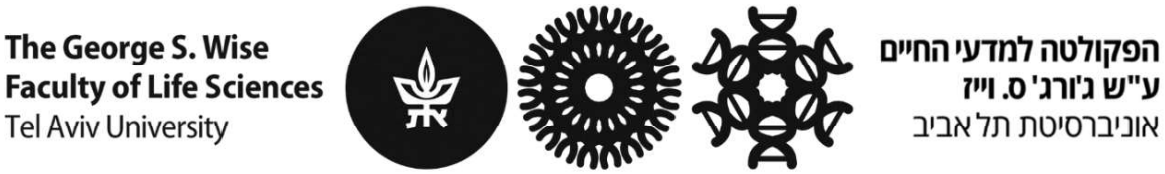

TEL-AVIV UNIVERSITY

GEORGE S. WISE FACULTY OF LIFE SCIENCES

SCHOOL OF ZOOLOGY

Assessing the extent and impact of plastic pollution in coral reefs

Thesis submitted for the degree "Doctor of Philosophy"

by

Gal Vered

Submitted to the Senate of Tel-Aviv University

March 2024

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This work was carried out under the supervision of

Professor Noa Shenkar

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ACKNOWLEDGMENTS

I would like to express my heartfelt appreciation to the amazing individuals and institutions who supported me along this journey.

First and foremost, I would like to express my deepest gratitude to my supervisor, Prof. **Noa Shenkar**, for her mentorship not only in the field of research but also in the journey of life itself. Noa, your unwavering belief in me, even during moments of self-doubt, has propelled me to succeed. Thank you for nurturing my passion for science and granting me the freedom to pursue my ideas. Your patience, wisdom, and genuine care have been invaluable to me. Your guidance provided me with the confidence and a safe environment to overcome obstacles and break through barriers. This achievement would have been impossible without your persistent support.

I extend my sincere gratitude to my esteemed committee members, Professors **Roi Holzman**, **Gitai Yahel**, and **Dror Avisar**, for their invaluable guidance throughout this research journey. Your expertise, insights, and encouragement have played a pivotal role in shaping my research into one that I am proud of.

To all Shenkar lab members, past and present, you are the best! And to the legendary lab manager **Lion Novak**, for the friendship and for being a fineshmaker in food and science.

I am grateful to the members of the Water Research Center lab group, **Aviv Kaplan**, **Gefen Ronen Eliraz**, **Olivia Nordland**, and **Igal Gozlan**, for their friendship, delightful collaboration, endless patience, and willingness to share their expertise in analytical chemistry with a biologist.

Special gratitude goes to Dr. **Gilad Antler** for graciously providing a home for my work and experiments at the NEGEVE Lab at IUI.

Thank you, **Lachan Roth**, for introducing me to the *Millepora* and also for being Lachan Roth. Thank you to Dr. **Jessica Bellworthy** and Dr. **Dor Shefy** for teaching me how to grow baby *Stylophora*.

Countless thank-yous to the best TAU-IUI office members of all times Dr. **Or Ben Zvi**, Dr. **Raz Tamir**, and Dr. **Ronen Liberman**. Thank you for your friendship, support, counsel, and always being willing to help.

A huge thank you again to Dr. **Ronen Liberman** for introducing me to *Rhytisma fulvum* and for much more.

My sincerest gratitude to Dr. **Tom Shlesinger** for his encouragement and wisdom.

Many thanks to Prof. **Yossi Loya**. Even though I haven't been part of the Loya lab, I always felt like a part of the Loya scientific family.

To Prof. **Yoni Belmaker**, Prof. **Moshe Kiflawi**, Prof. **Yehuda Benayahu**, Prof. **Micha Ilan**, and Prof. **Oren Levy**. I greatly appreciate their valuable advice and support throughout the years of my studies.

Thanks to the Interuniversity Institute for Marine Sciences in Eilat (IUI) staff for their assistance and friendship over the years. Special thanks to the staff at IUI Dive Center, **Ofir Ha'Meiri**, **Genadi Zalzman**, and **Nachumi Sela**.

This work would not have come to be without the assistance of friends and colleagues. Thank you to the supportive student communities at Zoology in Tel Aviv and IUI in Eilat. Heartfelt thanks to all of my dive buddies, with apologies for any inconvenience caused (you all survived).

I thank **Hagit Talmor** and **Meitar Termin** for their care, support, and assistance, and **Naomi Paz** for her valuable edits and suggestions throughout these years.

I thank Dr. **Itai Epstein** and **Michael Klein** for the expert analysis with microFTIR.

Finally, endless thanks to my mother, **Rachel Vered**, for consistently making the impossible possible throughout our lives, and to my life partner, **Raoul Saar**, for reminding me to enjoy life. **Lily** and **Michael**, your existence makes it all matter.

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ABSTRACT

Despite the growing awareness of the harmful effects, the continuous increase in plastic influx into natural environments still poses a constant threat, endangering unique marine ecosystems such as coral reefs. These reefs, which are essential ecologically and sustain livelihoods for over a billion people worldwide, face severe degradation due to anthropogenic activities, climate change and the pervasive issue of plastic pollution. Plastic enters the oceans through various land- and sea-based human activities, breaking down into microplastics and releasing chemical additives into the environment. Marine plastic debris has detrimental effects on corals, increasing their vulnerability to pathogens and causing issues like entanglement, suffocation and the ingestion of microplastics, resulting in energy loss. Additionally, plastic additives leaching into the environment can harm marine organisms, with chemicals such as bisphenols and phthalates disrupting endocrine systems. The primary motivation of my research was to address the main knowledge gaps identified by the United Nations Environment Programme concerning plastic pollution in coral-reef ecosystems. I achieved this by conducting controlled experiments and comprehensive field surveys, seeking to understand the extent and impact of plastic pollution in coral reefs.

My research encompasses an examination of the effects of plastic additives on coral-reef organisms; the characterization of plastic debris levels in the Gulf of Aqaba; and an exploration of plastic additives in coral-reef invertebrates residing on natural substrates compared to those residing on plastic debris. The findings from my study contribute to the development of a comprehensive understanding of marine plastic pollution and its implications for coral-reef ecosystems.

In Chapter 1, I examine the reproductive products of four tropical coral-reef invertebrates exposed to environmentally-relevant concentrations in seawater of four prevalent plastic additives: dibutyl phthalate (DBP), dimethyl phthalate (DMP), 4-nonylphenol (4-NP) and bisphenol A (BPA), as well as to 10^3 higher laboratory concentrations of these four plastic additives. The findings reveal that apart from the significant negative effect of the 1 μg/L of 4-NP on the settlement of the soft coral *Rhytisma fulvum*, none of the other tested materials demonstrated a significant effect on the exposed organisms at environmentally relevant concentrations in the seawater. Regarding the high laboratory concentrations: the 4-NP (1,000 μg/L) had significant negative effects on all the examined species; the BPA concentration (1,000 μg/L) significantly reduced fertilization success in the solitary ascidian *Herdmania momus*, up to its complete failure to reproduce; and the DMP concentration (100 μg/L) had a significant negative effect on planulae settlement of the stony coral *Stylophora pistillata*. The study's findings demonstrate the negative and selective effects of plastic additives on the development and reproduction of coral-reef organisms; and, specifically, the significant effect found following exposure to 4-NP. Consequently, if we wish to fully understand the impact of these contaminants on this endangered ecosystem, their actual concentrations within the living organism tissues need to be determined in order to produce relevant risk assessments for brooding coral species.

The Gulf of Aqaba in the northern Red Sea is considered a potential coral-reef refuge from climate change. However, it is subjected to increasing levels of plastic contamination. In Chapter 2, I characterized the levels of benthic plastic debris, microplastics and plasticizers within the coral-reef's surrounding seawater and sediments, over the course of

2 years. I found that concentrations of benthic plastic debris lay within the level of the reported data from other tropical coral reefs, with boating and fishing debris comprising the most abundant categories. Unlike other studies that have investigated the relationship between depth and the extent of plastic pollution, I found that the deep mesophotic reefs featured significantly higher levels of benthic plastic debris compared to the shallower reefs. The microplastic levels in the reef's surrounding seawater revealed similar concentrations to those reported for the central Red Sea surface seawater, with none of the studied plasticizers exceeding the limits of quantification. These findings provide an important scientific database for developing regional policies and management strategies for battling plastic pollution and making the reefs of the northern Red Sea a coral refuge protected from both global and local anthropogenic stressors.

In Chapter 3, I explore the differences in the presence of plastic additives among coral-reef invertebrates, particularly by comparing organisms residing on natural substrates with those on plastic debris. The connection between the occurrence of plastic additives in corals and encrusting invertebrates residing on plastic debris has not been explored to date, nor contrasted with those residing on natural substrates. My analytical approach involved utilizing Accelerated Solvent Extraction (ASE), Gas Chromatography-Mass Spectrometry (GCMS), and High-Performance Liquid Chromatography coupled to both UV and Mass Spectrometry (HPLC/UV/MS). These methodologies were applied to explore the occurrence of seven targeted plastic additives in samples of three prevalent coral-reef invertebrate species: *Stylophora pistillata*, *Rhytisma fulvum* and *Crella cyathophora,* collected from both a protected reef within a marine nature reserve and an adjacent reef outside the reserve. The study not only scrutinized the presence of plastic additives but also

investigated potential patterns across various species, substrates and sites. My findings revealed the widespread presence of plastic additives in 52% of the examined organisms, encompassing all the studied species and substrates, with Dibenzylamine and bis (2-ethylhexyl) phthalate (DEHP) emerging as the only detected compounds. Statistical analyses indicated no significant difference in their presence between the natural substrate and plastic debris. However, a statistically significant difference was observed in pollution levels between sites, with the protected reef site exhibiting lower contamination. Notably, *R. fulvum* within the marine reserve demonstrated significantly reduced pollution levels compared to its counterparts in the unprotected adjacent reef. This underscores the protective role of marine reserves in mitigating the impact of chemical pollutants on coral-reef ecosystems. The findings from this research add to the growing body of evidence regarding the widespread presence of plastic-related chemicals, and highlight the protective role of marine reserves in mitigating the impact of chemical pollutants on coral-reef ecosystems.

The data acquired and presented in my dissertation contribute to promoting a global understanding of the extent and impact of plastic pollution on coral-reef ecosystems. They provide essential support for the science-based management of marine plastic pollution and, consequently, may play a crucial role in the preservation of coral reefs.

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Abbreviations

4-NP	4-nonylphenol
ASE™	Accelerated Solvent Extraction
BPA	Bisphenol A
BPD	Benthic plastic debris
DBP	Dibutyl phthalate
DDW	Double distilled water
DEHP	Bis (2-ethylhexyl) phthalate
DMP	Dimethyl phthalate
EDCs	Endocrine-disrupting compounds
FSW	filtered seawater
GCMS	Gas Chromatography-Mass Spectrometry
GESAMP	Joint group of experts on the scientific aspects of marine environmental protection
GoA	Gulf of Aqaba
HDPE	High Density Polyethylene
HPLC	High-Performance Liquid Chromatography
IUI	Interuniversity Institute for Marine Sciences in Eilat
LC50	lethal concentration for 50% of the population
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MeOH	methanol
Microplastics	plastic particles smaller than < 5 mm
PAs	plastic additives
PP	Polypropylene
QA/QC	Quality assurance/quality control
UNEP	United Nations Environment Programme
US-EPA	US Environmental Protection Agency

INTRODUCTION

Marine plastic pollution

Plastic pollution constitutes a global threat, affecting nearly every marine and freshwater ecosystem (Rochman 2018; Persson et al. 2022). Despite the rise in public awareness, the amount of plastic waste entering our oceans is increasing at an alarming rate. Projections indicate that the global growth in plastic waste entering the ocean and freshwater ecosystems could reach up to 90 million tons per year by 2030 if the current waste generation trends persist without any improvements in waste management practices (Jambeck et al. 2015; Borrelle et al. 2020). Humankind heavily depends on the ecosystem services provided by the oceans, in regulating climate and CO_2 levels and in providing sustenance and livelihoods for over a billion people worldwide (Halpern et al. 2012). These marine ecosystems and biodiversity, however, are in danger due to plastic pollution, as well as other threats, such as climate change, overfishing, and oil spills (Halpern et al. 2019).

Plastic debris enters the marine environment from various sources, including marine activities such as fishing and boating, and land-based sources like water-treatment effluents, recreational activities on the coast, illegal coastal dumping, and river runoffs (GESAMP 2015; Jambeck et al. 2015; Thushari and Senevirathna 2020). When plastic debris is exposed to UV light and the physical forces of swell and currents, it breaks down into smaller particles called secondary microplastics, which are plastic particles smaller than 5 mm (Arthur et al. 2009). In addition, the influx of primary microplastics, such as industrial granules, synthetic fibers or abrasive components found in cosmetics, contributes to the accumulation of plastic debris in various shapes, sizes and polymer compositions

(Arthur et al. 2009). The global oceanic circulation plays a crucial role in dispersing plastics on a large scale, leading to their widespread presence and reaching even the ocean's greatest depths (Miyagi et al. 2018). The physical properties of these plastics, along with their interactions with biological activity, promote their vertical transport to the seafloor (Galloway et al. 2017; Choy et al. 2019). For example, giant larvaceans (phylum: Chordata, Class: Appendicularia) contribute to the vertical flux of microplastics through the rapid sinking of fecal pellets and discarded houses. Larvaceans, and potentially also other abundant pelagic filter-feeders, can transport microplastics from the surface waters, through the water column, down to the greatest depths of the ocean (Katija et al. 2017). Another example of biota influencing the vertical transport of plastic debris is that of how biofouling, and even early biofilms, alter the sinking behavior of larger plastic debris, making buoyant low-density polymers, such as polyethylene, end up in marine sediments (Kaiser et al. 2017; S. Zhao et al. 2021). Marine organisms also participate in physical-biological processes, such as benthic-pelagic coupling by benthic organisms, and contribute to the biogeochemical cycling of oxygen, carbon, and nutrients (Griffiths et al. 2017). Harris et al. (2021) reported a decrease in sinking and resuspension velocities of marine mussel biodeposits containing microplastics, which can result in increased dispersal distances, thus decreasing in-bed nutrient input and increasing nutrient subsidies for other communities. The capture of plastic particles by filter-feeding benthic mussels and other key species, such as solitary ascidians, involved in benthic-pelagic coupling, enhances the bioavailability of microplastics to sediment-dwelling organisms. This, in turn, may contribute to altering large-scale ecosystem processes (Galloway et al. 2017). This widespread contamination affects marine ecosystems globally, spanning tropical and polar

waters, isolated islands and even depths as extreme as the Mariana Trench, reaching down to 11,000 meters (Bergmann et al. 2017; Rochman 2018). Quantifying and monitoring the extent of plastic pollution is thus essential for understanding its impact on biological systems and its potential effect on biogeochemical cycles in the oceans.

Plastic debris poses a threat to marine life through entanglement and suffocation. Parton et al. (2019) conducted a review of 26 scientific papers documenting instances of sharks and rays entangled in anthropogenic marine debris. The results revealed 47 entanglement events of sharks and rays from 34 different species (N = 557). Ghost fishing gear accounted for the majority of entanglements (74% of animals), followed by polypropylene strapping bands (11% of animals). In a study by Lamb et al. (2018), a survey of 159 coral reefs in the Asia-Pacific region indicated that ca. 11.1 billion plastic items are entangled on coral reefs across the western Pacific Ocean. The projection suggested an anticipated 40% increase by 2025. These entanglements can inflict physical damage and elevate the occurrence of pathogens up to 20-fold in reef-building corals. Marine organisms are also susceptible to ingesting plastic debris and microplastics. In loggerhead turtles, plastic ingestion has resulted in reduced food consumption and energy availability, leading to a lower condition index and reproductive output, which could contribute to population decline (Marn et al. 2020). The ingestion of particles can also lead to a reduction in feeding rates, as demonstrated in the copepod *Centropages typicus* (Cole et al. 2013). Certain smaller particles (< 100 nm), referred to as 'nanoplastics' are predicted to penetrate biological barriers and enter animal tissues, posing an even greater hazard to marine organisms (Mattsson et al. 2015). Moreover, plastic may introduce hazardous chemicals into organisms (Amelia et al. 2021).

Plastic materials are not composed solely of pure polymers but also contain chemical additives that can migrate into the environment due to their weak bonds with the polymer matrix. Plasticizers increase flexibility, while stabilizers and antioxidants prevent degradation from UV light and oxygen and flame retardants reduce flammability (Wiesinger et al. 2021). However, some additives have endocrine-disrupting properties and can bio-concentrate in living organisms (Hahladakis et al. 2018; US-EPA 2022). Despite their endocrine-disrupting properties, bisphenols, nonylphenols and phthalate esters are commonly used as plastic additives (Groh et al. 2019; Darbre 2020). Exposure to bis (2-ethylhexyl) phthalate (DEHP) accelerated the start of spawning and decreased egg production in exposed marine madaka (*Oryzias melastigma*) females (Ye et al. 2014). Additionally, even low concentrations of dibutyl phthalate (DBP) were found to be lethal to zebrafish (*Danio rerio*) embryos (Chen et al. 2014). The leaching of nonylphenols from food-grade plastic is toxic to the coral-reef fish species *Pseudochromis fridmani* (Hamlin et al. 2015). In a study of the early life stages of a solitary ascidian, *Ciona robusta*, the LC50 for bisphenol A (BPA) was estimated to be 1,190 µg/L, and larval malformations in the pigment cells appeared at 23 µg/L (Messinetti et al. 2019). Researchers employ controlled exposure experiments to test the effects of plastic leachate rather than of specific compounds, owing to the considerable variation in the complex chemical composition of plastic materials among polymers and products (Groh et al. 2019). Exposure to leachates from inflatable toys was shown to cause delayed development and morphological changes in sea urchin larvae (Oliviero et al. 2019). Leachates from car tire rubber and Polyvinyl Chloride (PVC) were found to be toxic to algal growth and mussel early-stage development (Capolupo et al. 2020).

In addition, as ecosystem engineers, coral reefs create complex structures that are more likely to become entangled in large plastic debris. In a review by Carvalho-Souza et al. (2018), entanglement was reported in 418 species of corals across eight taxa. Fishing and tourism activities significantly contribute to marine plastic debris in coral ecosystems, as shown for the southern Great Barrier Reef (Wilson and Verlis 2017). However, a comprehensive quantification of plastic pollution associated with coral reefs is lacking, with reports to date varying across regions and oceanic conditions. Furthermore, many of the studies conducted on marine plastic pollution often fail to include all the different types of plastic pollutants, including large plastic items, microplastics, and chemical additives.

The key role of coral reefs

Although coral reefs cover less than 0.1% of the ocean floors, they are among the most diverse ecosystems, hosting thousands of species of organisms (Spalding et al. 2001). These extraordinary biological structures play a critical role in marine ecology and serve as crucial providers of ecosystem services to millions of people around the world (Moberg and Folke 1999). A third of the world's marine fish species depend on healthy coral reefs, and as much as 10% of the fish consumed are caught from reef areas. Coral reefs protect coastlines from storms and erosion and provide food and jobs for local communities, in addition to their cultural importance to indigenous peoples (Moberg and Folke 1999; Hoegh-Guldberg et al. 2017; Eddy et al. 2021). Over half a billion people depend on these reefs for food, income, and protection. Fishing, diving, and snorkeling on and near reefs add hundreds of millions of dollars to local businesses (UNEP, ISU, ICRI, and Trucost 2018).

Unfortunately, these vital services that coral reefs provide are at risk of severe degradation due to anthropogenic activities and climate change (Hoegh-Guldberg et al. 2017; Hughes et al. 2017; Eddy et al. 2021). Over the past four decades there has been a significant decline in coral cover on tropical reefs. This is mainly due to anthropogenic ocean warming, which has triggered mass bleaching and disease outbreaks, leading to the loss of coral cover on nearly every tropical reef in the ocean. (Adkins 2017; Hoegh-Guldberg et al. 2017; Hughes et al. 2018). Local drivers like pollution and overfishing contribute to coral loss, but regional stressors and their combined implications for the state of coral reefs on a global scale are not fully understood. For example, overfishing of coral-reef-associated fish has led to a decline in their abundance in many regions (Eddy et al. 2021).

The global community has recognized the importance of the ecological role and the ecosystem services that coral reefs provide. This is reflected in the commitment to achieve the United Nations (UN) Sustainable Development Goals (SDGs). SDG2 includes the aims of ending hunger, achieving food security, and promoting sustainable agriculture; and SDG14 seeks to conserve and sustainably use the oceans, seas, and marine resources to ensure their durable development (Eddy et al. 2021). Despite the social and environmental importance, research in this area has been limited to recent years, and there are still many unknowns regarding the extent and impacts of marine plastic pollution on reef organisms and on the ecosystem as a whole (Lamb et al. 2018; Lasut et al. 2018; Saliu et al. 2019; UNEP 2019).

Current knowledge regarding plastic pollution in coral reefs

Over 800 coral-reef species have experienced interactions with marine debris, with the majority of this being plastic debris. In 2019, the United Nations Environment Program (UNEP) published a report focusing on the impacts of plastics on shallow-water coral reefs. The report sought to promote mitigation management, awareness-raising, and scientific efforts in order to enhance our understanding of plastic pollution in coral reefs UNEP 2019).

Plastic marine debris poses a threat to corals and other sessile marine invertebrates. When coral comes into contact with plastic, its susceptibility to pathogens increases, leading to a rise in infection rates from 4% to 89% (Lamb et al. 2018). Additionally, plastic debris can block sunlight and water circulation, which are essential for coral survival, thus causing physical damage to the colony (de Carvalho-Souza et al. 2018; Lamb et al. 2018). Microplastics interact with the corals through both active ingestion and passive surface adhesion. Hall et al. (2015) were the pioneers in demonstrating the ingestion of microplastics ranging from 100 μm to 2 mm by shallow tropical corals. In a 48-hour laboratory exposure treatment, 20% of their studied colonies of *Dipsastraea pallida* were found to have ingested microplastics. Later studies confirmed the phenomenon of widespread microplastic ingestion across other cnidarian species (Allen et al. 2017; Rotjan et al. 2019). Microplastic ingestion is likely to be species-specific. For example, the coral species *Pocillopora verrucosa* and *Pocillopora damicornis* were reported to show varying levels of tissue necrosis when exposed to microplastics. *Acropora humilis*, *Acropora millepora,* and *Porites cylindrica* bleached under the same exposure conditions, while *Porites lutea* showed no adverse effects (Reichert et al. 2018). Field evidence further

confirms the presence of microplastic pollution on cnidarians in reef ecosystems, at levels exceeding 100 particles per coral polyp (Ding et al. 2019; Martin Corona et al. 2019; Carmona et al. 2023). Phthalate esters are among the chemicals commonly used as plasticizers in a wide range of plastic products. Both microplastics and phthalate esters have been detected in different coral species and the surrounding waters from the Maldives (Saliu et al. 2019; Montano et al. 2020) and the Persian Gulf (Ranjbar Jafarabadi et al. 2021b), with a negative correlation between phthalate concentrations in coral tissues and the density of the endosymbiotic algae in the coral.

The knowledge gaps identified by the UN in 2019 highlight crucial areas in which an understanding of the impact of plastics on reef ecosystems remains limited. These gaps include the need for a comprehensive understanding of: (1) the scale of mismanaged waste in coral-reef environments; (2) the patterns of plastic pollution around coral-reef environments; (3) the impacts of leaching chemicals from plastics into coral-reef organisms; (4) the broader ecological effects of plastics on coral reefs; and (5) the interactions of the plastic debris with benthic invertebrates; as well as (6) quantification of the impact of ghost gear and its impact on coral-reef communities; (7) an exploration of the role of plastics in transporting invasive species and potential pathogens globally; (8) standardized measurements of microplastics across coral-reef ecoregions; (9) assessment of the risk microplastics pose to reef organisms; (10) addressing the challenges in detecting microplastics in coral-reef organisms and their surrounding environment; and (11) evaluating the reliability of current assays and validating models for assessing the likelihood of false negatives and positives in plastic counts, as essential for advancing our understanding of plastic pollution in reef ecosystems (UNEP 2019).

In this dissertation, I have addressed several of these knowledge gaps by combining controlled laboratory exposure experiments with comprehensive field surveys. I investigated various plastic pollutants, including large items, microplastics, and chemical additives, with the purpose of contributing to a greater understanding of the extent and impacts of plastic pollution in coral reefs.

Research objectives

The overarching goal of my study was to build upon and expand the current scientific knowledge base regarding marine plastic pollution in coral-reef ecosystems. In order to achieve this goal, I conducted three independent explorations, each aimed at addressing a different research gap:

Chapter 1: Examining the possible effects of plastic additives on the early life stages of coral-reef organisms.

Here I address the lack of published experiments that test the exposure effects of common plastic-associated chemicals on the fecundity and development of tropical coral-reef invertebrates. Phthalate acid esters, bisphenols, and nonylphenols are chemical compounds integrated into the plastic during manufacturing. They are present in marine environments and associated with endocrine-disrupting processes. However, our understanding of the impact of these endocrine-disrupting plastic additives on coral-reef organisms is limited. In this chapter, I exposed the reproductive products of four tropical coral-reef invertebrates to environmentally relevant concentrations of four prevalent plastic additives in seawater, and at a 10^3 higher laboratory concentration.

Chapter 2: Characterizing over time the levels of benthic plastic debris, microplastic, and plasticizers within the coral-reef's surrounding seawater and sediments in the Gulf of Aqaba/Eilat, Red Sea.

I performed surveys applying a broad inspection to assess the extent of various forms of plastic pollution in a coral-reef environment, including anthropogenic benthic debris (man-made benthic debris larger than 2.5 cm), microplastics (0.25-5 mm plastic particles) in the reef surrounding seawater, and phthalate esters in the reef surrounding seawater and sediments. This involved monitoring those plastic pollutants in direct contact with benthic coral-reef organisms, as well as those in the surrounding seawater and sediment. Additionally, although the Gulf of Aqaba has been suggested as a potential coral refuge from climate change in the coming decades, plastic pollution in the Gulf has been little studied to date. In these surveys my goal was to gather comprehensive information on all forms of marine plastic pollution in the coral reefs of Eilat. These data can serve as a scientific basis for establishing effective preservation policies for the coral reefs in the Gulf, as well as for developing strategies for efficient plastic pollution management.

Chapter 3: Exploring plastic additives in coral-reef invertebrates on natural or plastic substrates.

Limited information exists regarding the presence and exposure pathways of plastic additives within coral-reef biota. In this chapter, I sought to test the hypothesis that plastic substrates leach plastic additives into the resident organisms. An analysis was performed to assess the presence of various plastic additives in coral-reef invertebrates residing on natural substrates and compare this with those residing on plastic debris.

CHAPTER 1

Assessing the Effects of Plastic Additives on the Early Life Stages of Coral-reef Organisms

The results of this chapter were published in *Environmental Pollution* 314: 120285 (2022):

Vered G. and Shenkar N. (2022). Limited effects of environmentally-relevant concentrations in seawater of dibutyl phthalate, dimethyl phthalate, bisphenol A, and 4-nonylphenol on the reproductive products of coral-reef organisms. *Environmental Pollution*, 314, 120285. https://doi.org/10.1016/j.envpol.2022.120285

Author contributions:

GV and NS designed the experiments. GV led and performed the field sampling, carried out the experiments and statistical analysis, drew the figures, and drafted the manuscript with NS. NS supervised the study and interpreted the data together with GV.

1.1 Introduction

Plastic debris has now polluted nearly every habitat on earth, dispersing thousands of types of synthetic chemicals into the natural environment (Cózar et al. 2017; Hahladakis et al. 2018; Miyagi et al. 2018; Napper et al. 2020). Plastic materials are not pure substances, as their polymer building blocks are combined with chemical additives (e.g., antioxidants, plasticizers, and flame retardants) to improve product properties. The majority of these plastic additives (PAs) are not covalently bound to the polymer matrix but, rather, linked by weak secondary bonds. Thus, they are more likely to migrate from the plastic material into the surrounding environment (Hahladakis et al. 2018). Some PAs have a moderate-to-high log octanol-water partition coefficient (log Kow) indicating their low affinity for water, but are partially soluble in biological fluids and have the potential to bio-concentrate in living organisms (US-EPA - ExpoBox 2022). Bisphenols, nonylphenols, and phthalate esters are widely used as PAs despite their endocrine-disrupting properties (Groh et al. 2019; Darbre 2020). Endocrine-disrupting compounds (EDCs) alter the hormonal and homeostatic systems responsible for reproduction, and developmental processes (Diamanti-Kandarakis et al. 2009).

Coral reefs provide a vast variety of biodiversity and ecosystem goods and services that benefit society, from food security and coastal protection to novel drug sources (Cinner 2014; UNEP, ISU, ICRI, and Trucost 2018). Climate change, ocean acidification, and ongoing anthropogenic stressors put these exceptional ecosystems at existential risk (Hughes et al. 2017). As about 80% of marine plastic debris originates from waste generated by coastal populations (Jambeck et al. 2015), biota-debris interactions are more

likely to occur in coastal marine habitats (Galloway et al. 2017), and even more so in those featuring high biological activity, such as fringing reefs. Lamb et al. (2018) surveyed 159 coral reefs in the Asia-Pacific region and found that corals in contact with plastic debris are up to twenty times more susceptible to pathogens. de Carvalho-Souza et al. (2018), based on field and literature investigations, estimated that at least 418 reef species had encountered anthropogenic marine debris.

Reef population structure is directly linked to the reproduction, offspring development, and settlement of coral-reef organisms. Plastic-associated EDCs have been shown to affect the development of early life stages in a variety of marine invertebrates, with large differences among phyla (Oehlmann et al. 2009; Gandara e Silva et al. 2016; Messinetti et al. 2019). The particular life stage at which the organism is exposed to the EDC is of great importance. A developing organism in its larval stage or during metamorphosis undergoes various sensitive processes that can be affected by EDCs, some of which may only become visible upon maturity (Diamanti-Kandarakis et al. 2009; Darbre 2020).

Corals and other coral-reef invertebrates can be either broadcast-spawners or brooders (Shlesinger and Loya 1985; Harrison 2011). Broadcast-spawners release eggs and sperm into the water column, where external fertilization and embryogenesis take place; whereas in brooders, fertilization and embryonic development take place internally, and the fully developed larvae are released into the water column. Consequently, the particular reproduction strategy may be a key factor in a species' vulnerability to EDCs, in particular if these EDCs accumulate within the parent's tissues. To date, no published experiments exist regarding the possible effects of common plastic-associated EDCs on the fecundity and development of tropical coral-reef invertebrates.

