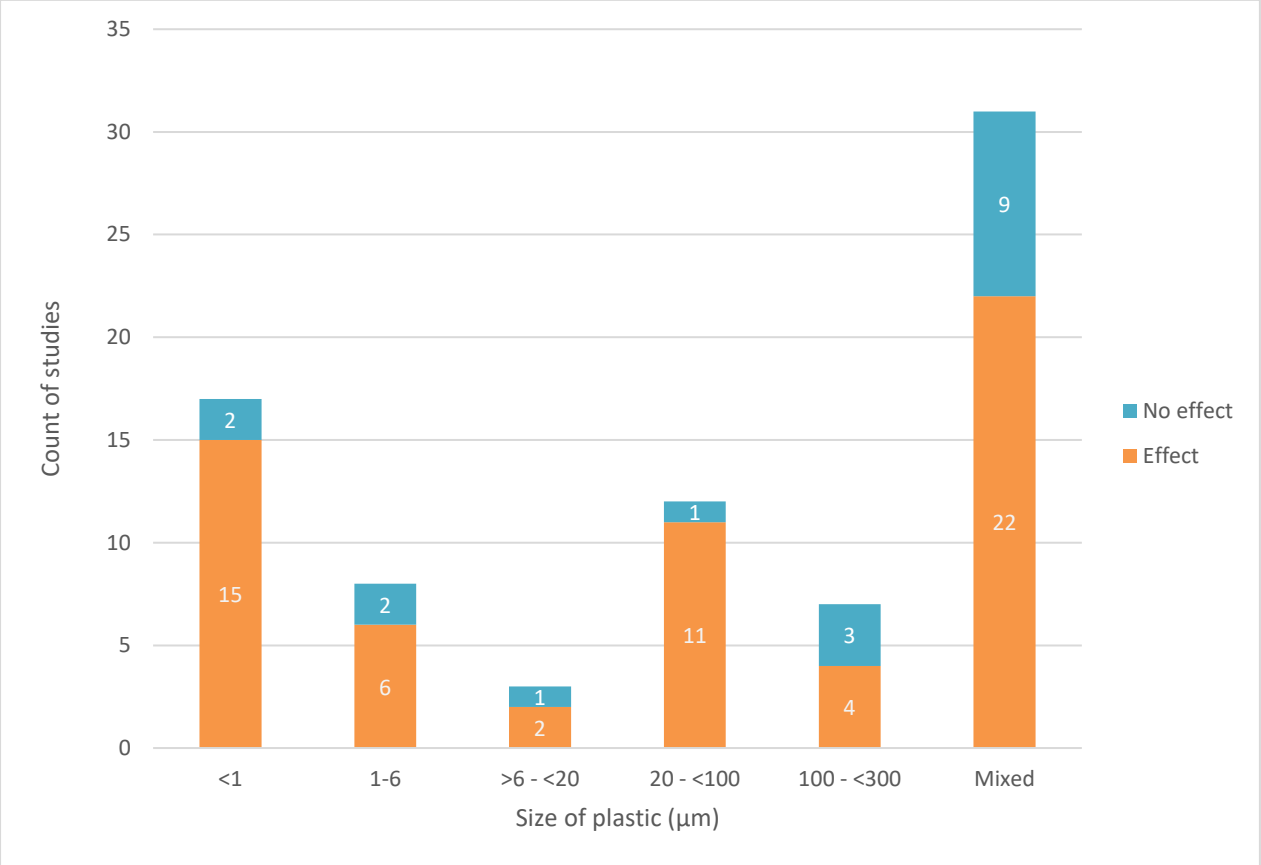

Figure 3: Different plastic sizes (µm) used in 78 reviewed studies on the physiological effects of micro/nanoplastics on bivalves: some found effects (orange bars) and others did not (blue bars). Some studies used a range of sizes which spanned multiple size classes and were designated "Mixed".

2.6.1.4 *Relevance of plastic concentration*

Determining what constitutes an environmentally relevant microplastic concentration is not a simple matter since environmental data on microplastics concentrations is highly varied (Paul-Pont et al 2018). This is because concentrations vary with location, habitat, and height in the water column (e.g. Noren, 2007; Besseling *et al.*, 2014; Lusher *et al.*, 2015; Song *et al.*, 2015; Paul-Pont *et al.*, 2018; Dahms, van Rensburg and Greenfield, 2020). Additionally, marine micro- and nanoplastic densities experience temporal changes, e.g., increases after extreme weather events, or differences between

dry and rainy seasons (Lo *et al.*, 2020; Weideman *et al.*, 2020; C. Li *et al.*, 2021). Maybe most importantly, microplastics of different sizes occur at different concentrations, and sampling bias (e.g. mesh size) can affect estimates of the overall concentration of environmental microplastics (Lindeque *et al.*, 2020). Small microplastics and nanoplastics are far more abundant than larger (>333 µm) microplastics, yet the majority of microplastic sampling studies only collect plastic debris >300 µm (Enders *et al.*, 2015; Ter Halle *et al.*, 2017; Paul-Pont *et al.*, 2018; Baroja *et al.*, 2021). Studies that use nets with multiple mesh sizes to collect water samples from the same location find that the smaller the mesh size, the more microplastics that are collected (Di Mauro, Kupchik and Benfield, 2017; Lindeque *et al.*, 2020). One study collected microplastics from the Gulf of Maine and English Channel using 500, 333, and 100 µm mesh sizes: they found that on average (mean), 100 µm nets (6.03 microplastics/m^3; 10.0 microplastics/m^3) collected ten times more microplastics than 500 µm nets (0.60 microplastics/m^3; 1.03 microplastics/m^3), and 2.5 times more than 333 µm nets (0.54 microplastics/m^3; 4.08 microplastics/m^3) (Lindeque *et al.*, 2020). A study in the Gulf of Mexico found that including microplastic of all sizes had a significantly different result. Microplastics >335 µm had concentrations ranging from 3.6 to 6.4 particles/m^3, but when all sizes were included, concentrations ranged from 60 000 to 157 000 particles/m^3 (Di Mauro, Kupchik and Benfield, 2017). Across the globe, mean nanoplastic concentrations in aquatic systems range from 0.283 – 563 µg/l, however, concentrations as high as 1588 µg/l have been recorded (Materić *et al.*, 2022; Shi *et al.*, 2024). Therefore, current estimates of environmental microplastic concentrations are largely unreliable due to sampling bias or are highly specific to the systems in which they were measured.

The reviewed studies chose the concentrations of their microplastic exposures with different intentions, many intentionally including extreme doses. Some studies purposely used a range of concentrations to include both environmentally relevant and heavily polluted or future levels (Green, 2016; Green *et al.*, 2017; Luan *et al.*, 2019; Harris and Carrington, 2020; X. Wang *et al.*, 2020; Z. Li *et al.*, 2020; Abidli *et al.*, 2021, 2023; Hamm and Lenz, 2021; Weber *et al.*, 2021; Contino *et al.*, 2023; Walkinshaw *et al.*, 2023; Wang *et al.*, 2023). Others purposely used higher than present concentrations to asses current locations that are highly polluted, or to examine future levels that are estimated to be higher than the current (J. Li *et al.*, 2020; Shi *et al.*, 2020, 2022; Sıkdokur *et al.*, 2020; Tang *et al.*, 2020; Lu *et al.*, 2024). Some used a range of concentrations which included extreme values to determine tipping points for ecotoxicological effects, or mechanistic responses that are more easily captured at high doses (Von Moos, Burkhardt-Holm and Köhler, 2012; Magara *et al.*, 2018, 2019; Pittura *et al.*, 2018; Tallec *et al.*, 2018, 2020; Parolini *et al.*, 2020; Webb *et al.*, 2020; Trestrail *et al.*, 2021; Bringer *et al.*, 2022). Others comparing microplastics to natural particles

used concentrations similar to natural seston levels instead of microplastic levels (Yap *et al.*, 2020; Barkhau *et al.*, 2022; Hamm *et al.*, 2022). However, many used extreme doses with no justification.

An initial problem encountered when comparing experimental microplastics to environmental microplastics is that there are several units used to assess microplastic concentrations in exposure experiments, as well as environmental microplastic surveys, making direct comparisons difficult. Previous reviews have used different criteria for classifying environmentally relevant concentrations. Qu *et al.* (2023) considered a study to be environmentally relevant if it used concentrations lower than 15 µg/l or if the study used concentrations that had a realistic reference from an environmental survey; in bivalves this ranged from 0.008 - 230 µg/l (weight/volume studies) and 10 – 10^6 particles/l (particles/volume studies). Cunningham and Sigwart (2019) classified exposure studies as either high (>100 MP/l water; >100 MP/kg sediment) or low (</=100 MP/l water; </=100 mp/kg), the cut off was determined using the highest recorded surface concentration at the time (100 MP/l as recorded by Burns and Boxall (2018). Baroja *et al.* (2021) considered microplastic concentrations to be environmentally relevant if they were equal to or less than 102 particles/l, the highest aquatic concentration recorded at the time as measured by Noren (2007). My review chose to take into consideration the fact that smaller microplastics are found at higher concentrations and used different cut-off points for different size ranges (Table 10). Although environmental surveys and exposure studies both report microplastic concentrations in various units without a standard consensus, the two most common units are particles/volume and weight/volume. As microplastic concentrations cannot be easily converted between these two units, surveys reported in each unit were needed for comparisons. For this review, I considered an exposure study to be environmentally relevant if it used doses that were equal to or lower than the highest environmental concentration recorded for its size class, or authors provided a realistic and relevant reference from an environmental survey. For example, if an exposure study used microplastics between 100 and <300 µm in size, they needed to use a concentration of equal to or less than 31 particles/l or 2.67 µg/l to be considered environmentally relevant, as these are the highest concentrations recorded by environmental surveys which collected microplastics of this size (Table 10).

Table 10: Micro/nanoplastics of different sizes occur at different concentrations in aquatic environments: highest concentrations per size range were used to determine environmental relevance of plastic concentrations used in bivalve exposure studies and are reported in the two most common survey units

Size range of plastic collected in environmental survey (µm)	Highest environmental concentration recorded (particles/l)	Highest environmental concentration recorded (µg/l)	References for highest concentrations (particles/l; µg/l)
<1	$0.2x10^7 – 4.7x10^7$	1588	Xu *et al.*, 2022; Materić *et al.*, 2022
1 - <100	187	69.9	Leslie *et al.*, 2017; Gunaalan, Fabbri and Capolupo, 2020
100 - <300	31	2.67	Song *et al.*, 2015; Reisser *et al.*, 2015

In total, 38.5% of the reviewed studies used concentrations that were considered as environmentally relevant; 29.5% used a range of concentrations or sizes, at least some of which were environmentally relevant; 30.8% used concentrations that are higher than those found in the environment; and one study could not be classified due to its units (Figure 4). Studies that used microplastics between 1 – 100 µm had the greatest proportion considered not to be environmentally relevant relative to the other size classes (Figure 5). Interestingly, using size-specific relevance criteria categorised more studies as environmentally relevant than other reviews did. In contrast to our findings, other reviews concluded that the vast majority (81.5 - 94%) of microplastic exposure studies on bivalves (and other aquatic organisms) used experimental concentrations that exceeded those considered environmentally relevant (Cunningham and Sigwart, 2019; Baroja *et al.*, 2021; Leistenschneider *et al.*, 2023). This is because previous reviews used only one concentration criteria to classify studies, even though microplastics of different sizes occur at different concentrations in the environment. This may have caused studies using small microplastics to be mistakenly classified as environmentally irrelevant. In any case, studies should carefully consider the size or the microplastics they will be using. Local environmental surveys should be conducted as pilot studies when choosing exposure concentrations.

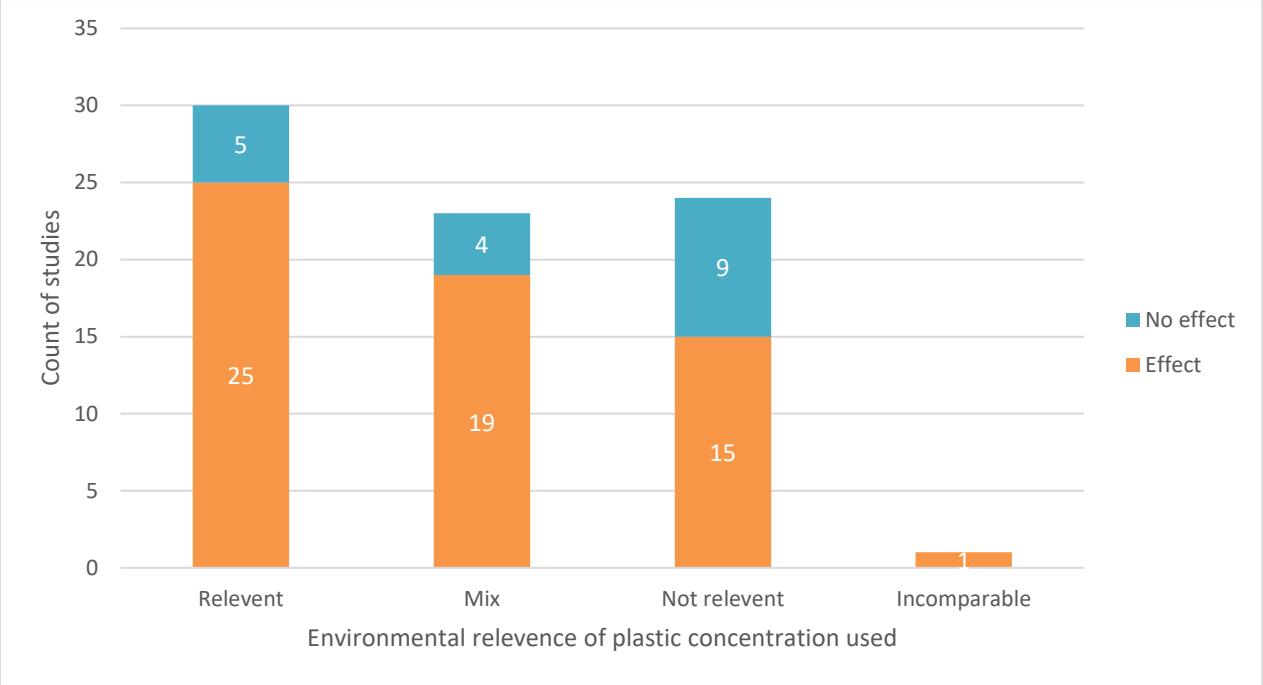

Figure 4: Environmental relevance of plastic concentrations used in 78 reviewed studies on the physiological effects of micro/nanoplastics on bivalves: some found effects (orange bars) and others did not (blue bars).Studies either used environmentally relent concentrations; used a mix of concentrations, some of which were environmentally relevant; used concentrations which were higher than considered environmentally relevant, or used units that were incomparable to environmental surveys.

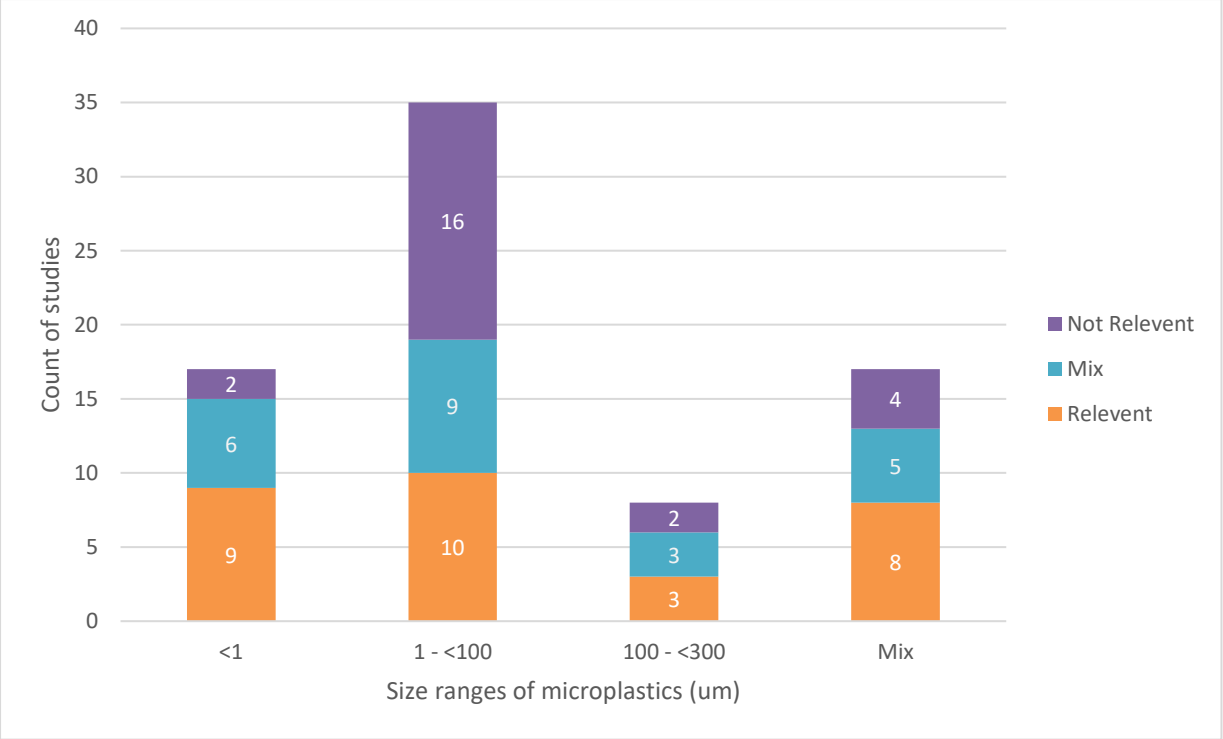

Figure 5: Different plastic sizes used in 78 reviewed studies on the physiological effects of micro/nanoplastics on bivalves. Studies either used environmentally relevant concentrations (orange bars); a mix of concentrations, some of which were environmentally relevant (blue bars); concentrations which were higher than considered environmentally relevant (purple bars); or units that were incomparable to environmental surveys (1 study, not included in figure).

2.7 Knowledge gaps, limitations, and future directions

For experimental studies assessing the impacts of macro and microplastic on marine organisms, environmental plastics should be collected from areas naturally inhabited by the study species. Virgin plastics have very different physiochemical surface qualities compared to naturally weathered plastics found in aquatic environments (Liu *et al.*, 2020). Additionally, virgin plastics make up a small proportion of marine debris and are seldom encountered by aquatic life (Ryan, 2008; Liu *et al.*, 2020). Even though virgin plastics are more easily accessible to experimenters, using environmentally sourced plastics ensures that a study will reflect real-world exposures. If microplastics cannot be collected directly from natural habitats, environmental macroplastics could be ground and milled as an alternative. In addition, for both macro and microplastics, baseline surveys should be conducted to determine local plastic levels. This will ensure that exposure concentrations reflect environmental conditions, which has become a common concern (Qu *et al.*, 2023). Using plastic concentrations orders of magnitude higher than current or predicted levels is

unhelpful to policymakers, who need to make informed decisions based on the realistic harms caused by plastics, not hypothetical extremes.

Currently, literature on microplastic exposure is commonly not directly comparable due to inconsistently reported units of measure among studies. Some studies have provided both the particles per volume and weight per volume of their exposure, a practice that should be applied as standard (Paul-Pont *et al.*, 2016; Rist *et al.*, 2016; Santana *et al.*, 2018; González-Soto *et al.*, 2019; Revel *et al.*, 2020; Sıkdokur *et al.*, 2020; Wang *et al.*, 2024). Some studies claim environmentally relevant doses based on weight per volume survey data, but as they've used smaller microplastics than those in the survey, their particle per volume doses end up much higher than actual environmental levels. Reporting both units would prevent this error and enable comparisons.

When designing microplastic exposure studies, properly controlling variables is essential to clearly identify the source of any effects. When testing one aspect of microplastic exposure (such as polymer functional groups), other aspects (such as plastic size and concentration) should be kept constant, or multi-factor treatments should be fully crossed and interactive effects investigated. For example, Luan *et al.* (2019) aimed to examine if PS with carboxylated (size 200 nm) and aminated (size 100 nm) groups had different effects on *M. meretrix.* They, however, used microplastics of different sizes, and smaller microplastics are known to have more toxic effects on bivalves (González-Fernández *et al.*, 2018; Contino *et al.*, 2023; Liu *et al.*, 2024). This makes it impossible to determine if results between treatments are different due to the different functional groups or due to the different plastic sizes. Furthermore, within the experimental protocol, attention should be given to how microplastics concentrations are maintained when more than a single dose is issued. For example, Paul-Pont *et al.* (2016) renewed microplastics at the same time as feeding, while others renewed plastics separately or did not specify this process (Table 5). This is an important consideration, as co-exposing bivalves to food at the same time as microplastics has been shown to mitigate some of the effects of the microplastic compared to when bivalves are fed separately (Wang, Zhong, *et al.*, 2021; Weber *et al.*, 2021). Even though the effects of microplastics have been well documented in the literature, there are still numerous gaps relating to the types of microplastic doses used compared to realistic environmental levels.

