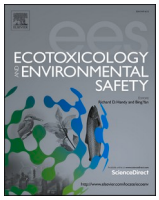
Ecotoxicology and Environmental Safety 274 (2024) 116184

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Biodegradable microplastics: Uptake by and effects on the rockpool shrimp *Palaemon elegans* (Crustacea: Decapoda)

Lukas Miksch [a,*], Chiau Yu Chen [b], Maria E. Granberg [b], Anna-Sara Krång [b], Lars Gutow [a], Reinhard Saborowski [a]

[a] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany
[b] IVL Swedish Environmental Research Institute, Kristineberg Marine Research Station, Kristineberg 566, Fiskebäckskil 45178, Sweden

ARTICLE INFO

Handling Editor: Mohamed Abdel-Daim

Keywords:
Biodegradable plastics
Microplastics
Ingestion
Oxidative stress
Enzyme activities
Shrimp

ABSTRACT

Ingestion of microplastics can lead to deleterious consequences for organisms, as documented by numerous laboratory studies. The current knowledge is based on a multitude of effect studies, conducted with conventional fossil-based and non-degradable plastics. However, there is a lack of information about the acceptance and the effects of novel bio-based and biodegradable plastics. Biodegradable plastics are considered an alternative to conventional plastics and are showing rapidly growing production rates. Biodegradable plastics can disperse into the environment in the same way as conventional plastics do, becoming available to marine organisms. This study aims to provide new insights into the uptake and effects of biodegradable microplastics on marine invertebrates. Rockpool shrimp, *Palaemon elegans,* were fed with algal flakes coated with polylactic acid (PLA), polyhydroxybutyrate-co-valerate (PHBV) and conventional low-density polyethylene (LDPE) microparticles. Live observations showed that all of the different types of microplastics were ingested. After dissection of the shrimp, less LDPE particles were found in the stomachs than PLA and PHBV particles. This indicates a longer retention time of biodegradable microplastics compared to conventional microplastics. Presumably, less LDPE particles were ingested or evacuated from the stomach, probably by regurgitation. The ingestion of microparticles of all types of plastics induced enzymatic activity of short-chain carboxylesterases in the midgut glands of the shrimp. However, only PLA induced enzymatic activity of medium-chain carboxylesterases. *Palaemon elegans* showed no oxidative stress response after ingestion of microparticles, irrespective of polymer type. From our results we conclude that biodegradable plastics might have different effects than conventional plastics. The longer retention times of biodegradable plastics might enhance exposure to leaching additives and other harmful substances. Our study provides new insights into how biodegradable plastics might affect aquatic fauna and indicate that the use of biodegradable plastics needs to be reconsidered to some extent.

1. Introduction

Plastic pollution is a perpetual problem across the globe. The large-scale production and use of plastic items generate unprecedented amounts of persistent waste. Improperly disposed plastics are released into the environment and are dispersed by wind, waterways, sewage systems, or surface runoff. Jambeck et al. (2015) estimated that between 4.8 and 12.7 million tons of persistent plastics enter the oceans each year. In the environment, plastic debris is exposed to environmental forces such as UV radiation, waves, and temperature changes, leading to a disintegration into numerous small fragments. These are called microplastics when they become smaller than 5 mm (Andrady, 2011). Microplastics are ubiquitous in aquatic ecosystems (Farady, 2019) with an estimated 24 trillion pieces in the upper layers of the world's oceans (Isobe et al., 2021). They can be found even in remotest areas and habitats such as deep-sea sediments (Van Cauwenberghe et al., 2013) or Arctic sea-ice (Peeken et al., 2018). In the oceans, microplastics can interact with organisms and are easily ingested due to their small size, either intentionally when they are mistaken for food or accidentally during feeding (Ory et al., 2017; Sussarellu et al., 2016). Uptake of microplastics has been reported for a multitude of marine invertebrates, including annelids, cnidarians, crustaceans, echinoderms, and mollusks (Lusher et al., 2017 and literature cited therein). The effects of microplastic ingestion vary greatly between species depending on their