Here I have studied four types of PAs detected in marine environments and considered to be environmental pollutants of emerging concern (Hermabessiere et al. 2017). (1) Dibutyl phthalate (DBP) and (2) dimethyl phthalate (DMP) are both phthalate acid esters and can be found in consumer products such as paints, adhesives, and cosmetics, however, 80% of all produced phthalates are used to soften plastic, making them ubiquitous contaminants in natural environments (Net et al. 2015a). Isa et al. (2022) measured the bioconcentration of phthalate esters in soft corals and found that DBP and DMP were the most represented in soft-coral tissues. (3) 4-nonylphenol (4-NP) is used for the production of non-ionic surfactants in detergents and cleaning products, and is also a common antioxidant and stabilizer used in rubber and plastic food packaging (Fernandes et al. 2008). (4) Bisphenol A (BPA) appears to be both an estrogen receptor agonist and an androgen receptor antagonist and is a major monomer and additive of epoxy resins and polycarbonate (Flint et al. 2012; Groh et al. 2019).

While the effects of these PAs have been investigated in humans, commercial species, and model organisms (Flint et al. 2012; Mankidy et al. 2013; Groh et al. 2019), their endocrinal and systemic effects on marine wildlife are less well known, and reports on their environmental concentrations in tropical seas featuring coral reefs are scarce (Kawahata et al. 2004; Malem et al. 2019; Saliu et al. 2019; Montano et al. 2020; Ranjbar Jafarabadi et al. 2021b).

The present study examined the effects of exposure to the above-mentioned PAs on the early life stages of four tropical coral-reef invertebrates common in the Red Sea: (1) the soft coral *Rhytisma fulvum* (Cnidaria, Alcyonacea) is an octocoral with a wide distribution in the Red Sea and the Indian Ocean. It is a gonochoric surface-brooder, with the oocytes

being fertilized within the coral tissues, and the developing planulae then brooded on the surface of the female colony (Benayahu and Loya 1983); (2) The stony coral *Stylophora pistillata* (Cnidaria, Scleractinia) is a branching coral with small polyps and a high surface/volume ratio, and is a hermaphroditic internal-brooder highly abundant in the reefs of the Red Sea (Rinkevich and Loya 1979); (3) The calcifying hydrocoral, *Millepora dichotoma* (Cnidaria, Hydrozoa), is highly abundant and serves as an important reef-builder in the northern Red Sea. It is a gonochoric broadcast-spawner, and its life cycle features either a male or female planktonic medusa stage. The gametes are shed into the open water shortly after the medusa spawns (Shlesinger and Loya 2019); and (4) *Herdmania momus* (Chordata, Ascidiacea) is a solitary ascidian, a sessile marine filter-feeder, known for its ability to thrive in both polluted and clean habitats in tropical environments around the world. It is a hermaphroditic broadcast-spawner, and as an invertebrate chordate its larval stage shares characteristics with vertebrates, making it an ideal model system for evo-devo studies (Gallo and Tosti 2015). The selected organisms play an important role in tropical coral-reef ecology, and any interference in their reproduction could initiate a cascade of deleterious changes in the community structure of the reef.

1.2 Materials and Methods

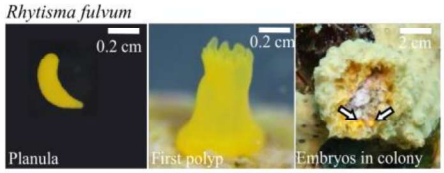
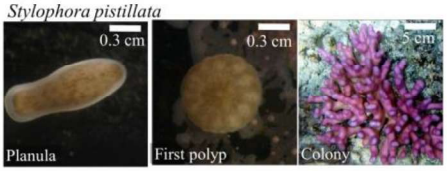
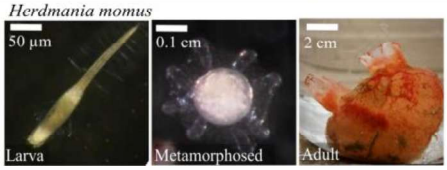
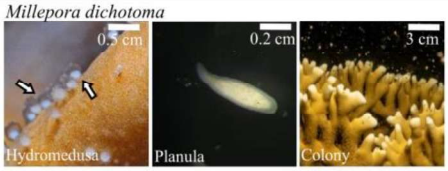
General Approach

A series of exposure experiments were conducted to examine the impact of plastic additives (PAs) on the early life stages of four common coral-reef invertebrates. All experiments were performed at the Interuniversity Institute for Marine Sciences in Eilat (IUI), Israel, and the organisms were collected from the tropical coral reefs in the Gulf of Aqaba, Red Sea (Supplementary Figure S1). As it is known that some endocrine-disrupting compounds (EDCs) have a non-monotonic dose response and that their effects at low doses are not predicted by effects at higher doses (Vandenberg et al. 2012), I have tested PA effects at an environmentally relevant concentration for seawater, and at a higher laboratory concentration (10^3 higher).

Each of the chosen organisms represents a major group found in tropical coral reefs around the world. Furthermore, the selected organisms vary in both their feeding behaviors and their reproduction strategies (Table 1.1). The measured parameters were: (1) larval survival, settlement, and metamorphosis of the solitary ascidian *Herdmania momus*, the soft coral *Rhytisma fulvum*, and the stony coral *Stylophora pistillata*; and (2) egg fertilization and larval development of *H. momus* and the calcifying hydrozoan *Millepora dichotoma*. Exposure experiments were conducted separately according to each of the species' reproductive periods. *H. momus* and *S. pistillata* were exposed to PAs in 50 mL glass Petri dishes, and *R. fulvum* and *M. dichotoma* in 150 mL glass beakers. Experimental setups are provided in detail in Figure 1.1.

Exposure experiments were performed under controlled conditions based on average environmental conditions measured at the sampling sites (25 ± 2°C, 40‰ salinity). The different treatments comprised one environmentally-relevant concentration for seawater (Table 1.2), and a higher laboratory concentration (10^3 higher) of each of the following PAs: bisphenol A (BPA), dibutyl phthalate (DBP), dimethyl phthalate (DMP), and 4-nonylphenol (4-NP). The chemical and physical properties of the selected PAs and their common uses and functions in plastic products are detailed in Supplementary Table S1.

Table 1.1: Feeding and reproduction characteristics of the tested organisms and images of their different life stages.

Species	Reproduction strategy	Adult feeding strategy	larvae feeding strategy	Notable role in coral reefs
Rhytisma fulvum 	Gonochoristic, surface-brooder	Mutual photo-autotrophy and heterotrophy	Parents transmit photosynthetic symbionts to planulae	Common in both shallow and mesophotic indo-pacific reefs
Stylophora pistillata 	Hermaphrodite brooder	Mutual photo-autotrophy and heterotrophy	Parents transmit photosynthetic symbionts to planulae	Ecosystem engineer
Herdmania momus 	Hermaphrodite, broadcast spawner	Suspension feeder	Non-feeding larvae	Responsible for benthic–pelagic coupling processes
Millepora dichotoma 	Gonochoristic, spawner of hydromedusa	Mutual photo-autotrophy and heterotrophy	Parents transmit photosynthetic symbionts to planulae	Ecosystem engineer

**M. dichotoma* photos: G. Koplovitz and T. Shlesinger, *R. fulvum* photos: R. Liberman

Table 1.2: Environmental concentrations of dibutyl phthalate (DBP), dimethyl phthalate (DMP), 4-nonylphenol (4-NP), and bisphenol A (BPA) in marine waters. * Coral reefs sites.

PA	Concentration [µg/L]	Study site	Reference
DBP	0.002 – 0.013	M2 seamount, NW Pacific Ocean*	(Zhang et al. 2019)
	0.23 – 0.77	Pradu Bay, the Tropical Eastern Coast of Thailand NW Pacific Ocean *	(Malem et al. 2019)
	0.071 – 0.319	South China Sea, NW Pacific Ocean	(Cao et al. 2022)
DMP	0.32 – 0.008	M2 seamount, NW Pacific Ocean*	(Zhang et al. 2019)
	0.003 – 0.142	Spanish coast, NW, Mediterranean Sea	(Sánchez-Avila *et al.*2012)
	0.003 – 0.012	South China Sea, NW Pacific Ocean	(Cao et al. 2022)
4-NP	1 – 1.24	Amur Bay, Japan, East Sea, NW Pacific Ocean	(Cherniaev et al. 2016)
	0.006 – 0.497	Cape D'Aguilar Marine Reserve, Hong Kong, NW Pacific Ocean *	(Xu et al. 2014)
	0.004 – 0.026	Kenting National Park, Taiwan, NW Pacific*	(Kung et al. 2018)
BPA	0.002 – 0.049	East China Sea, NW Pacific Ocean	(N Zhao et al. 2021)
	13 – 15	Turkish coast, Eastern Mediterranean Sea	(Ozhan and Kocaman, 2019)
	0.006 – 0.407	Cape D'Aguilar Marine Reserve, Hong Kong, NW Pacific Ocean *	(Xu et al. 2014)

Tested solutions

All PAs were purchased from Sigma-Aldrich, Chemical Company Ltd, Dorset, Kent, UK (4- nonylphenol No. 46405, PESTANAL®, dimethyl phthalate No. 41320, PESTANAL®, bis(2-ethylhexyl) phthalate No. 36735, PESTANAL®, and bisphenol A No. 42088, TraceCERT®). HPLC-grade methanol 99.9% and acetone were purchased from Bio-Lab (Israel). Water solubility and log Kow for all four PAs can be found in Supplementary Table S1. To avoid phase separation, to improve the mixture of the

chemicals in the seawater, and to ensure unity between the treatments I have used methanol (MeOH) as the carrier solvent and as the solubilizer for all PAs. The fresh working stock was prepared for each experiment one hour before exposure. Together with the different PAs, all working stocks were composed of DDW and MeOH 1:1 v/v. The PA concentrations in the stocks were calculated so as to achieve the desired exposure concentration by adding 1 mL stock solution to the volume of the test vessel (Petri dishes/beakers). In addition to the PA treatments, two negative control treatments were applied: (1) DDW-control: to control for the change in salinity by applying 1 mL DDW to each DDW-control vessel; and (2) MeOH-control: to control for the carrier solvent effect by applying 0.5 mL of DDW and 0.5 MeOH to each MeOH-control vessel. The concentration of both DDW and MeOH in all treatments was 1:100 v/v in the Petri dishes and 1:300 v/v in the beakers. All treatments other than the DDW-control that contained 1:50 DDW and no MeOH. I am able to report only the nominal concentrations since I was limited by the analytical methods for these volumes (Table 1.3).

Table 1.3: Nominal concentrations for each treatment (representing an environmental concentration and a high laboratory concentration = environmental $*10^3$).

Treatment	Type	Nominal conc.
Control - DDW	Control	1:100 v/v
Control - MeOH	Control	1:100 v/v
Dibutyl phthalate (DBP)	Environmental	0.1 µg/L
	High	100 µg/L
Dimethyl phthalate (DMP)	Environmental	0.1 µg/L
	High	100 µg/L
4-nonylphenol (4-NP)	Environmental	1 µg/L
	High	1000 µg/L
Bisphenol A (BPA)	Environmental	1 µg/L
	High	1000 µg/L

Exposure of *Herdmania momus* gametes and larvae

For the solitary ascidian *Herdmania momus*, two different exposure experiment series were conducted: (1) exposure of eggs and sperm to test for egg fertilization, larval development, and larval settlement success; and (2) exposure of 12-hour-old larvae to test for survival and settlement success. For both experiments mature *H. momus*, measuring ca. 7 cm in length between siphons along the mid-ventral line, were collected from the Dolphin Reef (29°31'33.9"N 34°56'17.6" E) and the Eilat Marina (29°33'11.1"N 34°57'37.4" E) from July 2021 to October 2021. Both early life stages were obtained in the laboratory by means of artificial fertilization following the methods of Gewing et al. (2019). In brief, artificial fertilization was performed by manually separating eggs and sperm from dissected gonads of the hermaphrodite ascidians. Gametes were transferred into a beaker containing 100 mL filtered seawater (FSW) filtered through a 0.8/0.45 µm AcroPak 1,000 Capsule. Using a filter mesh of 100 µm, the larger eggs (> 200 µm) remained at the bottom of the beaker while the sperm were collected into a separate beaker.

The gamete exposure experiment was initiated by mixing 50 eggs and 5 mL from the sperm beaker with 50 mL FSW and the different treatment solutions in each of the plates. Samples were kept in a dark room at 25°C. At 12- and 26-hours post-fertilization, eggs were examined under a stereomicroscope and noted as fertilized or unfertilized; hatched larvae were regarded as fertilized eggs, and each larva was noted as either developed, undeveloped, metamorphosed, settled, or dead. (Supplementary Figure S2).

The larvae exposure experiment was initiated with 8 larvae placed in each of the prepared Petri dishes containing the different treatments. Samples were kept in a dark room at 25°C. At 24 hours post-fertilization, plates were examined under a stereomicroscope and

each larva was noted as either swimming, settled, or dead. Larvae that had fully lost their tail and settled were considered settled larvae, while motionless or missing larvae were recorded as dead.

Exposure of *Stylophora pistillata* planulae

In May 2021, planulae of *Stylophora pistillata* were collected by SCUBA divers from large colonies displaying a size range of 15-25 cm in diameter, in the IUI shallow reefs (3-8 m depth) (29°30'05.1"N 34°55'02.3" E). Following the methodology described in Shefy et al. (2018), planulae were collected using collector cups (Zakai et al. 2006), comprising a floating plastic container, a 100 μm plankton net, and a plastic funnel. The collector cups were placed over the colonies at sunset and retrieved the following morning.

To avoid interspecies variation, for each experiment planulae from different parent colonies were divided evenly between the treatment plates. Glass Petri dishes were prepared with 50 mL fresh seawater, the treatment solution, and a preconditioned glass slide. The glass slides were biologically preconditioned for one month in running seawater to enable the development of the microbial films and algae that induce planulae settlement (Bellworthy et al. 2018).

Exposure was initiated with six planulae placed on each of the prepared plates. Samples were kept in 60% shade. Petri dishes were covered to minimize water evaporation and 2/3 were submerged in a water table with running ambient seawater flow to maintain seawater temperature. Observations were continued for up to 5 days, based on previous studies' conclusion that most planulae have settled by 48 hours post-release (Rinkevich and Loya

1979). Samples were examined daily and each planula was noted as either swimming, settled, malformed, or dead (Supplementary Figure S3).

Exposure of *Rhytisma fulvum* planulae

On July 2021, three days after the onset of a surface-brooding event, embryos of *Rhytisma fulvum* were collected by SCUBA divers from the surface of a female colony found at 10 m depth in the IUI reef (29°30'11.9"N 34°55'07.7" E). Following the methods of Liberman et al. (2021), embryos were carefully removed from the surface of the colony and placed in a Ziploc bag. In the lab, the embryos were gently washed twice with FSW. Glass beakers containing 150 mL FSW, the treatment solution, and a 4 cm^2 piece of preconditioned terracotta tile, were prepared. The terracotta tiles had been preconditioned in ambient seawater in the shade for two months.

Exposure was conducted with 50 planulae placed in each of the prepared beakers, which were covered to minimize water evaporation. Beakers were kept in 60% shade and 2/3 submerged in running ambient seawater flow to maintain temperature. For each beaker 1/3 seawater was changed on days 4 and 8. Seawater exchange was kept to a minimum to avoid turbulence in the beakers that might interfere with the metamorphosing planulae. Observations were carried out on days 7 and 13, and each planula was noted as either swimming, settled, or dead (Supplementary Figure S4).

Exposure of *Millepora dichotoma* gametes

Millepora dichotoma gametes were collected following the methods of (Roth, 2021). In August 2021, a synchronized hydromedusa release event occurred 350 m north of the IUI (29°30'16.0"N 34°55'08.5" E), and the gonad-bearing medusae were collected from 20

different colonies. Each colony was sampled into one Ziploc bag and within one hour had arrived at the lab. In the lab, the bags were inspected and the sampled bags were marked as male or female. Glass beakers containing 50 mL FSW and the treatment solution were prepared. Ten eggs from each of the eight sampled female colonies were added to each beaker, together with 10 mL of seawater from the bags of each of the ten sampled male colonies. The beakers were kept in 60% shade and 2/3 submerged in running ambient seawater flow to maintain seawater temperature. As (Roth 2021) had shown that planulae hatch about 48 hours post-medusae release, observations were conducted at 24 hours and 48 hours post-release. At 48 hours, intact and swimming planula were counted and documented (Supplementary Figure S5).

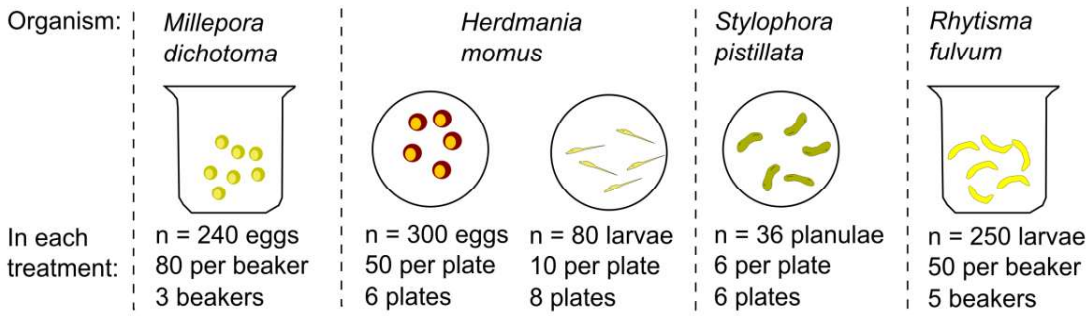


Figure 1.1: Summary of experimental design. Gamete exposure experiments on *Millepora dichotoma* (a), and *Herdmania momus* (b), and larvae exposure experiments on *Stylophora pistillata* (c), *Rhytisma fulvum* (d), and *Herdmania momus* (e).

Contamination control

To avoid contamination by laboratory PAs, all Petri dishes, beakers, measuring equipment, and solvent bottles were made of glass. All glassware underwent a cleaning protocol before each experiment, following Vered et al. (2019), comprising a wash with double-distilled water (DDW) and then acetone, followed by heating up to 200°C for 12 hours, and then washed again with methanol (MeOH) and then DDW.

Statistical analysis

The number of fertilized eggs, developed larvae, and settled and fully metamorphosed larvae were quantified to test for significant effects of the different treatments. Comparisons between the solvent control treatment (MeOH) and each of the PA treatments were performed using a generalized linear mixed-effects model (GLMM) with a binomial distribution. The GLMM analyses were performed using the R package 'lme4' (Bates et al. 2015). Successes were defined as fertilized eggs, fully developed and actively swimming larvae, and fully metamorphosed and settled larvae, for the fertilization, larval development, and settlement experiments, respectively; while non-fertilized eggs, undeveloped or dead larvae, metamorphosed larvae but not attached to the substrate, and attached but only partly metamorphosed larvae, were defined as failures. Since dead larvae degrade quickly missing larvae were recorded as dead larvae. As *H. momus* larvae develop quickly their survival was examined at a single time point at the end of the experiment (24 hours post-initiation) and analysis was performed using GLMM.

S. pistillata and *R. fulvum* planulae host symbionts and therefore can remain motile for several days before settling (Bellworthy et al. 2018; Shefy et al. 2018; Liberman et al. 2021). *S. pistillata* and *R. fulvum* planulae were exposed to various treatments and cultivated for five days and 13 days, respectively. Planulae survival rate was assessed in the different treatments and the analysis was performed using Kaplan-Meier (KM) log-rank survival analysis with the 'survival' R package (Therneau and Lumley 2015). The PA effects on planulae health were analyzed using mixed-effect Cox models. The models were fitted using the 'coxme' package in R (Therneau 2020). Actively swimming larvae (Figure 1.4.a and Figure 1.4.c), and settled fully metamorphosed larvae (Figure S2.f, Figure S3.b,

and Figure S4.d) were recorded as successes, while larvae with distorted borders (Figure 1.4.b, and Figure 1.4.d), motionless larvae, and missing larvae were recorded as failures.

For all mixed effect models, both GLMM and Cox, the treatment and control were set as the fixed effect, and the experiment vessels (beaker / Petri dish) and the parent organisms (female / colony) were set as random effects. Statistical significance was determined at a *p-value* ≤ 0.05.

To better understand the magnitude of the PAs effects I have calculated a standardized effect size for each treatment and the MeOH control. Effect sizes are reported as unpaired Cohen's $d = |M1 - M2|/SD$ (where M1 is the mean of group 1 (treatment), M2 is the mean of group 2 (control), and the SD is the pooled SD), and its 95% confidence interval (CI). These were calculated using 5,000 bootstrap samplings, with a CI not overlapping with zero considered a significant trend. I considered ≥ 0.20 as a small effect, ≥ 0.50 as moderate, ≥ 0.80 as large, and ≥ 1.20 as a very large effect (Cohen 1988). Analysis was performed using the R package 'dabestr' (Ho et al. 2019).

1.3 Results

General

For all experiments, no significant difference was found between the MeOH control and the DDW control.

Gamete exposure experiment in *Herdmania momus* and *Millepora dichotoma*

Compared to the solvent control (MeOH), none of the environmental concentrations of the tested PAs had a significant effect on fertilization or larval development in *Herdmania momus* (n = 300) or *Millepora dichotoma* (n = 240) (GLMM, $p \geq 0.05$). The 4-NP and BPA high laboratory concentration treatments (1,000 µg/L) significantly and negatively affected *H. momus* egg fertilization success (GLMM, p = 0. 003; p = 0.018, respectively), corresponding to a moderate-size effect with Cohen's d = -0.6 (95% CI -0.77; -0.43), and a small-size effect with Cohen's d = -0.27 (95% CI -0.44; -0.11), respectively. Larval development was assessed visually, and only actively swimming and intact larvae were considered fully developed healthy larvae. Similarly, to the fertilization results, *H. momus* larval development from the exposed gametes was negatively affected by the 1,000 µg/L 4-NP (GLMM, p = 0.001) and the 1,000 µg/L BPA (GLMM, p = 0.0003) treatments, which corresponded to a moderate-size effect with Cohen's d = -0.52 (95% CI -0.67; -0.36), and a large-size effect with Cohen's d = -0.8 (95% CI -0.92; -0.69), respectively (Figure 1.2.a-d).

The results for *M. dichotoma* reflect the presence of fully developed and active larvae, irrespective of the fertilization success itself. The 1,000 µg/L 4-NP treatment had a significant negative effect compared to the MeOH control, with only three *M. dichotoma*

larvae in total having developed out of 240 eggs, compared to 47 larvae in the MeOH control (n = 240, GLMM, p = 0.0124), demonstrating a small negative effect size with Cohen's d = -0.34 (95% CI -0.4; -0.27) (Figure 1.2.e, and Figure 1.2.f).

Settlement and metamorphosis of Herdmania momus, Stylophora pistillata, and Rhytisma fulvum larvae

Apart from the 1 µg/L 4-NP treatment, which affected *Rhytisma fulvum* settlement significantly and negatively (n = 250, GLMM, p = 0.022) with a small effect size (Cohen's d = -0.32 95% CI -0.5; -0.14), no environmental concentration effect significantly differed from that of the MeOH control. The 1,000 µg/L 4-NP treatment significantly and negatively affected settlement in all species: *Stylophora pistillata* (n = 36, GLMM, p < 0.0001), *R. fulvum* (n = 250, GLMM, p < 0.0001), and *H. momus* (n = 64, GLMM, p < 0.0001), corresponding to a very large effect size on *S. pistillata* settlement with Cohen's d = -2.45 (95% CI -3.94; -1.53), and large effect size on *R. fulvum* and *H. momus* settlement with Cohen's d = -1.02 (95% CI -1.19; -0.84), and Cohen's d = -1.13 (95% CI -1.67; -0.72), respectively. For *S. pistillata* settlement, a negative significant effect was also found between the 100 µg/L DMP treatment and the MeOH control (n=30, GLMM, p = 0.016) corresponding to a large effect size with Cohen's d = -1.02 (95% CI -1.59; -0.52) (Figure 1.3).

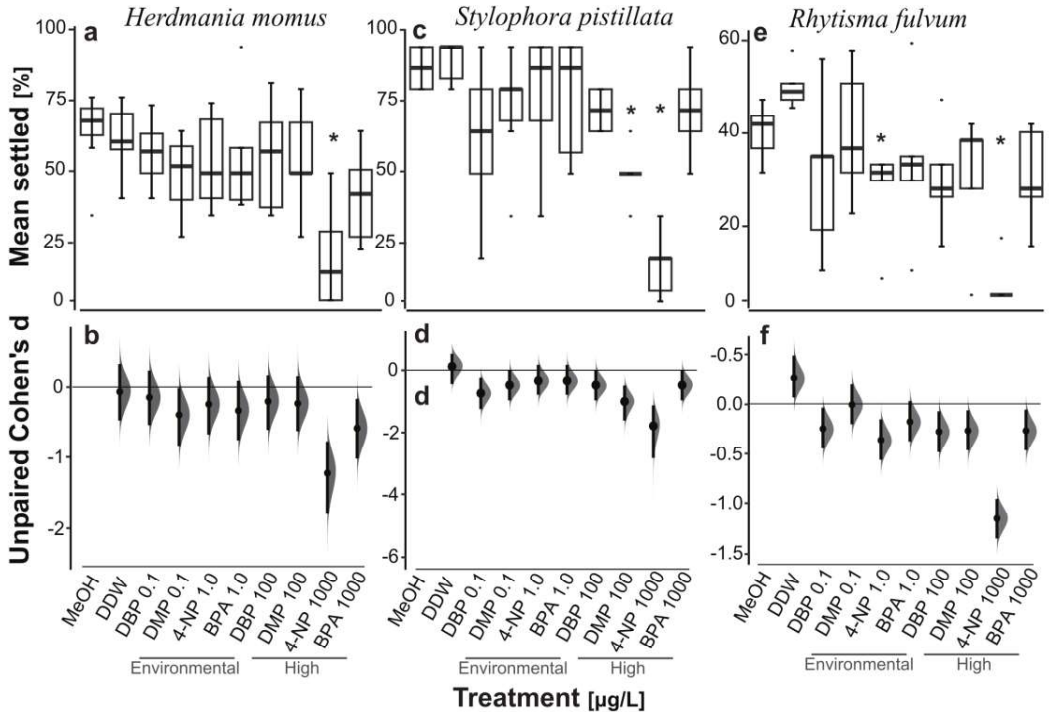


Figure 1.2: Larval settlement success in *Herdmania momus* (a, b), *Stylophora pistillata* (c, d), and *Rhytisma fulvum* (e, f). Box plots of mean percentage of success in Petri dishes/beakers under the different PA treatments and the controls. Line in box = median; box = 25th to 75th percentiles; bars = min and max values excluding outliers; dots = outliers (a, c, e). Shared control estimation plots present effect size as unpaired Cohen's d and its 95% CI for each treatment effect on settlement success minus the MeOH control (b, d, e). Asterisk marks treatments that significantly differed from the MeOH control (*p-value* ≤ 0.05, GLMM)

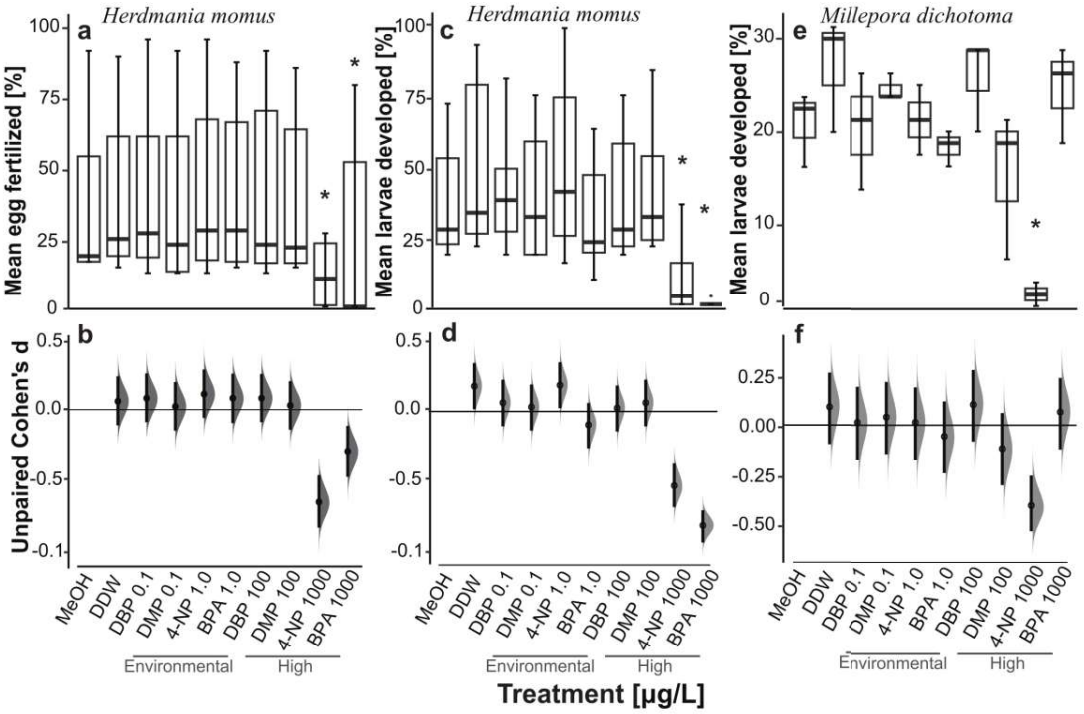


Figure 1.3: Egg fertilization success (a, b) and larval development (c, d) in *Herdmania momus*, and larval development in *Millepora dichotoma* (e, f). Box plots of mean percentage of success in Petri dishes/beakers under the different PA treatments and the controls. Line in box=median; box=25th to 75th percentiles: bars=min and max values excluding outliers (a, c, e). Shared control estimation plots present effect size as unpaired Cohen's d and its 95% CI for each treatment minus the MeOH control (b, d, e). Asterisk marks treatments that significantly differed from the MeOH control (*p-value* ≤ 0.05, GLMM).