While a large number of studies have been conducted on the effects of microplastics on bivalves, uncertainties remain. Currently, only a couple of studies used controlled laboratory experiments to examine macroplastics' effects. The paucity of literature on the effects of macroplastics on bivalves makes it difficult to discern general and patterns and potential trends. For example, it is difficult to tell if differing methodologies, such as surveys versus manipulative experiments, may be

contributing to the variation seen in results. This may be an important point in smothering experiments, as it has been pointed out that surveys that examine the effects of naturally occurring macroplastic debris usually do not monitor or control how long plastic has been in place (Clemente, Paresque and Santos, 2018). In these cases, if no effect of plastic is found, it may be due to plastic only recently being deposited. There is therefore a great need for further controlled manipulative multi-factorial experiments focusing on the effects of macroplastic on bivalves. Differences may also arise from inconsistent, broad, and arbitrary categorisation of macroplastic debris. Some papers identified plastic polymers, while others used broader categories such as classifying plastic only as either “styrofoam” or “other plastic”. As macroplastic condition has been shown to impact oyster settlement, future research should consider whether the use of virgin or weathered macroplastic is appropriate, and to include this in their methodology as it often unreported. Additionally, most studies (both macro and micro) have studied effects only at the individual level. This means that despite most bivalves forming aggregates that provide numerous ecosystem services, little is known about the effects of plastics at population or community levels.

2.8 Conclusion

The effects of both macro and microplastics on bivalves are highly variable (Figure 6). This can partially be attributed to methodological differences because study species and properties of plastics (size, shape, polymer type, concentration) differ between studies. Few studies have investigated the effect of macroplastic on bivalves, and even fewer of them have used controlled, manipulative experiments. Despite the effects of macroplastics on bivalves being incredibly understudied, there is evidence that they are affecting bivalves and their communities through smothering, habitat modification, provision of artificial substrata for fouling communities, and as a source of leachates and microplastics. Microplastics are known to induce various physiological changes in bivalves, such as reduced filtration rate, reduced food assimilation efficiency and digestion ability, increased respiration, reduced reproductive success, increased oxidative stress, decreased growth and byssus production, and increased immune- and genotoxicity. However, often no effects or limited effects have been reported. Microplastics can act as vectors for other pollutants, and co-exposing microplastics with dissolved pollutants increases their toxicity and effects on bivalve physiology. While this review generally considered the properties of microplastics used in experimental studies to be environmentally relevant, several types of microplastics were found to be understudied compared to their prevalence in the environment. For instance, very few studies use PP or microfibres, despite these being among the most common polymer types and

shapes. Additionally, many (30.8%) studies exclusively use microplastic concentrations that exceed those recorded in aquatic environments. Future experimental studies thus need to carefully choose microplastic concentrations (and other properties) that are environmentally relevant for bivalves.

To address one of the most apparent gaps identified in this review, the following thesis chapter aims to elucidate the effects of macroplastics on bivalve aggregates and their associated communities through a controlled, manipulative experiment.

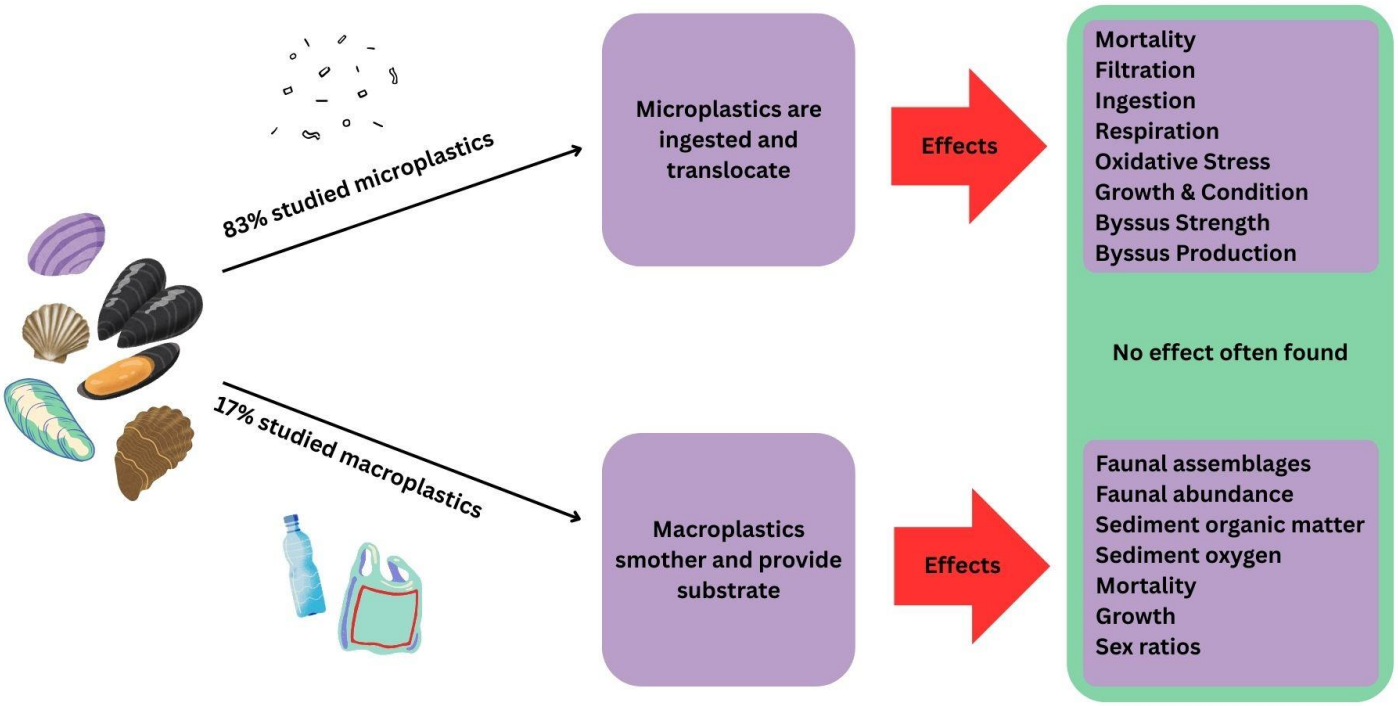

Figure 6: Summary of a literature review of 94 studies on the effects of plastic on bivalves.

3 Chapter 3 – The effects of macroplastics on *Mytilus galloprovincialis* aggregates and their associated fauna

3.1 Introduction

Despite having high recycling rates (46.3% in 2018; Plastics SA 2018) South Africa ranks 11th in the world for mismanaged plastic waste produced per year, producing 0.63 million metric tons per year (Jambeck et al 2015). It should be noted that this figure has been disputed and has shown to be an over-estimate of the amount of plastic mismanaged by South Africa (Vester and Bouwman 2020). Plastic makes up between 74% - 98.9% of litter on South Africa shores and averaged 93.8% across 82 beaches in 2015 (Chitaka and Blottnitz 2019; Plastics SA 2015; Plastics SA 2021; Weideman et al 2020). This shows a significant increase from litter surveys in the 1990s where plastic made up 81.7%–88% of coastal debris in South Africa (Chitaka and Blottnitz 2019, Madzena and Lasiak 1997, Ryan and Moloney 1990). Sandy beach surveys in South Africa show that plastic debris is consistently concentrated around large cities, and that most litter is likely from local, land-based sources (Ryan and Moloney, 1990; Ryan *et al.*, 2018; Chitaka and von Blottnitz, 2019; Ryan, 2020). Unlike sandy beaches, rocky shore anthropogenic debris has only recently been surveyed for the first time in South Africa (Weideman *et al.*, 2020).

South Africa's focus on sandy beach debris is not a local phenomenon. Despite growing concerns about plastic pollution, research on marine debris has overwhelmingly focused on sandy shores, leaving the impacts on intertidal systems such as rocky shore habitats understudied globally (Browne *et al.*, 2015; GESAMP, 2019). The limited research on rocky shores indicate that plastic makes up the majority of debris and is often trapped in the intertidal zone by sand inundation and biotic interaction (Moore *et al.*, 2001; Thiel *et al.*, 2013; Weideman *et al.*, 2020). Some of the most common kinds of plastic debris on rocky shores are plastic fragments, plastic bags, food packaging, and fishing gear (Moore *et al.*, 2001; Thiel *et al.*, 2013; Kuo and Huang, 2014; McWilliams, Liboiron and Wiersma, 2018; Weideman *et al.*, 2020). The dominant type of debris varies with distance from urban areas. Derelict fishing gear is commonly the dominant form of plastic debris in remote areas (Slip and Burton, 1990, 1991; Jones, 1995; Walker *et al.*, 1997; Convey, Barnes and Morton, 2002). New forms of plastic pollution on rocky shores have emerged in recent years: plastiglomerates, 'rocks' of plastic composed of a mixture of melted plastic and natural sediment; plasticrusts, thin plastic layers encrusting onto and keeping with the texture of intertidal rocks; plastiskins, plastic encrusting onto the surfaces of living intertidal organisms (mussels and macroalgae) which are

capable of excluding endoliths on the surfaces' of mussels (Corcoran, 2015; Gestoso *et al.*, 2019; Zardi *et al.*, 2024). Rocky shore biota interact with plastic debris by attaching to it as epibionts, becoming entangled in it, and ingesting it (Weideman *et al.*, 2020). Despite the lack of research, plastic debris can be abundant in rocky shores and frequently encountered by organisms such as mussels.

Mussels are filter-feeding bivalve molluscs found in both freshwater and marine habitats around the world (Brinkman, Dankers and Van Stralen, 2002; Smit and Kaeser, 2016). Most Mytilid mussels, such as *M. galloprovincialis*, are found on exposed shores in the intertidal and occasionally the shallow subtidal zone (Buschbaum *et al.*, 2009). Mussels are capable of secreting byssal threads, allowing them to attach to each other in layered mats as well as various substrates to form aggregates (Yonge, 1962). Aggregated living is beneficial to mussels as it aids them in preventing dislodgement from waves, and offers protection from drilling and crushing predators, desiccation and dislodgement from winter ice (Seed, 1969; Bertness and Grosholz, 1985; Okamura, 1986; Lin, 1991; Carrington *et al.*, 2008; Casey and Chattopadhyay, 2008). There are, however, some negatives to living in an aggregate. At high densities, despite reduced mortality from predation and environmental pressures, individual growth rate is often decreased in smaller individuals (Bertness and Grosholz, 1985).

Due to their ability to form multilayered beds, mussels are important ecosystem engineers in the intertidal zone that create suitable microhabitats for diverse infaunal assemblages (Suchanek, 1985; Tsuchiya and Nishihira, 1986; Jacobi, 1987; Lintas and Seed, 1994; Seed, 1996; Arribas *et al.*, 2014). These microhabitats remain moist and thermally stable during low tides, facilitating suitable conditions for hundreds of species while also offering protection from dislodgement by waves and predators (Seed, 1969, 1996; Suchanek, 1985; Stephens and Bertness, 1991; Stewart, Miner and Lowe, 1998; Crooks and Khim, 1999; Borthagaray and Carranza, 2007). Species richness and biomass within mussel beds are generally higher than on similar surrounding substrate without mussels (Ricciardi, Whoriskey and Rasmussen, 1997; Stewart, Miner and Lowe, 1998; Crooks and Khim, 1999; Borthagaray and Carranza, 2007). In addition to being ecosystem engineers, mussels also provide a number of other ecosystem services (Beaumont *et al.*, 2007; Gundersen *et al.*, 2017). These include provisioning food and recreational harvesting (Hockey, Bosman and Siegfried, 1988; Rius, Kaehler and Mcquaid, 2006; Britz, 2007), water filtration (MacIsaac, 1996; Durand *et al.*, 2020), and use as biomonitors of water quality by scientists (Li *et al.*, 2019). In South Africa, mussels are an important source of food and protein for subsistence harvesters (Hockey aet al 1988, Rius et al 2006) and are

also collected recreationally and grown for aquaculture (Adeleke et al 20121, Kaehler and Mcquaid 2006, Britz 2007, Santa Marta et al 2020).

The four most common mussel species recorded in South Africa include three indigenous species – *Choromytilus meridionalis, Perna perna*, and *Aulacomya ater* – and the invasive *M. galloprovincialis* (Branch and Steffani, 2004). *Mytilus galloprovincialis* was first detected in South Africa in the 1970s and became the dominant mussel species on the west coast in only 20 years (Grant and Cherry, 1985; Griffiths *et al.*, 1992; Robinson *et al.*, 2005). *Mytilus galloprovinciailis* has replaced *A. ater* as the dominant mussel on the west coast, presumably because *M. galloprovincialis* has a higher fecundity, recruitment rate, growth rate, and tolerance to desiccation and indigenous parasites (Van Erkom Schurink and Griffiths, 1990, 1991, 1992; Griffiths *et al.*, 1992; Calvo-Ugarteburu and Mcquaid, 1998b, 1998a; Robinson *et al.*, 2005). In South Africa, *M. galloprovincialis* aggregates support invertebrate faunal communities on both sandy and rocky shores (Robinson *et al.*, 2007a,b). The infaunal communities in invasive *M. galloprovincialis* beds differ from those in indigenous mussels beds (Robinson *et al.*, 2007a,b). There is a positive correlation in abundance of infauna and epifauna associated with the mussels as *M. galloprovincialis* has spread (Griffiths *et al.*, 1992; Robinson *et al.*, 2005; Robinson *et al.*, 2007a). The invasion of *M. galloprovincialis* has affected other marine species besides mussels. *Mytilus galloprovincialis* has also displaced several limpet species, particularly *Scutellastra argenvillei* which used to form mono-specific, high-density belts on semi-exposed and exposed shores (Bustamante, Branch and Eekhout, 1995; Branch and Steffani, 2004). In this way, *M. galloprovincialis* has had mixed impacts on the habitat it has invaded. It has both displaced indigenous species as well as provided an ideal habitat for faunal communities that live within *M. galloprovincialis* aggregates (Van Erkom Schurink and Griffiths, 1990; Griffiths *et al.*, 1992; Bustamante, Branch and Eekhout, 1995; Branch and Steffani, 2004; Hammond and Griffiths, 2004).

Many studies have investigated the physiological effects of plastics on mussels and other bivalves, with the majority of studies focusing on microplastics (Zhang *et al.*, 2020; Sendra *et al.*, 2021; Khanjani, Sharifinia and Mohammadi, 2023; Saraswati, 2023; Xu *et al.*, 2024). Microplastics induce various physiological changes in bivalves including reductions in filtration rate, food assimilation efficiency, digestion ability, reproductive success, growth and byssus production, and increases in respiration, immunotoxicity, and genotoxicity (Avio *et al.*, 2015; Rist *et al.*, 2016; Balbi *et al.*, 2017; Revel *et al.*, 2019; X. Wang *et al.*, 2020; Abidli *et al.*, 2021; Hamm and Lenz, 2021; Wang, Zhong, *et al.*, 2021; Wang *et al.*, 2023). Results are, however, highly variable both between and within studies. In many cases, studies conclude that no effects or inconclusive results are found (Santana *et al.*, 2018; Pedersen *et al.*, 2020; Barkhau *et al.*, 2022; Joyce and Falkenberg, 2022). In addition to studies

examining physiological effects, there is also an increasing interest in evaluating the suitability of using mussels as biomonitors of microplastics and other pollutants (Li *et al.*, 2019; Bendell, LeCadre and Zhou, 2020; Chahouri *et al.*, 2023). Unlike for microplastics, there is a dearth of literature on the effects of macroplastics on mussels. Only a single manipulative study has been performed on the impacts of macroplastic, in which plastic bags were secured on the surface of mud flats for 9 weeks, after which infaunal assemblages were compared to uncovered plots (Green *et al.*, 2015).

The recent focus on microplastics has probably contributed to a relative lack of attention and understanding of the effects of macroplastics on mussels and other marine invertebrates (Li, Tse and Fok, 2016; Bucci, Tulio and Rochman, 2020; Naidoo, Rajkaran, and Sershen, 2020; Li *et al.*, 2021). Additionally, there are hardly any studies that examine the effects of plastic (either macro or micro) on mussels at an assemblage or community level, despite the fact that they generally live as aggregates and have associated communities (Green *et al.*, 2015, 2017, 2019). The single study that addresses the effect of macroplastic on mussels at a community level revealed that the presence of conventional and bio-degradable bags changed the quality of the sediment, reduced infaunal abundance and altered the composition of the infaunal community, causing the exclusion of *Mytilus edulis* mussels entirely (Green *et al.*, 2015b). This highlights the utility of filling the gaps in research surrounding the effects of marine plastic debris on ecosystem engineering mussels, as knowledge of the effects on higher levels of biological organisations is considered to be of critical importance for decision makers in informing policy measures (Green *et al.*, 2015). Given the growing amounts of macroplastics in the environment, this constitutes a problem that needs to be addressed as a basis for effective management actions. This chapter therefore aims to elucidate the little-understood effects of macroplastics on bivalve aggregates and their associated communities through a controlled, manipulative experiment.

The objective of this chapter is to investigate the effects of different shapes (i.e. film versus filament) and quantities of macroplastics on the physiology and physical structure of aggregates of the mussel *M. galloprovincialis*. Furthermore, the experiment explores the effects of macroplastics on the composition of faunal communities associated with mussel aggregates by examining epibiont and mobile faunal abundance, taxonomic richness and diversity.

3.2 Methods

3.2.1 Study Area

False Bay's shoreline consists of both rocky and sandy beaches (Day, 1970). Mussels were collected from Wooley's Tidal Pool and later deployed in a marina in False Bay Yacht Club (Figure 7). Wooley's

Tidal Pool is a rocky intertidal shore on the lee of a kelp forest and is adjacent to a railway line and human settlements. The False Bay Yacht Club is a small marina with berthing facility for yachts. The marina has a sandy seabed interspersed with boulders and is protected from swells by a breakwater.

3.2.2 Mussel collection

Mussels (*Mytilus galloprovincialis*) with shell lengths between 25 mm and 35 mm were collected in October 2020 at low tide from Wooley's Tidal Pool, Kalk Bay (34°07'56.88''S, 18°26' 43.54''E) (Figure 7). Mussels were collected from this area as it is easily accessible and has an abundance of *M. galloprovincialis* mussels. Enough mussels to form 4 to 5 aggregates (experimental units) were collected and processed each day, so that the collection, aggregation, deployment, and re-collection of mussel aggregates was staggered across several days. Collection and all subsequent experimental phases were staggered due to logistical constrains. Mussel aggregates were processed in the same sequence for each phase. Scissors were used during collection to carefully snip connecting byssus threads, avoiding damage to the mussels. In the laboratory, mussels were cleaned of epibionts and their byssus threads cut at the shell margin. The length of each mussel was measured to the nearest mm using callipers, and their wet weights were measured to the nearest mg using a precision scale (Mettler Toledo AE 100 Analytical Balance; measured to 4 decimal places). To quantify the surface area of mussels, a sub-sample was sorted by length into ten 1- mm length classes. Five individuals from each length class were stored in a freezer (-40°C) for one day, after which imprints were made of the mussel shells by painting them red and carefully rolling the entire surface of the mussel onto a paper towel. Images of the imprinted paper towels were scanned using an office scanner along with a ruler for size reference. ImageJ 1.53a (Schneider, Rasband and Eliceiri, 2012) was used to quantify the surface areas of the imprints. The mean surface area (cm^2) of each length class was used for later analyses.