Larval survivorship in Herdmania momus, Stylophora pistillata, and Rhytisma fulvum

A significantly lower survival rate of planulae was recorded for the 1,000 µg/L 4-NP treatment in both *S. pistillata*, with 14% survival compared to 100% in the MeOH control (Figure 1.4.d), and *R. fulvum*, with 2% survival compared to 44% in the MeOH control (KM log-rank test: $p < 0.0001$) (Supplementary Figure S6 and Figure S7). *H. momus* larval survivorship was not significantly affected by any of the treatments (GLMM, $p \geq 0.05$) (Supplementary Figure S8), although larvae in both the MeOH and DDW controls displayed 100% survival while those in the 1,000 µg/L 4-NP treatment revealed a high survival variance among females, down to 50% survival (Figure 1.4.b).

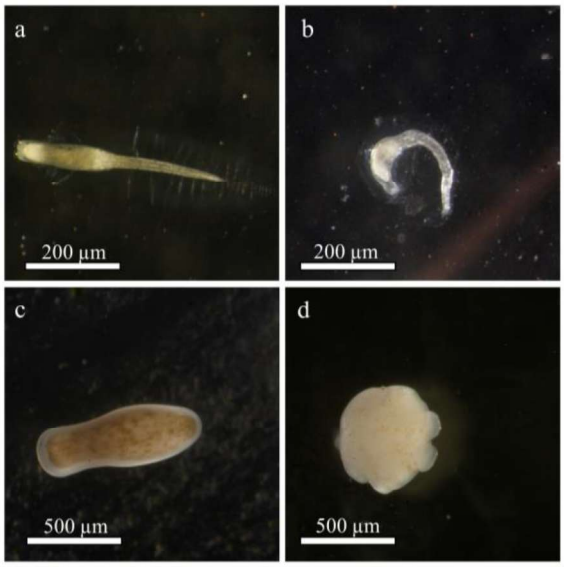


Figure 1.4: Larva of *Herdmania momus* (a, b) (scale bar: 200 µm) and planula of *Stylophora pistillata* (c, d) treated with the MeOH control (a, c) and with the 1,000 µg/L 4-nonylphenol treatment (b, d). Healthy developed *H. momus* tadpole larvae 24 hours post-fertilization (a), malformed *H. momus* larva 24 hours post-fertilization (b), healthy developed *S. pistillata* planula on day 3 (c), and malformed *S. pistillata* planula on day 3 (d). (scale bar: 500 µm).

1.4 Discussion

While the majority of studies testing the effects of plastic additives (PAs) on the development and reproduction of species have focused on vertebrates (Chen et al. 2014; Forner-Piquer et al. 2019; Sun et al. 2021), model organisms in ecotoxicology (Bejgarn et al. 2015; Shen et al. 2019), or commercial species (Gandara e Silva et al. 2016), coral-reef invertebrates have rarely been investigated. In this study, the effects of PAs on the reproduction, development, and settlement successes of prevalent coral-reef invertebrates were examined under both environmentally-relevant concentrations and a high laboratory concentration (10^3 higher).

The 1,000 µg/L 4-nonylphenol (4-NP) concentration had adverse effects on all four tested species and their reproductive success, with large negative effect sizes (Cohen's $d \leq -0.8$). In addition, significantly low survivorship was observed for both *Stylophora pistillata* and *Rhytisma fulvum* planulae treated with the high laboratory 4-NP concentration. Recently, in an environmental risk assessment of coastal ecosystems, 4-NP was found to be the most toxic compound (Tato et al. 2018). The 4-NP EC10 was estimated at 11.1 µg/L for the single cell alga *Isochrysis galbana*; 110.5 µg/L for the mussel *Mytilus galloprovincialis*; 53.8 µg/L for the sea urchin *Paracentrotus lividus*; and 29 µg/L for the copepod *Acartia clausi* (Tato et al. 2018). The US-EPA (US Environmental Protection Agency) recommends that 4-NP concentration in saltwater environments be lower than 1.7 µg/L for chronic exposure and 7 µg/L for acute exposure (US-EPA, 2016). However, I have found that already at a 1 µg/L concentration, 4-NP can put the settlement success of *R. fulvum* at high risk, indicating the potential hazard of this compound for this significant

octocoral species, abundant in both shallow and mesophotic reefs in the Red Sea. The majority of the world's coral reefs are located in the seas and oceans of developing countries, where the human population is rapidly expanding and where waste and wastewater management are limited (UNEP, ISU, ICRI, and Trucost 2018). Nonylphenols are found in plastic waste and are also prevalent in wastewater, with a direct relationship between their levels in seawater and the presence of poorly treated effluents (Chadha et al. 2018).

The well-known endocrine disruptor, bisphenol A (BPA), had a significant negative effect on the gametes of the solitary ascidian *Herdmania momus* at the high laboratory concentration (1,000 µg/L), resulting in a 48% reduction in fertilization success and zero healthy larval development compared to the control. BPA did not affect any of the other tested species. However, in a previous study on the early life stages of a different solitary ascidian species, *Ciona robusta*, BPA LC50 was estimated to be 1,190 µg/L, and larval malformations in the pigment cells appeared at 23 µg/L (Messinetti et al. 2019).

dimethyl phthalate (DMP) is a low molecular weight phthalate ester and has been shown to bioaccumulate in some coral species (Montano et al. 2020; Ranjbar Jafarabadi et al. 2021b; Isa et al. 2022). Lowest molecular weight phthalate esters display bioaccumulation factors greater than those predicted from a lipid-water partitioning model and have species-specific observed bioaccumulation (Isa et al. 2022). The DMP high laboratory concentration had a negative effect on settlement in *S. pistillata*. This reef-builder coral is a brooder species and the most abundant stony coral in the Red Sea (Loya 2004). As a brooder, the level of the compound within the organism's tissues, rather than the surrounding seawater concentration, may have a greater impact on *S. pistillata* larval

development. I suggest that DMP and other low molecular weight phthalates should be examined for additional adverse effects on *S. pistillata*.

We found a limited effect of the tested PAs when examining environmentally-relevant concentrations for seawater, other than the negative effect of 4-NP on the settlement of *R. fulvum* planulae. All reported concentrations are nominal and were not measured in the test vessels as the analytical ability to quantify these compounds was limited by the small volumes used. Consequently, the reproductive products exposed to the different compounds might have experienced lower levels than the reported nominal concentrations due to loss via compound adhesion to the glassware.

When assessing the environmental risk of a compound, a comparison is often conducted between the toxicity thresholds obtained from controlled experiments and the reported environmental concentrations (Tato et al. 2018). The environmentally-relevant concentrations used in the experiments (Table 1.3) were based on reported levels in seawater samples from marine environments, including tropical locations featuring coral reefs (Table 1.2). However, I hypothesize that the environmentally-relevant concentrations in seawater are not biologically relevant for brooding organisms, in light of the bioaccumulation of PAs in corals having already been demonstrated (Ranjbar Jafarabadi et al. 2018; Isa et al. 2022), and of gametes and brooding planulae experiencing the contamination level from within the body of the organism rather than from the environmental concentration in seawater.

A study on the bioaccumulation of polycyclic aromatic hydrocarbons in corals revealed two orders of magnitude higher compound accumulation in the coral mucus than in the

tissues (Han et al. 2020). In addition, phthalate metabolism was found to be related to the lipid content of the corals, and to be species-specific and not related to colony size (Isa et al. 2022). The coral mucus thus presents a higher accumulation potential as it contains lipid compounds (Han et al. 2020). PAs, which are also highly lipid-soluble, are expected to act similarly. Accumulation of PAs in coral mucus is particularly significant for the surface-brooder *R. fulvum*. Female colonies brood the fertilized eggs and develop planulae in a mucus layer on the surface of the colony (Benayahu and Loya 1983). Hence, *R. fulvum* embryos will probably be impacted directly by the PA concentrations that have accumulated in the colony's mucus rather than in the water column. The advantage provided by parental protection in brooders might, in this case, become a disadvantage when the contaminants accumulate.

Environmental concentrations measured in marine invertebrates' tissues are commonly reported as units per dry weight (Net et al. 2015a; Vered et al. 2019; Ranjbar Jafarabadi, et al. 2021b). These measurements, however, do not reflect the actual concentrations within the living organism's tissues and systems, particularly in those species featuring hydrostatic skeletons. The transition from units per dry weight to concentration in seawater, as used in controlled laboratory experiments, may therefore not accurately reflect the environmentally-relevant exposure levels for brooded planulae and developing gametes. Saliu et al. (2020a) recently proposed an in-situ measurement for plastic-associated contaminants in marine invertebrate tissues, using a non-lethal solid phase microextraction (SPME) coupled with liquid chromatography mass spectrometry (LC/MS) analysis (Saliu et al. 2020a; Saliu et al. 2020b). I suggest that this methodology can offer better insights into the concentrations within these types of organisms, and thus should be considered

when evaluating the risk posed by such chemicals to these organisms' reproduction products.

It is important to note that different PAs have varying levels of water solubility and partition coefficients. These differences play a role in the diverse impacts these substances have on marine organisms and the variations in concentrations found in seawater and coral tissues. Additionally, some PAs may be present in particulate matter within seawater. Microplastics have been suggested as vectors carrying PAs into the environment and organisms, however, this idea is still under debate (Amelia et al. 2021; Koelmans et al. 2022). Consequently, the primary path of PAs into marine organisms' tissue should be further investigated in order to examine their effects on these organisms' reproductive output. Another advantage of SPME techniques is they enable measuring the presence of free analytes in the matrix with no interference from particulate matter or microplastics.

Coral reefs must contend with numerous threats, such as the rise in ocean temperatures, ocean acidification, light pollution, and nutrient enrichment. Marine plastic pollution, with its introduction of PA contamination, can thus drive corals towards a critical tipping point and cause community shifts in coral-reef assemblages. To date, only a few studies have assessed the impact of plastic-associated chemicals on coral-reef organisms (Aminot et al. 2020; Montano et al. 2020; Ranjbar Jafarabadi et al. 2021a), despite the significant role of this important ecosystem and its endangered status.

My findings suggest the negative and selective effects of PAs on the development and reproduction of coral-reef organisms; and, specifically, the significant effect found following exposure to 4-NP, which consequently requires future acts of monitoring and

management. Environmental concentrations of DBP, DMP, and BPA had a limited effect on the organisms tested here. The 100 µg/L DMP, 1,000 µg/L BPA, and 1 µg/L 4-NP concentrations elicited a species-specific response, adding to the accumulating knowledge indicating that plastic pollution can selectively affect certain coral-reef species more than others. I suggest that studying the environmental concentrations of PAs in seawater is not biologically relevant for coral reproduction and risk assessment development, particularly for brooding organisms, which harbor their larvae during embryogenesis. As a more environmentally-relevant concentration, therefore, the level of PAs within an organism's tissues should thus be measured and used in future investigations, in order to assess the true risk of these contaminants.

CHAPTER 2

Characterizing and Measuring Levels of Benthic Plastic Debris, Microplastics and Plastic Additives Over Time in the Coral Reefs of the Gulf of Aqaba

The results of this chapter were published in *Science of the Total Environment* 905: 167791 (2023):

Vered G. and Shenkar N. (2023). Plastic pollution in a coral reef climate refuge: Occurrence of anthropogenic debris, microplastics, and plasticizers in the Gulf of Aqaba. *Science of the Total Environment*, 905, 167791.

https://doi.org/10.1016/j.scitotenv.2023.167791

Author contributions:

GV and NS designed the fieldwork and analysis. GV led and performed the fieldwork, carried out the laboratory experiments, sample analysis and statistical analysis, drew the figures, and drafted the manuscript with NS. NS supervised the study and interpreted the data together with GV.

2.1 Introduction

Despite occupying less than 1% of the ocean, coral reef ecosystems feature the highest biodiversity in the marine realm. Regrettably, even under a scenario of low greenhouse gas emissions, climate change poses a severe threat that could lead to the extinction of the majority of the world's tropical coral reefs by 2050 (Hoegh-Guldberg et al. 2017). The Red Sea features fringing coral reefs spanning its entire coastline. The natural selection process that has taken place in these reefs, due to a thermal barrier at the southernmost tip of the Red Sea, has led to the development of coral genotypes that are less susceptible to thermal stress. Consequently, the Gulf of Aqaba (GoA), located in the northernmost part of the Red Sea, has been considered a coral reef protected refuge from the impacts of climate change in the coming decades (Fine et al. 2013). Nonetheless, despite their resilience to global stressors, this considered refuge is under threat from local stressors originating from both land and marine associated human activities. Due to recent advancements in desalination and energy technologies, the Red Sea region is experiencing an increase in the human population, resulting in a rise in coastal development, fisheries, and pollution that directly impacts its coral reefs (Fine et al. 2019; Kleinhaus et al. 2020; Diem et al. 2023).

Plastic pollution is globally recognized as a major threat to coastal and marine ecosystems, including coral reefs. Plastic debris stresses coral through physical damage, light deprivation, toxin release, and anoxia. Coral colonies in contact with large items of marine plastic debris show an increase in disease infection, rising from 4% to 89% when corals come into contact with plastic (Lamb et al. 2018). Microplastics (plastic debris < 5 mm) interact with the corals through both active ingestion and passive surface adhesion.

These particles can be mistaken for prey and enter the mesenterial tissues of the coral gut cavity (Hall et al. 2015), causing a species-specific impact that results in compromised coral growth and health (e.g., bleaching, tissue necrosis, parasites) in certain taxa while other species are left unaffected (Reichert et al. 2019). Furthermore, plastic materials are not pure substances, being composed of polymer building blocks combined with chemical additives. These chemicals can leach out of plastic into the surrounding environment (Ambrogi et al. 2017). Phthalate esters are among the chemicals commonly used as plasticizers in a wide range of plastic products. Both microplastics and phthalate esters have been detected in different coral species and the surrounding water from the Maldives (Saliu et al. 2019; Montano et al. 2020; Raguso et al. 2022) and the Persian Gulf (Ranjbar Jafarabadi et al. 2021b), with a negative correlation between phthalate concentrations in coral tissues and the density of the corals' endosymbiotic algae. Furthermore, some phthalate ester compounds have been found to possess endocrine-disrupting properties that can interfere with hormonal systems in living organisms (Puri et al. 2023). Exposure to 100 µg/L of the plasticizer dimethyl phthalate was shown to have a significant negative effect on the planulae settlement of *Stylophora pistillata*, a highly common branching scleractinian coral in the Red Sea (Vered and Shenkar 2022).

There is a significant knowledge gap regarding the extent of plastic pollution in the Red Sea, particularly in the GoA region, which is undergoing continuous development and rapid population growth as recent improved desalination and energy technologies are introduced. The most recent study regarding large items of marine litter in the GoA was carried out in Aqaba, Jordan, by Al-Najjar and Al-Shiyab (2011), who published abundances of litter per m^2 based on a one-day survey in 2006. Their study raised

awareness of the quantity of metal cans and plastic litter in the area. More recently, Martí et al. (2017) measured floating plastic items in the size range of 0.2-500 mm along the eastern Arabian coast of the central Red Sea using net tows, and revealed a relatively low level of microplastics in its surface seawater (0.37 ± 0.12 microplastics/m^3). The authors suggested that this is a result of the extensive filtration of particulate materials by the coral reefs of the Red Sea, making them a potentially important sink for microplastics. In a later study, the same research group demonstrated that the passive removal of microplastics through adhesion to the coral surface was 40-fold higher than that of active removal through suspension feeding (Martin Corona et al. 2019). Regarding plastic additives, no data currently exists on the presence of phthalate ester plasticizers in seawater or sediment of the Red Sea. However, an assessment of phthalates and microplastics in solitary ascidians (Chordata, Ascidiacea) sampled from Eilat, Israel, in the GoA, revealed high levels of phthalates in these organisms (Vered et al. 2019).

It is important to note that sampling only the upper layer of the water column provides limited insight into the true extent of pollution experienced by benthic communities. Moreover, there is no published data on the levels and types of plastic pollution present in the deeper mesophotic coral reefs of the GoA (depth range 30 – 150 m). These mesophotic reefs display lower fluctuations in temperature, light pollution, sedimentation, and a decrease in shore-related disturbances (e.g., diving, fishing, boating) than adjacent shallower reefs (Eyal et al. 2019), and have been shown to provide settlement grounds for larvae from shallow reef communities (Kramer et al. 2019). However, they are not necessarily safe from other anthropogenic threats.

Acquiring data on plastic pollution in this region is vital in order to establish effective conservation policies and strategies for managing plastic pollution. In the present study, an inclusive approach was employed to track and analyze a range of plastic pollutants over time – both those in direct contact with benthic coral-reef organisms and those in the surrounding seawater and sediment. This study conducted an assessment of the quantity and characteristics of anthropogenic benthic debris, at depths of down to 100 m, and explored their interactions with coral-reef organisms. Additionally, it investigated the presence of microplastics in the surrounding seawater and examined the occurrence and concentration of phthalate esters in reef water and sediments down to 30 m.

2.2 Materials and Methods

Study area and sampling sites

Sampling activities were carried out by SCUBA divers at four designated sites during four sampling periods over a two-year span: June-July 2020, November-December 2021, May 2021, and December 2021. . Each site was sampled at two different depth ranges: 3-10 m and 22-30 m. The selected sampling sites varied in their proximity to the city of Eilat and tourist facilities: (A) the Northern Beach is located near the Israel-Jorden border, it's about 1-3 km away from Eilat and Aqaba and receives agriculture run-off and seasonal floods through the Kinet canal outlet; (B) the City Center Beach is at the center of the city of Eilat its touristic facilities and city runoff; (C) the Oil Jetty is about 3 km south of Eilat and is adjacent to an active oil port; and (D) the Nature Reserve is 8 km out of the city of Eilat and is located within a the Coral Reef Beach Nature Reserve near the Israel-Egypt border. (Figure 2.1). Additional information is provided in Supplementary Table S2.

Anthropogenic benthic debris

Anthropogenic benthic debris was assessed through a SCUBA-diving survey, comprising a total number of 112 benthic belt transects (90 m^2) positioned along depth contours. Two SCUBA divers visually inspected the benthic debris by scanning a 3 m wide belt along a 30 m line transect, resulting in a scanned area of 30 x 3 m per transect. The divers swam at a steady pace, ensuring a thorough coverage and documentation of all visible debris within the designated belt. Each anthropogenic item larger than 2.5 cm was documented by the divers in a table comprising the following data for each item: general description, size, material, interactions with organisms, and interacting taxa. The size range of the items was determined using a measuring tape. Randomly placed transects were

spaced approximately 20 m apart to ensure a representative sampling of the area in accordance with globally standardized protocols (GESAMP 2019). The random placement of transects was achieved by swimming for 5 minutes at a 20-degree angle to the right from the end of the previous transect. Additionally, photographs of notable debris items were taken to provide a visual record and assist in subsequent data validation and analysis.

In addition, photographs taken by Tamir et al. (2019) of the benthos at various depths (5-100 m) at various depths down to 100 m along the coast of Eilat during 2016-2018 were analyzed. A total of 1,257 images from 33 photographed belt transects were analyzed, and anthropogenic debris was classified and quantified per square meter.

Each piece of anthropogenic benthic debris larger than 2.5 cm was identified and classified according to the Marine Strategy Framework Directive (MSFD) Joint List (Fleet et al. 2021). Items were identified with a general description based on visual inspection, such as a bottle, hair band, rod, rope, fishing line, etc. Classification comprised the material (plastic, paper/cardboard, textile, concrete, glass, metal, and processed wood), use (personal items, fishing/boating, packaging, consumables, cigarette buds, construction, and undefined), and size range (2.5-30 cm, 31-100 cm, and >1 m). The minimum 2.5 cm size limit was chosen because of the limitation of the onsite visual identification of smaller items. Items were included in the sample if more than half of their overall size lay within the scanned belt. Ropes and strings were recorded independently each time they entered the belt, even if they constituted part of a particular item.

The interaction of benthic marine debris with organisms was described using four categories: no interaction, covering, entanglement, or colonization. The interacting benthic

organisms were documented based on the dominant category: comprising worms and/or mussels, ascidians and/or Bryozoa and/or sponges, soft corals, branching stony corals, massive stony corals, and moving organisms (e.g., fish, echinoderms, mobile gastropods, etc.).

Microplastics in the reef's surrounding seawater

The reef's surrounding seawater was sampled for microplastics based on Genin et al. (1995) methodology for plankton sampling. In this method, a plankton net with a circular mouth opening is towed by SCUBA divers as close as possible to the reef without causing damage. A total of 65 sampling tows were conducted at two different depth ranges: 3-10 m and 22-30 m, each along a 180 m line transect positioned along depth contours. The net used was a 100 μm plankton net with an opening of a 0.25 m radius. The volume of seawater sampled per tow was calculated as 37.04 m^3, with tow length determined as the mean length from measurements of a pre-calibrated T.S.K flowmeter for all sites and seasons (n=10, mean=189 m, SD ± 3 m). The mouth of the net was tightly choked and folded before and after each sampling tow, to avoid loss or added material to the sample. Upon retrieval from the water, the sampled particles were washed with double distilled water (DDW) and collected from the net into pre-cleaned glass jars. The jarred samples were then incubated at 4°C for 10-15 months to allow for the slow decomposition of biological matter.

Given that the Red Sea is an ultra-oligotrophic system (Zarubin et al. 2017), with relatively low levels of organic matter in its water, it was possible to avoid the use of digestive solvents for extracting the plastic particles from the towed samples. Following incubation, the samples were vacuum-filtered onto glass fiber filters (47 mm, glass

microfiber, Whatman®, Grade GF/B, pore size 1.0 μm). Each filter was placed in a covered glass Petri dish and left to dry at room temperature. Once dry, the filters were examined under a stereo-microscope, and visually identified microplastics larger than 0.25 mm were documented, quantified, and characterized in terms of shape (e.g., fiber, film, foam, and fragment), color, and size.

The visual identification of items as microplastics was further confirmed by an analysis using micro-Fourier-transformed infrared spectroscopy at Dr. Itai Epstein's Laboratory, Department of Physical Electronics, School of Electrical Engineering, Faculty of Engineering, Tel-Aviv University. A Nicolet iS50 Fourier-Transform Infra-Red (FTIR) spectrometer (Thermo Scientific, Waltham, MA, USA) coupled with a microscope was applied to a random subset of items (n = 20). Thirty-two scans were performed per item. Wave numbers ranged between 4,000 and 650 cm^{-1}, and the background spectrum was subtracted from the object spectrum. The spectra were verified using the Primpke Spectra Library. Unfortunately, this could not be applied to fibers, therefore, identification has remained supported by visual inspection for the lack of cellular or organic structures, the homogeneity of color, and the fiber being equally thick throughout its entire length, (microplastics features based on Hidalgo-Ruz et al. (2012)).

Phthalate esters in seawater and sediment

Phthalate esters were extracted and quantified in the reef's surrounding seawater and sediment. Six phthalate esters commonly used as plasticizers in plastic products (Groh et al. 2019) were targeted. The chosen phthalate esters, along with their names, abbreviations, formulas, molar masses, and molecular structures, are provided in Supplementary Figure S9. Standards of the targeted components were purchased from Sigma-Aldrich, Chemical

Company Ltd. Bis-2-ethylhexyl ester-3,4,5,6-d4 labeled isotopic (A2S technologies Ltd) (DEHP-d4) was used as the internal standard (IS), and Pesti-S grade n-hexane (C_6H_{14}) was purchased from Bio-Lab (Israel). All analytical methods were performed in the Hydro-Chemistry Laboratory, School of Geosciences, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel Aviv University.

A total of 37 seawater samples of 1 liter were collected 15 cm above the reef corals directly into glass bottles. Phthalate extraction was carried out by liquid-liquid extraction (LLE) using n-hexane as the solvent. Each seawater sample was mixed with 10 mL n-hexane in a separation funnel, shaken for 10 min, and the solvent phase was then collected. The extract was diluted to a final volume of 10 mL with n-hexane.

Sediment samples, totaling 51, were collected from the top 1 cm layer of the sediment into 50 mL glass vials. Phthalate extraction from sediment was carried out according to the method described by Avisar et al. (2019). Briefly, extraction was performed by an accelerated solvent extractor (Thermo ASE™ 350) with n-hexane at 120°C and 1500 psi. The extract was diluted to a final volume of 25 mL with n-hexane.

Both sediment and seawater extracts were analyzed using a Hewlett-Packard (HP) 6890 gas chromatograph equipped with a Restek Rxi-1MS column (15m×0.25mm×0.25 mm) and an HP 5973 mass spectrometer (GCMS). The GC oven was programmed from 70°C for an initial 1 min, followed by a temperature increase to 320°C at a rate of 15°C/min. Quantitative analysis was conducted in a Single Ion Monitoring (SIM) mode. The target ion, for native compounds, was m/z 149 and 153 for DEHP-d4.

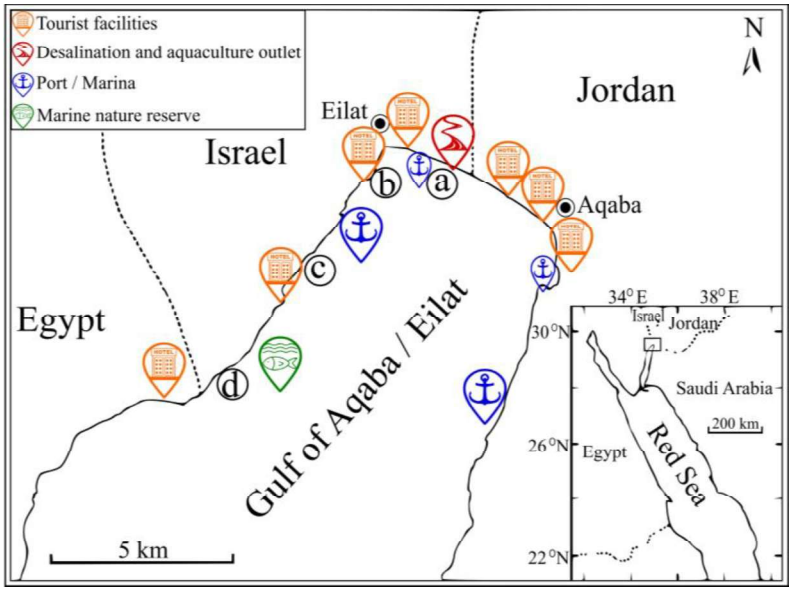


Figure 2.1: A map of sampling sites, Northern Beach (a), City Center Beach (b), Oil Jetty (c), and Nature Reserve (d).

Control of background contamination

Throughout the study, great care was taken to avoid microplastic and phthalate background contamination. Minimal use of gloves was employed, and plastic labware was avoided. All tools and glassware were thoroughly rinsed with distilled water and then acetone, heated to 200°C overnight and washed again with n-hexane before sample handling. Clean glassware was kept covered in aluminum foil, and samples were covered to prevent air exposure. Personnel used protective cotton lab coats. Blanks were prepared for all extraction procedures. Additionally, the ASE™ instrument and cells underwent a pre-wash and blank extraction before each sample extraction. Blanks were collected during the microplastic sampling and extraction to control for every stage of the field and laboratory work. For every 10 vacuum filtrations, a blank filter was filtered with 300 mL of double-distilled water. Petri dishes containing blank filters were set up during the microplastic sample filtering and microscopy to account for airborne contamination.

Statistical analysis

Statistical analyses were conducted using R version 4.2.2 (R Core Team 2021). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. In cases where these assumptions were not met, permutation tests were utilized. Differences in pollutant abundance among the sampling sites, two depth ranges, and sampling periods were evaluated using permutation ANOVA with the 'predictmeans' package, Luo et al. (2022) and permutation pairwise tests with Bonferroni correction with the 'RVAideMemoire' package, Hervé (2022). Data are presented as mean ± standard deviation (SD) and all tests were set with a significance level of α=0.05.

2.3 Results

Anthropogenic benthic debris

Analysis of the diving transects resulted in a total of 940 recorded items classified as anthropogenic benthic debris. Of these, 70% were identified as benthic plastic debris (BPD), with a concentration of 0.063 ± 0.056 BPD/m^2 for all samples (n=112) (Supplementary Figure S11). A similar pattern was observed in the phototransects (n = 33), where 93 recorded items were classified as anthropogenic benthic debris, and 72% were identified as plastic. In both the diving and photo transects the most common items were ropes and fishing lines, which were categorized as fishing and boating waste (Figure 2.2.a).

Analysis of the diving transect data revealed no significant differences between the sampled seasons. However, there was a significant variation in benthic plastic debris (BPD) abundances between the 3-10 m and 20-30 m depth ranges. The mean BPD/m^2 was 0.04 ± 0.04 (n=56) for the 3-10 m depth range and 0.08 ± 0.06 (n=56) for the 20-30 m depth range,

indicating lower BPD abundances in the former (*p-value* = 0.0002, permutation ANOVA test, n=4999 permutations). Moreover, significant differences in BPD abundances were observed between the different sites, the Nature Reserve site exhibited significantly lower BPD abundance compared to the City Center Beach and the Oil Jetty sites (*p-value* ≤ 0.05, pairwise permutation t-test, n=999 permutations) (Figure 2.2.b). Conversely, the data obtained from the phototransects did not show significant differences between sites. However, a significant difference was found in BPD abundances between the shallow reef (5-30 m) and the mesophotic reef (30-100 m), with mean BPD/m^2 was 0.11 ± 0.13 (n=22) for the shallow reef and 0.28 ± 0.25 (n=11) for the mesophotic reef, indicating lower BPD abundances in the former (*p-value* = 0.0108, permutation ANOVA test, n=4999 permutations).

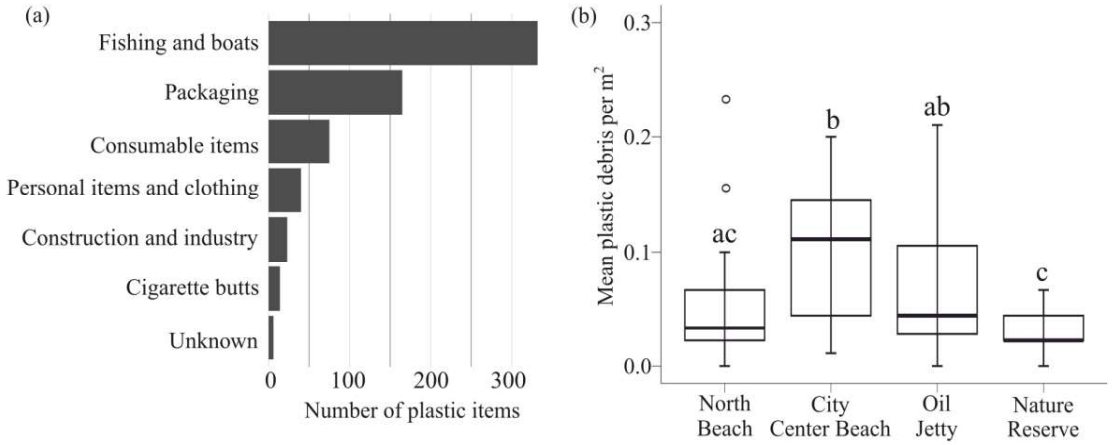


Figure 2.2: Bar plot (a) shows the number of plastic debris items in each category for all items recorded during the diving transects. Box plots (b) of the mean abundance of benthic plastic debris larger than 2.5 cm at each of the four sampling sites: Northern Beach (n=27), City Center Beach (n=27), Oil Jetty (n=31), and Nature Reserve (n=27). Line in box=median; box=25th-75th percentiles: bars= min and max values, excluding outliers. Different letters mark significantly different abundances (pairwise permutation t-test, *p-values* ≤ 0.05 for all pairs, n=999 permutations).