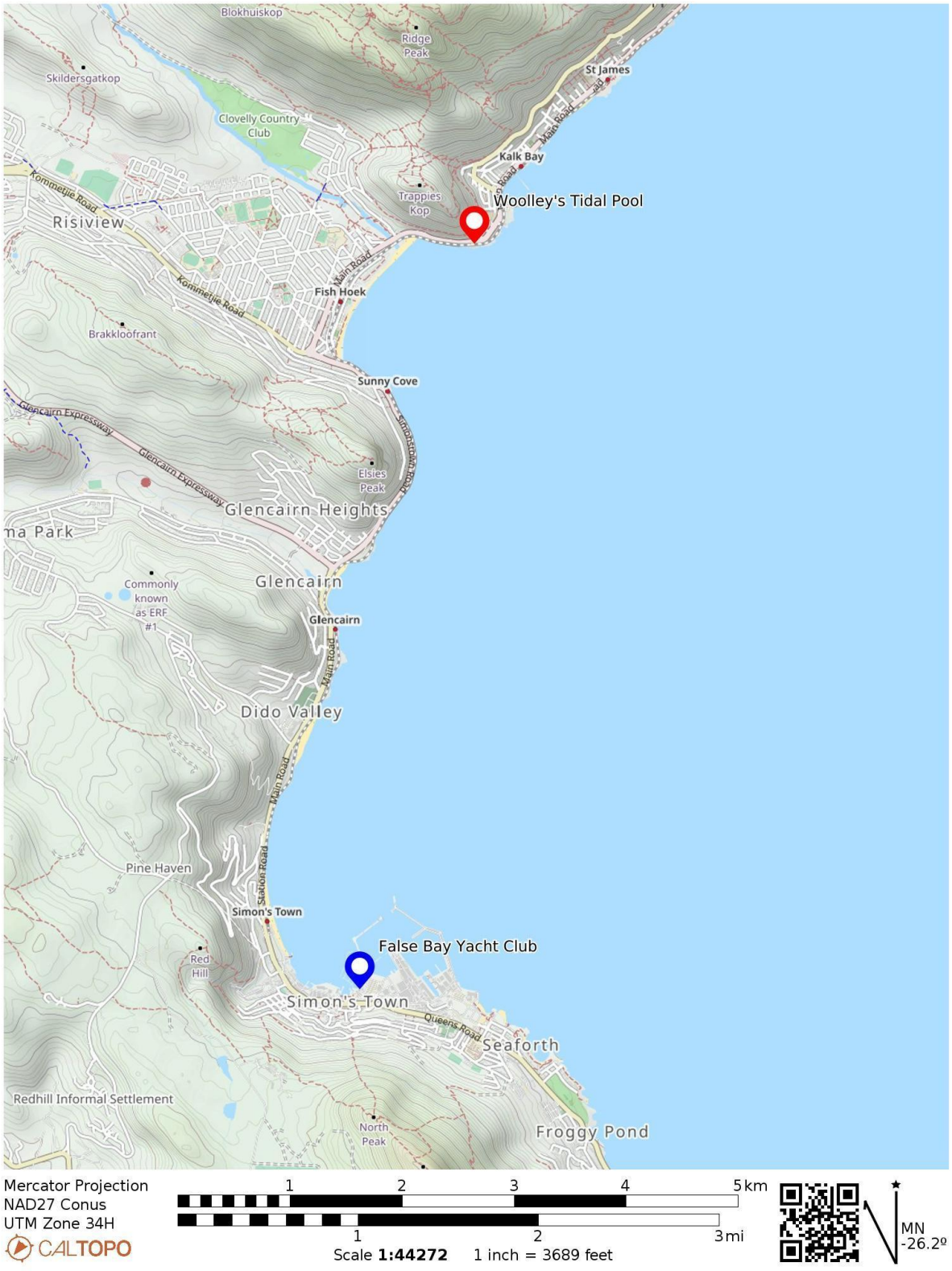

Figure 7: *Mytilus galloprovincialis* were collected from Woolley's Tidal Pool. Mussel platforms were placed in a marina from December 2020 to March 2021 at False Bay Yacht Club (34°11'31.82''S, 18°26'2.35''E) in Cape Town, South Africa.

3.2.3 Mussel aggregation and experimental design

In the laboratory after collection, mussels were randomly sorted into 35 groups containing 30 mussels each. Groups were placed onto individual experimental platforms where they formed aggregates with different plastic treatments (Figure 8). These platforms consisted of 20 cm x 20 cm PVC plastic base plates made of 0.3 cm thick plastic, each constituting an experimental unit (Figure 9a). To be able to suspend the platforms in the water column, holes (4 mm in diameter) were drilled 1.5 cm from the edge of the plate in each corner and at the centre of each plate for the attachment of plastic ski rope (PP). Ropes (1 m long) were attached to the holes in each corner and tied together at the ends of their lengths, forming a pyramid shape (Figure 9b). Holes were also drilled 1 cm from the edge of the plastic at the midpoint of each side to attach fencing to the plate with cable ties. Fencing prevented loose mussels from being washed from the platforms. Fencing was made of plastic (PE) gutter guard (7 cm high) and surrounded the entire base plate. Plastic cable ties (nylon) were used to attach pieces of fencing together.

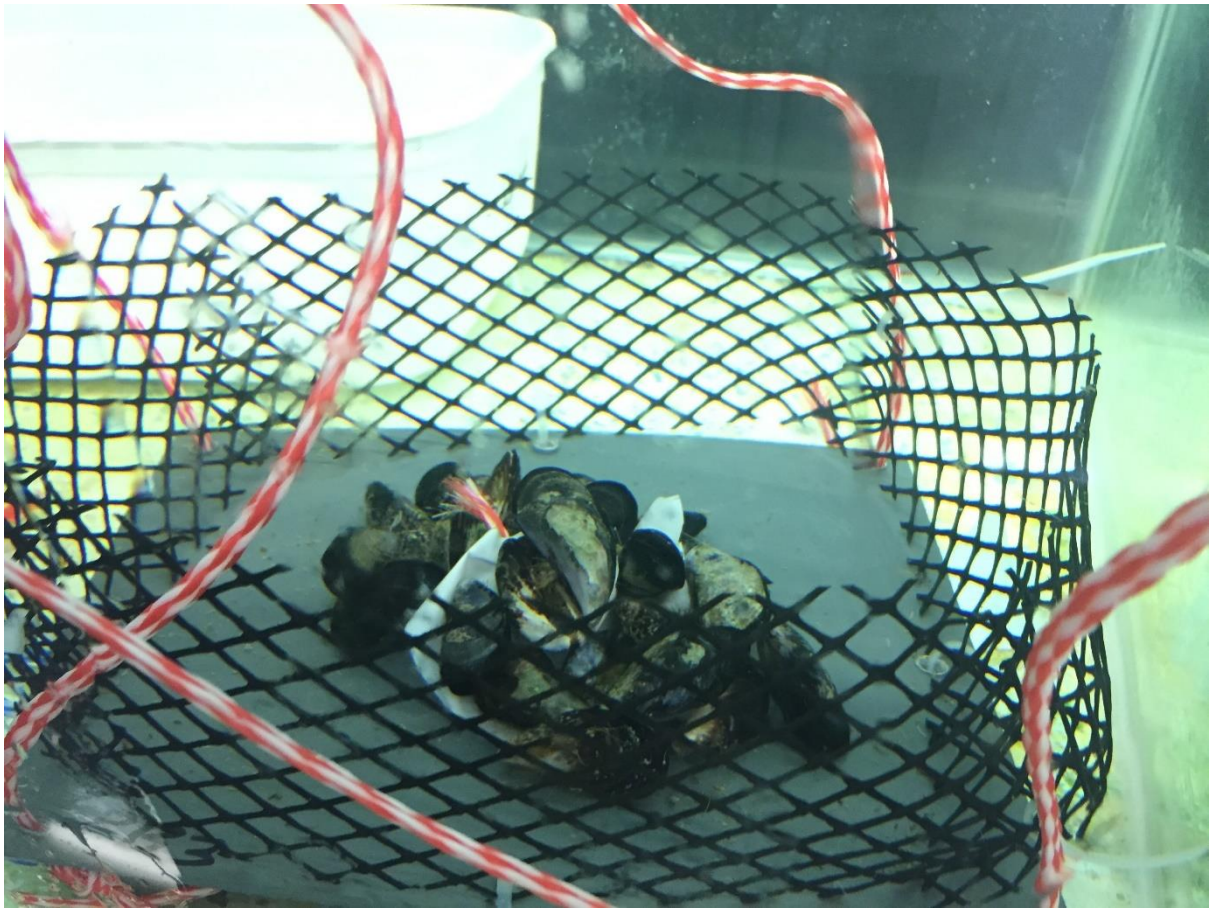

Figure 8: Mussel aggregate on an experimental platform within the laboratory aquarium several days after a piece of polyester bag was added to the aggregate during the aggregation phase.

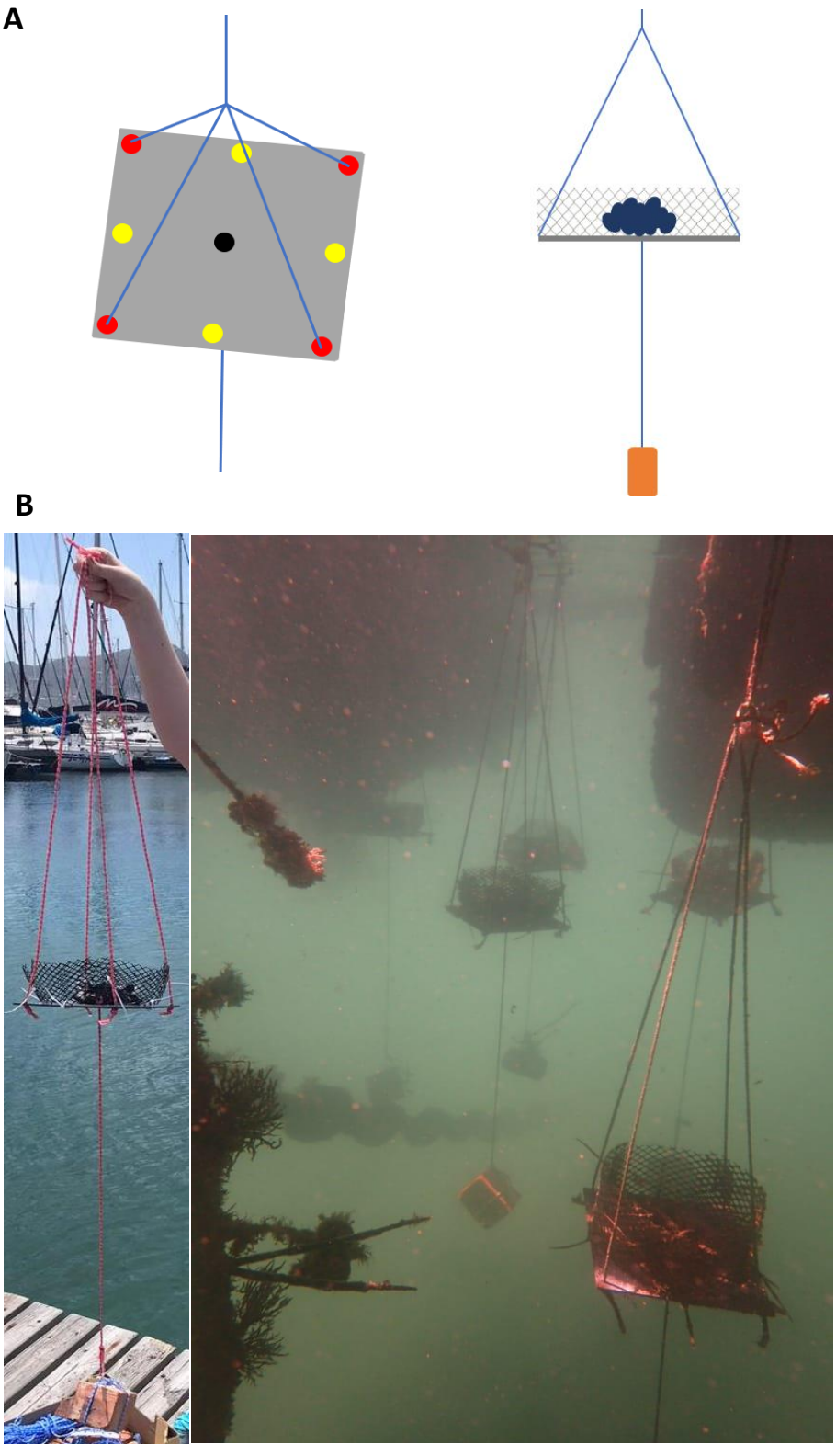

Figure 9: Platform design detailing A) the construction and rope attachment points (fencing not shown) and B) fully constructed platforms as deployed in the marina. Rope was attached at red points, fencing attached at yellow points and secured at corners using zip ties.

The experimental design for the study had two factors that were fully crossed (Table 11) and a control. The first factor, Shape, had two levels, Film and Filament, which described the shape of the plastic used. A single piece of plastic was added to each treated aggregate. Pieces of typical single-use PE plastic bags (60 cm^{2} - 143 cm^{2}) were used for aggregates containing film-shaped plastic, and clear nylon monofilament fishing line for those with filament-shaped plastic (63 cm^{2} - 143 cm^{2}; equivalent to 3.36 m -7.61 m of rope). Plastic bags and fishing line were chosen as they are common debris items on rocky shores that have been recorded to become entangled in and fouled by mussel aggregates in Cape Town (Weideman *et al.*, 2020) and elsewhere (Moore *et al.*, 2001; Thiel *et al.*, 2013). Plastics of different shapes were used to determine if differently shaped plastics could cause different stress induced physiological or structural effects or different degrees of effect. For example, film shaped plastics may be more likely to smother and act as a barrier or substrate, while filament shaped plastics may be more likely to entangle mussels. The second factor, Amount, also had two levels, low and high, and described the amount of plastic added to the mussel aggregates. The amount of plastic added was either 20% (low) or 40% (high) of the total shell surface area of a mussel aggregate. The total shell surface area of an aggregate was calculated as the sum of the shell surface areas of the individual mussels within the aggregate. This allowed for standardised amounts of plastic to be used relative to the size of each aggregate, as mussels of different sizes (25-35 mm length) made up each aggregate. All plastic added was of macro size and had surface areas ranging from 60 cm^{2}–143 cm^{2}.This size range existed due to some aggregates receiving low amounts of plastic (20%), and others high (40%) The amounts of plastic used (20% and 40%) were chosen arbitrarily, as no previous studies existed to inform the selection. Local surveys have not specified the average size of plastic debris found on rocky shores, however one study found that the average weight of litter items was 18.5 g (median = 1.2 g), and that 72% of items weighed less than 5 g (Weideman et al., 2020). Another local study on sandy beaches found that 97% of plastic debris weighed less than 5 g, and that items weighing 0.1–1.0 g contributed from 61% - 85% by count (Chitaka and von Blottnitz, 2019). The weight of plastic used in each aggregate was less than 5 g and therefore similar to plastic fragments found locally. The control treatment (no plastic) had mussel aggregates with no plastic added to them. Each of the five treatments (four experimental and one control) had seven replicate aggregates and each aggregate contained 30 mussels, totalling 1050 mussels across the 35 experimental units.

As weathered plastics have different surface properties compared to virgin plastics, all plastic was weathered in the False Bay Yacht Club basin for one week prior to the start of experimentation following Paul-Pont et al. (2018). By undergoing the same weathering processes that 'natural' marine plastic debris does, the study plastic was allowed to more closely resemble plastic debris

found in marine environments. There was visual evidence that biofilm had accrued on the plastic after weathering, indicating that a week was sufficient time for a biofilm to form, as previously demonstrated by Lobelle and Cunliffe (2011). Biofilm was deemed present if it was visibly detectable to the naked eye on at least half the plastic's surface area.

Mytilus galloprovincialis mussel aggregates had plastic items of different shapes and amounts added to them at the beginning of an aggregation phase, allowing the plastic to be integrated into their three-dimensional structures. Plastic was added to aggregates by a 'sandwich method' where plastic was gently placed between two loose layers of mussels. First, 15 mussels were placed onto the base plate of an experimental platform. Immediately afterward, a piece of plastic was placed on top of the mussels, followed by another 15 mussels placed on top of the plastic. The mussels were then left to re-position and attach to one another, tightly trapping the plastic within the center of the aggregate (Figure 8). Aggregation of the mussels took place over one week in temperature-controlled laboratory aquaria at 18°C, with a flow-through system comprising continuously aerated seawater (1 µm filtered). Seawater was sourced from the Marine Research Aquarium, which collects its seawater from the ocean. Mussels were checked daily. Any dead mussels were recorded and replaced, and any mussels that separated from their aggregate were placed back onto the aggregate to encourage re-attachment. To evaluate whether mussels were dead, any widely gaping mussels were gently tapped, and if they did not close in response to this stimulus they were classified as dead. By the end of the aggregation phase, all mussels within an aggregate were attached by byssal threads either to the base plate of the experimental platform or to each other.

Table 11: The treatment combinations of the two factorial, fully crossed experimental design showing the plastic shape and amount added to *M. galloprovincialis* aggregates in a laboratory setting in preparation for being deployed into the marina. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic was either film (plastic bag) or filament (plastic fishing line) in shape. Each treatment contained seven replicates.

Shape / Amount	**Film**	**Filament**
Low	Low Film	Low Filament
High	High Film	High Filament

3.2.4 Field deployment: sea exposure phase

After a week of aggregation in the laboratory, experimental platforms containing attached mussels aggregates were transported for one hour to the deployment site while submerged in containers filled with laboratory aquarium water. Mussel platforms were placed in a marina from December 2020 to March 2021 at False Bay Yacht Club (34°11'31.82''S, 18°26'2.35''E) in Cape Town, South Africa (Figure 7). The marina exposure period lasted for four months due to logistical constrains. During marina exposure the mean daily sea surface temperature in False Bay ranged between 17.7 °C and 20.7 °C, with mean monthly temperatures of 18.8 °C, 18.7 °C, 20 °C and 19.1 °C for December, January, February, and March respectively (*Sea Temperature Info*). The False Bay Yacht Club was selected as the deployment site for its practicality, safety, and accessibility. Its jetty provided suitable attachment points for the platforms, and as a private club with restricted public access, it could ensure that club members did not interfere with the experiment. The site also provided safe and convenient access to the platforms. Platforms were suspended beneath a jetty in the False Bay Yacht Club marina. Suspension was necessary to protect aggregates from benthic predators such as spiny starfish *Marthasterias glacialis*. At the marina, single building bricks were attached by rope to the ends of the central lines at the bottom of the base plates to act as moorings to restrict movement in the water column. Additionally, extra rope was attached to the top of each 'pyramid' of rope as needed to ensure all platforms sat at the same depth in the water column (approximately 1 m to ensure constant shallow submersion). Platforms were spaced at least 1 m apart so they were not in contact with each other. Suspending each experimental platform simply required the addition of brick and extra rope before tying a platform to the jetty, taking less than five minutes to complete and deploy a platform. The deployment of platforms into the sea followed the same staggered sequence as mussel collection and aggregation. Platforms were deployed randomly along the jetty to reduce bias caused by variability in environmental conditions. Randomization was done by assigning a unique identifier to each replicate and using a list randomizer in R (Posit team, 2022). Replicates were then assigned a number from 1 to 35, depending on the order they appeared in the list, and were aggregated and deployed in the order assigned to them. The number they were assigned also determined which treatment they received so that different treatments were randomly deployed and located along the exposure area. The platforms were cleaned of fouling organisms once a week using a brush while snorkelling. Care was taken to remove only fouling organisms from the platforms and their rigging, but not the aggregates themselves. Platforms were also repaired if necessary during the cleaning sessions.

3.2.5 Lab measurement phase

After the marina exposure phase, the mussel aggregates were retrieved in the same order they had been deployed. Due to time and labour constraints, only three or four aggregates were removed and processed per day, so that total ocean deployment time of each aggregate ranged between 85 and 91 days. During retrieval, experimental platforms were briefly placed in sealed plastic bags to prevent mobile fauna from escaping while the platforms were being detached from the jetty and removed from the marina. During transportation, mussels were kept at ambient temperature and were out of the water for no more than 1.5 hours. In the laboratory, the mussels quickly opened upon being placed in aquaria (18 °C, oxygen saturated, 1 µm filtered seawater). The platforms housing the mussels were deconstructed and removed so that only the base plates with the attached mussels remained. Platforms were deconstructed while submerged by snipping cable ties and ropes, a non-invasive process completed in under five minutes without touching or jostling the mussels on their base plates.

Some measurements and samples were taken on the day of removal (measurements of filtration rate, respiration rate, byssus strength, and wet weight; collection of mobile fauna and particulate matter; estimates of abundance and identification of soft-bodied epibionts) and others were done at a later date (measurements for calculating the body condition index, particulate matter measurement, estimation of abundance and identification of hard-bodied epibionts and mobile fauna). After initial measurements were taken on the day of removal, mussels were frozen and stored at -40 ^{o}C until further measurements could be taken. While awaiting initial processing, mussel aggregates were housed in oxygenated, temperature controlled (18^{o}C) flow-through seawater tanks for no more than three hours.

3.2.5.1 Filtration rate

Filtration rate was measured as it is an indicator of fitness. Filtration is directly linked to mussel feeding and decreased filtration can negatively impact bivalves' ability to grow, function, and reproduce, and is considered the single most important factor controlling growth in bivalves (Griffiths and Griffiths, 1987; Sussarellu *et al.*, 2016; Webb *et al.*, 2020; Walkinshaw *et al.*, 2023). A decreased filtration rate might therefore be an indication of decreased fitness, while increased filtration may be an indication that mussels are needing to compensate for the effects of a stressor to maintain metabolic balance (Griffiths and Griffiths, 1987; Riisgård, Bøttiger and Pleissner, 2012; Hartmann *et al.*, 2016; Martinez, Mayer-Pinto and Christofoletti, 2019). If energetic costs are not met through feeding, then somatic growth, reproduction, and/or energy storage are reduced to still be able to meet the energetic costs of metabolism (Widdows and Johnson, 1988). Feeding is

commonly estimated using clearance rate (Griffiths and Griffiths, 1987). Therefore, the clearance rate of the mussels was measured to determine if macroplastics were affecting the mussels' ability to feed and maintain normal metabolism, thus impacting their fitness.

The filtration rate of the aggregates was measured using the rate of decline of suspended *R. salina* algae (Harris *et al.*, 1998) obtained from the Seapoint Research Aquarium. Mussels were placed in an 18-l tank filled with filtered (1 µm) seawater fitted with an aquarium pump for aeration and water circulation. Once mussels were placed inside the tank they were allowed to acclimatize for ten minutes so that they would open and actively filter. *Rhodomonas salina* algae were added to the tanks to reach a starting concentration of 16 666 cells.ml^{-1}. The first water sample of 2 mL was taken using a pipette immediately after the algae were added. Samples were then taken every three minutes over a 12-minute period; five samples were taken in total. Samples were placed in cryovials that contained 20 µl of glutaraldehyde and stored at -80^{o}C. The water in the experimental tank was replaced with new filtered (1 µm) oxygen-saturated seawater after the filtration rate measurement of each aggregate was complete.

Two blanks were also measured to ensure algal settling rates were negligible. For these, a carrier plate without mussels was placed in the tank and readings were taken using the same methods as when mussels were present.

Samples were later analysed using a FACSymphony flow cytometer to count the number of algal cells.mL^{-1} of each sample. The filtration rates *m* were then calculated using the equation (Coughlan, 1969):

$$m = \frac{V}{n.t}.log_e \frac{Conc_0}{Conc_t}$$

Where *V* (ml) is the volume of suspension, *n* = number of animals used in the experiment, *t* = duration of run (min), $Conc_0$ = initial particle concentration, and $Conc_t$ = final particle concentration.

3.2.5.2 Respiration rate

Respiration was measured as it can be used as a proxy for metabolic rate in mussels (Griffiths and Griffiths, 1987). This, and its ease of measurement, makes it a common biomarker of stress in mussels (Gough, Gascho Landis and Stoeckel, 2012; Ganser, Newton and Haro, 2013; Haney, Abdelrahman and Stoeckel, 2020; Curley *et al.*, 2021). Increased respiration may be an indicator of stress as the organisms try to maintain physiological homeostasis (Smolders, Bervoets and Blust,

2002; Green, 2016; Wang, Zhong, *et al.*, 2021; Jiang *et al.*, 2022). For these reasons, the respiration rates of the mussels were measured to determine if macroplastic exposure caused stress and thus changes in their metabolism.

Respiration rate measurements took place after filtration rate measurements. Mussel aggregates were placed in an 18-l seawater (1 µm filtered, 18 °C) tank with an initial *Rhodomonas salina* concentration of 16 666 cells.mL^{-1}. As respiration and filtration (feeding) are simultaneously operated by the gills , algae were used to stimulate valve opening and active respiration as in Tang and Riisgard (2018). Mussel filtration rate is independent of algal concentrations between 1 500 and 30 000 cells.mL^{-1} : concentrations outside of this range are suboptimal, and filtration and respiration will begin to decrease due to valve closure (Riisgard and Randlov, 1981). Therefore, using algae in this study was a suitable way to ensure that mussels would be open and actively respiring during the experiment without impacting the respiration rates.

An aquarium pump provided constant water circulation during the experiment. After sealing and covering the holding tanks so that the mussels were in complete darkness, the first oxygen consumption reading was taken after two minutes to the nearest 1 mgO_2.L^{-1} using a dissolved oxygen meter (SD400 Oxi L). Readings were then taken every ten minutes for 30 minutes. The water in the experimental tank was replaced with new filtered (1 µm) oxygen-saturated seawater after the respiration rate measurement of each aggregate was complete. Two blanks were also measured. For these a carrier plate without mussels was placed in the tank and readings were taken using the same methods as when mussels were present.

A linear regression was fitted to the measurements of dissolved oxygen concentration over time. The mean respiration rate (R, mg O_2.min^{-1}) of each mussel in the aggregate was calculated using the regression slope and the number of open (i.e. alive and actively filtering) mussels using the equation (Tang and Riisgard, 2018):

$$R = \frac{V \times b}{n}$$

where V (l) is volume of the tank, b is the slope of the regression for the reduction in dissolved oxygen concentration with time (mg O_2.L^{-1}.min^{-1}) and n is the number of actively filtering mussels.

3.2.5.3 *Byssus tenacity*

The byssal tenacity (strength) of the mussels was measured to determine if their ability to attach to each other and the substrate had been affected. If their ability to form aggregates was affected, both their fitness and function as ecosystems engineers would also be affected. Byssal threads are important to the fitness (specifically defence) of mussels as they allow attachment to solid surfaces, preventing dislodgement by waves and predators (Steffani and Branch, 2003; Moeser, Leba and Carrington, 2006; Carrington *et al.*, 2008; Casey and Chattopadhyay, 2008; Sui *et al.*, 2017; Hamm and Lenz, 2021). Stress can induce mussels to increase the number and strength of their byssal threads (Reimer and Tedengren, 1997; Kobak, Kakareko and Poznańska, 2010; Seguin-Heine *et al.*, 2014). However, byssal production is metabolically costly and can be compromised when mussels are subjected to environmental stressors (Sui et al 2017). Therefore, byssal strength was measured to investigate whether macroplastic exposure had acted as a stressor on the mussels, causing them to allocate additional energy to byssal growth, or reducing their fitness by hindering their defence mechanisms and ability to form aggregates.

Five non-adjacent individual mussels were chosen from each aggregate. Each chosen mussel was carefully gripped in a clamp attached to a spring balance and pulled off the aggregate. At the moment of detachment, the spring balance was read, and the mass needed to remove the mussel was recorded to the nearest mg. The mass was converted into newtons to calculate the force (*F*, kg $m.s^{-2}$) needed to remove a mussel from an aggregate. The mean force required was then calculated for each aggregate and used for statistical analyses. The force *F* needed to remove a mussel was calculated using the equation:

$$F = m \text{ x } a$$

Where *m* is mass (kg), and *a* is acceleration due to gravity ($m.s^{-2}$).

3.2.5.4 *Total mortality and body condition index (BCI)*

Total mortality was measured to determine if macroplastic exposure induced lethal effects in the mussels. Body condition index (BCI) is closely related to the fitness of mussels and so was measured to determine if mussel fitness was affected by the macroplastic exposure (Griffiths and Griffiths, 1987). Body condition index has long been used to detect stress in mussels, as changes can indicate that energy storage has been affected (Gilek, Tedengren and Kautsky, 1992; Granby and Spliid, 1995; Lundebye, Langston and Depledge, 1997; Ciparis, Rhyne and Stephenson, 2019). This is because when mussels are under stress they are capable of reducing their growth and tissue condition, so that they are able to maintain metabolism (Bayne, Salkeld and Worrall, 1983; Russell-Hunter and Buckley, 1983). Body condition index also correlates with other measures of well-being in mussels,

such as scope for growth, which also decreases with increasing stress (Martin *et al.*, 1984). Therefore, the total mortality and BCI of the mussels was determined to determine if macroplastic exposure caused stress responses or reduced the fitness of the mussels.

The total numbers of mussels remaining in each aggregate were counted; any missing mussels were recorded as both missing and dead. Mussels were also visually inspected; empty mussels without body tissue were recorded as dead. Fifteen mussels were randomly chosen per aggregate. The shell length of each mussel was measured using callipers to the nearest mm. The soft body tissue of each chosen mussel was oven dried in pre-weighed aluminium containers at 90 °C until constant mass was reached after approximately 48 hours. The dried body tissue was then weighed to the nearest 0.0001 g using a precision scale (Mettler Toledo AE 100 Analytical Balance).

BCI ($mg.cm^{-3}$) was calculated as:

$$BCI = \frac{W}{L^3}$$

where W (mg) is the dried soft tissue and L (cm) is the shell length (Uneputty and Evans, 1997).

3.2.5.5 Spatial complexity

As a measure of physical structure, the spatial complexity of the mussels was quantified to determine if the spatial arrangement of the aggregates was being changed by macroplastic exposure. This could occur either through mussels rearranging themselves in response to stress, or through the plastic physically forcing the mussels to position themselves differently. Mussels typically increase their aggregation behaviour and decrease the space between conspecifics (thereby changing the spatial complexity of the aggregates) in response to stress (Reimer and Tedengren, 1997; Cote and Jelnikar, 1999; Nicastro, Zardi and McQuaid, 2007; Kobak, Kakareko and Poznańska, 2010; de Jager, Weissing and van de Koppel, 2017). This study used bidimensional rugosity to determine changes in spatial complexity. Rugosity is the 'roughness' of the aggregate's spatial organisation, and can influence small-scale water movement. This in turn can influence nutrient and oxygen transfer between the aggregate and the water column, and the propagule settlement of mussels (Bulleri, 2005; Parr *et al.*, 2014; Sadchatheeswaran *et al.*, 2019).

Since *M. galloprovincialis* are bioengineers, changes in their traits may have cascading effects on their associated fauna. For example, the composition of macrofaunal assemblages can change with increased spatial complexity within mussel beds (Gestoso *et al.*, 2013). This is because changes in structural complexity can change the size and shapes of interstitial spaces, as well as the total surface area of the aggregates (Thompson *et al.*, 1996; Crooks, 2002). Changes to the surface area of aggregates would cause a change in the available space for attachment by epibionts (Crooks, 2002; Munguia *et al.*, 2011). Interstitial spaces between mussels provide shelter from physical stress and predators to associated fauna, so a change could affect the number and size of individuals inhabiting the aggregates (Tsuchiya and Nishihira, 1986; Firstater *et al.*, 2011). The spatial complexity of the aggregates was therefore measured to determine if macroplastic exposure caused stress in the mussels, as well as to determine if the habitat of their associated fauna was being altered.

The spatial complexity of the mussel aggregates was assessed using the bidimensional rugosity (BR) index as calculated in Gestoso *et al.* (2013). The greater the BR the greater the spatial complexity of the aggregate.

The bidimensional rugosity (g^{-1}) was calculated as:

$$BR = \frac{\left(\frac{C1 \times C2}{L1 \times L2}\right)}{\left(\frac{DW}{n}\right)}$$

Where *C1* and *C2* (mm) are the bidimensional projections of an aggregates' contours, *L1* and *L2* (mm) are the bidimensional projections of an aggregate's base, *DW* (g) is the dry weight of mussel shells in an aggregate, and *n* is the number of mussels in an aggregate.

To measure *C1* and *C2*, two pieces of damp string were laid in two diagonals across an intact aggregate while it was attached to the base plate. The strings were carefully bent and sculpted around the mussels, following the shape of the mussels as closely as possible. The string was cut, pulled straight, and measured to the nearest mm using a ruler to determine the contoured length across each diagonal. Before removing the string from the mussels, a permanent marker was used to mark where the measurements for the diagonals started and ended. *L1* and *L2* were measured after aggregates had been disassembled and removed from the plates. *L1* and *L2* were measured to the nearest mm as the flat diagonal lengths between the points previously marked on the plates.

To obtain *DW*, the shells of 15 mussels (selected for calculations of body mass index) were oven dried in pre-weighed tin containers at 90 °C until constant mass was reached, after approximately 48 hours. The dry shells were then weighed to the nearest 0.0001 g using a precision scale (Mettler Toledo AE 100 Analytical Balance). The dry mass of the 15 shells was then used to estimate the dry weight of the full *n* shells by finding the mean dry mass of one shell and multiplying the mass by *n*.

3.2.5.6 Particulate organic matter (POM) and mobile fauna

As another measure of physical structure, particulate matter was collected to determine whether macroplastic exposure influenced organic matter accumulation within the mussel aggregates. Mussels are able to redistribute organic particles from the water column to benthic habitats through filter feeding and the subsequent biodepostion of faeces and pseudofaeces (Tsuchiya and Nishihira, 1986; Griffiths and Griffiths, 1987; Kautsky and Evans, 1987). This increases the organic matter mass around mussel beds, increasing the availability of POM to other benthic organisms (Prins *et al.*, 1996; Stewart, Miner and Lowe, 1998; Daunys *et al.*, 2006). The presence of plastic could influence POM accumulation, either through changes to mussel filtration and biodepostion or through physically retaining POM by preventing faeces and pseudofaeces from being washed away. Increased POM retention could possibly provide increased nutrients to associated fauna or potentially be toxic to the mussel aggregates and their associated fauna if accumulation is excessive. Particulate organic matter was therefore collected to determine if its accumulation was a mechanism in plastic exposure affecting mussel physiology and associated fauna.

As mussels aggregates provide habitat for hundreds of species, mobile fauna were collected to determine if macroplastics in aggregates affected not only the mussels but the organisms they provide habitats for (Suchanek, 1985; Tsuchiya and Nishihira, 1986; Jacobi, 1987; Lintas and Seed, 1994; Seed, 1996; Arribas *et al.*, 2014). Since *M. galloprovincialis* are bioengineers, changes in their traits may have cascading effects on associated fauna. For example, the composition of macrofaunal assemblages changes with increased spatial complexity within mussel beds (Gestoso *et al.*, 2013). Therefore, the mobile fauna inhabiting the mussel aggregates were evaluated to determine if macroplastic affected the broader benthic community, either by altering the aggregates or by directly affecting the associated fauna.

Before processing in the laboratory, mussel aggregates were rinsed with one litre of filtered (1 µm) seawater. The rinsing water was passed through a 500 µm sieve and collected for measurements of POM and mobile fauna. The water containing the collected POM was filtered through pre-weighed 2 µm filter papers assisted by vacuum pumps. Samples were rinsed with 30 ml of Milli-Q once all water

was finished filtering. The total amount of water (ml) filtered per sample was recorded. Filter papers were placed in a drying oven on pre-weighed aluminium containers at 80 ^{o}C for 48 hours. The dried POM was weighed using precision scales (Mettler Toledo AE 100 Analytical Balance) to the nearest 0.0001 g.

Mobile fauna collected in the 500 µm sieves were placed in 99% ethanol for later identification. Additionally, the bags used to collect the aggregates were visually searched for mobile fauna and rinsed with water that was passed through a 500 µm sieve. Using a stereo microscope, mobile fauna were counted and identified to species level (or to the next lowest taxonomic level) based on (Branch *et al.*, 1994). Juvenile mussels were classified as mobile fauna instead of epibionts as they were not found attached to the exterior of mussel aggregates but were found in highly dense clumps within the matrix of the mussel aggregates. This meant that they could not be measured as epibionts which were measured by the surface area of the mussel they covered. Taxonomic richness was determined as the sum of the number of different mobile fauna taxa present. Shannon diversity index *H* was calculated in R (Posit team, 2022) using the 'vegan' package (Oksanen, J *et al.*, 2022) using the equation:

$$H = -\Sigma p_i * ln(p_i)$$

Where p_i is proportion of the entire community made up of species *i*.

3.2.5.7 Epibiont cover

As mussel aggregates provide habitat for hundreds of species, epibionts were collected to determine if macroplastic exposure affected not only the mussels but the organisms they provide habitats for (Suchanek, 1985; Tsuchiya and Nishihira, 1986; Jacobi, 1987; Lintas and Seed, 1994; Seed, 1996; Arribas *et al.*, 2014). Mussels can potentially change their spatial arrangement and thus their habitable surface area in response to stress (Reimer and Tedengren, 1997; Cote and Jelnikar, 1999; Nicastro, Zardi and McQuaid, 2007; Kobak, Kakareko and Poznańska, 2010; de Jager, Weissing and van de Koppel, 2017). Additionally, as marine plastics have been shown to act as substrate for fouling organisms, epibiont abundance may increase if the plastic within the aggregates provides additional surface area for attachment (Gündoğdu, Çevik and Karaca, 2017; De-la-Torre *et al.*, 2021). Therefore, the epibionts of the mussel aggregates were evaluated to determine if macroplastic affected the broader benthic community, either by altering the available surface area or otherwise affecting the associated fauna.

The same 15 mussels randomly chosen to calculate BCI were searched for hard-bodied epibionts. Epibionts were identified to the lowest taxonomic level. The percentage cover of each taxon of

epibiont was recorded per mussel. Soft bodied epibionts were identified on all mussels in an aggregate before freezing occurred.

Taxonomic richness was determined as the sum of the number of different epibiont species present. Shannon diversity index *H* was calculated in R (Posit team, 2022) using the 'vegan' package (Oksanen, J *et al.*, 2022) using the equation:

$$H = -\Sigma p_i * ln(p_i)$$

Where p_i is proportion of the entire community made up of species *i*.

3.2.5.8 Data analyses

Evaluating the effects of plastic on the mussel aggregates

Data were analysed using the computing software R version 4.2.2 (Posit team, 2022). All necessary model assumptions were checked prior to finalising analyses. The assumption of normality of residuals was checked using histograms. Homogeneity of variances was checked by comparing the largest and smallest variances; if the largest variance was less than ten times larger than the smallest (Uneputty and Evans, 1997), variances were considered homogeneous.

Firstly, the effects of plastic presence on the respiration rate, filtration rate, byssus tenacity, BCI, mortality, spatial complexity, and POM concentrations of *M. galloprovincialis* aggregates were evaluated using one-way analyses of variance (ANOVA) for each of these variables; the control treatment was included in the analyses as baseline. Secondly, the effects of plastic shape and plastic amount on the same physiological and structural response variables were evaluated using a two-way ANOVA; the control treatment was not included in these analyses, which focused on determining potential interactions between plastic amount and shape. Posthoc Tukey comparisons were used where interactions were significant. As mortality data did not meet the assumptions for ANOVA, a Welch-adjusted ANOVA was used to evaluate for the same effects. Particulate organic matter (POM) mass and respiration data were log transformed prior to analyses to conform to model assumptions.

Evaluating the effects of plastic on the associated fauna of the mussel aggregates

Firstly, the effects of plastic presence on the abundance, area covered, taxonomic richness, and diversity (Shannon diversity index) of epibionts and mobile fauna associated with the *M. galloprovincialis* were evaluated using a one-way ANOVA. The control treatment was included in these analyses. Secondly, the effects of plastic shape, amount, and their interaction on the cover (i.e.

abundance), taxonomic richness, and Shannon's diversity of the epibionts and mobile communities associated with the *M. galloprovincialis* were individually evaluated using two-way ANOVAs. The control treatment was not included in these analyses to prevent perfectly aliased coefficients. As Shannon diversity data for epibionts did not meet the assumptions for ANOVA, a Kruskal-Wallis test was used to evaluate for the same effects. Mobile fauna abundances were log transformed, area covered by epibionts were cube root transformed, and epibiont Shannon diversity index values were cube root transformed prior to analyses to conform to model assumptions.

The Bray-Curtis similarity index was used to quantify the level of similarity among treatments for both the epibiont and mobile fauna community composition. Abundances of mobile fauna were cube-root transformed prior to calculating the Bray-Curtis index to reduce the influence of abundant species. Results are presented as non-metric multidimensional scaling plots (NMDS).

Two-way permutational multivariate analyses of variance (PERMANOVAs) with 999 free permutations were used to determine the effect of plastic shape and amount (and their interaction) on the community composition of epibionts and associated mobile fauna. Homogeneity of variances was tested visually by producing boxplots showing the multivariate dispersion, as well as performing ANOVAs on the distances to the group centroids (multidimensional means). Abundances of mobile fauna were cube-root transformed prior to analyses to conform to model assumptions.

Analyses of similarities (ANOSIMs) were used to determine the effect of plastic presence, plastic shape, and plastic amount on the community composition of epibionts and mobile fauna. Homogeneity of variances was checked by comparing the largest and smallest variances; if the largest variance was less than ten times larger than the smallest, variances were considered homogeneous (Frias and Nash, 2019). Abundances of mobile fauna were cube root transformed prior to analyses to conform to model assumptions.

3.3 Results

The mussel aggregates spent between 85 and 91 days in the marina. During marina exposure, one of the control replicates was lost to predation by the spiny starfish *Marthasterias glacialis*, but all other replicates were recovered. In general, by the end of the marina exposure period the mussel aggregates exposed to film shaped plastic had completely surrounded the plastic, which was crumpled in the centre of the aggregate and no longer visible. The mussels on the outermost layer of the aggregate were no longer in contact with the plastic. During the marina exposure, several replicates ejected the film shaped plastic from the aggregate completely. Rejection was evident as

the plastic was no longer within the aggregates, nor were any mussels attached to the plastic. Ten response variables were measured during the laboratory phase. Five response variables related to mussel physiology (respiration rate, filtration rate, byssus strength, BCI, and mortality), two to the physical structure of the aggregates (POM mass, spatial complexity) and three to the community composition of epibionts and mobile fauna living in and upon the aggregates (cover or abundance, taxonomic richness and Shannon diversity). Thirty-four full aggregates had their respiration rates, filtration rates, mortalities, POM masses, and spatial complexities measured. Five individuals per aggregate (170 mussels in total) were subsampled to measure the strength of their byssus threads. Fifteen individuals per aggregate (510 mussels in total) were processed further to obtain body condition indices.

3.3.1 Effects of plastic on the mussel aggregates

All response variables from the mussel aggregates were highly variable. Most data needed to be transformed to meet the assumptions of statistical testing, which contributed to relatively low statistical power. Thus, even though some patterns were visible within the data, they were not necessarily statistically significant. Filtration rate values were deemed unreliable and were discarded from further analysis.

The impacts of plastic presence were assessed by comparing the controls (no plastic) to the experimental treatments (one-way ANOVA). Plastic presence had no significant effect on any of the response variables (

Figure 10 and Figure 11, Table 12). However, the controls consistently had lower or equal mean POM concentration, rugosity, and byssus strength per individual than treatments that included plastic. Conversely, mean respiration rate per individual and BCI for the controls were higher than or equal to the means of treatments including plastic.