Analysis of the interactions between anthropogenic debris and benthic organisms found that of all the benthic plastic debris (BPD) observed, 48% had no interactions with organisms, 31% were colonized by organisms, 15% were entangled with organisms, and 5% were documented as covering benthic organisms (Figure 2.3). Various taxa were found to colonize BPD, while entanglement and covering interactions were exclusive to stony corals. Specifically, 74% of the interactions involving BPD and branching stony corals were entanglements, 13% involved BPD covering the corals, and 13% consisted of corals colonizing the BPD. For massive stony corals, 35% of the interactions involved items covering the corals, 33% were entanglements, and 33% represented massive corals colonizing the BPD. Soft corals predominantly exhibited growth on the plastic items, with 75% of occurrences being colonization, 20% entanglement, and 5% covering (Supplementary Figure S10).

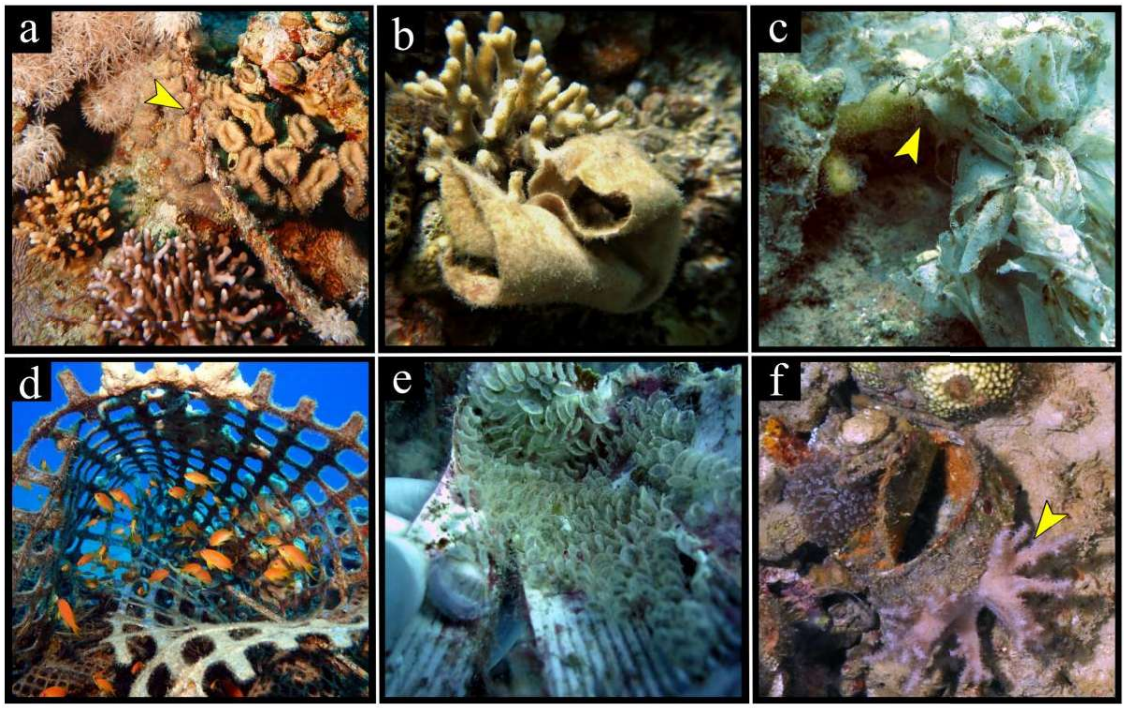


Figure 2.3: Examples of interactions of anthropogenic benthic debris with reef organisms. Polymer rope entangled within a stony coral (a), wet-wipe covering a stony coral (b), plastic bag covering a stony coral (c), a school of reef fish colonizing a ghost net (d), gastropod egg capsules laid in a single-use plastic cup (e), and mesophotic soft corals growing on a metal can (f).

Microplastics in the reef's surrounding seawater

A total of 1,316 plastic items (290 non-fibers and 1,026 fibers) were recorded as within the size range of 0.3-5 mm, with an abundance of 0.516 ± 0.317 microplastics/m^3 (Supplementary Figure S12). No significant difference was observed among the sampled seasons or between the two depth ranges. However, the Nature Reserve site exhibited significantly lower microplastic abundances compared to the three other sites (*p-value* = 0.012 for all pairwise comparisons, pairwise permutation t-test, n = 999 permutations) (Figure 2.4.a). The most abundant microplastic recorded was that of black fibers, which accounted for 78% of all recorded microplastics. Additional details on the color and shape

distributions of microplastics are provided in Figure 2.4.b, and images of microplastics collected from the reef surrounding seawater are provided in Figure 2.5.

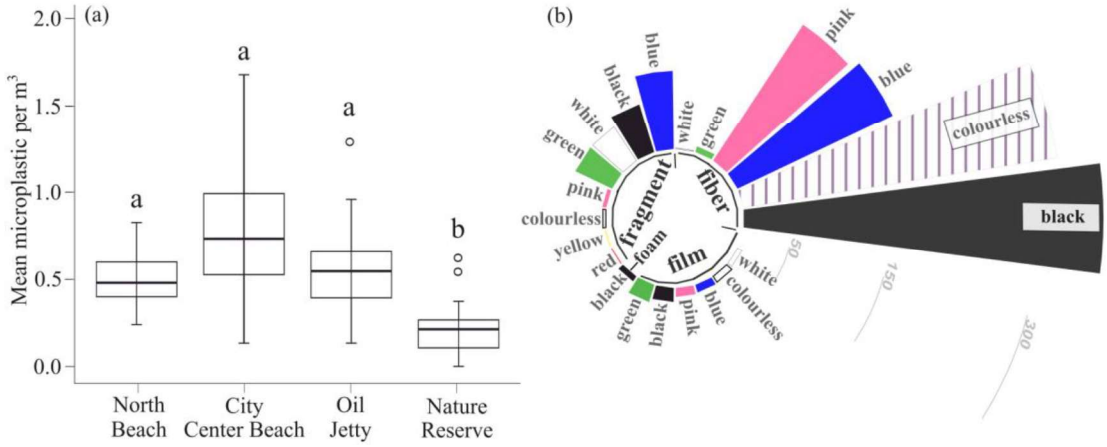


Figure 2.4: Box plots (a) of the mean abundance of microplastics smaller than 5 mm at each of the four sampling sites: Northern Beach (n=15), City Center Beach (n=18), Oil Jetty (n=17), and Nature Reserve (n=14). Line in box=median; box=25th-75th percentiles: bars=min and max values, excluding outliers. Different letters mark significantly different abundances (pairwise permutation t-test, *p-values* ≤ 0.05 for all pairs, n=999 permutations). Circular bar plot (b) illustrating microplastic color and shape distribution of recorded items.

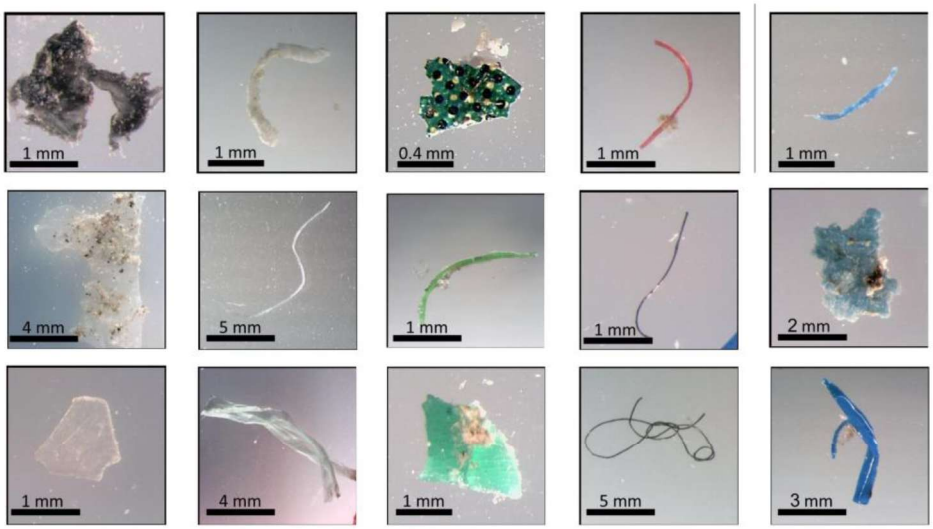


Figure 2.5: Photographs of microplastics collected from the reef water.

Phthalate esters in the reef's surrounding seawater and sediments

For all six analytes (Supplementary Figure S9), the limit of detection (LOD) was determined based on the minimum concentration of standards at which the peak signal-to-noise ratio exceeded 10. For the liquid-liquid extraction (LLE) method used for seawater samples, the LOD was set at 4.9 ng/mL. The LLE procedural blanks showed a mean level of 4.6 ± 4.5 ngDMP/mL (n=5). For the accelerated solvent extraction (ASE) method used for sediment samples, the LOD was set at 4.9 ng/g dry weight. The ASE™ procedural blanks resulted in levels of 2.1 ± 1.1 ngDMP/mL, and 0.5 ± 0.54 ngDEHP/mL (n=6). In similar studies, a three-fold LOQ/LOD ratio is commonly applied as a criterion (Avisar et al. 2019). Consequently, the limit of quantification (LOQ) for seawater and sediment samples was set at 14.7 ng/L and 14.7 ng/g, respectively. To account for the DMP levels that appeared in the LLE blanks, the LOD of DMP in seawater samples was set as 25 ngDMP/mL.

None of the targeted phthalate compounds were above the LOQ in the seawater samples (n=37) (Supplementary Table S4). Furthermore, DBP, DnOP, and DMP were not detected in any of the sediment samples (n = 51). DEP was detected in only one sample from the shallow site at the Oil Jetty, while DDP was detected in all three samples from the Eilat Marina sediments. DEHP was detected and quantified in 22 out of the 51 sediment samples, with concentrations ranging from 16 ng/g to 277 ng/g. (For further details and comprehensive results of the sediment phthalate analysis see Supplementary Table S3).

2.4 Discussion

The concentrations of both benthic plastic debris (BPD) and microplastics in seawater were significantly lower in the Nature Reserve sampling site (Figures 2.2 and 2.4). These may be attributed to the fact that this site is the furthest away compared to other sites (8 km and 1-3 km, respectively) from the cities of Eilat and Aqaba, as well as tourist facilities. The abundances of BPD observed at the study sites fall within the reported range found in other studies conducted in tropical coral reefs (Table 2.1). The relatively low abundance of BPD found at the North Beach site can be attributed to the absence of a complex seabed, as this site is predominantly composed of sand. I suggest that the lack of reef structures at this site prevents the entrapment of BPD, causing it to roll down the slope to deeper areas.

Our findings regarding the interactions of benthic debris with reef organisms align with those of previous studies that demonstrated the species-specific nature of plastic pollution (Maximenko et al. 2019; Reichert et al. 2019; Tang et al. 2021). Specifically, entanglement with debris is more commonly observed among branching corals (Lamb et al. 2018), while certain species of soft corals, ascidians, and sponges may benefit from the presence of debris as a substrate for settlement.

The phototransects revealed higher levels of pollution compared to the non-phototransects. This disparity could be attributed to several factors: the time gap between the two datasets, which are four years apart, the different methodologies used, and differences in sites and depth ranges that varied between the two datasets. Due to these significant differences, I cannot compare data from these two datasets directly.

In the phototransects, elevated pollution levels were found in the mesophotic reefs, which are located at dim-light ocean depths ranging from 30-150 m. These mesophotic reefs are believed to experience relatively less anthropogenic disturbance compared to shallower reefs (Weinstein et al. 2021), and to provide settlement grounds for larvae as the shallow reefs encounter increasing impact from climate change (Kramer et al. 2019). These findings present evidence that the mesophotic coral reefs in the Gulf of Aqaba are more impacted by benthic plastic debris compared to the shallower reefs. This finding highlights the previously overlooked vulnerability of these deeper reefs to plastic pollution. This is an important issue and of serious concern, particularly considering the endocrine-disrupting properties associated with plastic additives (Darbre, 2020), and their possible bioconcentration in soft coral tissues (Isa et al. 2022).

Fibers were the predominant microplastic type found in the seawater samples. Notably, the blank samples were free of fibers, providing strong evidence that the observed black fibers were the most abundant microplastics present in the coral-reef's surrounding waters.

The microplastic abundances detected in the reef's surrounding seawater align with the lower range of microplastic abundances observed in various coastal and marine ecosystems worldwide, which typically range from 0.001-140 microplastics/m^3 (Thushari and Senevirathna 2020). Martí et al. (2017) reported plastic abundances in the size range of 0.2-500 mm for surface waters of the central Red Sea collected by net tows. In their study they found the mean abundance reported was 0.37 ± 0.12 microplastics/m^3 and 55.1% of the plastic documented was of the size fraction of 0.5-1 mm. In comparison, the largest plastic retrieved in the net tows was 9 mm in size, however, there were less than 9 items larger than 5 mm from all the samples so this size fraction was disregarded. Martí et al.

(2017) concluded that the Red Sea exhibited the lowest microplastic abundances among all coastal seas reported at that time, with abundance levels two orders of magnitude lower than those recorded in the Mediterranean Sea. One suggested explanation for these low levels is the role of coral-reef structures as sinks for plastic particles via the mechanism of adhesion (Martin Corona et al. 2019). A study carried out by Saliu et al. (2018) around an unhabituated coral-reef island in the Maldives reported an abundance of 0.32 ± 0.15 microplastics/m^3 in surface water. Later the same research group investigated microplastics in coral colonies. The 25-150 μm size range was explored and abundances as high as 8.9 microplastics/g were found in the corals (Raguso et al. 2022). In the Maldives studies, fibers accounted for less than 10% of the detected microplastics for both the surface water and corals.

Surrounded by deserts, the regional climate of the GoA is extremely arid with an average annual rainfall of under 30 mm/year. However, the gulf receives several flash floods each winter (Katz et al. 2015) that may be the dominant source of plastic debris in the marine environment and drive benthic litter to the deep (Pierdomenico et al. 2019). Despite being classified as a coastal sea, due to its narrow width, the Red Sea exhibits certain characteristics of a deep sea, including an unstable seasonal thermocline and an annual deep mixing process (Lindell and Post, 1995). The Red Sea has an average width of 220 km and a maximum depth of about 3000 m, and the GoA has an average width of 16 km and reaches 1825 m depth (Rasul et al., 2019). These factors may contribute to the removal of particles from the upper water column, potentially explaining the relatively lower microplastic abundances observed in the Red Sea.

Historically, due to the hot and arid climate, the human populations along the Red Sea's coasts were sparse, however, with improving desalination and energy technologies and increased economic interest, coastal development and population are rapidly increasing. While stretches of the coast are still sparsely populated, larger towns and cities continue to grow along the Red Sea resulting in sewage, agricultural, and pesticide runoff that may be contributors of microplastics and chemicals into the marine environment (Kleinhaus et al. 2020).

The absence of phthalate esters in all the seawater samples may have been due to the small sample volume (300-800 ml), hindering the detection of very low concentrations, below the limit of detection and quantification. In the sediment samples, I did detect a few of the tested phthalates, but no clear pattern was found, suggesting that the presence of these pollutants in the sediment of the Gulf of Aqaba is not homogeneous. This variability could be attributed to microplastics in the sediment, which may have released the chemicals during the extraction process. Although I did not test for microplastics in the sediment samples due to limited facilities, it is important also to examine microplastics in the sediment to better understand the inconsistent concentrations of the analyzed phthalate esters at these sites. A previous assessment of sediment contamination in the Red Sea, including polycyclic aromatic hydrocarbons, metals, and microplastics, indicated that metal and polycyclic aromatic hydrocarbon levels were generally low compared to the international guidelines, while the higher levels of microplastics were associated with industrial or densely populated areas (Ruiz-Compean et al. 2017).

Throughout the study, I observed various interactions between the anthropogenic debris and reef organisms, including entanglement, colonization, and covering, with branching

corals being particularly vulnerable to entanglement. My findings provide valuable insights into the state of plastic pollution in the Gulf of Aqaba, revealing relatively lower abundances of benthic plastic debris (BPD) and microplastics compared to other coral-reef regions. Currently, the United Nations Environment Program (UNEP) is taking action through the development of a Global Plastic Treaty, which seeks to reduce the negative effects throughout the entire lifecycle of plastic. The Treaty is essential to help protect unique environments such as the coral reefs of the Red Sea, particularly since the majority of countries in the region are low- to middle-income countries that are experiencing rapid economic growth together with limited waste management infrastructure. This study has further emphasized the need for increased efforts to implement pollution prevention policies at the national level. I have shown the effectiveness of marine nature reserves that are located a distance from crowded urban centers which apply active operative management aimed at reducing the presence of plastic debris. Finally, I highlight the importance of implementing coral-reef preservation policies and local management strategies, which are crucial for protecting the reefs of the northern Red Sea from both global and local anthropogenic stressors.

Table 2.1: Large anthropogenic debris concentrations (Mean ± SD) from other studies performed in coral reefs and those that resulted in this study. *Range of mean plastic debris in 159 reefs in Australia, Indonesia, Myanmar, and Thailand.

Location (year)	**Sampling method (bottom depth)**	**N**	**Anthropogenic debris [item/m^2]**	**Plastic debris [%]**	**Reference**
Gulf of Aqaba, Northern Red Sea (2020-2021)	Scuba diving, belt transects (3-30 m)	112	0.093 ± 0.091	68	This study
Gulf of Aqaba, Northern Red Sea (2016-2017)	Photographs, belt transects (5-100 m)	31	0.2 ± 0.2	69	This study
Gulf of Aqaba, Northern Red Sea (2003-2004)	Cleaning campaigns (3-10 m)	3	2.8	32 – 96	(Abu-Hilal and Al-Najjar 2009)
Darvel Bay, Malaysia, Western Pacific Ocean (2019)	Video, belt transects (5-15 m)	17	0.107 ± 0.038	91	(Santodomingo et al. 2021)
Mayotte, South Western Indian Ocean (2018-2019)	Scuba diving, belt transects (3-4 m)	44	0.008 ± 0.003	92	(Mulochau et al. 2020)
Asia-Pacific region (2011-2014)	Belt transects (3-4 m)		0.004 - 0.256*	NA	(Lamb et al. 2018)

CHAPTER 3

Occurrence of plastic additives in coral-reef invertebrates on natural or plastic substrates

Author contributions:

GV and NS designed the experiments. GV led and performed the field sampling. Sample analysis was carried out by GV with the support of the technical staff at the Water Research Center. GV conducted the statistical analysis, drew the figures, and drafted the manuscript. NS supervised the study and interpreted the data together with GV.

3.1 Introduction

Plastic products incorporate plastic additives (PAs) throughout their manufacturing process, to improve or modify their properties. Plasticizers increase flexibility, stabilizers and antioxidants prevent plastic degradation from factors such as UV light and oxygen exposure, and flame retardants are employed to reduce the product's flammability (Wiesinger et al. 2021). However, despite their functional benefits, these additives are known to pose risks, as many of them are pervasive and persistent environmental pollutants (Hahladakis et al. 2018; Groh et al. 2019). This risk is amplified when waste management technologies and recycling processes fail to consider additives unintentionally released into the environment or incorporated into new products.

Although studies have explored the leaching of additives from plastic products under laboratory conditions, confirming the substantial release of hazardous additives (Bejgarn et al. 2015; Oliviero et al. 2019; Dimassi et al. 2023; Jiang et al. 2023), our understanding of the environmental fate and impact of additives released from plastic pollution in marine ecosystems remains limited (Bridson et al. 2021). Furthermore, measuring PAs in environmental samples is challenging due to their low concentrations (often in the parts per billion or trillion range), a diverse range of chemical compounds, complex matrices, and procedural contamination (Net et al. 2015b). The necessity for highly sensitive analytical instruments often limits the environmental investigation of PAs (Rochman, 2015; Hahladakis et al. 2018; Groh et al. 2019).

As the complex chemical composition of plastic materials varies considerably among polymers and products (Groh et al. 2019), researchers conduct controlled exposure experiments to test the effects of plastic leachate rather than specific compounds (Oliviero

et al. 2019; Capolupo et al. 2020; Dimassi et al. 2023; Jiang et al. 2023). For example, Oliviero *et al.* (2019) found that exposure to the leachates from inflatable toys on sea urchin larvae (*Paracentrotus lividus*) induced delayed development and morphological alterations in the larvae. Additionally, Capolupo *et al.* (2020) assessed the chemical content of leachates produced by different polymers and tested their impact on aquatic organisms: the microalgae *Raphidocelis subcapitata* (freshwater) and *Skeletonema costatum* (marine) and the Mediterranean mussel *Mytilus galloprovincialis*. They found that leachates from car tire rubber and Polyvinyl Chloride (PVC) were the most toxic to algal growth and mussel early-stage development.

The unpredictable nature of PAs makes it challenging to fully determine how these various compounds enter biological systems, specifically marine organisms. While it is debated whether microplastics play a major role in introducing additives into marine organisms (Amelia et al. 2021; Koelmans et al. 2022), the leaching of PAs from submerged plastic items such as buoys can increase the chemical levels in the surrounding marine environment (Pan et al. 2023). In addition, PAs can transfer into those marine organisms that grow directly on plastic items and then serve as a substrate, whether through absorption due to their proximity or contact with the plastic material itself. Jang *et al.* (2016) investigated the marine mussel*, Mytilus galloprovincialis*, inhabiting Styrofoam and found that flame retardants transferred from the Styrofoam substrate to the mussels at concentrations of up to 5160 ng/g lipid weight. Further investigation into the transfer of specific additives from plastic substrates to organisms is needed in order to more fully understand the role of plastic debris and submerged plastic structures as sources of PAs in organisms.

Coral reefs are critical ecosystems that contribute to the biodiversity of the ocean. Stony coral species function globally as ecosystem engineers in tropical and sub-tropical shallow oceanic beds (Wilkinson, 2004). Coral-reef organisms currently face multiple threats from climate change, ocean acidification, and other anthropogenic stressors, including plastic pollution (Hughes et al. 2017). Corals and other sessile invertebrates can be contaminated by PAs in dissolved forms through diffusion by contact or through ingesting microplastics (Gandara e Silva et al. 2016). Microplastics and PAs have been detected in coral species in the Maldives (Saliu et al. 2019; Montano et al. 2020; Raguso et al. 2022) and the Persian Gulf (Ranjbar Jafarabadi et al. 2021b), raising concern due to the potential bio-concentration of these additives in coral tissues (Isa et al. 2022). (Ranjbar Jafarabadi et al. 2021a) found a negative correlation between PA concentrations in coral tissues and the density of their endosymbiotic algae. This could have long-term detrimental effects on the health and survival of corals and the reef ecosystem as a whole. Moreover, some PAs, such as phthalate plasticizers and bisphenols, are known to interfere with the endocrine system (Darbre 2020), and their presence in coral-reef organisms can harm fertilization success and cause developmental distortion (Vered and Shenkar 2022).

Our primary objective was to assess the presence of various PAs in coral-reef invertebrates residing on natural substrates compared to those residing on plastic debris. Despite the extensive settlement of corals, sponges, and other benthic organisms on large plastic debris (de Carvalho-Souza et al. 2018; Vered and Shenkar 2023), the relationship between the presence of PAs in corals or in encrusting invertebrates that grow on plastic debris has not been investigated and compared with those growing on natural substrates.

3.2 Materials and Methods

Study workflow

Initially, a literature-based list of plastic additives (PAs) was compiled to select candidate compounds for investigation. Seven reference compounds commonly used as PAs were validated in this study. A comprehensive study workflow was developed and validated, including an Accelerated Solvent Extraction (ASE™) method for both organic and aqueous extractions, a full-scan analysis using Gas Chromatography-Mass Spectrometry (GCMS), a Single-Ion Monitoring (SIM) analysis for specific compounds using GCMS, and a secondary scan utilizing High-Performance Liquid Chromatography coupled to both UV and Mass Spectrometry (HPLC/UV/MS). This validated workflow was then applied to screen for PAs in coral-reef invertebrates collected from both natural substrates and plastic debris within and outside a marine nature reserve. The screening was conducted on samples from various sites, species, and substrates, and significant patterns were identified through statistical analysis.

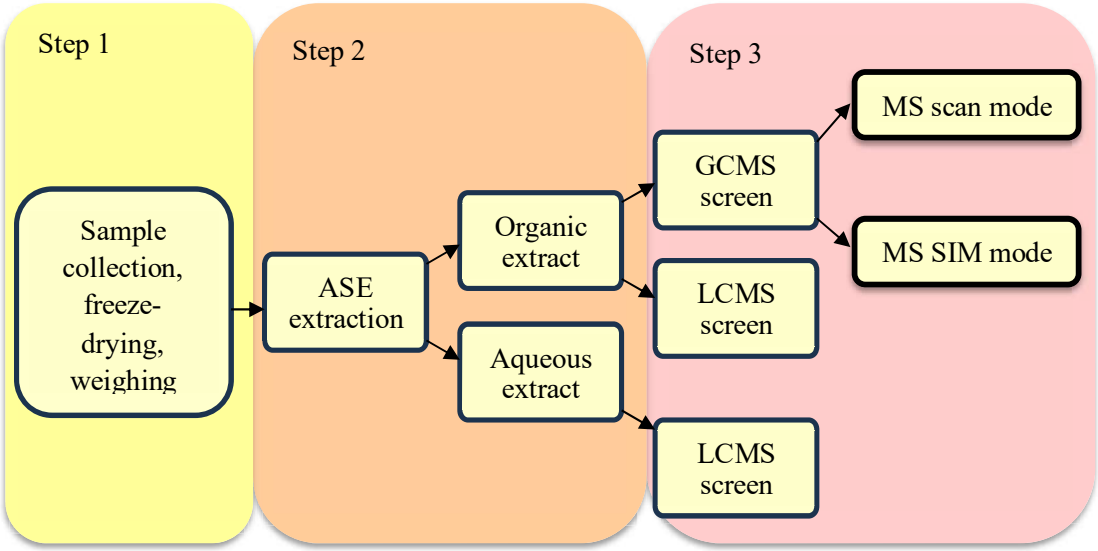


Figure 3.1: The study workflow comprised (1) sample preparation, (2) extractions, and (3) instrumental methods.

The study workflow is presented in Figure 3.1. All analytical methods detailed in this section were developed and performed at the Water Research Centre, School of Geosciences, Raymond and Beverly Sackler Faculty of Exact Sciences, at Tel Aviv University.

Targeted compounds

A literature-based list of plastic additives (PAs) was compiled, with a specific focus on those used in Polypropylene (PP) and Polyethylene (PE). All PAs on the list are known synthetic compounds with no natural origin. The list comprises: the CAS number, commercial name, formula, logP, molecular mass, common uses, and relevant references linking to PP or PE. The structural and identifying properties of the compounds were obtained from the 'PubChem Substance and Compound databases' (https://pubchem.ncbi.nlm.nih.gov/), and the logP was calculated using the ACD/ChemSketch software. The list is provided in Supplementary Table S5: 'List of plastic additives suspected to be found in items made of polypropylene and polyethylene'.

Based on the compiled PA list, the certified reference material, 'Extractables and Leachables Screening Standard for LC' (95636), was selected and procured from TraceCERT®, containing 21 compounds in acetonitrile, manufactured by Sigma-Aldrich Production GmbH, Switzerland. Among the 21 compounds in the reference material, 11 were additives with a natural origin. However, these are not reported in the present study since their presence in the organisms could not be solely attributed to pollution. Out of the 10 synthetic non-natural PAs, I was able to report on seven compounds that met acceptable method validation and quality assurance/quality control (QA/QC) standards. A list of the targeted compounds of interest, including their CAS numbers, molecular mass, retention

times, selected m/z values for GCMS analysis, observed masses for HPLC/UV/MS analysis, and limits of detection (LOD), is detailed in Table 3.1. Additional information on the compounds is available in the aforementioned PA list (Supplementary Table S5).

Table 3.1: Targeted compounds in GCMS SIM mode (a), and HPLC/UV/MS (b) their CAS, molecular weight, retention time, and selected observed masses for each instrument.

Commercial Name	CAS	MW	RT [min]	Observed masses			LOD [ng/g]
(a) GCMS				**m/z1 (main)**	**m/z2**	**m/z3**	
Dibenzylamine	103-49-1	197	8.98	91	106	196	100
Antioxidant 754	88-26-6	236	9.35	221	236	205	100
Drometrizole	2440-22-4	225	11.29	225	93	168	100
Bis(4-chlorophenyl) sulfone	80-07-9	286	12.15	158	286	111	100
Bis (2-ethylhexyl) phthalate	117-81-7	390	14.03	149	167	279	100
(b) HPLC/UV/MS							
Irganox 1010	6683-19-8	1178	10.26	M+18 = 1194.84			10
Irganox 3114	27676-62-6	784	9.81	M+18 = 801.57			10

Sample attributes: species, substrates, and sites

I focused on three prevalent coral-reef invertebrate species in the Gulf of Aqaba sampled from a variety of substrates and sites (1) *Stylophora pistillata*, a branching Scleractinia coral; (2) *Rhytisma fulvum*, an encrusting octocoral; and (3) *Crella cyathophora*, an encrusting sponge (Figure 3.3). During November 2022, organisms were collected by SCUBA divers from a natural substrate and a plastic substrate (High Density Polyethylene (HDPE) pipes and Polypropylene (PP) ropes), at depths ranging from 5 to 20 m at two sites: (1) a protected reef within The Coral Reef Beach Nature Reserve; and (2) an unprotected reef adjacent to the reserve, approximately 3 km away The unprotected site is in the vicinity of the port, the oil jetty, and tourist facilities (Figure 3.3).