A second analysis (two-way ANOVA) showed that plastic amount (low, 20% or high, 40%) also had no clear effect on physiology, structure, and associated fauna of mussel assemblages, except for respiration rates per individual, which were lower for low plastic than high plastic ($p > 0.05$) (

Figure 10 and Figure 11, Table 13). Similarly, plastic shape had a statistically negligible ($p > 0.05$) impact on the suite of response variables, although respiration rates per individual and BCIs per individual appeared lower for filament shaped plastic than for film shaped plastic. There was, however, a significant interaction between shape and amount for epibiont cover ($p < 0.05$) (Figure 11C, Table 13), with posthoc Tukey tests confirming that low amounts of filament shaped plastics led to decreased epibiont cover (adjusted $p = 0.0289$). While not statistically significant (Table 13), it was

noticeable that for all the response variables relating to associated fauna (Figure 11), treatments without plastic consistently had means higher than, or equal to treatments with plastic. Plastic presence appeared to have a greater impact on epibionts than mobile fauna, as epibionts tended to have lower cover, richness, and diversity when plastic was incorporated. In contrast, mobile fauna abundance, richness, and evenness all had treatments with similar means, except for low filament, which had smaller means and more variable results than the other treatments.

Table 12: Summary of one-way ANOVA results testing for the effect of plastic presence on physiology, structure, and epibionts and mobile communities associated with *Mytilus galloprovincialis* aggregates. Degrees of freedom (effect, total) are shown in subscript next to F values. The test statistic for epibiont diversity is chi2 (not F) as a Kruskal-Wallis test was performed as assumptions were not met for an ANOVA. F is the F-Statistic and P is the significance value of each ANOVA result.

Response variable	F_{df}	P
PHYSIOLOGY		
Respiration rate	$1.022_{4,33}$	0.413
Byssus strength	$0.635_{4,33}$	0.642
BCI	$0.859_{4,33}$	0.500
Mortality	$0.584_{4,33}$	0.680
STRUCTURE		
Spatial complexity	$0.118_{4,33}$	0.975
POM	$0.219_{4,33}$	0.926
EPIBIONTS & MOBILE FAUNA		
Epibiont cover	$1.809_{4,33}$	0.154
Epibiont richness	$0.529_{4,33}$	0.715
Epibiont diversity	$1.449_{4,33}$	0.840
Mobile fauna abundance	$0.830_{4,33}$	0.517
Mobile fauna richness	$1.159_{4,33}$	0.349
Mobile fauna diversity	$0.301_{4,33}$	0.875

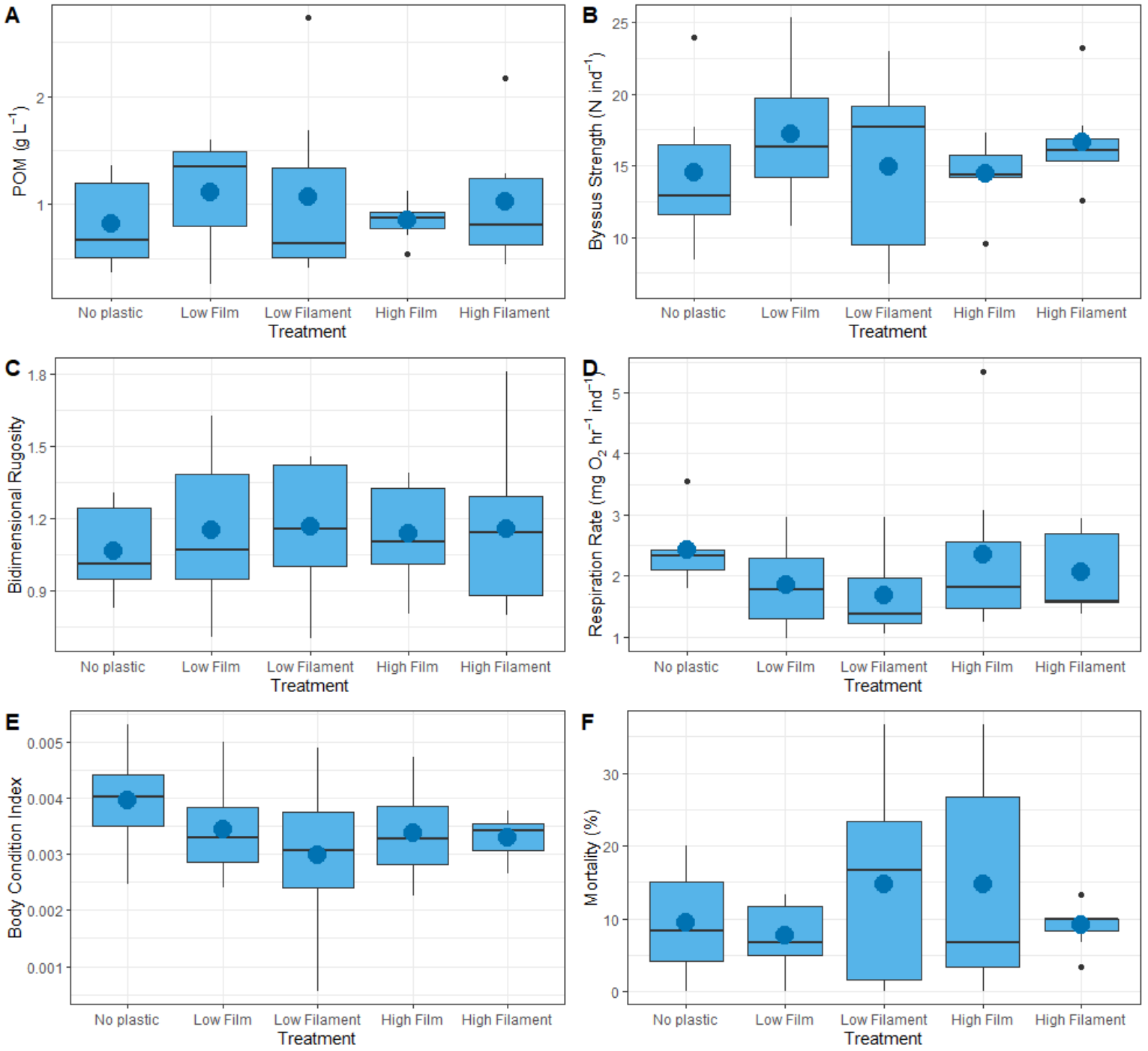

Figure 10: Effects of plastic presence, shape and amount on A) POM mass, B) individual byssus strength, C) bidimensional rugosity, D) individual respiration rate, E) body condition index (BCI), F) mortality of *M. galloprovincialis* aggregates after 85-91 days of marina exposure. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was either film (plastic bag) or filament (plastic fishing line). Each treatment had n=7 replicates, except the No plastic control group which had n=6. Blue dots show the means of each treatment, and horizontal lines the medians. Black dots indicate outliers. The boxes show the interquartile ranges and the whiskers the minimum and maximum values. No significant differences were detected between the control (no plastic) and plastic treatments.

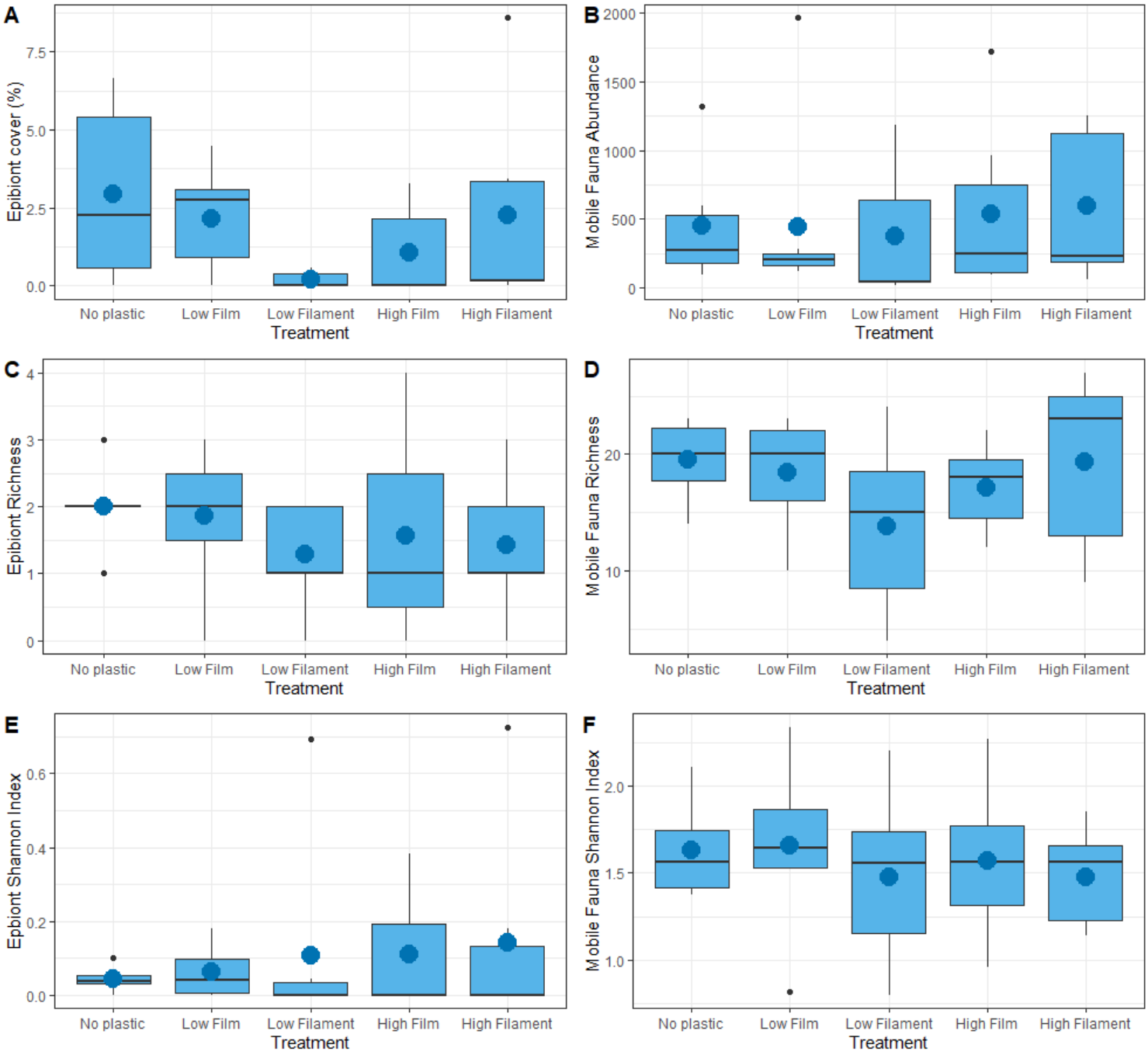

Figure 11: Effects of plastic presence, shape and amount on A) epibiont cover, B) mobile fauna abundance, C) epibiont richness, D) mobile fauna richness, E) epibiont diversity (Shannon index), and F) mobile fauna diversity (Shannon index) of *M. galloprovincialis* aggregates. Each aggregate contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amount of plastic (40% aggregate surface area). Plastic was either film (plastic bag) or filament (plastic fishing line) in shape. Each treatment had n=7, except the no plastic control group of n=6. Blue dots show the means of each treatment, and horizontal lines the medians. Black dots indicate outliers. The boxes show the interquartile ranges and the whiskers the minimum and maximum values.

Table 13: Summary of two-way ANOVA results testing for the effect of plastic shape, plastic amount, and their interaction on physiology, structure epibionts and mobile fauna in Mytilus galloprovincialis aggregates. The aggregates contained either low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Significant results highlighted by **. Degrees of freedom (effect, total) are shown in subscript next to F values. F is the F-Statistic and P is the significance value of each ANOVA result.

Response variable	Source of variation					
	Plastic Amount		Plastic Shape		Amount: Shape	
	F	P	F	P	F	P
PHSIOLOGY						
Respiration rate	$1.552_{1,27}$	0.225	$0.197_{1,27}$	0.662	$0.020_{1,27}$	0.889
Byssus strength	$0.014_{1,27}$	0.908	$0.061_{1,27}$	0.807	$2.111_{1,27}$	0.159
BCI	$0.128_{1,27}$	0.723	$0.539_{1,27}$	0.470	$0.294_{1,27}$	0.593
Mortality	$0.249_{1,27}$	0.622	$0.087_{1,27}$	0.771	$0.022_{1,27}$	0.883
STRUCTURE						
Spatial complexity	$0.047_{1,27}$	0.831	$0.054_{1,27}$	0.819	$0.143_{1,27}$	0.708
POM	$0.043_{1,27}$	0.837	$0.011_{1,27}$	0.916	$0.210_{1,27}$	0.651
EPIBIONTS & MOBILE FAUNA						
Epibiont cover	$0.087_{1,27}$	0.770	$0.341_{1,27}$	0.565	$4.691_{1,27}$	0.041**
Epibiont richness	$0.029_{1,27}$	0.867	$0.721_{1,27}$	0.404	$0.260_{1,27}$	0.615
Epibiont diversity	$0.065_{1,27}$	0.938	$0.044_{1,27}$	0.0835	$0.235_{1,27}$	0.632
Mobile fauna abundance	$1.610_{1,27}$	0.217	$0.370_{1,27}$	0.549	$0.902_{1,27}$	0.352
Mobile fauna richness	$0.847_{1,27}$	0.367	$0.291_{1,27}$	0.594	$2.225_{1,27}$	0.149
Mobile fauna diversity	$0.085_{1,27}$	0.774	$0.727_{1,27}$	0.402	$0.070_{1,27}$	0.793

3.3.2 Effects of plastic on the community composition of the mussel aggregates

Community composition of epibionts and mobile fauna were recorded during the experiment. Even though care was taken to initially rinse aggregates of mobile fauna, a large number (8093) of organisms were recovered during subsequent processing of the mussel aggregates. Organisms which escaped the original collection (6810 being juvenile *M. galloprovincialis*) were smaller than half a centimetre. In total, 16 450 mobile faunal organisms were collected and identified. Aggregates had little epibiont cover (between 0 - 8.6% per aggregate) and negligible algal growth (Table 14). Most (82%) aggregates had barnacle (*Notomegabalanus algicola*) growth, and all aggregates had juvenile *M. galloprovincialis*, amphipods, and various bivalves present (Table 15). Most of the juvenile *M. galloprovincialis* were smaller than 1 mm. Five epibiotic taxa and 50 taxa of mobile fauna were recorded. Juvenile *M. galloprovincialis* had the highest total and mean abundance of all mobile fauna (mean ± standard deviation of 286 ± 363), ranging from 3 to 1349. Four aggregates had no epibionts after marina exposure. These comprised two aggregates with high amounts of filament plastic, one aggregate with a low amount of filament plastic, and one aggregate with a low amount of film plastic. They are excluded from the MDS plots (Figure 14 A, C, E). The NMDS stress values were 0.1267 for mobile fauna (Figure 14 B, D, F) and 0.0388 for epibionts (Figure 14 A, C, E). The NMDS plots show that mobile fauna did not group into any patterns for treatment, plastic shape, or plastic amount. However, epibiont treatments for low and high filament formed separate clusters along the y axis (Figure 14 A). Additionally, high filament and high film clustered apart along the y axis (Figure 14 A). The divide in the epibiont NMDS is caused by aggregates which had an epibiont cover consisting only of barnacles (*N. algicola)*, these aggregates grouped on the right of the ordination. Barnacles were the only hard-bodied epibionts found during the study.

ANOSIM detected a significant difference among the epibionts compositions of the five experimental treatments ($R = 0.1407$, $p = 0.035$) (Table 16). When applying a 2-way crossed PERMANOVA, epibiont composition was found to differ significantly with plastic shape ($F = 1.2163$, $p = 0.048$), but did not with plastic amount (Table 17). Plastic presence, amount, and shape had no significant impact on the community composition of mobile fauna associated with *M. galloprovincialis* aggregates (Table 16, Table 17).

Table 14: Total % cover of epibionts on *Mytilus galloprovincialis* aggregates. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Each treatment had n=7, except the no plastic control group which had n=6.

Treatment	No Plastic	Low Film	Low Filament	High Film	High Filament
Ciona intestinalis	17.43	14.82	1.21	7.07	14.05
Ascidiacea a	0.10	0.07	0.16	0.29	0.83
Clavelina lepadiformis	0.07	0	0	0.17	0
Ascidiacea b	0	0.17	0	0	0
Notomegabalanus algicola	0.05	0.09	0.05	0.04	0.91
Total Cover (%)	17.65	15.15	1.42	7.57	15.79

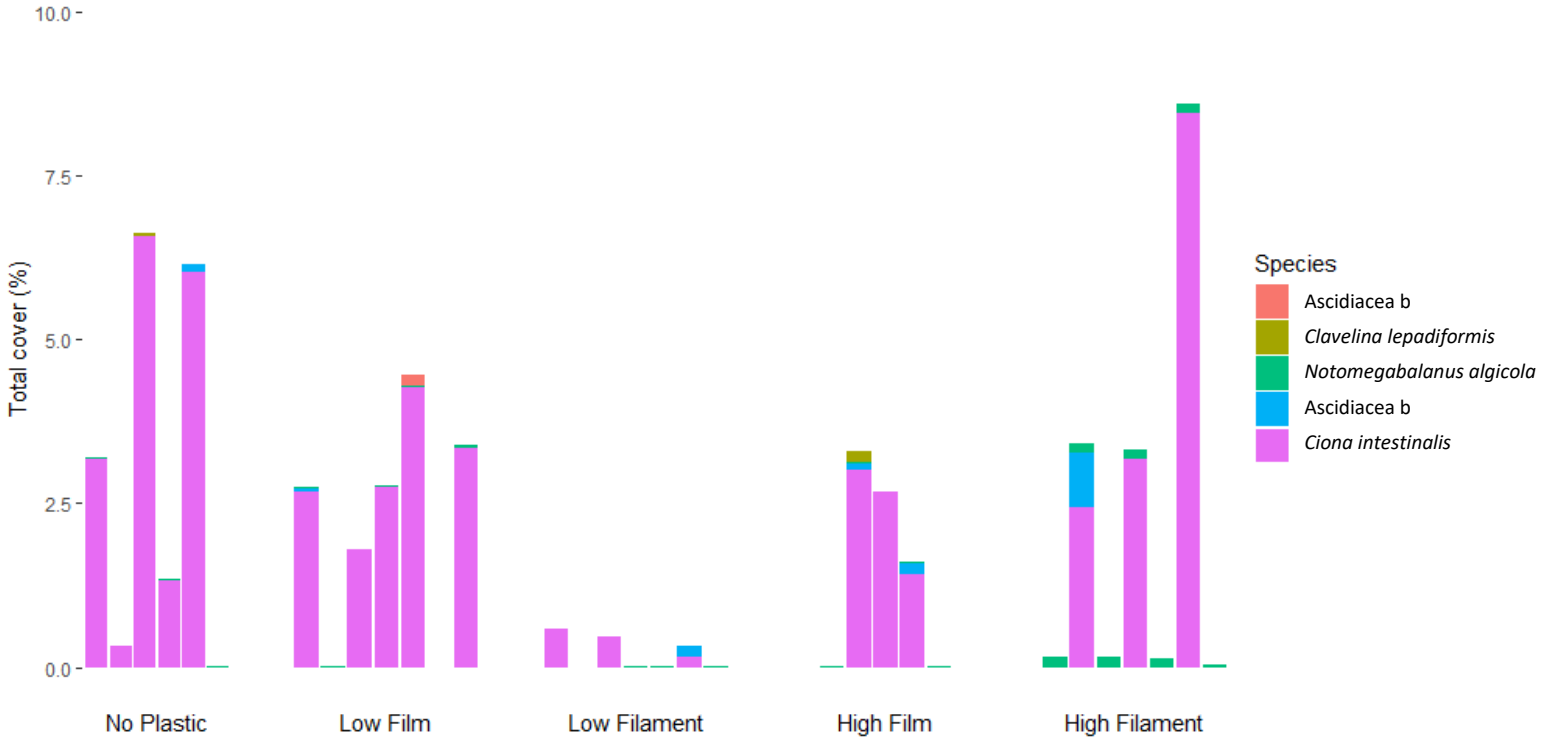

Figure 12: Total % cover of epibionts on *Mytilus galloprovincialis* aggregates. Each bar represents an individual mussel aggregate. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Each treatment had n=7, except the no plastic control group which had n=6.

Table 15: Total abundance of mobile fauna associated with *Mytilus galloprovincialis* aggregates. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Each treatment had n=7, except the no plastic control group which had n=6.