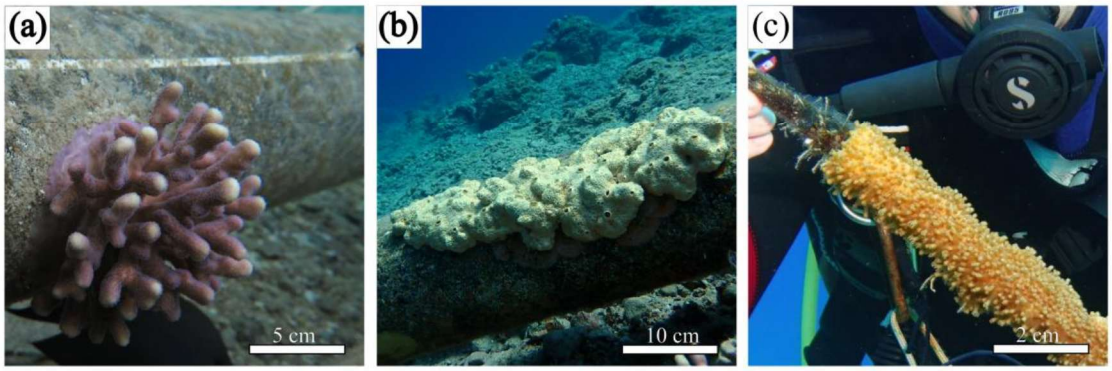


Figure 3.2: Photographs of the studied species on plastic substrates. *Stylophora pistillata* (a) and *Crella cyathophora* (b) growing on a HDPE pipe, and *Rhytisma fulvum* growing on a PP rope. Photographs by G. Vered (a,b) and R. Liberman (c).

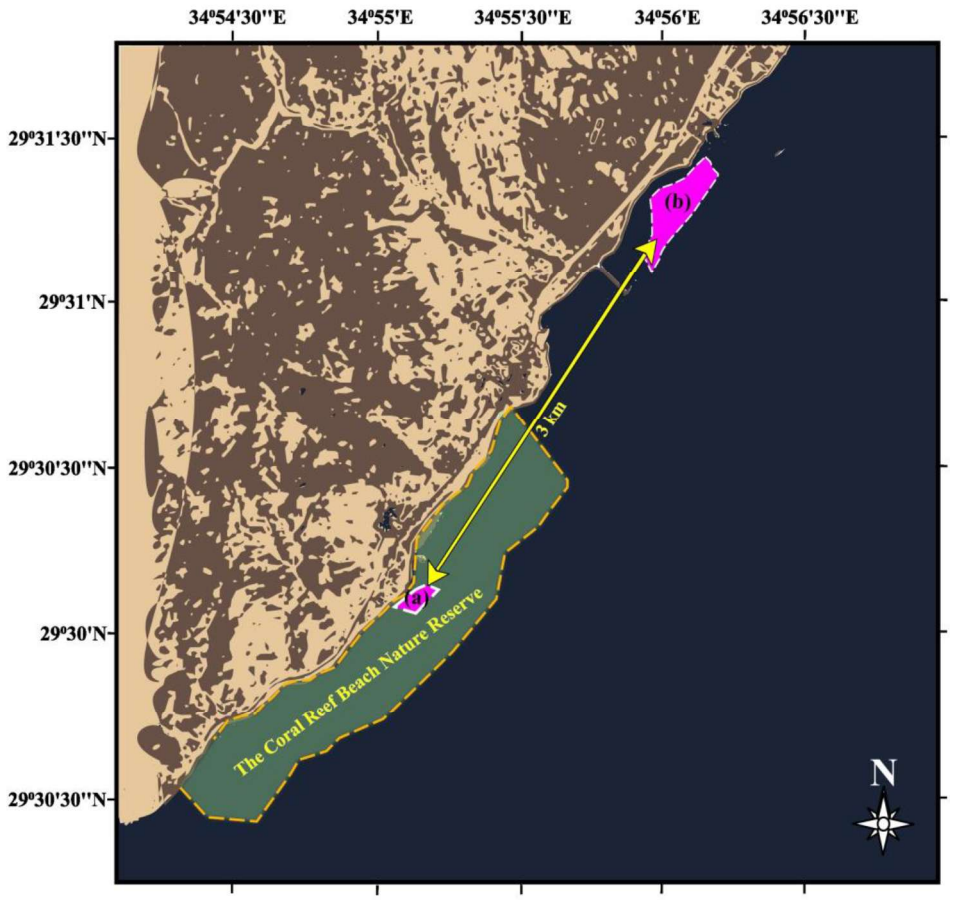


Figure 3.3: A map of the sampling sites: a protected reef within The Coral Reef Beach Nature Reserve (a), and an unprotected reef adjacent to the reserve (b).

Extraction methods

Initial sample preparation involved freeze-drying the samples, followed by extraction of 0.5-1.5 gr dry weight of each sample. Extraction was performed by an accelerated solvent extractor (Thermo ASE™ 350) utilizing acetonitrile and n-hexane as extraction solvents. Two extraction phases were obtained from each sample: (1) an organic solvent extract and (2) an aqueous extract. Detailed ASE™ extraction conditions are provided in Table 3.2.

Table 3.2: Table ASE™ extraction conditions

ASE™ conditions	**Extract 1 (vial A)**	**Extract 2 (vial B)**
Sample dry weight [gr]	0.5-1.5	0.5-1.5
Cell vol. [mL]	10	10
Filer type	Glass fibers	Glass fibers
Filler	Diatomaceous earth	Diatomaceous earth
Temp [C]	120º	120º
Cycles	2	2
Static time [min]	5	5
Solvent	Acetonitrile	n-hexane
Rinse volume	50%	50%
Purge time [sec]	90	60
Volume obtained [mL]	~20	~20

Following the ASE™ extractions, the samples underwent further processing as follows: the n-hexane extract (vial B) was evaporated down to 0.5 mL and the acetonitrile extract (vial A) was then added to vial B. Vial A was rinsed with an additional 0.5 mL acetonitrile, which was then added to vial B. The combined extracts were evaporated down to 1 mL, and 1 mL of methyl acetate was introduced into vial B, followed by vortexing and a 10-

minute settling period for precipitation. To collect the organic extract fraction, the supernatant from vial B was transferred to a clean vial and centrifuged at 3900 rpm for 15 min. For the aqueous extract fraction, the remaining precipitate in vial B was dissolved in 1 mL H2O pH 2.0 plus 1 mL acetonitrile and methyl acetate [1:1 v/v], vortexed, and left in the hood for 10 min without a cap to allow evaporation of the organic solvents. The resulting aqueous supernatant was then transferred to a clean vial and centrifuged at 3900 rpm for 15 min. For each sample, the organic solvent extract underwent analysis by both GCMS and LCMS, while the aqueous extract was analyzed by LCMS only.

Chromatographic method

The extracts from the samples underwent qualitative screening for targeted PAs using two analytical instruments: GCMS (Agilent 6890 GC coupled with an Agilent 5973 mass spectrometer); and HPLC/UV/MS (Agilent 1200 HPLC coupled with a Thermo Executive mass spectrometer). Three distinct instrumental methods were applied for the screening process: (1) GCMS in scan mode; (2) GCMS in single-ion monitoring (SIM) mode with selected m/z values for each compound of interest; and (3) HPLC/UV/MS in scan mode. Only the organic extracts were subjected to analysis using the two GCMS methods, while both the organic and aqueous extracts from samples were analyzed using HPLC/UV/MS.

GCMS scan mode analysis: The following parameters were employed in the GCMS scan mode method: 1µL of the sample was injected by pulsed splitless injection mode with a pulse time of 1.00 min. A Restek Rxi-1MS column (20 m, 180 µm film thickness, 0.18 mm internal diameter) was employed. Hydrogen was utilized as the carrier gas at a constant flow rate of 0.4 mL/min (linear velocity of 42 cm/sec). The column oven gradient was programmed as follows: initial temperature of 70°C (hold 1 min), ramp 15°C/min to 330°C

(hold 8 min). The total run time for the GC method was 26 min. MS acquisition began after a solvent delay of 4.2 min, with the mass range in scan mode defined as m/z = 41-800.

GCMS SIM mode analysis: All GC parameters utilized in the SIM mode method were the same as those utilized in the scan mode method (see previous section). MS acquisition in SIM mode also began after a solvent delay of 4.2 min. Starting from 4.2 min, the selected m/z values for the target compounds were grouped into 11 separate SIM segments according to compound retention time (Table 3.1). Depending on the number of masses included in each segment, a dwell value of 25 or 40 was used for each m/z. Cycles/sec for each SIM segment remained > 5. Additional information on the GCMS method conditions can be found in Supplementary Table S6.

HPLC/UV/MS scan mode analysis: A Kinetex EVO C18 column (50 x 2.1 mm, 2.6 µm,100 Å) with a C-18 precolumn was utilized. The mobile phases were acetonitrile: H2O 1:10 v/v (+ 0.1% formic acid) (solvent A), and isopropanol (+ 0.1% formic acid) (solvent B). The solvent gradient was programmed as follows: starting condition of 100% A (hold 1 min), gradient from 100% A to 100% B (7 min), hold 100% B (5 min). The total run time of the HPLC method was 21 min (including gradient program, return to starting conditions, and method post-time). The mobile phase flow rate was kept constant at 0.35 mL/min. The sample injection volume was 10 µL, and the column temperature was set at 50°C. UV detector wavelengths were set at λ= 220 nm, 254 nm, and 280 nm (bandwidth: 4). A post-column makeup flow containing ammonium format was used to help improve MS sensitivity. Additional information on the HPLC/UV/MS method conditions can be found in Supplementary Table S7.

Methods validation

Calibration curves spanning from 0.005 to 10 µg/mL were prepared for each instrumental method utilizing the certified reference material. The linearity of each calibration curve was verified through the calculation of linear regression and correlation coefficients for every compound. The limit of detection (LOD) for each compound was established as the lowest point on the calibration curve producing a signal-to-noise ratio greater than 3, while the limit of quantification (LOQ) was identified as the lowest point with a signal-to-noise ratio exceeding 9. The LODs for each compound and method are provided in Table 3.1. Percentage recovery from sample preparation was assessed by spiking a mixed sample of the three organisms collected with a known quantity of the reference material. The complete Accelerated Solvent Extraction (ASE^{TM}) procedure was then performed, and the extracts were analyzed using the three instrumental methods. The applicable calibration curve for each instrumental method was utilized for the calculation of recovery for the spiked reference material. Acceptable recovery was defined as within the range of 70% to 120% based on this assessment procedure.

Quality assurance and quality control

Numerous quality assurance and control (QA/QC) measures were incorporated throughout the experimental process. For example, analytical-grade solvents were used for all lab work, and six blanks, consisting solely of diatomaceous earth filler, were subjected to the entire extraction process alongside the actual samples. Extracts from the blanks underwent analysis through the three instrumental methods to detect any contamination peaks originating from the extraction procedure or solvents. Glassware was rigorously cleaned before each use, involving a thorough rinsing with n-hexane and 8 hours in a drying

oven at 200°C. The ASE™ instrument and cells underwent a pre-wash before each sample extraction, involving the loading of extraction cells with filler (no sample) and processing through the ASE™ extraction methods. Blank extracts were collected for every sample extraction series. Minimal use of nitrile gloves and avoidance of plastic labware were maintained throughout the study. During instrumental analysis, a blank comprising acetonitrile and methyl acetate (1:1 v/v) was injected after every five samples to monitor potential peak carry-over between samples.

The decision to employ three instrumental methods for sample analysis served as an additional layer of quality assurance and control in this study. Firstly, some compounds in the reference material, particularly those with higher molecular weights, were incompatible with the GCMS methods used. Therefore, LCMS was employed for a target screen of these compounds in the samples. Secondly, many compounds compatible with GCMS also worked well with LCMS. For these compounds, LCMS analysis acted to validate the results obtained through GCMS scan and SIM mode analyses. The use of two GCMS methods (MS scan and SIM modes) added another layer of QA/QC. Given the substantial biological "noise" in the samples, identifying the compounds of interest was challenging. Thus, GCMS SIM mode was employed for a target screen, enhancing the sensitivity of GCMS analysis and resulting in lower limits of detection (LOD) and limits of quantification (LOQ) for all target compounds.

Statistical analysis

Statistical analyses were performed using R version 4.3.1 (R Core Team 2021). The frequency of occurrence of compounds in samples was documented as a binary code (1 for

detected compound, 0 for undetected compound). Data are presented as mean ± standard deviation (SD) and all tests were set with a significance level of $\alpha=0.05$.

A nonparametric analysis of variance based on the Aligned Rank Transform using the 'ARTool' package (Elkin et al. 2021) was used to assess the impact of the independent variables: sampling site (protected reef site/unprotected adjacent reef site); species (*R. fulvum*/*C. cyathophora*); and substrate (natural/plastic) on the number of pollutants found in a sample (0, 1, 2, 3). Additionally, the generalized linear model (GLM) examined the effects of the variables: species (*R. fulvum*/*C. cyathophora*/*S. pistillata*) and substrate (natural/plastic) on the number of pollutants found in a sample (0, 1, 2, 3) from within the protected reef site. Where significant impact was observed ($p < 0.05$), post-hoc pairwise comparisons were conducted with the ART-C procedure (Elkin et al. 2021) and corrected with Holm's sequential Bonferroni procedure.

A GLM with a binomial link function (logistic regression) was employed to assess the impact of the independent variables: sampling site (protected reef site/unprotected adjacent reef site); species (*R. fulvum*/*C. cyathophora*); and substrate (natural/plastic) on the variations in the frequency of occurrence of compounds in samples of *R. fulvum* and *C. cyathophora*. Additionally, the GLM examined the effects of the variables: species (*R. fulvum*/*C. cyathophora*/*S. pistillata*) and substrate (natural/plastic) on the differences in the frequency of compound occurrence in organisms sampled within the protected reef site. Assumptions of parametric tests were verified for all data and, in cases of violation, permutation tests were conducted using the 'predictmeans' package (Luo et al. 2022). If the GLM model indicated statistical significance ($p < 0.05$), a pairwise comparison with Tukey's Honestly Significant Difference (HSD) was conducted to identify the specific

levels of the variables responsible for the observed significant differences using the 'emmeans' package (Lenth 2023). Statistical significance was determined at a *p-value* $\leq$ 0.05.

3.3 Results

Detection of plastic additives in coral-reef invertebrates

Plastic additives (PAs) were identified in 52% of the examined organisms (n=63), revealing that a significant portion of the sampled coral-reef invertebrates had been exposed to these chemicals. This widespread presence of plastic additives (PAs) was observed across all investigated species. Among the seven targeted PAs, two specific compounds were observed in the collected samples. Dibenzylamine was detected in 21 samples, and bis (2-ethylhexyl) phthalate (DEHP) in 20 samples.

Number of compounds in samples and the statistical significance across the independent variables: species, site, and substrate

To determine whether the number of compounds in samples was impacted by the various factors, each sample was graded 0, 1, or 2 according to the number of PAs detected. A nonparametric analysis of variance using the Aligned Rank Transform (ART) revealed a statistically significant effect of the site factor on the number of PAs detected in the samples only for *Rhytisma fulvum* ($F(1, 44) = 18.98$, $p = 0.00008$). Further examination of the factors and their interactions did not yield statistical significance ($n=46$, $p \leq 0.05$).

The quantity of PAs in *R. fulvum* samples from the protected reef site was significantly lower than in those from the adjacent unprotected reef, with mean ± SD of 0.375 ± 0.8 and 1.76 ± 0.9, respectively (pairwise comparison corrected with Holm–Bonferroni, $t(44) = -3.49$, $p = 0.03$). When exclusively comparing between samples from the protected reef site, no statistically significant effects on the number of PAs were observed among species (*R. fulvum*, *Crella cyathophora*, or *Stylophora pistillata*), substrates (natural or plastic), or

their interaction (n = 36, $p \leq 0.05$). Detailed post-hoc test results and significant outcomes are provided in Figure 3.4 and Supplementary Table S8.

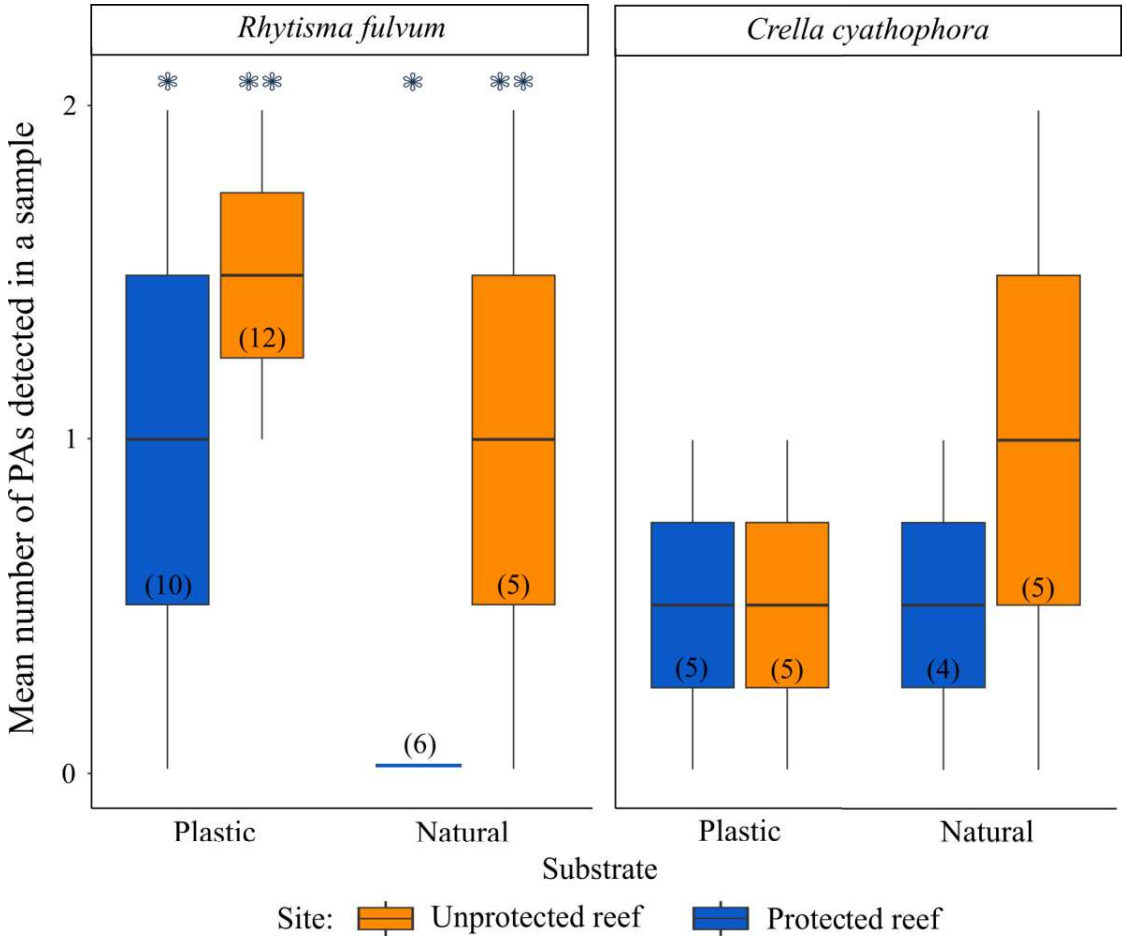


Figure 3.4: Box plot of the mean number of plastic additives detected in samples of *R. fulvum* (a) and *C. cyathophora* (b) from the different sites, including natural reefs and plastic substrates (pipes and ropes). The number of samples is indicated in parentheses. The line in the box represents the median; the box represents the 25th to 75th percentiles; bars indicate minimum and maximum values. An asterisk denotes significant differences between sample types (pairwise comparisons conducted with the ART-C procedure, and corrected with Holm's sequential Bonferroni procedure, $p \leq 0.05$).

Influence of the independent variables: species, site, and substrate, on plastic additive occurrence frequencies across samples

A statistically significant finding from all tests was that organisms collected from the protected reef within The Coral Beach Nature Reserve were less polluted than those from the adjacent unprotected reef.

A Generalized Linear Model (GLM) with a binomial distribution revealed a statistically significant effect of the sampling site on the frequency of occurrence of PAs in the samples ($\chi^2(1, n=51) = 14.11$, $p < 0.001$), and specifically on the frequency of DEHP occurrence in the samples ($\chi^2(1, n=51) = 23.57$, $p < 0.0001$). Post-hoc pairwise comparisons, conducted using Tukey's HSD test, indicated that the protected reef site exhibited a lower percentage of samples containing PAs (n = 25) compared to samples from the adjacent unprotected reef site (n = 27). No other pairwise comparisons presented statistical significance. The percentage of samples detected with DEHP or dibenzylamine is presented in Figure 3.5, and the percentage of samples detected with at least one compound is reported in Figure 3.6. Detailed results of pairwise comparisons are presented in Supplementary Table S9 and Table S10.

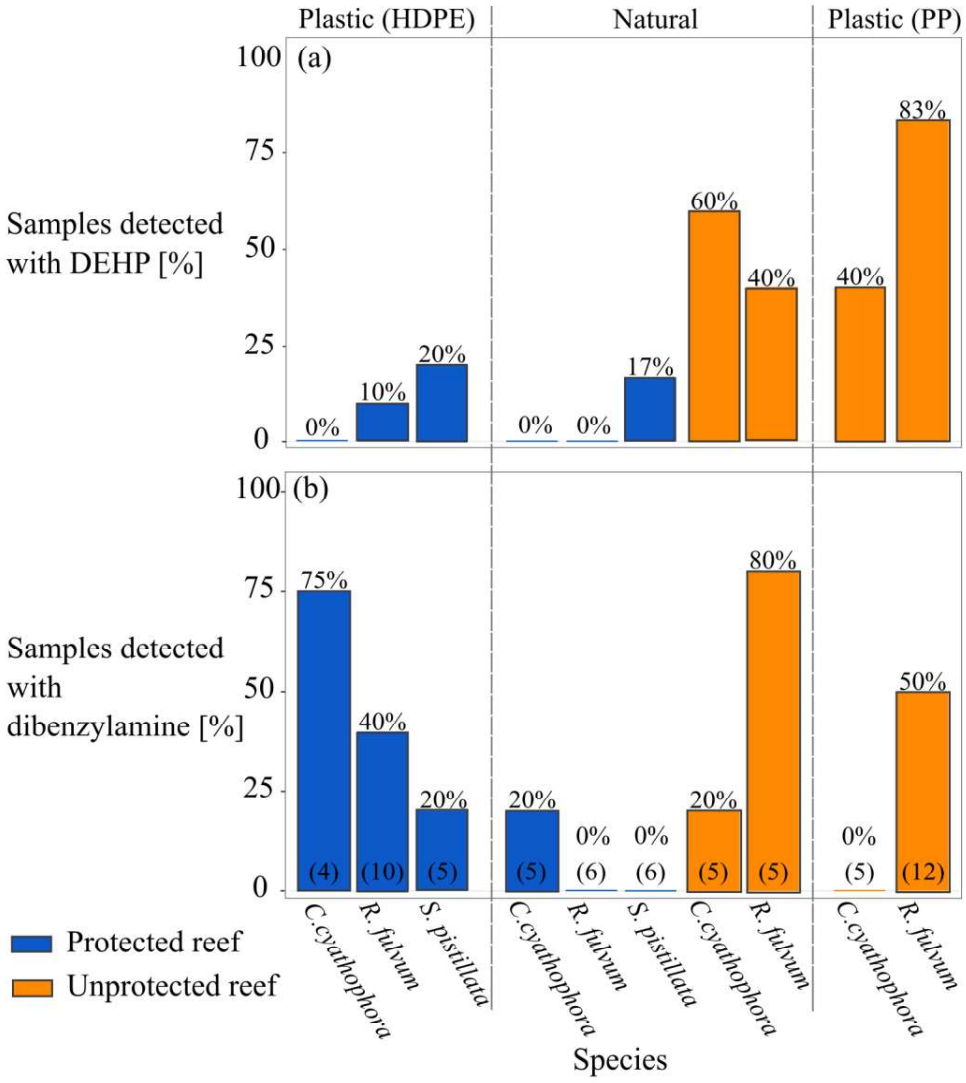


Figure 3.5: Bar plot of the percentage of samples detected with DEHP (a) or dibenzylamine (b). The samples are of various species, from different sites and both natural reefs and plastic substrates (pipes and ropes). Number of samples is given in parentheses.

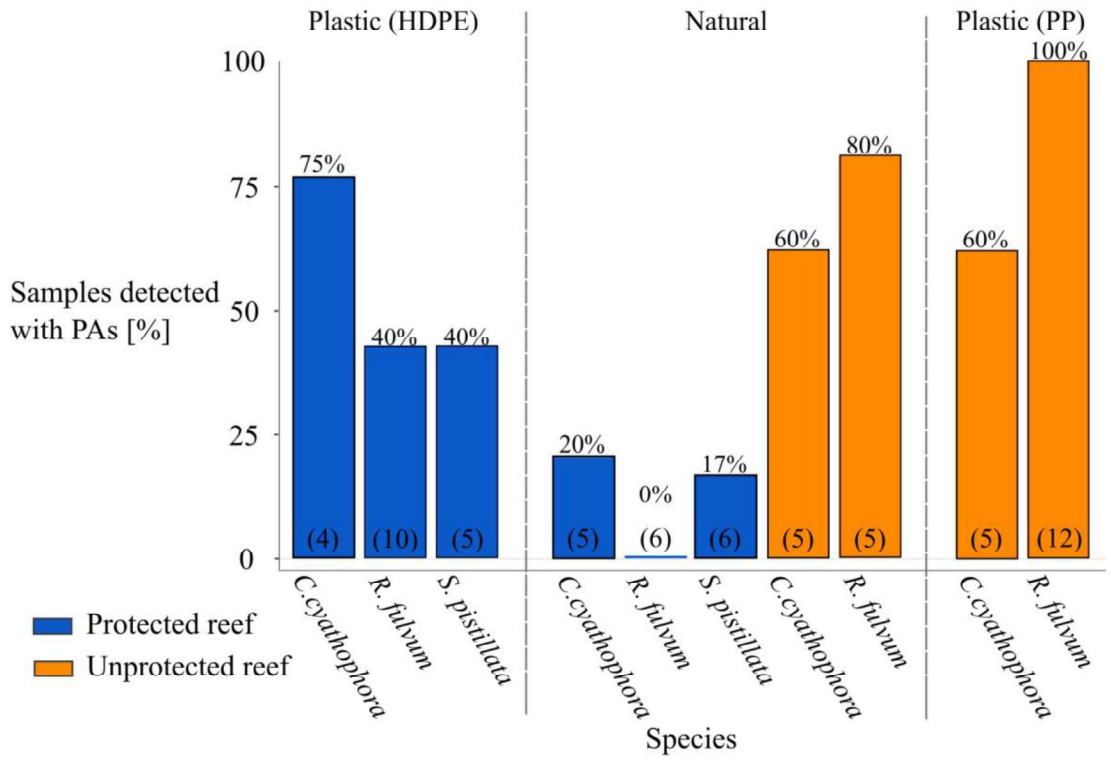


Figure 3.6: Bar plot of the percentage of samples detected with at least one compound. The samples are of various species, from different sites and both natural reefs and plastic substrates (pipes and ropes). Number of samples is given in parentheses.

3.4 Discussion

Plastic pollution has become a global problem, as evidenced also by the discovery of microplastics even on the pristine snows of Mount Everest (Napper et al. 2020) as well as the identification of plastic items in the deepest abysses of our oceans (Mohrig 2020). The current study's findings confirm the widespread presence of plastic-related contaminants. Even in the coral reefs of Eilat, which are considered relatively less polluted by plastic (Vered and Shenkar 2023), I detected the presence of man-made synthetic chemicals in organisms of all three of the studied species, inhabiting both natural and plastic substrates, from both the protected site within the nature reserve and the adjacent unprotected reef. This underscores the pervasive occurrence of plastic additives (PAs) in the coral-reef ecosystem, raising concerns about the potential impact on these marine organisms.

Out of the seven chemicals investigated, two were detected: dibenzylamine and bis (2-ethylhexyl) phthalate (DEHP). Dibenzylamine is a synthetic aromatic amine utilized primarily as an intermediate in the synthesis of various chemicals and as a vulcanization activator in the rubber and tire industries (Jiang et al. 2023). A vulcanization activator increases the speed and heat stability of vulcanization, a chemical process used to make rubber stronger, more durable, and more elastic. Dibenzylamine is also mentioned as a potential additive in packaging (Groh et al. 2019), and Müller *et al.* (2022) identified it as a leachable associated with tires. Since it is used in various industries, the introduction of dibenzylamine into marine environments might be linked to industrial discharge or wastewater, rather than to plastic debris. However, Eilat lacks a chemical industry and effluent discharge, while the Port of Eilat receives most of its revenue from importing cars

via cargo ships. Consequently, a more plausible source of dibenzylamine could be that of tire wear particles, which are assumed to be one of the major sources of microplastic pollution in the environment (Hartmann et al. 2019).

Alarmingly, according to the European Chemicals Agency (ECHA 2024), dibenzylamine is highly toxic to aquatic life and can have long-lasting effects. Predicted toxicity endpoints for three trophic levels of aquatic organisms have shown that it has medium toxicity for *Danio rerio* (LC50 of 5.78 mg/L) and high toxicity for *Daphnia magna* (LC50 of 0.769 mg/L) and the green alga, *Scenedesmus obliquus*, (LC50 of 0.494 mg/L) (Jiang et al. 2023). However, no studies to date have investigated its potential risk to coral-reef invertebrates.

On the other hand, DEHP has been reported as present in numerous marine ecosystems (Hermabessiere et al. 2017), as well as in coral-reef organisms from various locations (Ranjbar Jafarabadi et al. 2021b; Isa et al. 2022; Saliu et al. 2019), including solitary ascidians (Chordata, Ascidiacea) from Eilat's coral reef (Vered et al. 2019). DEHP is a phthalate ester and a widely used plasticizer prevalent in plastic items and various polymers. It is widely manufactured and used throughout the world, with the majority applied to the production of Polyvinyl Chloride (PVC) plastics. Its primary purpose is to impart flexibility and durability to various products, including medical devices, toys, and building materials (ECHA 2024). Generally, phthalates are not covalently bonded to the polymer matrix but are attached to it by weaker secondary bonds, and may easily leach into the environment during manufacturing, use, and disposal (Net et al. 2015a). In 2015, global plasticizer use reached 8.4 million tons, with DEHP being the predominant plasticizer used, constituting 37.1% of the market (Hermabessiere et al. 2017).

DEHP is classified as an endocrine-disrupting chemical and as a substance of very high concern, and is included in the list of materials requiring authorization by the EU before use (ECHA 2024). Prenatal exposure to environmentally relevant levels of DEHP is associated with disruptions to testicular cell development in rats (Abdel-Maksoud et al. 2019). Furthermore, this PA can potentially damage the reproduction success and development processes of aquatic life (US-EPA 2012). One example is the exposure of female ricefish, *Oryzias melastigma,* to DEHP, which accelerated the onset of spawning and led to reduced egg production (Ye et al. 2014).

All of the above emphasize the importance of increasing our knowledge regarding the occurrence of these pollutants in coral reefs. Corals can absorb phthalates from their surroundings through seawater diffusion, direct contact with plastic items, adhesion and ingestion of microplastics, and ingestion of zooplankton (Montano et al. 2020; Isa et al. 2022). Ranjbar Jafarabadi et al. (2021a) observed a potential link between phthalate concentrations and the density of zooxanthellae in corals. In a bioconcentration study exploring phthalates among four soft-coral species, significantly different distributions of the compounds were found, suggesting a species-specific DEHP metabolic transformation among the surveyed soft-coral species (Isa et al. 2022).

In the current study, there were no discernible variations in the occurrence rates of dibenzylamine and DEHP across the different substrates. I suggest that these chemicals primarily reach the organisms through the water column, potentially via microplastics or trophic transfer. This is supported by the statistically significant differences found in the frequency of occurrence of these pollutants between the distinct sites. The variation in pollutant levels can be attributed to several factors, including the direction of water

currents, the proximity to potential pollution sources (e.g., ports), and local factors such as the structure of the reefs and human activities. Additionally, varying rates of PAs or debris degradation, as well as complex interactions within the reef ecosystem, may also play significant roles in the distribution and concentration of these pollutants. The sampled Polypropylene (PP) ropes and High Density Polyethylene (HDPE) pipes have been present in the marine environment for a period spanning several years and might have undergone substantial additive leaching (Dimassi et al. 2023). Plastic additives are indeed known to leach out over time (Hahladakis et al. 2018; Dimassi et al. 2023), suggesting that these plastics may have released a significant portion of their additives during their years of submersion. However, the question of whether the levels of PAs detected in corals are associated with the ingestion of plastic particles, microplastics trapped by coral mucus, or other mechanisms, remains unanswered.

PAs were detected in the three of the study species, with no significant difference found in pollution levels. While the results did not show significant variations among species, this finding warrants further investigation to understand the underlying factors contributing to PAs concentrations in biota. Such factors could include feeding behaviors or lipid content in the tissues. Expanding the analysis to include a broader range of species and environmental variables might reveal more patterns of PAs distribution. Reef-building corals serve as the architects of coral-reef ecosystems, providing habitat, supporting biodiversity, and contributing to marine health. *Stylophora pistillata*, a branching coral abundant in the Red Sea, is a model species in coral research (Shefy and Rinkevich 2021), and in recent years also in stress studies (Lanctôt et al. 2020). It features small polyps and a high surface/volume ratio. Because plastic debris has an eight-fold higher chance of

affecting corals with higher structural complexity (Lamb et al. 2018), *S. pistillata* is highly susceptible to plastic debris. Additionally, in a previous study I demonstrated that dimethyl phthalate can negatively affect the settlement success and survival of *S. pistillata* planulae (Vered and Shenkar 2022). Thus, the presence of PAs in *S. pistillata* adds to the growing evidence of the various stressors that are currently endangering coral-reef ecosystems, and may influence community structure.

Rhytisma fulvum samples within the nature reserve exhibited a significantly lower number of pollutants per sample compared to those from outside the reserve. Intriguingly, *R. fulvum* samples collected from natural substrates within the reserve did not show the presence of any compounds, a distinctive pattern not observed in the other sample types. Further emphasizing the reserve's protective effect, this trend was consistent for both detected PAs. In addition to earlier findings highlighting the vulnerability of *R. fulvum* larval development and settlement to 4-nonylphenol, a common antioxidant and stabilizer used in rubber (Vered and Shenkar 2022), the present study has revealed significant variations in pollution levels among the *R. fulvum* samples collected from two distinct sites. Interestingly, this pattern was not detected in the sponge *Crella cyathophora*. The distinctive findings of *R. fulvum's* accumulation of PAs observed at the different sites suggest that it may be a promising candidate for monitoring the presence of these chemicals in coral-reef environments. This encrusting soft coral is highly abundant between 3-40 m depth and has a wide distribution in the Red Sea and the Indo-Pacific (Benayahu and Loya 1983).

In conclusion, the findings from this study add to the growing evidence of the presence of PAs in various environments and in the wildlife that inhabit them. The identification of

dibenzylamine and DEHP in coral-reef organisms is noteworthy, given these chemicals' known toxicities to aquatic life and their potential impacts on coral fecundity.

The significant differences in pollutant frequency between different sites suggest that marine reserves play a protective role in reducing the introduction of chemical and plastic pollution to benthic coral-reef invertebrates, thus preserving coral-reef ecosystems. Furthermore, this study did not find significant differences in the occurrence rates of plastic additives in coral-reef invertebrates residing on natural versus plastic substrates. It is still uncertain whether the detected levels of PAs in organisms are a result of their diet, transfer through microplastics or other mechanisms.

GENERAL DISCUSSION

Coral reefs are of ecological importance, host valuable biodiversity, and provide irreplaceable services to humankind, such as coastal protection, natural materials and, mainly in developing countries, food and livelihood (Spalding et al. 2001). Despite their ecological significance, these systems face existential danger due to human activity, including various pollutants, overfishing, and climate change (Hoegh-Guldberg et al. 2017; Hughes et al. 2017). Although coral reefs have been studied in numerous research fields, it is only recently that attention has been given to the phenomenon of plastic pollution in these reefs. In 2019, the UN Environment Programme (UNEP) published a report emphasizing the need to address knowledge gaps and conduct research on the global impact and scope of plastic pollution on coral reefs. The report clearly states 11 knowledge gaps concerning the impact of plastic pollution on coral-reef environments. These include understanding the scale and patterns of mismanaged waste, the impacts of leached chemicals from plastics, and how plastics affect benthic invertebrates and coral-reef communities. Other gaps include assessing the risk that microplastics pose to reef organisms, and the need to improve microplastic detection methods and validate models for assessing plastic pollution accurately (UNEP 2019).

The overarching goal of my research was to broaden the understanding of plastic pollution within coral-reef ecosystems and address some of the gaps stated in the 2019 UNEP report. To achieve my aim I employed diverse methodological approaches, encompassing controlled experiments, comprehensive field surveys, and analytical analyses. My findings have demonstrated that plastic and plastic additives have a selective,

species-specific effect on coral-reef fauna, and revealed the widespread presence of plastic additives, even in corals and sponges from less polluted sites. This work also contributes crucial data to assist local regulators, enabling them to formulate science-based policies focused on the effective management and reduction of plastic pollution in coral reefs.

In Chapter 1, I focus on the current research gaps regarding the potential impacts of prevalent plastic additives on the reproductive products of tropical coral-reef invertebrates. My examination revealed a limited effect of the tested phthalate esters and bisphenol A when applying environmentally-relevant concentrations in seawater. The 4-nonylphenol was the only additive to demonstrate an effect at such environmentally-relevant concentrations, specifically impairing the settlement success of larvae of the soft coral *Rhytisma fulvum*.

Although only limited effects were found as a result of the exposure experiments at seawater environmental concentrations of dibutyl phthalate (DBP), dimethyl phthalate (DMP), and bisphenol A (BPA), the insights derived (as provided in Chapters 2 and 3) reveal that additives were indeed identified in both animals and sediments, albeit not in the seawater samples. Given that plastic additives are predominantly hydrophobic, with a higher affinity to biological fluids, and tend to accumulate in living organisms (Hahladakis et al. 2018; US-EPA 2022), I suggest that exposure experiments applying environmental concentrations in seawater may not accurately represent the existing risk, particularly for brooding corals safeguarding their larvae during embryogenesis.

Moreover, at higher concentrations of the other additives, negative and selective species-specific effects were observed on the development and reproduction success of the

tested coral-reef organisms. These findings align with other research findings, indicating that plastic pollution exerts selective and species-specific effects on various organisms within the reef. Reichert et al. (2019) for example, reported that microplastic exposure caused species-specific effects on coral growth and photosynthetic performance in tropical reef-building corals. Moreover, the measured metabolism of phthalate esters in soft corals was found to be related to the lipid content of the corals, to be species-specific, and not related to colony size (Isa et al. 2022). In addition to the exposure experiments carried out and presented in Chapter 1, the interactions between large plastic debris and reef fauna, as presented in Chapter 2, further support the species-specific nature of plastic impact on coral reefs. While branching stony corals are considered to be the most negatively affected by large debris, as found in the present study and by Lamb et al. (2018), who determined that plastic debris is eight times more likely to affect reef corals with greater structural complexity such as branching stony corals, soft corals have been recorded as mainly colonizing the plastic debris. Furthermore, Ranjbar Jafarabadi et al. (2021a) found a potential link between plasticizer concentrations and the density of zooxanthellae in stony and soft corals, and reported that soft corals were less stressed by the plasticizers compared to the stony corals. To conclude, my findings agree with the growing knowledge of plastic pollution possibly driving shifts in coral-reef communities.

The abundant soft coral *R. fulvum* was the only species negatively affected by 4-nonylphenol at its seawater environmentally-relevant concentration out of the four organisms I exposed to plastic additives (see Chapter 1). Furthermore, in Chapter 3 I note that the *R. fulvum* samples from within the marine reserve demonstrated significantly reduced pollution levels compared to their counterparts in the unprotected adjacent reef. I

consider this species as a prominent potential candidate for monitoring the presence and impact of plastic additives in coral-reef environments. The presence of various unknown compounds in consumer plastics poses a challenge when seeking to assess the source of pollutants detected in environmental samples. For example, a screening of recycled High-Density Polyethylene (HDPE) pellets from diverse regions resulted in the identification of 491 chemical compounds (Carmona et al. 2023). Thus, it is crucial to monitor and collect extensive data on plastic additives in key coral-reef species in order to achieve a comprehensive understanding of the distribution and impact of plastic pollution on coral-reef ecosystems.

The Gulf of Aqaba in the northern Red Sea is considered a potential coral-reef refuge from climate change (Fine et al. 2013). However, it is becoming subjected to increasing levels of plastic contamination (Diem et al. 2023). In Chapter 2, I present a crucial database detailing the state of plastic pollution in the coral reefs along the Israeli coast of the Gulf of Aqaba, and which may serve as a valuable scientific basis for designing regional policies aimed at reducing plastic pollution in the Gulf. My research findings of ropes and fishing line debris in the reefs being the most abundant items, and of the distribution patterns of such debris increasing with depth, contradict studies that were performed on other nearshore marine environments, where plastic debris densities decrease with depth and are dominated by packaging and consumable items (Morales-Caselles et al. 2021). However, these contradictions align with a comprehensive recent review on plastic pollution in coral-reef environments by Pinheiro et al. (2023). The elevated plastic pollution levels in the deeper reefs can be explained by the steep slope that is a feature of the Gulf of Aqaba, with a maximum width of 24 km and depth of 1,850 m. The mesophotic reefs, which are located

deeper, at dim-light ocean depths ranging from 30 to 150 m, are believed to experience relatively less anthropogenic disturbance compared to shallower reefs (Weinstein et al. 2021). My findings, however, present evidence that the mesophotic coral reefs in the Gulf of Aqaba are more impacted by benthic anthropogenic debris compared to the shallower reefs. This finding highlights the previously overlooked vulnerability of these deeper reefs to plastic pollution.

This two-year survey resulted in the observation of relatively low concentrations of large anthropogenic debris on the Israeli reefs in the Gulf of Aqaba compared to various studied tropical coral reefs in other parts of the world (Lamb et al. 2018; Santodomingo et al. 2021). Similarly, the microplastic concentration I found falls within the range found in the few other studies conducted in the Red Sea (Martí et al. 2017; Martin Agustí et al. 2019) and aligns with the lower range of microplastic abundances observed in other coastal and marine ecosystems worldwide (Martí et al. 2017; Thushari and Senevirathna 2020). It is crucial to note that these lower range observations are likely influenced by the population density in the region (Fine et al. 2019) rather than by the level of waste management practices. Municipal solid waste management in Israel is still most commonly (~80%) disposed of at landfill sites (Daskal et al. 2018). The forecast for the area indicates ongoing development projects and population growth (Fine et al. 2019; Kleinhaus et al. 2020; Diem et al. 2023). Jordan is planning to build the Aqaba-Amman Water Desalination and Conveyance National Project on the Gulf of Aqaba (Chenoweth and Al-Masri 2022); and in the Eilat-Eliot region of Israel over 70% of the electricity supply is derived from renewable energy sources, offering energy security that supports continuing socio-economic benefits consequent and population growth (Hamed and Bressler 2019). Without

effective planning, management, and regulation, therefore, the state of plastic pollution in the Red Sea is likely to worsen. Currently, the UNEP is taking action through the development of a Global Plastic Treaty (UNEP 2017). This treaty is crucial for safeguarding those valuable and less protected environments (Simon et al. 2021) such as the coral reefs of the Red Sea.

Plastic waste, a relatively recent addition to the planet, has seen over 8,000 million metric tons produced in less than a century, with approximately 79% ending up in landfills and the environment (Geyer et al. 2017). Persson et al. (2022) argue that we have surpassed the planetary boundary for novel entities and chemical pollution on the planet. The production and release of numerous new substances, including plastics and their additives, outstrip our ability to conduct reliable research in order to understand their potential effects on our ecosystems, challenging our ability to ensure safety and monitor materials in the environment.

In this dissertation I explore the potential effects of man-made synthetic substances on coral reefs, an ecosystem severely impacted by the surpassed planetary boundaries, including both global-scale processes like Climate Change and Ocean Acidification, and local-regional processes such as Biodiversity Loss, Global Phosphorus and Nitrogen Cycles, and Chemical Pollution (Rockström et al. 2009).

The findings of this study serve as scientific evidence of the impacts and magnitude of marine plastic pollution on coral reefs, which is essential for promoting science-based management and policies. The study offers insights into various forms of plastic pollution in coral-reef environments: the effects of plastic additives on the early life-stages of coral-

reef organisms; the magnitude and spatial distribution of plastic pollution in a coral-reef environment; the challenges involved in monitoring microplastics in the surrounding environment of coral-reef organisms; and the occurrence of plastic additives in coral-reef invertebrates regardless of whether they reside on natural or plastic substrates. Additionally, this research makes a significant contribution to filling in the knowledge gaps described by the UNEP in 2019, by providing a scientific foundation and relevant findings that support the development of a Global Plastic Treaty along with other regulations aimed at safeguarding the unique and endangered coral-reef ecosystem.

SUPPLEMENTARY INFORMATION

Supplementary Material for Chapter 1

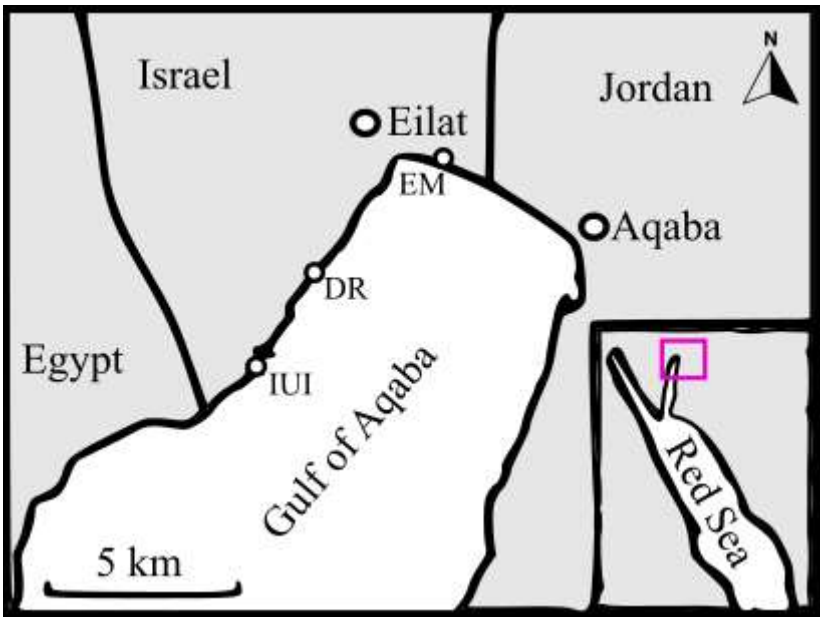


Figure S1: Map of the Gulf of Aqaba and the northern tip of the Red Sea. Sampling locations: EM = Eilat Marina, DR = Dolphin Reef, and IUI = Interuniversity Institute for Marine Sciences.

Table S1: Chemical and physical properties of the selected compounds and their common uses and functions.

Chemical compound (abbreviation)	CAS N°[1]	Mw (g/mol)[1]	Water solubility (mg/l)[1]	log Kow[1]	Functions in plastics[4]	Used in	Regulation[5]
Dibutyl phthalate (DBP)	84-74-2	278.34	11.2	4.57	Plasticizer; hardener; softener; solvent; filler; lubricant; colorant; adhesive	PVC (soft); food packaging; nitrocellulose; nitrile rubber; perfume oils; textile. [2, 3]	Restricted (EU, Brazil, Hong Kong, and Israel): less than 0.1% of plastic weight in toys and childcare articles.
Dimethyl phthalate (DMP)	131-11-3	194.2	4000	1.61	Plasticizer; hardener; softener; filler; colorant; adhesive	Polyethylene terephthalate; solid rocket propellants; lacquers; plastics; rubber coating [2, 4]	Restricted (EU, Canada, and South Korea): less than 0.1% of plastic weight for uses in cleaning, and cosmetic products.
4-nonylphenol (4-NP)	104-40-5	220.35	7	5.76	Antioxidant; plasticizer; solvent; surfactant; emulsifier; adhesive; monomer; catalyst; thermal stabilizer	Food packaging, clothes, polyurethane foam; epoxy PVC- floors [4]	Restricted (EU, Canada, and South Korea): less than 0.1% by weight for uses in textiles/leather processing, metal working, cleaning, and cosmetic products.
Bisphenol A (BPA)	80-05-7	228.29	120-300	3.32	Monomer; hardener; softener; solvent; filler; thermal stabilizer;	PVC; plastic toys; reusable plastic containers; polycarbonate and epoxy resins [2, 3]	Prohibited for use in baby bottles and drinking cups. (EU, Canada, Brazil, South Africa, India, and Israel)

[1] National Library of Medicine (NIH) PubChem compound summaries: https://pubchem.ncbi.nlm.nih.gov/compound/dibutylphthalate;https://pubchem.ncbi.nlm.nih.gov/compound/dimethylphthalate; https://pubchem.ncbi.nlm.nih.gov/compound/4-Nonylphenol; https://pubchem.ncbi.nlm.nih.gov/compound/bisphenol-a

[2] ECHA, 2019. S47 | ECHAPLASTICS | A list from the Plastic Additives Initiative Mapping Exercise by ECHA. https://doi.org/10.5281/ZENODO.2658140

[3] Net S., Sempéré R., Delmont A., Paluselli A., and Ouddane B. (2015a). Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. *Environmental Science & Technology*, 49(7), 4019–4035. https://doi.org/10.1021/es505233b

[4] Groh K. J., Backhaus T., Carney-Almroth B., Geueke B., Inostroza P. A., Lennquist A., Leslie H. A., Maffini M., Slunge D., Trasande L., Warhurst A. M., and Muncke J. (2019). Overview of known plastic packaging-associated chemicals and their hazards. Science of The Total Environment, 651, 3253–3268. https://doi.org/10.1016/j.scitotenv.2018.10.015

[5] International Panel on Chemical Pollution, and (IPCP) T. I. P. on C. P. (2017). Overview Report III: Existing national, regional, and global regulatory frameworks addressing endocrine disrupting chemicals (EDCs). https://wedocs.unep.org/20.500.11822/25636

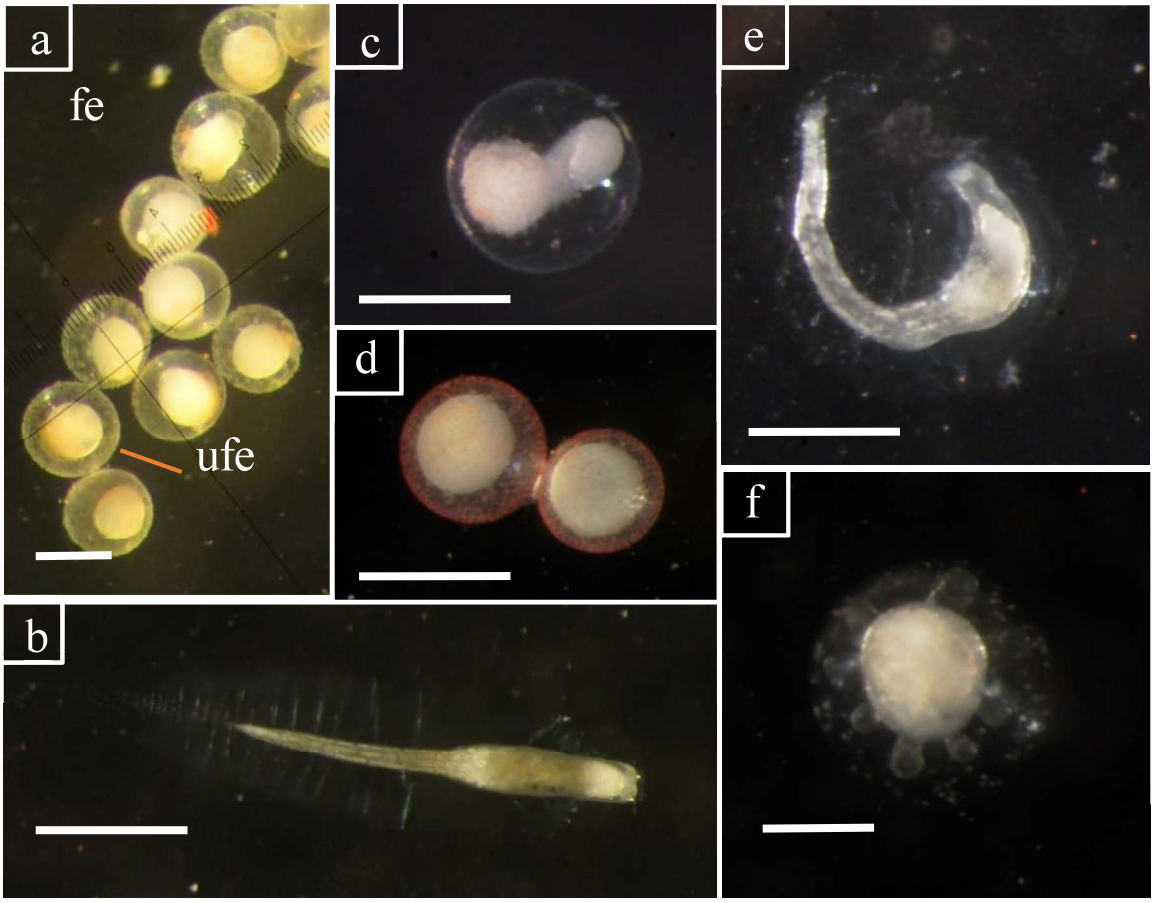


Figure S2: Eggs and larvae of *Herdmania momus*. Fertilized egg (fe) and unfertilized egg (ufe) 5 hours post-fertilization (a), a hatched and fully developed larva at 12 hours post-fertilization (b), an undeveloped larva at 12 hours post-fertilization (c), unfertilized eggs 12 hours post-fertilization (d), a malformed larva at 24 hours post-fertilization (e), and a metamorphosed settled *H. momus* at 24 hours post-fertilization (f). Scale bar: 200 µm.

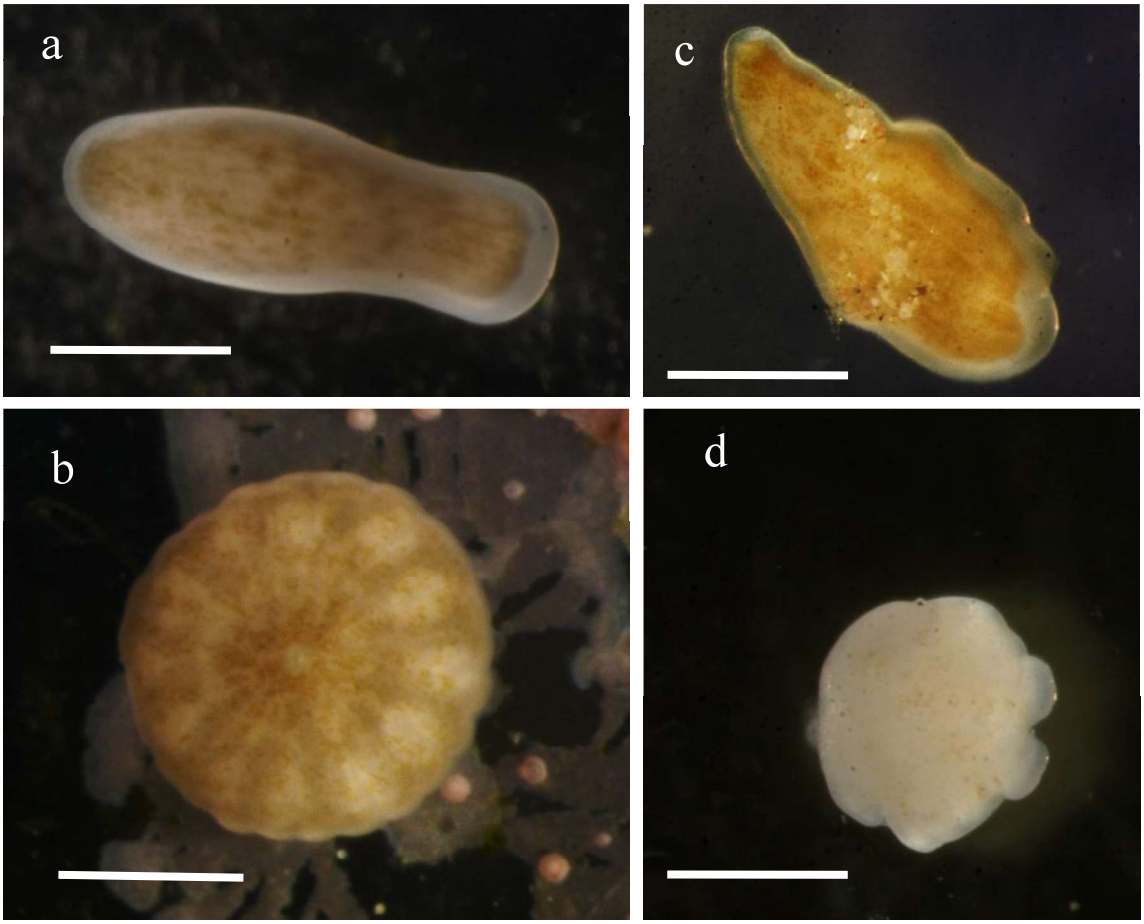


Figure S3: Planulae of *Stylophora pistillata* on day 4 post-exposure. A healthy developed planula (a), a settled planula (b), and a malformed planula (c, and d). Scale bar: 500 µm.

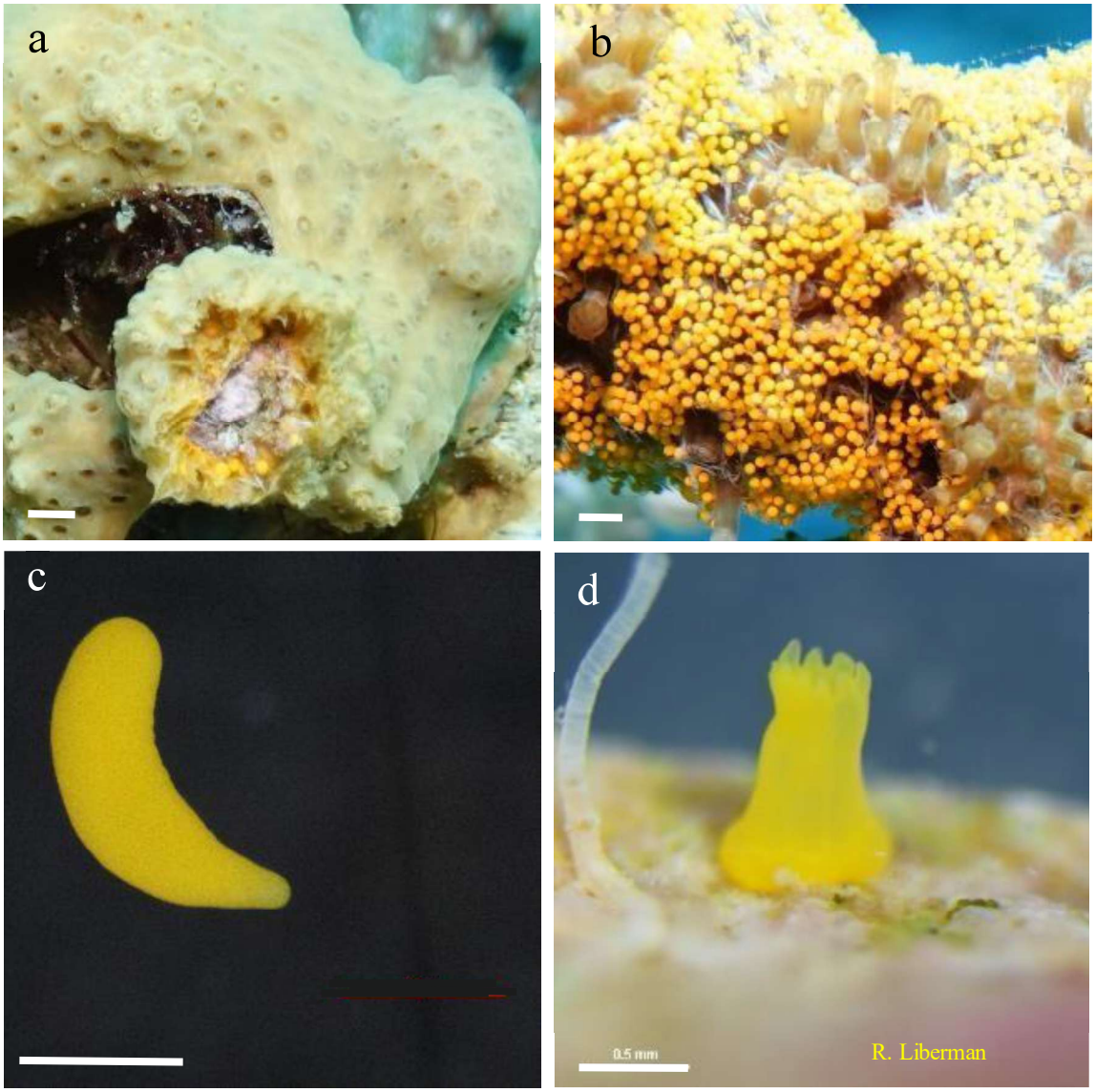


Figure S4: Early life stages of *Rhytisma fulvum*. Embryos still within coral tissue (a), embryos on colony surface during a surface-brooding event (b), a planula on the day of collection from the colony (c), a settled and metamorphosed polyp with short tentacles on day 13 (d), Scale bar: 500 µm.