	No Plastic	Low Film	Low Filament	High Film	High Filament
BIVALVIA					
Juvenile *Mytilus galloprovincialis*	1479	1444	1652	2352	2790
Limaria tuberculata	4	1	2	0	1
Venerupis corrugatus	8	11	10	8	19
Tellina gilchristi	0	0	0	0	1
Kraussina rubra	0	0	0	0	1
Pecten sulcicostatus	0	0	1	0	0
Tellina spp.	0	2	0	0	0
White shell a	124	159	102	318	242
White shell b	2	7	2	9	4
White shell c	49	34	29	62	50
White shell x	1	0	0	1	0
Striped shell	11	6	17	24	19
Small orange shell	8	11	4	8	9
Brown shell x	14	8	12	13	15
GASTROPODA					
Fissurella mutabilis	2	3	1	6	6
Afrilittorina knysnaensis	2	0	1	0	0
Black shell	0	0	1	1	2
Janolus capensis	0	1	0	0	0
Nudibranch b	7	1	0	0	1
THECOSTRACA					
Notomegabalanus algicola	14	27	20	21	17
MALACOSTRACA					
Caprella equilibra	44	51	43	19	42
Amphipoda a	458	567	342	445	370
Amphipoda b	307	531	223	275	327
Amphipoda c	30	52	30	30	66
Amphipoda d	1	0	0	0	0

Amphipoda f	1	7	0	2	2
Amphipoda e	3	12	23	6	22
Isopoda a	2	9	0	4	1
Isopoda b	0	0	0	0	1
Plagusia chabrus	0	1	0	0	0
POLYCHAETA					
Lepidontus semitectus	26	31	21	23	28
Ochetostoma capense	2	3	6	1	2
Pseudonereis variegata	78	71	41	97	68
Polydora spp.	0	0	1	0	0
Thelepus spp.	3	2	4	1	12
Segmented worm LF3	0	0	1	0	0
Segmented worm f	1	1	0	0	1
Segmented worm e	0	1	0	0	1
NEMERTEA					
Unsegmented worm a	1	0	0	0	0
Unsegemented worm b	1	1	1	0	1
Unsegemented worm LB1a	0	0	0	0	1
POLYCLADIDA					
Thysanozoon spp.	4	3	4	8	10
Planocera gilchristi	0	1	0	1	1
SABELLIDA					
Serpula vermicularis	10	9	1	7	6
HEXACORALLIA					
Actiniaria	1	4	5	4	5
ECHINAOIDEA					
Parechinus angulosus	20	21	14	30	24
CRINOIDEA					
Comanthus wahlbergi	4	26	4	2	5
OTHER					
Asteroidea	0	0	1	0	0
Porifera	1	0	0	0	0

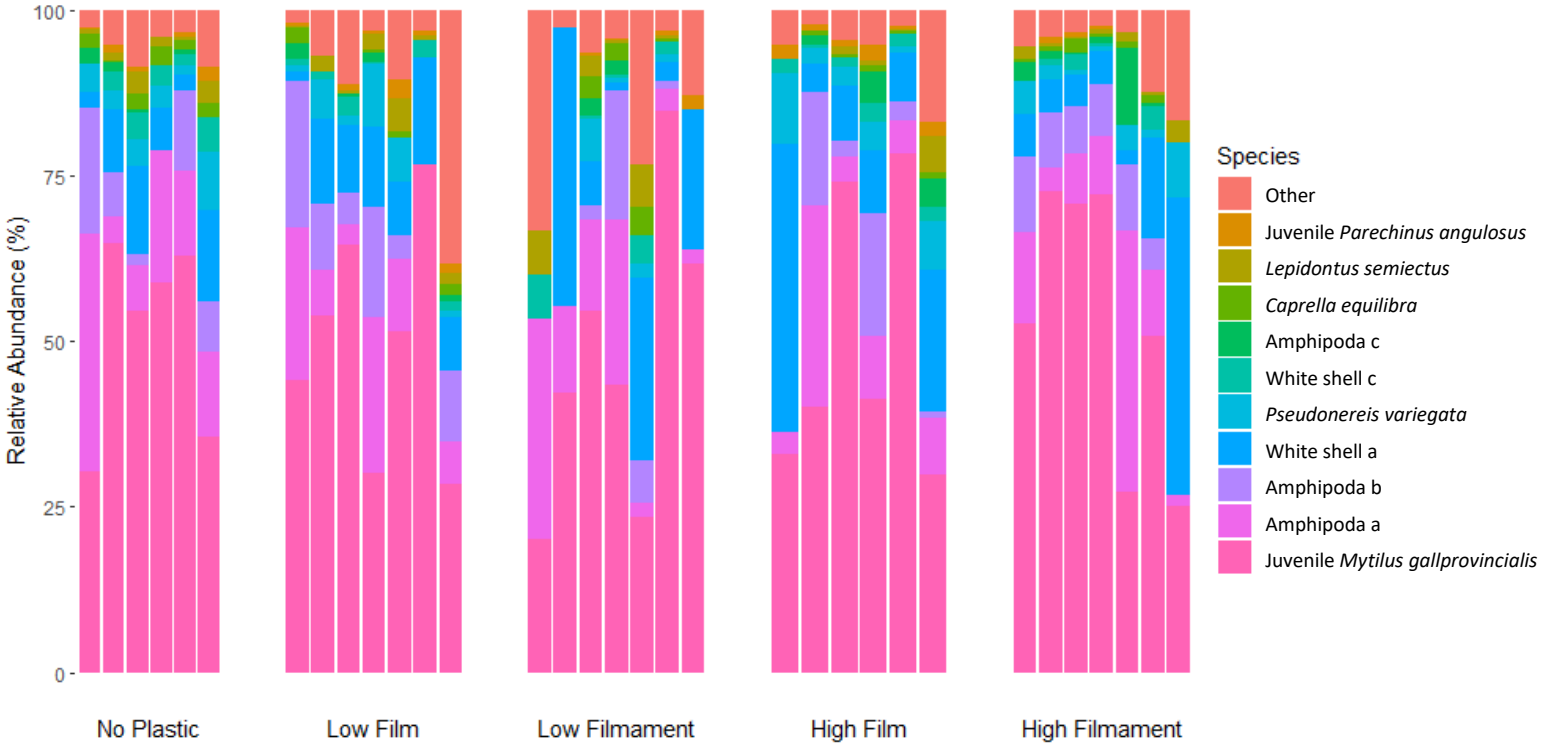

Figure 13: Relative abundance of mobile fauna associated with *Mytilus galloprovincialis* aggregates. Each bar represents an individual mussel aggregate. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Each treatment had n=7, except the no plastic control group which had n=6.

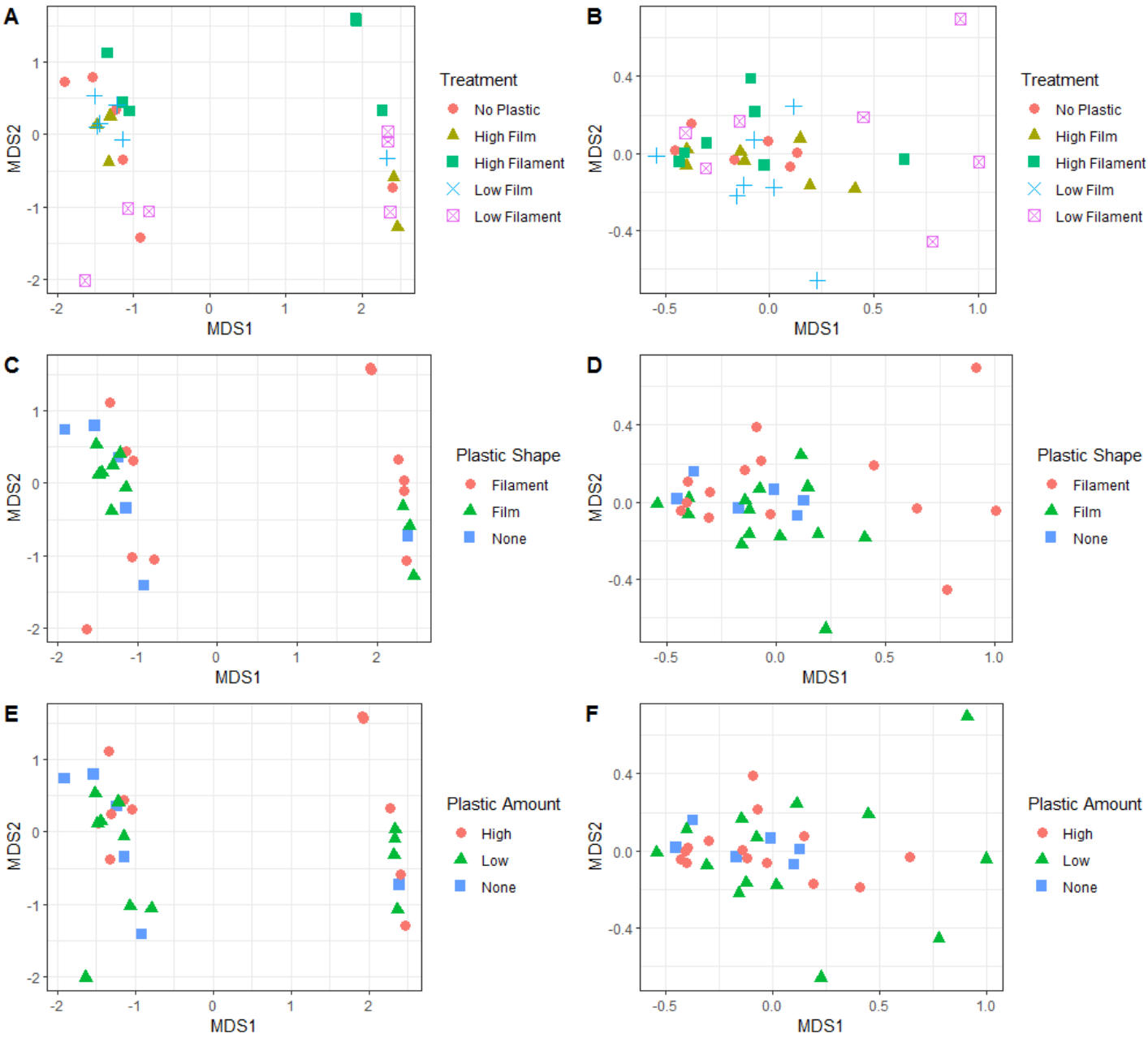

Figure 14: Non-metric dimensional scaling ordinations showing *M. galloprovincialis* aggregates grouped according to the community composition of epifauna (A, C, E) and mobile fauna (B, D, F) after 85-91 days of marina exposure. Each aggregate contained either no plastic, low amounts of plastic, or high amount of plastic. Plastic was either film or filament in shape. Each treatment had an n=7, except the No plastic control group of n=6. Colour and symbols have overlapping but different meanings. For plastic treatments in A) and B) red circles = no plastic, green triangles = high film, green squares = high filament, blue crosses = low film, and pink squares with crosses = low filament. For plastic treatments in C) and D) red circles = filaments, green triangles = film and blue squares = no plastic. For E) and F) red circles = high amounts of plastic, green triangles = low amounts of plastic, and blue squares = plastic.

Table 16: Summary of ANOSIM results of the effect of Treatment (High Film, High Filament, Low Film, Low Filament, No plastic) plastic shape, and plastic amount on the community composition of epibionts and mobile fauna of *Mytilus galloprovincialis* aggregates after 88 ± 3 days of marina exposure. The aggregates contained either no plastic, low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Significant results are highlighted by **. R is the R statistic and P is the significance value of each ANOSIM result.

Response variable	Source of variation					
	Treatment		Plastic Amount		Plastic Shape	
	R	P	R	P	R	P
Epibiont composition	0.1407	0.035**	0.1407	0.395	0.0615	0.14
Mobile fauna composition	-0.00757	0.524	-0.06602	0.913	-0.02273	0.646

Table 17: Summary of PERMANOVA results of the effect of plastic shape, plastic amount, and the interaction of the two factors on the community composition of epibionts and mobile fauna of *Mytilus galloprovincialis* aggregates after 88 ± 3 days of marina exposure. The aggregates contained either low amounts of plastic (20% aggregate surface area), or high amounts of plastic (40% aggregate surface area). Plastic shape was film (plastic bag) or filament (fishing line). Significant results are highlighted by ** and Degrees of freedom (effect, total) shown in subscript next to F values. F is the pseudo-F statistic and P is the significance value of each PERMANOVA result.

Response variable	Source of variation					
	Plastic Amount		Plastic Shape		Amount: Shape	
	F	P	F	P	F	P
Epibiont composition	$1.2163_{1,27}$	0.261	$1.2163_{1,27}$	0.048 **	$2.4672_{1,27}$	0.071
Mobile fauna composition	$1.0966_{1,27}$	0.337	$1.3415_{1,27}$	0.210	$0.9192_{1,27}$	0.455

3.4 Discussion

This study set out to examine the effects of macroplastics, firstly on the physiology and physical structure of *M. galloprovincialis* aggregates, and secondly on associated biota. The effects of plastic amount and shape were examined, using high and low amounts of both plastic bag fragments (films) and fishing line (filaments). Results show that the presence, amount or shape of plastic had no significant impacts on the physiology or structure of *M. galloprovincialis* aggregates. More specifically, there were no significant effects of these variables on respiration rate, byssus tenacity, body condition index, mortality, particulate organic matter mass, or spatial complexity. Plastic presence, amount and shape also did not affect the mobile fauna associated with *M. galloprovincialis* aggregates. Epibiont cover was greatest for aggregates without plastic, but the differences to other treatments were mostly not significant. Epibiont assemblages consisted of five species, and aggregates with filament shaped plastic had nine times more barnacle (*N. algicola*) cover than aggregates with film shaped plastic and 17 times more barnacle cover than aggregates with no plastic, suggesting the plastic shape influenced epibiont assemblage structure. In addition, aggregates with filament shaped plastic were unique from the other treatments in that they had no bell ascidians, *Clavelina lepadiformis*.

While the effects of macro- and microplastics on bivalves are not directly comparable—macroplastics primarily cause impacts through smothering and habitat alteration, whereas microplastics cause impacts through fundamentally different mechanisms including ingestion and translocation—microplastic studies were included to provide ecological context due to the absence of substantive macroplastic literature. Notably, the impacts of macroplastics on bivalves extend beyond physical disturbance. Similarly to microplastics, macroplastics act as a source of for toxic leachates (Jang *et al.*, 2016) which have repeatedly been shown to negatively impact bivalve physiology (Ke *et al.*, 2019; Gunaalan, Fabbri and Capolupo, 2020; Capolupo *et al.*, 2021; Delaeter *et al.*, 2022). Consequently, comparisons of physiological effects of the two plastic types may still provide valuable comparative insight and ecological context.

3.4.1 Mussel physiology and physical structure

3.4.1.1 Respiration rate

This study found that macroplastic exposure had no effect on the respiration rate of *Mytilus galloprovincialis* aggregates. This is similar to the findings of other studies on the effects of macroplastics on bivalve respiration (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). Here, results were also similar to those that have exposed mussels to microplastics. These studies generally found

there to be no effect of microplastic exposure on respiration (González-Soto *et al.*, 2019; Pedersen *et al.*, 2020; Webb *et al.*, 2020; Yap *et al.*, 2020; Barkhau *et al.*, 2022; Hamm *et al.*, 2022; Joyce and Falkenberg, 2022). It is possible that the performance of mussels in my study was not affected by macroplastics due to their resilience, as proposed by microplastic exposure studies (Pedersen *et al.*, 2020; Yap *et al.*, 2020; Barkhau *et al.*, 2022; Joyce and Falkenberg, 2022). This would be unsurprising as *M. galloprovincialis* are highly adaptive and outperform other mussels species when exposed to similar environmental stressors (Van Erkom Schurink and Griffiths, 1991; Griffiths *et al.*, 1992; Robinson *et al.*, 2005). There are some contradictory microplastic studies that did find mussel respiration to be affected: two found respiration to increase (*Mytilus* spp), and one found it to decrease (*P. viridis*) (Rist *et al.*, 2016; Wang, Zhong, *et al.*, 2021; Walkinshaw *et al.*, 2023). In these cases, increased respiration was thought to be an indication of stress caused by organism trying to maintain physiological homeostasis (Smolders, Bervoets and Blust, 2002; Green, 2016; Wang, Zhong, *et al.*, 2021; Jiang *et al.*, 2022). Conversely, decreased respiration in bivalves was thought to be caused by valve closure, which is commonly observed in mussels in the presence of potentially harmful particles (Ward and MacDonald, 1996; Bacon, MacDonald and Ward, 1998; Madon *et al.*, 1998; Rist *et al.*, 2016). Numerous microplastic exposure studies on bivalves report respiration to be unaffected but reproductive efforts reduced to provide the energy to maintain their metabolism (Xu *et al.*, 2017; Gardon *et al.*, 2018; Tallec *et al.*, 2021). Here, my findings suggest that macroplastic exposure does not induce respiratory stress in *M. galloprovincialis;* however, processes not measured in this study, such as gonadal development, were possibly sacrificed in an energy trade-off to maintain metabolism without impacting respiration, as observed in previous studies on bivalves. Further study is therefore needed to confirm whether macroplastic exposure causes reproductive or other stress in *M. galloprovincialis* mussels.

3.4.1.2 Byssus tenacity

Macroplastic exposure had no effect on the byssal tenacity of *M. galloprovincialis* aggregates. This is consistent with other studies which also found no change in byssal tenacity due to macroplastic exposure (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). When exposed to leachates or microplastics, effects on byssal strength are either negligible or species-specific. For example, plastic leachate impacts on byssal production varied across species, *P. perna* and *M. galloprovincialis* produced more threads after exposure, while *M. edulis* and *C. meridionalis* showed no change (Seuront *et al.*, 2021). In contrast, when *M. edulis* mussels are exposed to PE microplastics, both their byssal strength and production decrease by approximately 50% (Green *et al.*, 2019). Decreased byssus production has also been recorded in *M. edulis* (Hamm and Lenz, 2021), *P. canaliculus* (Webb *et al.*, 2020), and *P. viridis* (Rist *et al.*, 2016) mussels after exposure to microplastics. This suggests

that for certain species, exposure to leachate and microplastics acts as an anthropogenic stressor, eliciting responses similar to those induced by hypoxia, acidification, and decreased salinity. These stressors have been shown to negatively affect byssus production, with several studies reporting reduced byssus strength, production, and thickness in mussels exposed to both acute and chronic conditions (Clarke and McMahon, 1996; Wang *et al.*, 2012; O'Donnell, George and Carrington, 2013; Li *et al.*, 2015; Sui *et al.*, 2017; Manríquez *et al.*, 2021).

Byssus production is metabolically costly, and under stress, mussels may divert energy from byssus production to maintain growth and essential cellular functions, thereby compromising mussel tenacity (Sui *et al.*, 2017; Xu *et al.*, 2024). There is also limited evidence that reduced byssal thread secretion is caused by stress-induced reduction in myosin levels and gene expressions of byssal proteins (Sui *et al.*, 2017; Green *et al.*, 2019). Results in this study, however, found no effects on byssal thread tenacity, similar to that reported in *M. edulis* mussels after exposure to acidified seawater (Dickey *et al.*, 2018). This highlights the variability of results depending on species and the type of stressor.

3.4.1.3 Body condition index (BCI)

This study found that exposure to macroplastics did not affect the body condition index (BCI) of *M. galloprovincialis* aggregates. This result is consistent with other studies, which also reported no effect of macroplastic exposure on BCI (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). This is also consistent with microplastic studies, which usually report condition index of mussel to be unaffected after exposure (Revel *et al.*, 2019; Hamm and Lenz, 2021; Hamm *et al.*, 2022; Joyce and Falkenberg, 2022). In contrast, two studies have reported mussel condition to decrease after microplastic exposure, but in one of these studies condition was only decreased in comparison to natural particles (red clay) and not the control (Yap *et al.*, 2020; Barkhau *et al.*, 2022). Additionally, some studies on other pollutants (e.g. tributyltin, dibutyltin) and environmental stressors such as ocean acidification, hypoxia, shell damage, and non-lethal parasites, have also reported no effect on mussel condition, similar to the finding of my study (Dickey et al., 2018; O'Donnell et al., 2013; Lundebye et al., 1997; Sanders et al., 2014; Gilek et al., 1992). This suggests it is not unusual for stressors to have no effect on the BCI of mussels.

In this study, mussels were deployed beneath active jetties in a yacht club, where yachts were sometimes moored for extended periods (from weeks to months), reducing the light available to nearby mussel aggregates. Light availability can alter mussel shell growth without altering flesh growth (i.e. altering the BCI) (Griffiths and Griffiths, 1987). This variation in light conditions could have contributed to the observed high variability within treatment groups in results, masking

potential differences between treatments. Ultimately, the unchanged BCI suggests that macroplastic exposure did not induce stress or cause the mussels to use more physiological resources in response. However, potential stress effects could have been obscured by field variability, such as changes in light availability during deployment.

3.4.1.4 Total Mortality

This study found that macroplastic exposure had no effect on the mortality of *M. galloprovincialis* aggregates, further highlighting their physiological plasticity and resilience (Braby and Somero, 2006). While the results presented here are the first to record mortality after macroplastic exposure to mussels, when *Crassostrea virginica* oyster spat were settled and grown on either natural substrate or plastic, those grown on plastic had greater mortality rates (Sorini *et al.*, 2021). Generally, microplastic exposure has been reported to be non-lethal, with most bivalve studies finding no effect on mortality (Rist *et al.*, 2016; Santana *et al.*, 2018; Pedersen *et al.*, 2020; Yap *et al.*, 2020; Abidli *et al.*, 2021; Urban-Malinga, Jakubowska and Białowąs, 2021; Barkhau *et al.*, 2022; Hamm *et al.*, 2022; Joyce and Falkenberg, 2022; Walkinshaw *et al.*, 2023). There have, however, been cases where microplastic exposure caused an increase in bivalve mortality (Rist *et al.*, 2016; Rahim, Yaqin and Rukminasari, 2019; Thomas *et al.*, 2020; Phothakwanpracha, Lirdwitayaprasit and Pairohakul, 2021; Bringer *et al.*, 2022).This again highlights the variability in exposure effects on different bivalves.