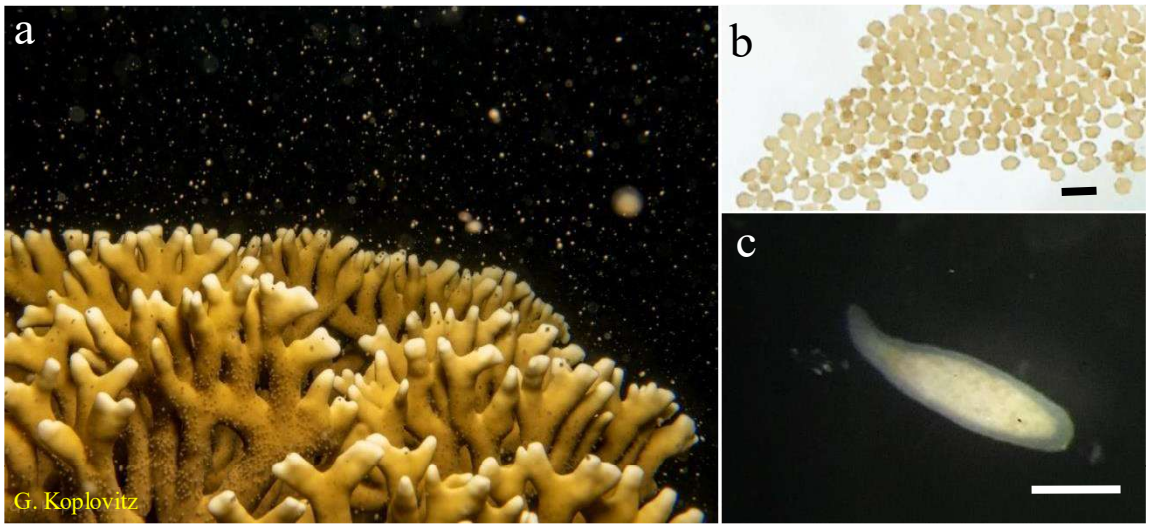


Figure S5: Early life stages of *Millepora dichotoma*. A colony releasing the hydromedusas into the water column (a), fertilized oocytes at 12 hours post-hydromedusa collection (b), and a planula on day 2 post-fertilization (c). Scale bar: 500 µm.

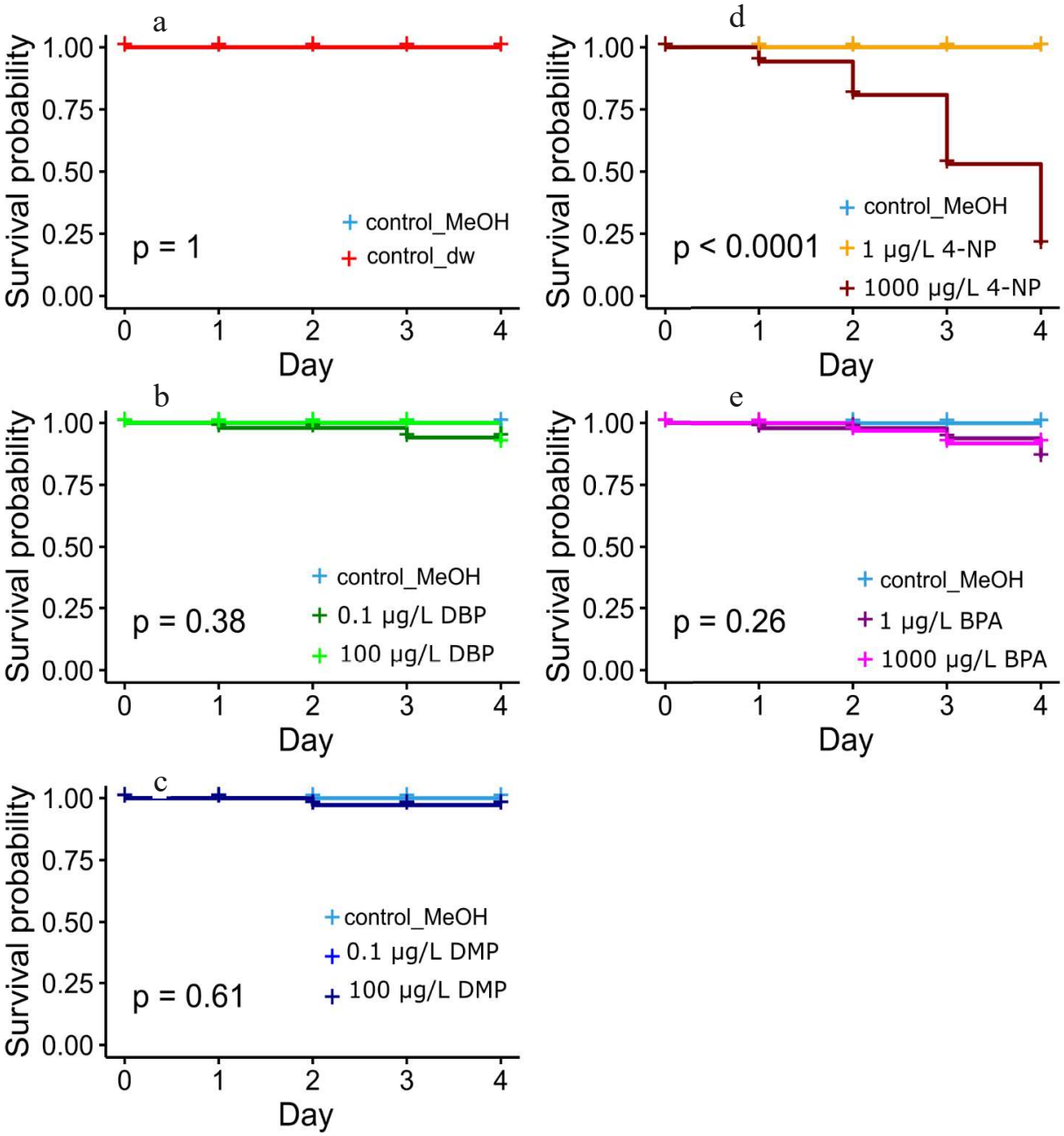


Figure S6: Kaplan-Meier log-rank survival probability of *Stylophora pistillata* planulae under the different treatments and control: MeOH control and DWW control (a), MeOH control, DBP environmental, and DBP laboratory high concentrations (b), MeOH control, DMP environmental, and DMP laboratory high concentrations (c), MeOH control, 4-NP environmental, and 4-NP laboratory high concentrations (d), MeOH control, BPA environmental, and BPA laboratory high concentrations (e). Cross marks represent the survival probability of each treatment on day 4.

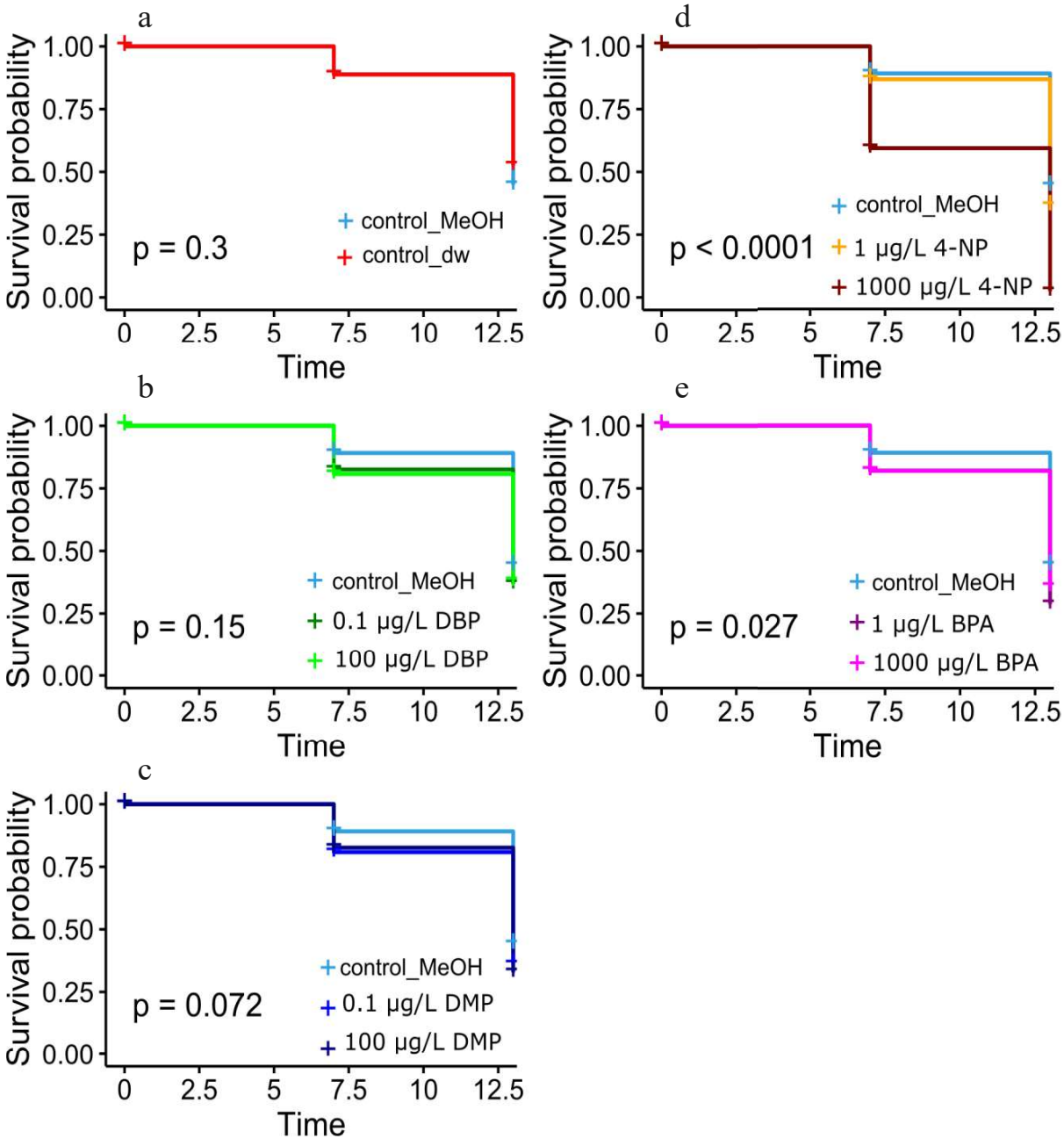


Figure S7: Kaplan-Meier log-rank survival probability of *Rhytisma fulvum* planulae under the different treatments and control: MeOH control and DWW control (a), MeOH control, DBP environmental, and DBP laboratory high concentrations (b), MeOH control, DMP environmental, and DMP laboratory high concentrations (c), MeOH control, 4-NP environmental, and 4-NP laboratory high concentrations (d), MeOH control, BPA environmental, and BPA laboratory high concentrations (e). Cross marks represent the survival probability of each treatment on day 13 post-collection.

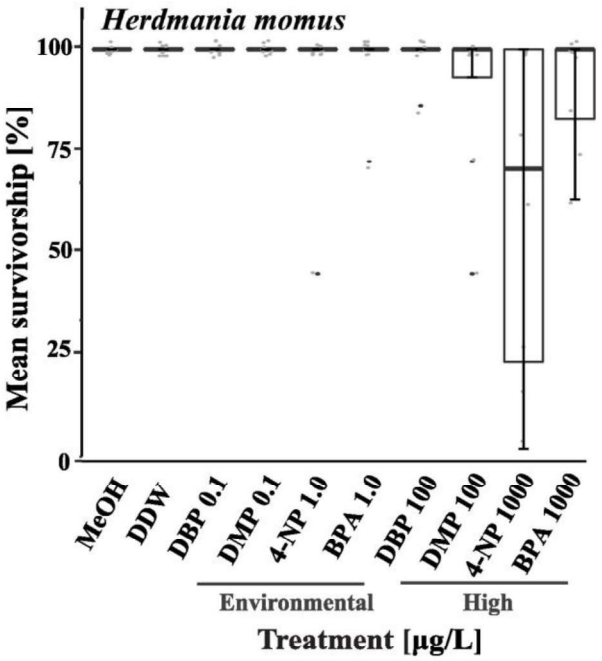


Figure S8: *Herdmania momus* larval survivorship. Box plots of the mean percentage of the surviving *H. momus* larvae in the Petri dishes under the different PA treatments and controls. Line in box = median; box = 25th to 75th percentiles; bars = min and max values excluding outliers; dots = outliers. No significant difference was found compared to the MeOH control ($p > 0.05$, GLMM).

Supplementary Material for Chapter 2

Table S2: A description of the sampling sites: coordinates, distance from the city center, and potential sources of influence within 1 km of radios. Correspond to Figure 2.1.

Site name (symbol on map)	Potential sources of influence within 1 km	Depth range (m)	Substrate	Slope
Northern Beach (a)	Kinet canal, fish farms, agriculture wash-off, the border with Jorden	3-10	Seagrass-dominated	Flat
		20-30	Sand-dominated, some seagrasses	Flat
City Centre Beach (b)	Hotels, watersport dock, touristic facilities, a military port, city runoff	3-10	Sand-dominated, some seagrasses	moderated ≤ 20°
		20-30	Coral reef	moderated ≤ 20°
Oil Jetty (c)	Port, tourist facilities	3-10	Sand and coral knolls	Flat
		20-30	Coral reef	Steep ≥ 20°
Marine Nature Reserve (d)	Marine nature reserve, the border with Egypt	3-10	Sand and coral knolls	Flat
		20-30	Many coral knolls and few seagrasses	Flat

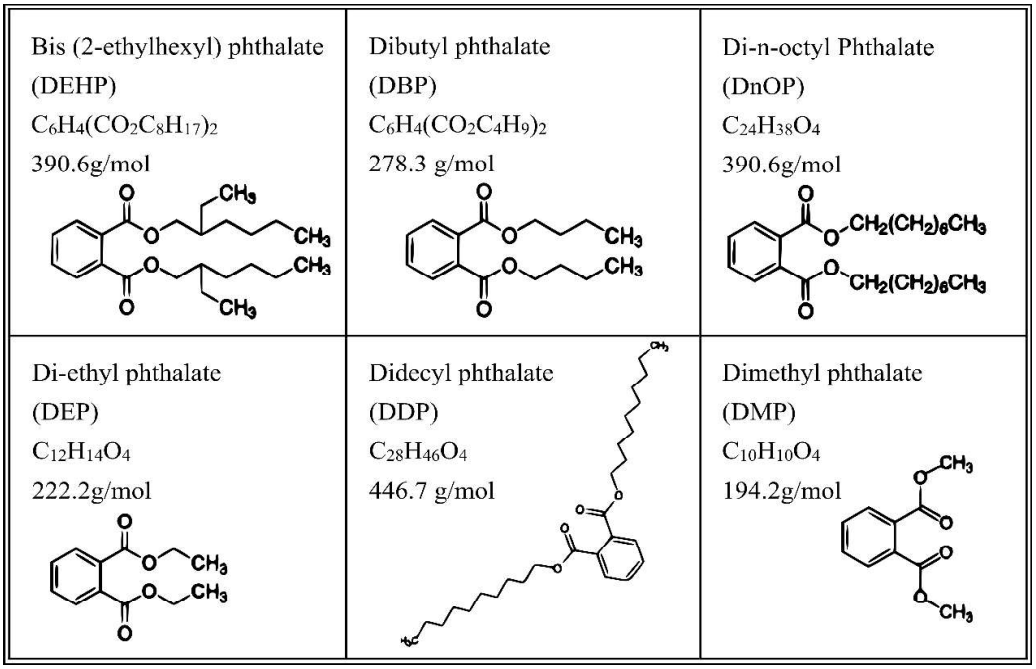


Figure S9: Targeted compounds: The chosen phthalate esters and their properties include the name, abbreviation, formula, molar mass, and molecular structure.

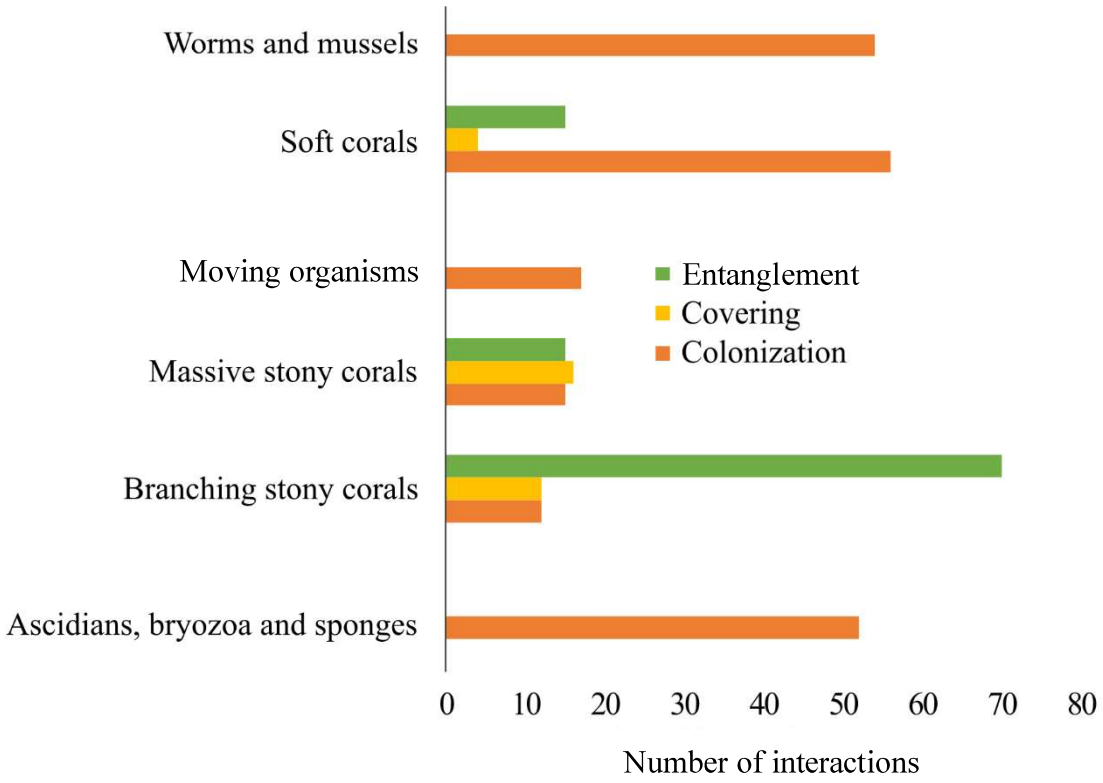


Figure S10: Bar plot presenting the number of each type of debris-organism interaction for all recorded interactions.

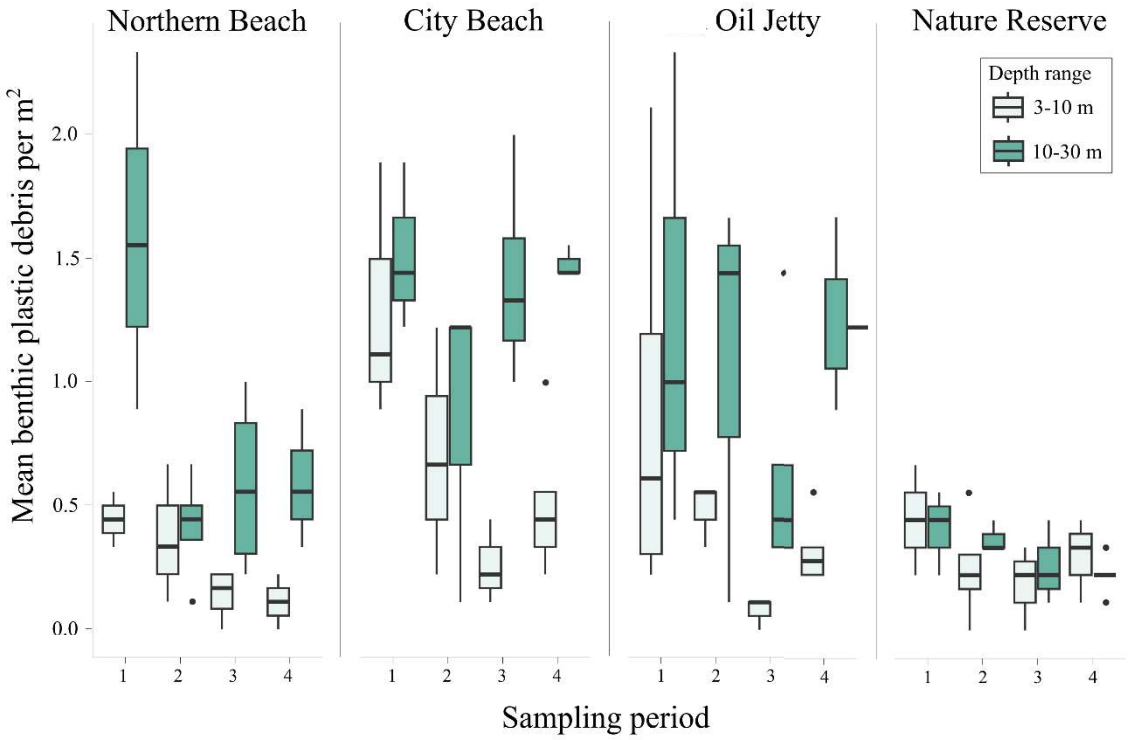


Figure S11: Box plots of the mean concentration of benthic plastic debris larger than 2.5 cm documented during the diving belt transects (n = 112). The x-axis displays the four sampling periods (1-4), with the sampling sites indicated at the top of the plots. The light and dark colors distinguish the shallow and deep depth ranges, respectively. Line in box = median; box = 25th to 75th percentiles: bars = min and max values excluding outliers. Outliers are marked by dots.

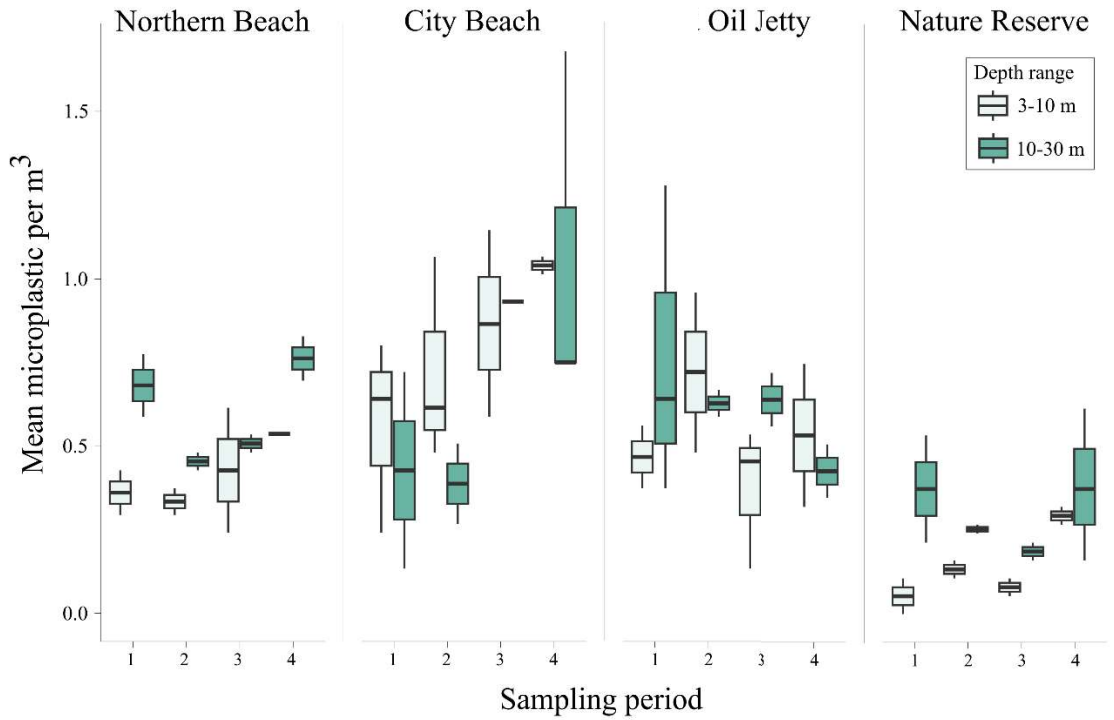


Figure S12: Box plots of the mean concentration of microplastics smaller than 5 mm. The x-axis displays the four sampling periods (1-4), with the sampling sites indicated at the top of the plots. The light and dark colors distinguish the shallow and deep depth ranges, respectively. Line in box = median; box = 25th to 75th percentiles: bars = min and max values excluding outliers. Outliers are marked by dots.

Table S3: Phthalate levels in all sediment samples (LOQ = 14.7 ng/g). Concentration [ng/g]. BLQ = Below Quantification Limit.

Site	Replica	Depth [m]	Date	Weight [g]	DMP	DEP	DBP	DEHP	DnOP	DDP
Blank sample of ASE	1	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of ASE	2	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of ASE	3	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of ASE	4	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of ASE	5	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of ASE	6	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	25	27/05/2021	27	BLQ	BLQ	BLQ	249.31	BLQ	BLQ
Nature Reserve	2	25	27/05/2021	26	BLQ	BLQ	BLQ	157.78	BLQ	BLQ
Nature Reserve	3	25	27/05/2021	26	BLQ	BLQ	BLQ	45.48	BLQ	BLQ
Oil Jetty	1	5	27/05/2021	32	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	2	5	27/05/2021	31	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	3	5	27/05/2021	30	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	1	21	10/05/2021	26	BLQ	BLQ	BLQ	77.93	BLQ	BLQ
Oil Jetty	2	21	10/05/2021	25	BLQ	BLQ	BLQ	141.71	BLQ	BLQ
Oil Jetty	3	21	10/05/2021	24	BLQ	BLQ	BLQ	277.16	BLQ	BLQ
City Beach	1	6	10/05/2021	33	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	6	10/05/2021	28	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	3	6	10/05/2021	27	BLQ	BLQ	BLQ	54.71	BLQ	BLQ
North Beach	1	5	09/05/2021	28	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	5	09/05/2021	28	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	3	5	09/05/2021	26	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	24	06/05/2021	26	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	24	06/05/2021	25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	3	24	06/05/2021	21	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eilat Marina	1	3	05/05/2021	22	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eilat Marina	2	3	05/05/2021	20	BLQ	BLQ	BLQ	BLQ	BLQ	14.96
Eilat Marina	3	3	05/05/2021	17	BLQ	BLQ	BLQ	44.80	BLQ	15.78
City Beach	1	29	04/05/2021	26	BLQ	BLQ	BLQ	52.64	BLQ	BLQ
City Beach	2	29	04/05/2021	26	BLQ	BLQ	BLQ	51.23	BLQ	BLQ
City Beach	3	29	04/05/2021	25	BLQ	BLQ	BLQ	40.97	BLQ	BLQ
North Beach	1	23	04/05/2021	25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	23	04/05/2021	25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	3	23	04/05/2021	24	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	5	02/05/2021	32	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	5	02/05/2021	31	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	3	5	02/05/2021	31	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	5	23/12/2020	30	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Nature Reserve	**2**	**5**	**23/12/2020**	**29**	**BLQ**	**BLQ**	**BLQ**	**205.44**	**BLQ**	**BLQ**
Nature Reserve	3	5	23/12/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	5	22/12/2020	30	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	5	22/12/2020	30	BLQ	BLQ	BLQ	70.62	BLQ	BLQ
City Beach	3	5	22/12/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	1	5	22/12/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	5	22/12/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	3	5	22/12/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	7	03/12/2020	32	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	7	03/12/2020	32	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	3	7	03/12/2020	31	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	25	17/11/2020	25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	25	17/11/2020	24	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	3	25	17/11/2020	24	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	1	21	17/11/2020	26	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	21	17/11/2020	25	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	3	21	17/11/2020	23	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	1	7	16/11/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	2	7	16/11/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	3	7	16/11/2020	29	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Table S4: Phthalate levels in all seawater samples (LOQ = 14.7 ng/l). BLQ = Below Quantification Limit.

Site	Replica	Depth [m]	Date	Vol. [ml]	DMP	DEP	DBP	DEHP	DnOP	DDP
Oil Jetty	1	5	16/11/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	2	5	16/11/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	3	5	16/11/2020	400	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	25	17/11/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	25	17/11/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	1	5	22/12/2020	300	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	5	22/12/2020	300	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	5	22/12/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	5	22/12/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	5	23/12/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	5	23/12/2020	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	1	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	2	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	3	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	5	02/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	29	03/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	29	03/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	3	29	03/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	1	23	04/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	2	23	04/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
North Beach	3	23	04/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eilat Marina	1	3	05/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eilat Marina	2	3	05/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eilat Marina	3	3	05/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	1	24	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	24	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	3	24	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	1	6	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	2	6	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
City Beach	3	6	06/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	1	21	12/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	2	21	12/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Oil Jetty	3	21	12/05/2021	300	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Nature Reserve	1	25	27/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Nature Reserve	2	25	27/05/2021	800	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of LLE	1	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of LLE	2	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of LLE	3	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of LLE	4	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Blank sample of LLE	5	n.a	n.a	0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Supplementary Material for Chapter 3

Table S5: List of plastic additives suspected to be found in items made of polypropylene and polyethylene: Properties including CAS number, commercial name, formula, logP, molecular mass, common uses, and relevant references linking to PP or PE. The structural and identifying properties of the compounds were obtained from the 'PubChem Substance and Compound databases' (https://pubchem.ncbi.nlm.nih.gov/), and the logP was calculated using the ACD/ChemSketch software.