It is unsurprising that total mortality was unaffected as all sub-lethal measures of stress in this study were not significant. If the mussels were under enough stress that energy reserves became depleted to the point that essential life functions were affected (such as ATP synthesis), mortality may have increased (Rist *et al.*, 2016). In South Africa, mortality rates of *M. galloprovincialis* vary seasonally, with mass mortality events thought to be caused by a combination of increased sand and wave stress (Paine and Levin, 1981; Carrington, 2002; Zardi, McQuaid and Nicastro, 2007; Nicastro, Zardi and McQuaid, 2008). Mortality in mussel populations is generally thought to occur when several physical and biological stressors coincide (Burdon *et al.*, 2014; Capelle *et al.*, 2021). However, mussels are highly physiologically resilient, and are adapted to survive in stressful and fluctuating environments such as the intertidal zone (Roberts, Hofmann and Somero, 1997; Payne, Miller and Shaffer, 1999; Collins *et al.*, 2020). Not only are *M. galloprovincialis* highly resilient, they also outperform local species, which is partially what has allowed them to become prolific invasives (Branch and Steffani, 2004; Robinson *et al.*, 2005). *Mytilus galloprovincialis* have higher re-colonisation rates, stronger responses to predators (moving away; aggregation), stronger responses to mass mortalities (re-aggregating and moving into safer arrangements after losing neighbours), greater resistance to trematode parasites, faster growth rate, and greater resistance to desiccation

and heat than various indigenous species such as *P. perna* (Griffiths *et al.*, 1992; Hockey and Van Erkom Schurink, 1992; Calvo-Ugarteburu and Mcquaid, 1998b; Braby and Somero, 2006; Nicastro, Zardi and McQuaid, 2007). *Mytilus galloprovincialis* therefore stand out as a particularly resilient species within an already resilient taxon. This study found that macroplastic exposure had no effect on mussel mortality, presumably because *M. galloprovincialis,* are highly resilient and are adapted to living in stressful, fluctuating environments. It is unsurprising that stress levels were below the critical threshold to cause lethal effects as no sub-lethal effects were found either.

3.4.1.5 Spatial complexity

This study found that macroplastic exposure had no effect on the spatial complexity of *M. galloprovincialis* aggregates. This study not only investigated whether the presence of plastic caused changes in spatial complexity due to stress, but whether the plastic was physically forcing spatial complexity to change due to its presence. By the end of the marina exposure period, most aggregates surrounded the plastic bag fragments, causing the plastic to form a small scrunched up ball at the centre of the aggregate. Several aggregates ejected the plastic bag pieces entirely. This exemplifies the ability of mussels to self-organize, allowing the maintenance of spatial patterns on multiple scales, thus enhancing their resilience to disturbance (Frost *et al.*, 2005; van de Koppel *et al.*, 2005). Mussel aggregates are able to reorganise to increase stability in response to stressors such as wave disturbance or small-scale differences in food availability (Hunt and Scheibling, 2001; Commito *et al.*, 2014). This ability to form denser arrangements allows them protection from wave action and predation (Petraitis, 1987). No aggregates managed to displace the filaments, which supports records and personal observations of fishing line being thoroughly entangled in natural mussel beds (Weideman *et al.*, 2020).

Finding no effect on spatial complexity concurs with previous studies where different types and amounts of macroplastic were used (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). However, exposure to rigid plastic bottles created greater spatial complexity within mussel aggregates than soft plastic bags when higher plastic amounts of plastic were used (40% and 80%) than in my study (Kumpitsch, 2021). Although my study found no change in spatial complexity, it is not surprising that Kumpitsch (2021) found a difference between rigid and soft plastic. Hard plastics cannot be crumpled by the mussels so their effects cannot be minimised as with the plastic bags. This would force the aggregates to take on different arrangements than if they were attached to a flat surface. Direct comparisons of the results reported here with the literature are difficult due to the various methods used to assess the structural complexity of marine habitats (Sadchatheeswaran *et al.*, 2019).

Very few studies have examined the effect of stressors on the bidimensional rugosity of mussel beds (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). Some studies measure a form of spatial complexity closely linked with mobility that is termed 'aggregation behaviour'. This is either quantified as the number of solitary mussels versus those forming aggregates, or can be measured by how close mussels are to each other (Manríquez *et al.*, 2021; Seuront *et al.*, 2021). Studies on the effects of plastic leachates and other stressors on mussel aggregation behaviour have had variable results. One study found that *P. perna* and *M. galloprovincialis were* unaffected by PP leachates while *M. edulis* and *Choromytilus meridionalis* increased their mobility and aggregation behaviour (Seuront *et al.*, 2021). This suggests leachate effects are species-specific. A different study on *M. edulis* mussels also found that PP leachate exposure increased attraction to conspecifics, but only in small mussels (Uguen *et al.*, 2023). It is typical for mussels to increase their aggregation behaviour and decrease the space between conspecifics in response to stress, resulting in more densely packed beds (Reimer and Tedengren, 1997; Cote and Jelnikar, 1999; Nicastro, Zardi and McQuaid, 2007; Kobak, Kakareko and Poznańska, 2010; de Jager, Weissing and van de Koppel, 2017). Increased mussel density reduces both the surface area of aggregates and the size of interstitial spaces, thus reducing the available habitable space for associated fauna (Thompson *et al.*, 1996; Crooks, 2002; Munguia *et al.*, 2011). Such changes are reported to affect the community composition, number, and size of organisms inhabiting aggregates (Tsuchiya and Nishihira, 1986; Firstater *et al.*, 2011; Gestoso *et al.*, 2013).

As reported here and by Seuront *et al.* (2020), *M. galloprovincialis* do not change their spatial complexity in response to either macroplastic or plastic leachate exposure. This is similar to a study on the effects of increased sea water temperature and acidification on *Perumytilus purpuratus,* which found that mussel aggregation behaviour was unchanged by either stressor (Manríquez *et al.*, 2021). This further reinforces the resilience of mussels when exposed to potential stressors. It should be noted that spatial complexity is difficult to accurately measure, and it has been pointed out that when no relationship is found between surface complexity and an ecological variable it may be due to imprecise estimates based on too few replicates (Frost et al 2005). So while it would appear that soft macroplastics had no effect on the spatial complexity of the mussel beds, it is possible that the small samples size coupled with suboptimal measurements masked potentially interesting results.

3.4.1.6 *Particulate organic matter (POM) accumulation*

Mussels are able to redistribute organic particles from the water column to benthic habitats through filter feeding and the subsequent biodeposition of faeces and pseudofaeces (Kautsky and Evans 1987, Tsuchiya 1982). This redistribution and deposition of sediments is not only due to their

filtration but the physical presence of the aggregates (Kent *et al.*, 2017). Organic matter mass is 3-5 times higher around mussel beds than bare substrate or mussel shells (Stewart et al 1998), and one model estimates that zebra mussels, *D. polymorpha,* are able to deposit 10 – 30% of incoming total particulate matter in shallow lagoon ecosystems (Daunys *et al.*, 2006). This enhances the availability of POM to other trophic benthic groups (Prins *et al.*, 1996; Daunys *et al.*, 2006).

This study found that macroplastic exposure had no effect on the accumulation of particulate organic matter (POM) mass of *M. galloprovincialis* aggregates. Higher POM in aggregates exposed to plastic may have indicated that the plastics were reducing water flow and allowing organic matter to accumulate. Lower POM may have indicated a decrease in biodeposition resulting from a decrease in filtration rate. However, no differences suggest that bag pieces and fishing line shaped macroplastics do not affect the accumulation of POM within mussel aggregates. The fencing around the aggregates may also have masked any potential effects. Despite being cleaned once a week, the fences fouled quickly and may have prevented organic matter that was not trapped by the aggregates and integrated plastic from being naturally washed away.

Few studies have examined the effects of plastic on POM accumulation in mussel aggregates, and like my study, none found POM to be affected by plastic presence (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). However, one study reported that the plastic coverage amount had a significant influence on the POM mass that accumulated (Baensch, 2021). They found that mussel aggregates exposed to high amounts (40%) of plastic (straws and fishing line) contained 67% more POM than aggregates exposed to low amounts of plastic (Baensch, 2021). It is possible that straws were more effective at capturing and maintaining POM than the other shapes of plastic. Straws would have been the most likely shape used to trap POM as no other plastic type studied had entirely enclosed shapes as they were cut to maintain the same plastic amount within each aggregate (20 or 40%) relative to their surface area (Baensch, 2021; Berning, 2021; Kumpitsch, 2021). If full sized plastic bags had been used in my experiment it is possible that the POM accumulation would have been higher due to the bags' enclosed nature compared to plastic fragments. So while my study found that plastic bag pieces and fishing line do not affect POM accumulation in mussel aggregates, it is possible that other shapes of plastic would exert an effect. However, this would need to be studied further to confirm.

3.4.2 Associated fauna results in context

Epibiont composition differed among experimental treatments, however, aggregates without plastics were not consistently different from those containing high and low levels of plastic filaments

of films. Epibiont cover was influenced by the interaction of amount and shape, and low amounts of filament plastic reduced epibiont cover while other treatments had no effect. Plastic exposure had no effect on epibiont richness and evenness. Similarly, there was no effect on the abundance, richness, evenness, or composition of the mobile fauna recorded within the *M. galloprovincialis* aggregates. A similar study on the effects of macroplastics (straws and fishing line) on *M. trossulus* and *M. edulis* mussel aggregate communities also found no effect on associated fauna (Baensch, 2021). In contrast, Berning (2021) found that plastic amount (20% vs 40%) impacted epibiont cover but that plastic rigidness (soft vs rigid; bag vs bottle) affected mobile fauna abundance and composition. In contrast to my study, they found that high amounts of plastic had higher epibiont cover than low amounts, although neither exposure differed from the control. Additionally, they found that rigid plastic had lower mobile fauna abundance than either soft plastic or the control, and the species composition differed between rigid, soft, or no plastic. No other studies have examined the effects of macroplastics on the associated fauna of mussel beds. There have been some correlative and manipulative studies on the effects of macroplastics on the communities of other benthic habitats; some of these communities include bivalves, although only one study identified mussels within their communities (Uneputty and Evans, 1997; Green *et al.*, 2015; Clemente, Paresque and Santos, 2018, 2022).

Plastic affecting organisms at an assemblage level is a relatively novel finding, as previous studies which investigated the effect of macroplastics on both terrestrial and aquatic organisms usually examined the effects on lower levels of biological organisation, such as populations or individual organisms (Katsanevakis *et al.*, 2007; Moore *et al.*, 2009; Rochman *et al.*, 2016; Green *et al.*, 2017; Bucci, Tulio and Rochman, 2020; Clemente, Paresque and Santos, 2022). Few studies have examined the effect of marine debris at an assemblage level, with plastic sometimes making up a varying proportion of the debris in these studies (Uneputty and Evans, 1997; Katsanevakis *et al.*, 2007; Lewis *et al.*, 2009; Green *et al.*, 2015; Clemente, Paresque and Santos, 2018, 2022). In a meta-analyses by Rochman *et al.* (2016) that investigated the ecological impacts of marine debris, there were only two recorded studies demonstrating the effects of marine debris at an ecological level, the rest being at a suborganismal (48 studies) or organismal level (24 studies) (studies which used correlative evidence were not included in the meta-analysis). Since their review, only two new non-correlative studies on the topic have been published (Green *et al.*, 2015; Clemente, Paresque and Santos, 2022). There are therefore few studies to meaningfully compare to mine, with other studies varying in the kinds of debris they used, the kind of benthic environment, and organism composition of assemblages.

Subtidal studies which investigated the effects of marine debris at an ecological level found that marine debris (the majority of which was plastic) changed species composition, injured, and smothered organisms (Katsanevakis *et al.*, 2007; Lewis *et al.*, 2009). Katsanevakis *et al.* (2007) demonstrated assemblage-level impacts by placing plastic bottles (75% of debris used in the experiment) and glass jars (25% of debris used) into 10 x 10 m experimental plots of sediment at 16-20 m depth in a coastal soft bottomed environment. After a year it was found that the debris were acting as hard substrate in the soft bottom benthic habitat, thus altering the species compositions as well as increasing the number of hard-substratum sessile organisms compared to plots with no added debris. Additionally, mobile species used the marine litter as refuge and reproduction sites. Despite finding a difference in epibiont communities and abundance between treatments, this study found no epibionts attached to the macroplastics. All epibionts were attached to the mussels; however fouling organisms did grow and were cleaned from the bottom of the plates the mussels were deployed on. Epibionts growing on the suspended plates were not quantified, nor were they counted towards the diversity or abundance of epibionts of the mussels. Despite this, it was noted that there was a greater richness and abundance of epibionts growing on the plates than on the mussels themselves, lending support to Katsanevakis *et al.* (2007) finding that plastic can act as a hard substrate and increase the number of sessile organisms in a benthic environment. So while my study supports that plastic can act as substrate to fouling organisms, this does not explain the differences between treatments in my study.

Derelict fishing gear (active lobster traps) also has the ability to smother and cause the mortality of several species of corals and associated sessile fauna after just four months (Lewis et al 2009). These authors found that despite damaging fauna beneath the traps more than compared to surrounding quadrats there was no change in benthic faunal coverage. Traps mainly injured stony coral, octocoral, and sponges, demonstrating an assemblage-level impact by plastic structures. Here, no physical injury to mussels or associated fauna was observed. Lobster traps are rigid and far larger than the soft plastic pieces used in my study. The shape and rigidity of plastic are likely important to the effects it can have on marine life. Tight fishing line entangling mussel aggregates could, over time, injure soft epibionts as they grow larger. A longer term experiment would be needed to investigate this.

A limited number of studies on the effects of macroplastic on intertidal benthic assemblages have been conducted. One correlative study examined the tropical estuarine benthic communities (contained bivalves) in the sediment beneath, bordering, or distant from naturally deposited plastic bags (Clemente, Paresque and Santos, 2018). Similar to my study, they found that plastic bags had

no effect on macrofaunal density and diversity, but community structure differed between treatments (under, border or distant from bags). The taxonomic richness was also slightly higher beneath bags, contrasting with my results. In a different correlative study on tidal flats, meiofauna abundance was higher in littered quadrats, and assemblage composition differed between litter and litter-free quadrats (Uneputty and Evans, 1997).

In a manipulative study on mud flats, conventional and biodegradable plastic bags were secured on sediment for 9 weeks, and changes in the communities before and after placement were recorded (Green *et al.*, 2015). Infauna abundance was six times lower beneath bags. *Mytilus edulis* was entirely absent under conventional and biodegradable bags (Green *et al.*, 2015). The community composition of infauna also changed, which was attributed to changes in the sediment (lower organic matter, increased ammonium, reduced oxygen) caused by the plastic bags acting as a barrier (Green *et al.*, 2015). Plastic bags also significantly decrease macrofaunal abundance, taxonomic richness, and Shannon-Wiener diversity in tropical estuaries (Clemente, Paresque and Santos, 2022). These changes are due to the bags acting as barriers, reducing the organic matter and silt-clay percentage beneath the bags (Clemente, Paresque and Santos, 2022). Unlike in mud flats, however, community structure remained unaffected. In my study, plastic was not fastened in space and could be freely manipulated by the mussels such that no sealed barrier developed between aggregates and the water column. Additionally, my study found no changes in the particulate organic matter accumulation, supporting the evidence that the plastic did not act as a barrier between the mussels and the water column. While the effects of macroplastics on marine benthic communities vary, my results align with most studies in the literature, showing that species composition changes.

It is unsurprising that the mobile fauna in my study were not affected, as potentially relevant factors such as the POM and spatial complexity were unaffected. Spatial complexity of mussel beds affects the composition and abundance of associated assemblages, with richness and density typically increasing with complexity (Thompson *et al.*, 1996; McKindsey and Bourget, 2001; Crooks, 2002; Borthagaray and Carranza, 2007; Maggi *et al.*, 2009; Gestoso *et al.*, 2013; Sunday *et al.*, 2017; Sadchatheeswaran *et al.*, 2019; Veiga *et al.*, 2022). For example, a field study which measured similar sized mussel clumps consisting of different mussel species found that the spatial complexity of clumps differed between species, influencing their associated macrofaunal assemblages (Gestoso *et al.*, 2013). However, the associated fauna of mussel beds can remain unchanged, even when experimental changes are made to the beds. Firstater *et al.* (2011) exposed *Perumytilus purpuratus* mussel beds to increased nutrient supply and found that this had no effect on the composition or abundance of associated biota. The same study manipulated structural complexities by forming

aggregates with either only small or large mussels and found that despite having more than three times the amount of interstitial space, the composition and abundance of associated biota did not differ between large and small mussel beds. This shows that even when changes are made to the nutrient load or spatial complexity of a mussel bed it does not always influence the mobile fauna.

The lack of physiological and structural changes after macroplastic exposure makes it difficult to identify the mechanisms driving changes in epibiont communities and abundance in my study. The plastic may have degraded and released microplastics fragments and leachates. Plastic leachates are toxic and have been found to negatively affect a number of marine taxa, including those that made up the epibiont communities in my study (Bejgarn *et al.*, 2015; Nobre *et al.*, 2015; Gandara e Silva *et al.*, 2016). The toxicity may be from catalysts, additives and non-polymerised monomers used during plastic manufacturing (Oehlmann *et al.*, 2009; Teuten *et al.*, 2009). In my study, all treatments had *N. algicola* barnacles as epibionts, and in some aggregates they were the only epibiont present. Barnacles are negatively affected by plastic exposure in a number of ways. Barnacles settled on plastic have weaker shells than those settled on glass; shell weakness is possibly caused by leachates containing molecules that mimic mineralocorticoids impacting barnacle physiology and calcification (H.-X. Li *et al.*, 2016; Li, Tse and Fok, 2016). Plastic leachates also inhibit barnacle settlement and cause naupliar mortality (H. X. Li *et al.*, 2016). Microplastic exposure has negative intergenerational effects on barnacle by increasing offspring mortality and delaying larval development (Yu and Chan, 2020). In addition to barnacles, several ascidian species were epibionts on the mussel aggregates. Microplastic exposure reduces fertilisation rates and slows the metamorphosis of juvenile ascidians (Messinetti *et al.*, 2018; Anderson and Shenkar, 2021). Additionally, common plastic leachates cause larval malformations and affect ascidian neural development (Messinetti, Mercurio and Pennati, 2019; Mercurio *et al.*, 2022). It is possible that changes in epibiont abundance and community compositions were caused by plastic leachates or microplastic fragments from the macroplastics within the mussel aggregates. This can, however, not be confirmed as neither plastic leachates nor microplastic were measured in either the mussels or their associated fauna. Future studies wishing to understand the effects of macroplastics on invertebrate communities should therefore consider including measures of plastic leachates and fragmentation.

3.4.3 Crumpling of plastics by mussel aggregates

This study observed that mussels in aggregates containing plastic bag pieces would surround and crumple the plastic often moving it to the centre until it was no longer visible. Similarly, Kumpitsch (2021) observed that 79% of *M. edulis* aggregates contained crumpled plastic, with some mussels completely enveloping the plastic and experiencing no growth during the experimental exposure

period (73 ± 3 days). Weideman *et al.* (2020) also reported mussel beds trapping plastic waste in the intertidal zone, noting that fishing line became entangled in seaweeds and mussel beds. This encapsulating behaviour suggest that mussel beds act as temporary plastic sinks while also providing a source of microplastics within aggregates. Given this crumpling behaviour has been observed in two *Mytilus* species, monitoring mussel beds for plastic sequestration may be valuable. Additionally, as mussels die, they may release the trapped plastic, acting as reservoirs that eventually release the plastics back into the ocean.

3.4.4 Mussels and ecosystem services

Marine ecosystems provide an abundance of ecosystem services such as fisheries, tourism, and shoreline stabilisation (Barbier, 2017). All marine ecosystem services are negatively impacted by plastic to some extent (Beaumont *et al.*, 2019). Plastics are predicted to have implications for human health and wellbeing, particularly services relating to fisheries, heritage, and recreation, impacting human health and well being. Mussel beds provide food and habitat, filter water and have cultural importance (Beaumont *et al.*, 2007; Gundersen *et al.*, 2017). Microplastics may impair the biofiltration capacity of bivalves which usually alleviate the effects of coastal eutrophication. This is evident in the blue mussel, *M. edulis,* that experiences a 21% decrease in clearance rate after long term (39 days) exposure to plastic microfibres smaller than 100 µm (Christoforou *et al.*, 2020). It is notable that plastic changed the species composition of the mussel epibionts and aggregates with filament shaped plastic had no bell ascidians, *C. lepadiformis*, present in my study. Loss of biodiversity is one of the greatest risks to ecosystem service provision (Worm *et al.*, 2006; Cardinale *et al.*, 2012; Harrison *et al.*, 2014; Lefcheck *et al.*, 2015). When biodiversity in marine environments decline, rates of resource collapse increase and recovery potential, stability, and water quality exponentially decrease (Worm *et al.*, 2006). Because mussels provide such important ecosystem services there is a need to further understand the potential impacts on provision of goods as services as a result of plastics exposure and associated biodiversity declines.

The effects of plastic on fisheries and biodiversity (among other ecosystem services) are identified as understudied in South Africa (Arabi and Nahman, 2020). Negative effects of plastics on the biodiversity and habitats of commercial fishing stocks may result in negative economic impact and food security outcomes (Ostfeld and LoGiudice, 2003; Worm *et al.*, 2006; Cardinale *et al.*, 2012). Additionally, subsistence fishers that rely on mussels along South Africa's coast (Hockey, Bosman and Siegfried, 1988; Rius, Kaehler and Mcquaid, 2006) may also experience food insecurity if negative thresholds are reached that destabilise their harvesting populations. Despite macroplastics having minimal effect on the health of the mussels in this study, the collective effects of plastic

waste, climate change, ocean acidification, and over exploitation may significantly impact mussel aggregates. This possibility of cumulative effects makes plastic debris important to monitor for economic as well as environmental reasons, as Beaumont *et al.* (2019) have already estimated a 1–5% decline in ecosystem services as a result of marine plastics in 2011. The plastic problem will not cease any time soon with one study estimating that by 2060 mismanaged plastic waste accumulation will reach 155-265 million metric tonnes a year globally (Lebreton and Andrady, 2019). It is therefore imperative to understand the impact plastic will have on our ecosystems, both to be able to responsibly inform policy makers on best practices going forward as well as ameliorate the impacts that are already occurring. Globally, and in South Africa, plastic is a danger to ecosystem services (Arabi and Nahman, 2020; Kumar *et al.*, 2021; Sridharan *et al.*, 2021). Despite this danger, there have been few studies on the effects of plastics on multi-scale marine ecology. The effects of microplastics on individual organisms have been extensively studied, yet the effects on populations, communities and ecosystems remain largely unexplored (Agathokleous *et al.*, 2021). The same paradigm applies to the study of the effect of macroplastics on marine systems. Rochman *et al.* (2016) made similar points about marine debris in general, stating that "quantity and quality of current research regarding ecological impacts of marine debris requires improvement before any clear general ecological conclusions [can] be reached". There is a "pressing need" for quantitative information to predict the ecological impacts of marine debris as the "presence, sizes, frequencies, and nature of ecological impacts are currently largely unknown".

3.4.5 Limitations and recommendations for future studies

3.4.5.1 The challenges of identifying stressors in a resilient species that occur in naturally stressed environments

Identifying interactions among stressors in natural ecosystems provides several challenges caused by natural environmental variation and the ability of organisms to withstand and recover from environmental stressors (Carrie-Belleau et al 2024). When conducting experiments in natural systems there are variables that are unaccounted for caused by natural variation (in comparison to controlled laboratory settings), and decreases the ability to detect effects caused by experimentation (Lundebye, Langston and Depledge, 1997; Spicer, 2014). It is therefore possible that the high variability in the environmental conditions experienced by the mussels prior to and during this experiment accounts for the variation in recorded measurements, and contributed to my difficulty in identifying the statistically significant effects of the macroplastics.

Mussels are highly adaptable and have numerous mechanisms for dealing with stress. Despite, or perhaps because of these mechanisms, stress responses can be stressor- and species-specific and highly variable among individuals (Curley *et al.*, 2021). The differences among mussel species are likely driven by differences in physiology (Gough, Gascho Landis and Stoeckel, 2012; Ganser, Newton and Haro, 2013; Haney, Abdelrahman and Stoeckel, 2020; Curley *et al.*, 2021; Seuront *et al.*, 2021). For example, physiological responses of bivalve species to stressors such as hypoxia are species-specific, with intertidal species being more tolerant to hypoxia than subtidal species (Pörtner, Langenbuch and Michaelidis, 2005; Wang *et al.*, 2012; Sui *et al.*, 2017). *Mytilus galloprovincialis* are known to be a particularly resilient species capable of out-performing other species, which is partly why it is such a prolific invader (Branch and Steffani, 2004; Robinson *et al.*, 2005). This hardiness and adaptability may have masked potential stress responses after chronic exposure to macroplastics. It is possible that less adaptable indigenous species would be more impacted by macroplastic exposure.

Mussels have substantial intraspecific variation in response to stress (Gilek, Tedengren and Kautsky, 1992; Denny *et al.*, 2011; Miller and Dowd, 2019; Curley *et al.*, 2021). Mussels commonly deviate from normal metabolic functioning after stress exposure, however, the way metabolic function deviates varies between individuals (Curley *et al.*, 2021). For example, when exposed to air and high concentrations of suspended solids, some individuals increase while others depress their metabolic rates (Curley *et al.*, 2021). Individual variation is in part due to fine scale environmental differences in exposure to waves and compass orientation which subject individuals to different abiotic conditions (Helmuth and Hofmann, 2001; Jimenez *et al.*, 2015; Miller and Dowd, 2019). For example, mussels can increase the number and strength of their byssal threads when in more turbulent environments compared to sheltered ones (Seguin-Heine *et al.*, 2014). This can be highly localised, with individuals sheltering within the centre of aggregates having lower attachment strength than those on the fringes (Witman and Suchanek, 1984; Bell and Gosline, 1997; Zardi *et al.*, 2006). In addition to location within an aggregate, variations in stress responses may be caused by differences in reproductive stages. Reproduction in mussels is energetically costly, and depletes glycogen stores (Bayne, Salkeld and Worrall, 1983; Fearman, Bolch and Moltschaniwskyj, 2009). Therefore, after spawning, their energy reserves are low, making them less resilient to stress (Tremblay *et al.*, 1998; Uguen *et al.*, 2023). For example, a study which took place after spawning found intertidal mussels (*P. perna*, *M. galloprovincialis*, *M. edulis*, *C. meridionalis*) to be sensitive to plastic leachates, but a similar study which took place before spawning found *M. edulis* mussels to be resilient to leachates (Seuront *et al.*, 2021; Uguen *et al.*, 2023). It is therefore likely that inherent variability in individual

mussel responses contributed to the high variance in this study's measured responses and contributed to my difficulty in identifying statistically significant effects of the macroplastics.

3.4.5.2 Specific limitations caused by methods

This study had some limitations due to practical constraints of the methods. Measurements were taken the same day that mussels were removed from the marina and transported to the laboratory, which may have produced stress responses in the mussels and affected physiological measurements. However, as *M. galloprovincialis* is an intertidal species, any stress caused by the 1.5 hours of exposure during transport likely did not differ greatly from that which the mussels routinely experience during low tide exposure. Additionally, the mussels were allowed to acclimate for one hour before measurements were taken. Some measurements may not have been entirely accurate due to the way they needed to be measured. Particulate organic matter needed to be washed out of the aggregates and collected before further measurements could be taken in the lab. This meant that POM was potentially trapped in the interstitial spaces and not collected. The aggregates needed to remain intact for further measurement, rendering this the most practical solution, while providing the highest possible accuracy. Furthermore, despite using non-adjacent individuals, the pulling of the first mussels may have loosened the threads of other mussels, so that inaccurate measurements of byssal tenacity were taken by the time the last mussel was pulled. The methodological limitations of this study may therefore have added to the variation in the responses measured.

As filtration rate results were determined to be unreliable, this study was not able to successfully determine whether macroplastic exposure affects mussel filtration. The flow cytometry results for algal concentrations were inaccurate as determined by inspecting blank samples. Furthermore, initial measurements of algal concentrations were usually far lower than those that were initially added to tanks, and concentrations often increased during or by the end of measurements. It is possible that the aquarium pump used was not sufficiently mixing and homogenising the water in the experimental tanks, however this does not account for the consistently lower initial algal concentrations. The flow cytometer may have been detecting additional particles of similar size to the algae, such as faeces and pseudofaeces, causing them to be included in, or counted instead of, the algal counts.

Due to the nature of the GAME programme, platforms had to be constructed from materials that were readily available globally so that all participants could use similar materials. Additionally, materials needed to withstand prolonged submersion in salt water. For these reasons, the platforms were made out of plastic. Platforms being made from plastic may have affected the mussels, however, limited literature on the topic makes it is difficult to appraise possible effects. Known

effects of macroplastics on bivalves include smothering (Green *et al.*, 2015; McCoy *et al.*, 2020; Clemente, Paresque and Santos, 2022), and acting as sources of chemical leachates and microplastics when used as settlement material (Jang *et al.*, 2016). The mussels in this study were not at risk of being smothered by the platforms as the platforms were made from rigid materials that did not cover the mussels. It is possible that the platforms acted as a source of leachates or microplastics, but this was not measured as it is beyond the scope of this study. As all platforms in this experiment were identical, any effects caused by the platform bring plastic should not have caused differences between control and treatment groups.

3.4.5.3 Limitations due to the experimental design

This study failed to detect effects of macroplastic presence, shape and amount on the majority of response variables measured. However, some consistent patterns were visible that were not statistically significant. In factorial experiments, replication within treatments is commonly limited by the available resources and processing times, and this was also the case in this study. This study might thus have benefitted from increased statistical power by focusing on one factor (e.g. amount of plastic) and increasing replication. In addition, the study design could have benefitted from assessing trends in continuous treatment variables rather than limiting the assessment to two treatment levels (low/high). However, since natural environments are fraught with complex interactions of multiple stressors, a single-factor study would face other limitations in terms of applicability and assessment of interactive effects.

3.4.5.4 Recommendations and directions for future studies

Future studies on the effects of macroplastics on mussel aggregates would benefit from incorporating lessons learned in this study. As this study found the responses of the mussels to be highly variable, future studies should increase the number of replicates used to improve the ability to detect any effects veiled by high variability. Additionally, researchers may want to increase the number of mussels per aggregate to better replicate the size of natural mussel beds and make observations on mussels at the centre vs. the periphery of aggregates. I found that some mussel aggregates completely encapsulated the introduced plastic and moved it to the centre of the aggregates. Therefore, mussels at the centre of the aggregate may have been more affected than those on the fringes. It should be noted that the aggregates in this study were too small to always make definite boundaries between central and peripheral mussels. Because of this, researchers who want to investigate the differences in stress responses of mussels located in different parts of an aggregate should use more mussels in their aggregates than was used in this study (30 individuals). When measuring the spatial complexity of aggregates, different or additional methods could be used. For example, the displacement interstitial volume could additionally be used to determine the

interstitial volume of the aggregates now that we know that soft plastics are integrated into the aggregates. It would be interesting to determine whether the interstitial spaces were being reduced even if the spatial complexity of the mussels is unchanged. This could potentially reduce the living space for fauna and alter the hydrodynamics within the aggregates.

There are a number of ways this study could be expanded upon. It would be interesting to further investigate the effect of plastics with emphasis on different plastic characteristics such as different rigidities and shapes. This study, as well as others, point to different kinds of plastics providing different problems for marine organisms. Rigid plastics like plastic bottles change the spatial complexity of the aggregates (Kumpitsch, 2021) but soft film-shaped plastic like plastic bags can both smother sandy benthic habitats (Green *et al.*, 2015) and become incorporated into mussel aggregates as shown by this study and Kumpitsch (2021). Larger rigid plastic items (lobster traps) have been shown to damage corals and their associated sessile fauna (Lewis *et al.*, 2009). Smaller rigid plastic items (plastic bottles) have been shown to act as hard substrate in sandy benthic habitats, providing points for attachment and shelter for a different composition of species than would occur without the plastic (Katsanevakis *et al.*, 2007).

It would be valuable to test for the threshold at which mussel aggregates and their infauna start to be affected by plastic. This study only used fragments of plastic bags (surface areas 60 cm^2 - 143 cm^2), in future research a range of plastic sizes could be used. Full sized plastic bags would have greater smothering capabilities and would have a greater potential to affect infauna if mussels managed to incorporate them into their aggregates. Additionally, if full sized plastic bags were used POM accumulation would likely have been higher due to their enclosed nature compared to plastic fragments. It would also be interesting to examine the long-term effects of plastic on mussel aggregates. As plastics are highly durable (Zheng, Yanful and Bassi, 2005) they could stay within a mussel bed for years, affecting the entire life cycle of mussels. The age of mussel aggregates can affect the associated species diversity (Tsuchiya and Nishihira, 1986) and different infauna may be more resilient to plastic than others. It would therefore be interesting to look at the effect of plastic on aggregates of different ages. Future studies could therefore gain a better understanding of the effects of macroplastics on mussel beds by using plastics with different characteristics, using larger pieces or greater amounts of plastic, having a longer plastic exposure period, or using mussel aggregates composed of mussels of different age groups.

Further investigations may also want to expand upon the kind of stress responses measured in this study, so as to have a better understanding of the mechanisms underlying potential effects of macroplastic exposure on mussel aggregates. It would be interesting to examine if reproduction is

affected by macroplastic exposure. Mussels are capable of making metabolic compensations when stressed by adjusting their growth, reproduction, or energy allocation as needed (Bayne, Salkeld and Worrall, 1983; Russell-Hunter and Buckley, 1983). Despite this, this study found mussel physiology to be unchanged by macroplastic exposure. However, it is possible that reproductive efforts were sacrificed to maintain this physiology by maintaining body condition and respiration at the cost of gametogenesis (Xu *et al.*, 2017; Gardon *et al.*, 2018; Tallec *et al.*, 2021). It would therefore be interesting to measure reproductive health in addition to body condition in future studies, as stressors can negatively affect mussel reproduction in addition to body condition (Petes, Menge and Murphy, 2007).

Marine organisms are under pressure from multiple co-occurring anthropogenic sources, including marine debris and chemical pollutants, ocean acidification, and warming ocean temperatures (Harley *et al.*, 2006; van Dam *et al.*, 2011; Wernberg, Smale and Thomsen, 2012; Poloczanska *et al.*, 2013; Iñiguez, Conesa and Fullana, 2016; Rochman *et al.*, 2016). It is thus important to understand the effects of multiple additive stressors and their interactions (Pörtner, 2010; Breitburg *et al.*, 2015; Gobler and Baumann, 2016; Carrier-Belleau *et al.*, 2023). For example, when zebra mussels, *D. polymorpha*, are exposed to both nutrient enrichment and saltwater intrusion, stress thresholds occur at lower levels along the environmental gradients (Carrier-Belleau *et al.*, 2023). This means that if we only consider stressors separately in laboratory experiments, we may be overestimating the tipping points at which sub-lethal and lethal effects occur in natural settings. It is thought that the interactive effects of multiple stressors associated with global climate change will expose marine organisms to physiological challenges potentially exceeding their current abilities to acclimatize (Lesser 2016). When stressors consistent with future predictions of the marine environment, such as elevated temperatures and low pHs/elevated CO_2, are combined, the metabolism of *M. edulis* mussels are depressed (Zittier *et al.*, 2015; Lesser, 2016). This is caused by a lowered threshold for thermal stress and subsequent metabolic depression caused by persistent extracellular acidosis (Pörtner, Langenbuch and Michaelidis, 2005; Lesser, 2016). If multiple stressors that reduce respiration and feeding are compounded, there may be a serious impact on the invertebrate's abilities to obtain sufficient energy. This is currently seen in mass mortality events of mussels, which are caused when multiple natural stressor coincide or extreme abiotic conditions occur (Paine and Levin, 1981; Carrington, 2002; Zardi, McQuaid and Nicastro, 2007; Nicastro, Zardi and McQuaid, 2008; McDowell and Sousa, 2019; Carrier-Belleau *et al.*, 2023). The increased number and intensity of stressors brought about by climate change are anticipated to raise the yearly number of mortality events for mussels (Trenberth, 2011; McDowell and Sousa, 2019). It is therefore important that we properly understand how mussels respond to multiple coinciding stressors, as currently we are at

risk of underestimating the negative impacts of anthropogenic stressors such as climate change and pollution.

3.4.6 Conclusions

Outside of Masters theses conducted within the GAME (Globular Approach by Modular Experiments) project, no other researchers have examined the effects of macroplastics on mussel respiration, filtration, byssus tenacity, BCI, spatial complexity or POM accumulation. This study found that the physiology and structure of *Mytilus galloprovincialis* aggregates were unaffected by chronic exposure to macroplastics. However, natural stressors in marine environments are complex and variable, and mussels exhibit high interindividual variability in their stress responses. This variability may have masked any potential effects of stress after macroplastic exposure. Alternatively, the amount of macroplastic used in the study may have been insufficient to elicit detectable stress responses in the mussels. Although the mussels appeared unaffected, the abundance of epibionts decreased following exposure to low amounts of fishing line. Additionally, epibiont community compositions differed between treatments. This finding highlights that even if highly adaptive and resilient species like the invasive mussel *M. galloprovincialis* show no obvious effects from macroplastic exposure, broader impacts on faunal communities cannot be ruled out. With marine plastic pollution rapidly increasing, mussel beds and their associated fauna are likely to face growing exposure to macroplastics. Despite this, the functional responses of marine communities to inundation by macroplastics remain largely unknown. As macroplastics debris has the potential for considerable ecological ramifications, further research on the effect of macroplastics on marine benthic communities is urgently needed.

4 Chapter 4 - General discussion and conclusions

Despite the growing body of literature on the effects of plastics on marine life, there is little consensus on the effects on invertebrate assemblages. The second chapter of this dissertation aimed to critically review and highlight knowledge gaps of the effects of plastics on bivalves. The assessment revealed that microplastics can induce various physiological changes in bivalves, such as reduced filtration rate, reduced food assimilation efficiency and digestion ability, increased respiration, reduced reproductive success, increased oxidative stress, decreased growth and byssus production, and increased immune- and genotoxicity. Microplastics also act as vectors for other pollutants and can amplify pollutant effects on bivalve physiology when co-exposed. Despite studies often reporting no effects or limited effects, such potentially important results have been omitted from previous reviews on the topic firmly establishing biases in the syntheses of the literature. The effects of microplastics varies greatly among studies. However, evaluating the causes of this variability is made difficult in part due to the different study species and properties of microplastics (size, shape, polymer type, concentration) being used in studies. So, while there is some evidence that the effects of microplastics are species-, size-, and polymer-specific, this cannot be concluded without further research.

Research on the effects of macroplastics on bivalves are severely understudied. Only 16 studies have investigated the effect of macroplastic on communities containing bivalves, and bivalves were the primary study species in only five of these. Of those five, only three used controlled, manipulative experiments. There is, however, evidence that plastic can smother bivalves, reducing their abundance and altering community compositions by restricting the flow of oxygen and organic matter. Additionally, macroplastics serve as settlement substrata or rafting material in otherwise unsuitable habitats. Bivalves, however, that settled on plastic experience increased mortality and altered sex ratios. Additionally, the species composition of associated fouling communities can differ with polymer type. Bivalves settled on macroplastics are also subjected to increased chemical leachates and microplastic fragments as their macroplastic substrate weathers and breaks down. This highlights the important link between macroplastics, microplastics and plastic leachates. Even if macroplastics are not causing physical effects (e.g. smothering), they are acting as a source of leachates and microplastics, both of which have been repeatedly shown to negatively impact bivalves.

While this review generally found that microplastics used in experimental studies are environmentally relevant, several types of microplastics are understudied compared to their prevalence in the environment. Future experimental studies need to carefully choose microplastic

concentrations (and other properties) that are environmentally relevant for bivalves. Particular consideration needs to be given to the size of microplastics used to ensure environmental relevance, as environmental microplastic concentration and composition change with size, yet little is known about environmental microplastics smaller than 300 µm due to sampling biases. Additionally, studies should report both the particles per volume and weight per volume of their microplastic exposures, as it is currently impossible to make direct comparisons among studies due to differing units.

To address the most apparent gaps identified in through a comprehensive literature review (Chapter 2), the following chapter (Chapter 3) provided empirical evidence for the effects of macroplastics on bivalve aggregates and their associated communities. This study found that the physiology (respiration rate, byssus tenacity, BCI, mortality), physical structure (spatial complexity, POM), and associated mobile fauna community living within *Mytilus galloprovincialis* aggregates were all unaffected by exposure to macroplastics. While the performance of mussels appeared unaffected, the cover of epibionts was greatest in aggregates that contained no plastics. Epibiont community composition differed among experimental treatments, with patterns pointing to slight differences between low and high filament treatments, as well as between high filament and high film treatments. It is however possible that, even though highly adaptive and resilient species like the invasive mussel *M. galloprovincialis* show no obvious impacts from macroplastic exposure, effects on indigenous faunal communities cannot be ruled out, and these effects are likely context dependent. The functional responses of marine communities to macroplastics remain largely unknown. Outside of Masters theses conducted within the GAME (Globular Approach by Modular Experiments) programme, no other researchers have examined the effects of macroplastics on mussel respiration, filtration, byssus tenacity, BCI, spatial complexity or POM accumulation. As macroplastics debris has the potential for considerable ecological ramifications, further research on the effect of macroplastics on marine benthic communities is urgently needed.

This thesis advances knowledge on plastic's effects on bivalve physiology, structure and associated communities with a synthesis of the literature and an original experiment. It shows that there are striking inconsistencies in the methodologies, model organisms and results presented in the literature, and a large bias in current syntheses. Furthermore, it shows that plastics generally have a negligible effect on *M. galloprovincialis* physiology and structural dynamics but could play a role in associated in- and epifaunal community assembly. Nevertheless, research on the effects of plastic has been ongoing for a very short time, so continued research with well developed and standardised experimental designs are crucial to establish consensus on the effects of plastics on bivalves.

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