CAS	Commercial Name	Formula	logP	Mass [g/mol]	Common Uses	References linking to PP/PE
6683-19-8	Irganox 1010	$C_{73}H_{108}O_{12}$	10<	1178	Antioxidant in PVC, PA, PP, PE, and cellulosic polymers	Ambrogi et al. (2017)
13560-89-9	Dechlorane plus	$C_{18}H_{12}Cl_{12}$		653.7	chlorinates flame retardant in PA, PP and natural rubber	
35958-30-6	ANOX 29	$C_{30}H_{46}O_2$		438.7	Primary antioxidant	
621-07-8	N,N-Dibenzylhydroxylamine	$C_{14}H_{15}NO$	2.52	213.3	Primary antioxidant in PE and PP	
31570-04-4	Irgafos 168	$C_{42}H_{63}O_3P$	10	646.5	Catalysis, metallation, and secondary antioxidant in PVC, PS, PA, PP, PE and cellulosic	Chen et al. (2021) ;Ambrogi et al. (2017)
103-49-1	Dibenzylamine	$C_{14}H_{15}N$	2.97	197.3	Vulcanization activator in rubber, and non-intentionally added substance in packaging	(Jiang et al. 2023; Groh et al. 2019)
26523-78-4	Tris(nonylphenyl) phosphite	$C_{45}H_{69}O_3P$	10	689	Stabilizer in PE, packaging, rubber, tire, film and sheets	Chen et al. (2021)
27676-62-6	Irganox 3114	$C_{48}H_{69}N_3O_6$	10 <	784.1	UV stabilizer, antioxidant, oxidizing/reducing agent in PP and PE	Groh et al. (2019)
78-30-8	Tri-O-cresyl phosphate	$C_{21}H_{21}O_4P$	4.11	368.4	Non-intentionally added substance in PP	
1709-70-2	Irganox 1330	$C_{54}H_{78}O_3$	10<	775	Thermoplastic elastomers, PE and PP, and hot melt adhesives	
2082-79-3	Irganox 1076	$C_{35}H_{62}O_3$	10<	530.9	Stabilizer and antioxidant	
131-11-3	Dimethyl phthalate	$C_{10}H_{10}O_4$	1.96	194.2	Plasticizer	
80-07-9	Bis(4-chlorophenyl) sulfone	$C_{12}H_8C_{l2}O_2S$	3.58	287.2	Precursor in polysulfone, and used in pipes	
117-81-7	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	7.43	390.6	Plasticizer	

88-26-6	Antioxidant 754	$C_{11}H_{23}NO_2$	4.04	236.4	Antioxidant	Groh et al. (2019)
123-77-3	Azodicarbonamide	$C_2H_4N_4O_2$	0.96	116.1	Foaming agent in rubber, copolymer for PVC, and elastomers for PP and PE	
2440-22-4	Drometrizole	$C_{13}H_{11}N_3O$	3.27	225.2	Stabilizer, UV absorber, raw material for plastics production, solvent, and adhesive	

Ambrogi V., Carfagna C., Cerruti P., and Marturano V. (2017). Additives in Polymers. In C. F. Jasso-Gastinel and J. M. Kenny (Eds.), Modification of Polymer Properties (pp. 87–108). Elsevier. https://doi.org/10.1016/B978-0-323-44353-1.00004-X

Chen Q., Santos M. M. dos, Tanabe P., Harraka G. T., Magnuson J. T., McGruer V., Qiu W., Shi H., Snyder S. A., and Schlenk D. (2021). Bioassay guided analysis coupled with non-target chemical screening in polyethylene plastic shopping bag fragments after exposure to simulated gastric juice of Fish. Journal of Hazardous Materials, 401, 123421. https://doi.org/10.1016/j.jhazmat.2020.123421

Groh K. J., Backhaus T., Carney-Almroth B., Geueke B., Inostroza P. A., Lennquist A., Leslie H. A., Maffini M., Slunge D., Trasande L., Warhurst A. M., and Muncke J. (2019). Overview of known plastic packaging-associated chemicals and their hazards. Science of The Total Environment, 651, 3253–3268. https://doi.org/10.1016/j.scitotenv.2018.10.015

Jiang J. R., Chen Z. F., Liao X. L., Liu Q. Y., Zhou J. M., Ou S. P., and Cai Z. (2023). Identifying potential toxic organic substances in leachates from tire wear particles and their mechanisms of toxicity to *Scenedesmus obliquus*. *Journal of Hazardous Materials*, 458, 132022. https://doi.org/10.1016/j.jhazmat.2023.132022

Table S6: The GC/MS method parameters.

GC system:	Agilent, model 6890	
MS Detector:	Agilent, model 5973	
Chromatographic Conditions		
Column:	Restek	
Liner:	Restek double goose-neck 4 mm GC liner	
Carrier Gas:	Hydrogen	
Column max temperature:	350 'C	
Column mode:	constant flow	
Initial flow:	0.4 mL/min	
Nominal initial pressure:	1.05 psi	
Average velocity:	42 cm/sec	
Inlet/Injector		
Mode:	Pulsed Splitless	
Injection volume:	1 µL	
Pre-injection syringe washes:	5 (ethyl acetate)	
Pre-injection sample washes:	5	
Post-injection syringe washes:	5 (ethyl acetate)	
Injector temp:	250 C	
Injector Pressure:	1.05 psi	
Pulse pressure:	12.0 psi	
Pulse time:	1.00 min	
Purge flow:	9.9 mL/min	
Purge time:	1.00 min	
Total flow:	17.6 mL/min	
Initial Temperature Equilibration Time:	1.00 min	
Total run time:	26 min	
Gradient Segment	Temperature (C)	Time (min)
Initial temp	70	1
First ramp	15 C per minute	
Final Temp	330	8
MS Parameters		
MS transfer line heater temp:	280 C	
Solvent delay:	4.20 min	
EMVolts:	2082.4	
MS Quad:	150 C, maximum 200 C	
MS Source:	230 C, maximum 250 C	
TUNE PARAMETERS		
Emission:	34.61	
energy:	69.922	

repeller:	32.301
ionfocus:	72.125
entrance:	9.5
emvolts:	1776.471
amugain:	2680
amuoffset:	127
filament:	1
dcpolarity:	0
entlensoffs:	21.333
massgain:	504
massoffset:	-11
SCAN MODE – Scan Range	
Low mass:	41
High mass:	800
Threshold:	150
SIM MODE	MODE
Dwell:	25 or 40 for all entered masses
Cycles/sec:	> 5 (for all SIM segments)

Table S7: The HPLC/UV/MS method parameters.

Pumping system and UV detector:	**Agilent Technologies model 1200 series.**	
Chromatographic Conditions		
UV Detector Wavelengths:	λ= 220 nm, 254 nm, 280 nm (bandwidth: 4)	
Column:	Kinetex, EVO, C18, 50 x 2.1 mm, 2.6 µm,100 Å	
Precolumn:	C-18	
Mobile Phase:	A1 – Acetonitrile: Water 1:10 (+ 0.1% formic acid)	
	B1 – Isopropanol (+ 0.1% formic acid)	
Total run time:	21 min	
Flow:	0.35 mL/min	
Injection volume:	10 µL	
Column temperature:	50°C	
Gradient Program		
Time (min)	**%A**	**%B**
0	100	0
1	100	0
8	0	100
13	0	100
14	100	0
Post time (7.00)	100	0
Post-column Makeup and MS Parameters		
Post-column makeup pump:	Mobile phase – 25 mM Ammonium format solution, pH 3	
Flow:	0.2 mL/min	
MS method name:	TG_MS_Method.meth	
Acquisition time:	13 min	
Start delay:	None	
Positive/Negative mode:	Both	
Scan range:	80-1200 m/z	
Microscans:	2	
Maximum inject time:	250 ms	
Tune file:	Tune POS TG.mstune	
Sheath gas flow:	10	
Auxiliary gas flow:	1	
Sweep gas flow rate:	0	
Heater Temperature:	450°C (increase to 470°C to minimize condensation)	
POSITIVE MODE		
Spray voltage:	3.30 kV	

Spray current:	0.00 uA
Capillary temp:	350°C
Capillary voltage:	30.00 V
Tube lens voltage:	70.00 V
Skimmer voltage:	18.00 V
NEGATIVE MODE	
Spray voltage:	3.00 kV
Spray current:	0.00 uA
Capillary temp:	350°C
Capillary voltage:	-52.50 V
Tube lens voltage:	-95.00 V

Table S8: The number of compounds found in a sample, among samples with different variables. Results of the post-hoc pairwise comparisons conducted using the ART-C procedure, and corrected with Holm's sequential Bonferroni procedure to determine the significance of differences in measurements. *P-values*: * <0.05, ** <0.005, and ***<0.0005.

Factor contrasts (site, substrate, species)	**t ratio (df) and *p-value***
Protected reef site, plastic, *C. cyathophora* - Protected reef site, plastic, *R. fulvum*	t(44) = 0.93, p = 1
Protected reef site, plastic, *C. cyathophora* - Protected reef site, natural, *C. cyathophora*	t(44) = 1.59, p = 1
Protected reef site, plastic, *C. cyathophora* - Protected reef site, natural, *R. fulvum*	t(44) = 2.26, p = 0.61
Protected reef site, plastic, *C. cyathophora* - Unprotected reef site, plastic, *C. cyathophora*	t(44) = 0.43, p = 1
Protected reef site, plastic, *C. cyathophora* - Unprotected reef site, plastic, *R. fulvum*	t(44) = -1.61, p = 1
Protected reef site, plastic, *C. cyathophora* - Unprotected reef site, natural, *C. cyathophora*	t(44) = 0.04, p = 1
Protected reef site, plastic, *C. cyathophora* - Unprotected reef site, natural, *R. fulvum*	t(44) = -0.94, p = 1
Protected reef site, plastic, *R. fulvum* - Protected reef site, natural, *C. cyathophora*	t(44) = 0.95, p = 1
Protected reef site, plastic, *R. fulvum* - Protected reef site, natural, *R. fulvum*	t(44) = 1.76, p = 1
Protected reef site, plastic, *R. fulvum* - Unprotected reef site, plastic, *C. cyathophora*	t(44) = -0.47, p = 1
Protected reef site, plastic, *R. fulvum* - Unprotected reef site, plastic, *R. fulvum*	**t(44) = -3.45, p = 0.03 ***
Protected reef site, plastic, *R. fulvum* - Unprotected reef site, natural, *C. cyathophora*	t(44) = -0.95, p = 1
Protected reef site, plastic, *R. fulvum* - Unprotected reef site, natural, *R. fulvum*	t(44) = -2.15, p = 0.75
Protected reef site, natural, *C. cyathophora* - Protected reef site, natural, *R. fulvum*	t(44) = 0.64, p = 1
Protected reef site, natural, *C. cyathophora* - Unprotected reef site, plastic, *C. cyathophora*	t(44) = -1.23, p = 1
Protected reef site, natural, *C. cyathophora* - Unprotected reef site, plastic, *R. fulvum*	**t(44) = -3.75, p = 0.01 ***
Protected reef site, natural, *C. cyathophora* - Unprotected reef site, natural, *C. cyathophora*	t(44) = -1.65, p = 1
Protected reef site, natural, *C. cyathophora* - Unprotected reef site, natural, *R. fulvum*	t(44) = -2.68, p = 0.25
Protected reef site, natural, *R. fulvum* - Unprotected reef site, plastic, *C. cyathophora*	t(44) = -1.93, p = 1
Protected reef site, natural, *R. fulvum* - Unprotected reef site, plastic, *R. fulvum*	**t(44) = -4.77, p = 0.00 ***
Protected reef site, natural, *R. fulvum* - Unprotected reef site, natural, *C. cyathophora*	t(44) = -2.36, p = 0.52
Protected reef site, natural, *R. fulvum* - Unprotected reef site, natural, *R. fulvum*	**t(44) = -3.44, p = 0.03 ***
Unprotected reef site, plastic, *C. cyathophora* - Unprotected reef site, plastic, *R. fulvum*	t(44) = -2.29, p = 0.59
Unprotected reef site, plastic, *C. cyathophora* - Unprotected reef site, natural, *C. cyathophora*	t(44) = -0.42, p = 1
Unprotected reef site, plastic, *C. cyathophora* - Unprotected reef site, natural, *R. fulvum*	t(44) = -0.14, p = 1
Unprotected reef site, plastic, *R. fulvum* - Unprotected reef site, natural, *C. cyathophora*	t(44) = 1.11, p = 1
Unprotected reef site, plastic, *R. fulvum* - Unprotected reef site, natural, *R. fulvum*	t(44) = 0.72, p = 1
Unprotected reef site, natural, *C. cyathophora* - Unprotected reef site, natural, *R. fulvum*	t(44) = -0.32, p = 1

Table S9: The frequency of occurrence of plastic additives in a sample. Results of pairwise comparisons with Tukey's (HSD), examining the differences between the independent variables.

Factor contrasts (site, substrate, species)	**Z and *p-value***	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, natural	**Z = 3.33, p = 0.02**	*
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, natural	Z = 0.25, p = 1	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, natural	Z = 2.48, p = 0.204	
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *R. fulvum*, plastic	Z = -2.22, p = 0.339	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, plastic	Z = 0.93, p = 0.983	
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -1.32, p = 0.89	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, plastic	Z = 0.8, p = 0.993	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, natural	Z = -2.28, p = 0.305	
Protected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, natural	Z = 0.25, p = 1	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *R. fulvum*, plastic	**Z = -3.39, p = 0.016**	*
Protected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, plastic	Z = -2.22, p = 0.339	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -2.72, p = 0.117	
Protected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, plastic	Z = -1.32, p = 0.89	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *C. cyathophora*, natural	**Z = 3.33, p = 0.02**	*
Unprotected reef site, *C. cyathophora*, natural - Unprotected reef site, *R. fulvum*, plastic	Z = -1.93, p = 0.529	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *R. fulvum*, plastic	Z = 0.61, p = 0.999	
Unprotected reef site, *C. cyathophora*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -2.22, p = 0.339	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *C. cyathophora*, plastic	Z = 0.93, p = 0.983	
Protected reef site, *C. cyathophora*, natural - Unprotected reef site, *R. fulvum*, plastic	**Z = -3.12, p = 0.038**	*
Protected reef site, *C. cyathophora*, natural - Protected reef site, *R. fulvum*, plastic	Z = -1.93, p = 0.529	
Protected reef site, *C. cyathophora*, natural - Unprotected reef site, *C. cyathophora*, plastic	**Z = -3.39, p = 0.016**	*
Protected reef site, *C. cyathophora*, natural – Protected reef site, *C. cyathophora*, plastic	Z = -2.22, p = 0.339	
Unprotected reef site, *R. fulvum*, plastic - Protected reef site, *R. fulvum*, plastic	**Z = 3.33, p = 0.02**	*
Unprotected reef site, *R. fulvum*, plastic - Unprotected reef site, *C. cyathophora*, plastic	Z = 0.25, p = 1	
Unprotected reef site, *R. fulvum*, plastic - Protected reef site, *C. cyathophora*, plastic	Z = 2.48, p = 0.204	
Protected reef site, *R. fulvum*, plastic - Unprotected reef site, *C. cyathophora*, plastic	Z = -2.28, p = 0.305	
Protected reef site, *R. fulvum*, plastic - Protected reef site, *C. cyathophora*, plastic	Z = 0.25, p = 1	
Unprotected reef site, *C. cyathophora*, plastic - Protected reef site, *C. cyathophora*, plastic	**Z = 3.33, p = 0.02**	*

Table S10: The frequency of occurrence of DEHP in a sample. Results of pairwise comparisons with Tukey's (HSD), examining the differences between the independent variables.

Factor contrasts (site, substrate, species)	**Z and *p-value***	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, natural	**Z = 3.43, p = 0.014**	*
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, natural	Z = 1.04, p = 0.968	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, natural	**Z = 3.17, p = 0.033**	*
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *R. fulvum*, plastic	Z = -1.11, p = 0.956	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, plastic	Z = 2.27, p = 0.311	
Unprotected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -0.03, p = 1	
Unprotected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, plastic	Z = 2.27, p = 0.308	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, natural	Z = -2.35, p = 0.268	
Protected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, natural	Z = 1.04, p = 0.968	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *R. fulvum*, plastic	**Z = -3.27, p = 0.024**	*
Protected reef site, *R. fulvum*, natural - Protected reef site, *R. fulvum*, plastic	Z = -1.11, p = 0.956	
Protected reef site, *R. fulvum*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -2.39, p = 0.244	
Protected reef site, *R. fulvum*, natural - Protected reef site, *C. cyathophora*, plastic	Z = -0.03, p = 1	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *C. cyathophora*, natural	**Z = 3.43, p = 0.014**	*
Unprotected reef site, *C. cyathophora*, natural - Unprotected reef site, *R. fulvum*, plastic	Z = -1.62, p = 0.736	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *R. fulvum*, plastic	Z = 1.53, p = 0.792	
Unprotected reef site, *C. cyathophora*, natural - Unprotected reef site, *C. cyathophora*, plastic	Z = -1.11, p = 0.956	
Unprotected reef site, *C. cyathophora*, natural - Protected reef site, *C. cyathophora*, plastic	Z = 2.27, p = 0.311	
Protected reef site, *C. cyathophora*, natural - Unprotected reef site, *R. fulvum*, plastic	Z = -3.29, p = 0.022	
Protected reef site, *C. cyathophora*, natural - Protected reef site, *R. fulvum*, plastic	Z = -1.62, p = 0.736	
Protected reef site, *C. cyathophora*, natural - Unprotected reef site, *C. cyathophora*, plastic	**Z = -3.27, p = 0.024**	*
Protected reef site, *C. cyathophora*, natural - Protected reef site, *C. cyathophora*, plastic	Z = -1.11, p = 0.956	
Unprotected reef site, *R. fulvum*, plastic - Protected reef site, *R. fulvum*, plastic	**Z = 3.43, p = 0.014**	*
Unprotected reef site, *R. fulvum*, plastic - Unprotected reef site, *C. cyathophora*, plastic	Z = 1.04, p = 0.968	
Unprotected reef site, *R. fulvum*, plastic - Protected reef site, *C. cyathophora*, plastic	**Z = 3.17, p = 0.033**	*
Protected reef site, *R. fulvum*, plastic - Unprotected reef site, *C. cyathophora*, plastic	Z = -2.35, p = 0.268	
Protected reef site, *R. fulvum*, plastic - Protected reef site, *C. cyathophora*, plastic	Z = 1.04, p = 0.968	
Unprotected reef site, *C. cyathophora*, plastic - Protected reef site, *C. cyathophora*, plastic	**Z = 3.43, p = 0.014**	*

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List of publications

Publications arising from work directly related to this dissertation:

1. **Vered**, **G**., and Shenkar, N. (*under review*). Occurrence of plastic additives in coral-reef invertebrates on natural or plastic substrates.
2. **Vered**, **G**., and Shenkar, N. (2023). Plastic pollution in a coral reef climate refuge: Occurrence of anthropogenic debris, microplastics, and plasticizers in the Gulf of Aqaba. *Sci. Total Environ.*, 905:167791. https://doi.org/10.1016/j.scitotenv.2023.167791
3. **Vered**, **G**., and Shenkar, N. (2022). Limited effects of environmentally-relevant concentrations in seawater of dibutyl phthalate, dimethyl phthalate, bisphenol A, and 4-nonylphenol on the reproductive products of coral-reef organisms. *Environ. Pollut*., 314:120285. https://doi.org/10.1016/j.envpol.2022.120285
4. **Vered, G.,** and Shenkar, N. (2021). Monitoring plastic pollution in the oceans. *Curr. Opin. Toxicol*., 27:60–68. https://doi.org/10.1016/j.cotox.2021.08.005.

Publications emanating from my studies not directly related to my dissertation

1. Dutta, S., Saar, R., Lavie, Z., **Vered, G.**, Bradbury, H.J., and Antler, G. (2022). A method for a fast and economical in situ collection of pore water in sandy sediments. *Front. Mar. Sci.*, 9:1779. https://doi.org/10.3389/fmars.2022.968063
2. Shenkar, N., Bereza, D., Gordon, T., Koplovitz, G., Navon, G., Novak, L., **Vered, G.** (2022). Ascidians of the Red Sea: in peril and invasive. In: The Encyclopedia of Conservation. DellaSala D.A. and Goldstein M.I. (Eds.), p. 733-739. Elsevier. https://doi.org/10.1016/B978-0-12-821139-7.00232-4.
3. **Vered, G.**, Kaplan, A., Avisar, D., Shenkar, N. (2019). Using solitary ascidians to assess microplastic and phthalate plasticizers pollution among marine biota : A case study of the Eastern Mediterranean and Red Sea. *Mar. Pollut. Bull.*, 138:618–625. https://doi.org/10.1016/j.marpolbul.2018.12.013
4. Avisar, D., Kaplan, A., Ronen-Eliraz, G., **Vered, G.**, Shenkar, N., and Gozlan, I. (2019). Validated method for the detection of three phthalates derived from marine invertebrates. *Am. J. Anal. Chem.*, 10:445-458. https://doi.org/10.4236/ajac.2019.1010032

Hebrew abstract

תקציר

זיהום פלסטיק מהווה כיום איום נרחב על הסביבה הימית. פסולת של חלקי פלסטיק גדולים פוגעת בבעלי חיים המסתבכים בה ונחנקים, אורגניזמים קטנים יותר בולעים חלקיקי מיקרופלסטיק (חלקיקי פלסטיק קטנים מחמישה מ״מ) ועלולים לגווע ברעב, וכימיקלים המשמשים כתוספי פלסטיק זולגים לסביבה מפסולת פלסטיק המגיעה לים. תוספי פלסטיק הם חומרים כימיים המשולבים בפלסטיק בשלב היצור על מנת לשפר את תכונות החומר. רבים מהכימיקלים הללו ידועים כמשבשים הורמונאליים הפוגעים בחיות בר. פלסטיק ונגזרותיו מסכן מערכות אקולוגיות ימיות ייחודיות כמו שוניות אלמוגים, המכילות מגוון ביולוגי עצום, ומספקות שירותי מערכת אקולוגית לאדם כגון הגנה חופית, ומקור למזון ופרנסה לחצי מיליארד אנשים ברחבי העולם. פעילויות אנתרופוגניות המובילות לשינויי אקלים, דייג יתר וזיהום מעמידות כיום את שוניות האלמוגים ברחבי העולם בסכנה מיידית. נמצא כי לפסולת פלסטיק השפעות מזיקות על אלמוגים ודרי שונית אחרים, על ידי העלאת רגישותם לפתוגניים, פגיעה בחילוף גזים, חסימת אור שמש, ובליעה של מיקרופלסטיק, המובילים לתמותה. עם זאת, רק בשנים האחרונות החלו לחקור את ההיקף וההשפעות של זיהום פלסטיק ותוספיו על שוניות אלמוגים וידע בסיסי רב עוד חסר.

במהלך הדוקטורט, שאפתי לצמצם את פערי הידע שזוהו על ידי תוכנית הסביבה של האו״ם בשנת 2019, בנוגע לבסיס המדעי החסר באשר להשפעת זיהום פלסטיק במערכות אקולוגיות של שוניות האלמוגים. השגתי זאת על ידי ביצוע ניסויים מבוקרים וסקרי שטח מקיפים להבנת היקף התופעה.

עתיד שונית האלמוגים מתבסס על תהליכי רבייה, התגייסות של שלבים צעירים, והצלחתם להתפתח לפרט בוגר. כפועל יוצא, נוכחותם של משבשים הורמונליים עלולה להיות גורלית למבנה האוכלוסייה בשונית. בפרק הראשון בעבודת המחקר שלי, בחנתי את השפעתם של ריכוזים סביבתיים וריכוזי מעבדה גבוהים של ארבעה תוספי פלסטיק, על שלבי החיים הראשונים של מיני מפתח בשונית האלמוגים של אילת: אצטלן יחידאי *Herdmania momus*, אלמוג אבן מדגיר - *Stylophora pistillata*, הידרוזואה בונה שונית - *Millepora dichotoma*, ואלמוג שמונאי מדגיר - *Rhytisma fulvum*.

בריכוזי המעבדה הגבוהים הכימיקל 4-nonylphenol פגעה בהצלחת ההפריה וההתפתחות בכל האורגניזמים שנבחנו. לא נמצאה השפעה של הכימיקלים שנבדקו בריכוזים הסביבתיים מלבד nonylphenol שהיה הכימיקל היחיד שטיפול עם הריכוז הסביבתי נמצאה השפעה שלילית על התיישבות באלמוג הרך *R. fulvum*. ממצאי המחקר מדגימים לראשונה את ההשפעות השליליות של תוספי פלסטיק על חסרי חוליות בוני שונית. מעבר לכך, אני מציעה לבדוק ריכוזים בפועל בתוך רקמות האורגניזם החי כדי לייצר הערכות סיכונים רלוונטיות עבור מיני אלמוגים מדגירי עוברים.

מפרץ אילת בצפון הים האדום נחשב למקלט שוניות אלמוגים מפני שינויי אקלים. עם זאת, הוא נתון לרמות הולכות וגדלות של זיהום פלסטיק. בפרק השני בעבודת בדוקטורט שלי, סקרתי במשך שנתיים אתרים שונים בעומקים שונים במפרץ אילת וניטרתי שלושה מדדים: חלקי פלסטיק גדולים על הקרקעית, מיקרופלסטיק במי הים, ותוספי פלסטיק במי הים ובסדימנט. נמצא שפסולת שייט ודייג הייתה הפסולת הנפוצה ביותר, בדומה לממצאים של מחקרים מאתרים שונים בעולם. בשוניות המזופוטיות העמוקות (30-100 מטר) תועדו ריכוזים גבוהים יותר של פסולת פלסטיק בהשוואה לשוניות הרדודות (3-30 מטר). במחקר זה התגלה כי שונית האלמוגים באזור הדרומי ביותר של שמורת הטבע הימית נמצאה נקייה מחלקיקים ופסולת גדולה באופן מובהק ביחס לאתרים אחרים במפרץ. תוצאות אלו מספקות מסד נתונים מדעי חשוב לפיתוח מדיניות אזורית ואסטרטגיות ניהול למאבק בזיהום הפלסטיק, והפיכת השוניות של צפון הים האדום למקלט אלמוגים מוגן מגורמי לחץ אנתרופוגניים עולמיים ומקומיים כאחד.

הפרק השלישי מתמקד בנוכחות של תוספי פלסטיק בחסרי חוליות בשוניות האלמוגים, ומשווה בין אורגניזמים המאכלסים מצעים טבעיים לאלה השוכנים על פסולת פלסטיק. דוגמאות בעלי החיים נאספו בצלילה, יובשו בהקפאה, ועברו מיצוי באמצעות שני ממסים אורגנים במכשיר למיצוי מואץ תחת לחץ וטמפ' גבוהה (ASE). המיצויים עברו אנליזה בכרומטוגרפיה גזית מצומדת לספקטרומטר מסה (GCMS), ואנליזה נוספת בכרומטוגרפיה נוזלית בלחץ גבוה מצומדת לחיישן UV וספקטרומטר מסה (HPLC/UV/MS). השימוש במכשירים אנליטיים מגוונים איפשר לבחון את נוכחותם של שבעה תוספי פלסטיק בדגימות שלושה מינים נפוצים בשונית האלמוגים של מפרץ אילת: אלמוג רך מרפד מצע מהמין *R. fulvum*, אלמוג אבן שיחני מהמין *S. pistillata*, וספוג מרפד מהמין *Crella cyathophora*. הפרטים נדגמו הן משונית מוגנת בתוך שמורת הטבע הימית והן משונית סמוכה מחוץ לשמורה. בחנתי את נוכחותם של תוספי הפלסטיק וחקרתי דפוסים פוטנציאליים על מנת לזהות שונות מובהקת בנוכחות התוספים בין מינים, מצעים, תוך וחוץ

שמורה. נמצאה נוכחות משמעותית של תוספי פלסטיק במעלה ממחצית מהדגימות, ובהם פרטים מייצגים מכל המינים והמצעים שנבדקו. שתי התרכובות שזוהו בדוגמאות הן: Dibenzylamine, Bis(2-ethylhexyl) phthalate. לא נמצא הבדל בין המצע הטבעי למצע הפלסטיק; עם זאת, נצפה הבדל מובהק בנוכחות חומרים בבעלי חיים מחוץ לשמורה ובתוכה. בבעלי חיים שנדגמו בתוך השמורה נמצאו פחות פרטים המכילים תוספי פלסטיק מאשר מחוץ לשמורה. בעבודה זו מתגלה תפקיד המפתח של שמורות ימיות בהפחתת ההשפעה של מזהמים כימיים על מערכות אקולוגיות של שונית האלמוגים. מחקר זה תורם לשיח המתמשך על מקורם ודרך הפצתם של מזהמים הקשורים לפלסטיק ומחזק את החשיבות של שמורות ימיות בהפחתת ההשפעה של מזהמים כימיים על שוניות האלמוגים.

תוצאות עבודת הדוקטורט שלי מהוות תרומה משמעותית לצמצום הפער הקיים בעובדות וידע מחקרי לגבי היקפו והשפעותיו של זיהום פלסטיק על מערכות אקולוגיות של שוניות האלמוגים. גוף הידע הזה מציע בסיס מהמן לניהול מבוסס-מדע של זיהום פלסטיק , ועשוי למלא תפקיד מכריע בשימור שוניות בעתיד.

בחינת ההיקף וההשפעה של זיהום פלסטיק בשוניות אלמוגים

חיבור לשם קבלת התואר "דוקטור לפילוסופיה"

מאת

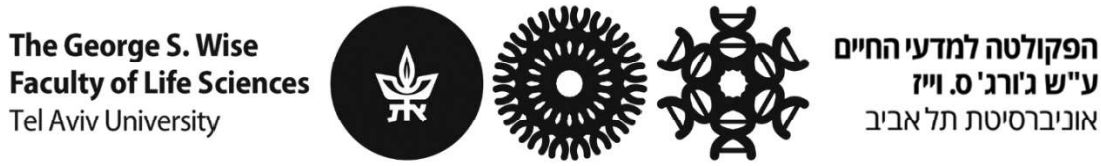
גל ורד

הוגש לסנאט אוניברסיטת תל-אביב

עבודה זו נעשתה בהנחיית

פרופסור נועה שנקר

מרץ 2024



אוניברסיטת תל-אביב

הפקולטה למדעי החיים ע״ש ג׳ורג׳ ס. וויז

בית הספר לזואולוגיה

בחינת ההיקף וההשפעה של זיהום פלסטיק בשוניות אלמוגים

חיבור לשם קבלת התואר ״דוקטור לפילוסופיה״

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