* Corresponding author.
E-mail address: Lukas.miksch@awi.de (L. Miksch).

https://doi.org/10.1016/j.ecoenv.2024.116184
Received 10 November 2023; Received in revised form 16 January 2024; Accepted 5 March 2024

intrinsic features such as feeding traits and digestive systems. While some organisms simply reject micro-particles apparently without harmful effect (Korez et al., 2022), others suffer from starvation or malnutrition due to clogging or damage of the digestive tract (Lei et al., 2018; Welden and Cowie, 2016). Moreover, ingestion of microplastics can increase mortality (Gray and Weinstein, 2017), reduce fecundity and growth (Cole et al., 2015; Lo and Chan, 2018), and induce oxidative stress (Browne et al., 2013; Mazurais et al., 2015). These toxic effects were caused by various conventional plastics based on polyethylene (PE), polystyrene (PS), polypropylene (PP), and other non-degradable petroleum-based polymers.

Recently, novel plastics that are biodegradable and/or based on renewable biomass prosper in development and production. They are commercially exploited as promising alternatives to conventional plastics, mitigating environmental pollution. However, recent studies suggest that these materials, although considered biodegradable, degrade slowly under marine conditions (Miksch et al., 2022; Nazareth et al., 2019). Similar to conventional plastics, biodegradable plastics are weathered and fragmented into countless microscopic particles, accumulating in the environment (Wei et al., 2022). Microplastics from biodegradable plastics have recently been found in marine environments, such as sediments (Okoffo et al., 2022), where they can interact with biota. However, studies about the uptake of biodegradable plastics by marine biota and their effects after ingestion are scarce.

Biodegradable plastics differ from conventional plastics in their chemical structure (Endres, 2017). Glycosidic-, peptide-, and ester-bonds in the polymer facilitate enzymatic degradation (Rosato et al., 2022). The biological consequences of ingestion by biota are unknown. Digestive enzymes might be capable of hydrolyzing these polymers. Enzymatic hydrolysis of biodegradable polymers can liberate cleavage products (Hakkarainen, 2002), which may be metabolized or alter digestive processes (Trestrail et al., 2021). To improve the specific product properties, biodegradable plastics, just like conventional plastics, contain potentially toxic chemical additives (Zimmermann et al., 2020). Due to their improved biodegradability, these plastics may release their chemical additives faster than conventional, non-degradable plastics. It is unclear, if chemical cues such as degradation products or additives might affect the perception and ingestion of biodegradable microplastics. Generally, there is considerable uncertainty as to whether and how biodegradable plastics differ in the effects on biota from conventional plastics. Due to the higher density, biodegradable plastics are more likely to sink in fresh and seawater, being available to organisms in benthic environments, such as decapod crustaceans.

Decapod crustaceans inhabit various marine ecosystems, where they occupy important ecological niches (Briones-Fourzán et al., 2020) and are economically relevant targets in fisheries and aquaculture. Decapods are key components of aquatic food webs, foraging on a wide spectrum of food items. Decapods have a broad range of highly active digestive enzymes that are capable of breaking down ester-, peptide-, and glycosidic-bonds (Saborowski, 2015; Vogt, 2021). Furthermore, decapods ingest microplastics as shown for a multitude of species (D'Costa, 2022), making this taxon particularly interesting for studying the uptake and the effects of the ingested particles.

The rockpool shrimp, *Palaemon elegans* Rathke, 1836, was selected as test organism for a comparative investigation of the uptake and effects of conventional and biodegradable microplastics. The presence and numbers of ingested microplastics in organs and feces of the shrimp after feeding were studied with fluorescent-dyed particles. Activities of digestive carboxylesterase activities and superoxide dismutase, as oxidative stress marker, in the midgut gland of the shrimp were measured at different times after microplastic ingestion. We hypothesize that ingested biodegradable microplastics induce different biochemical effects in *P. elegans* than conventional microplastics do.

2. Materials and methods

2.1. Plastic compounds

The biodegradable plastic compounds used in this study were provided as granules by the company Natureplast SAS (Ifs, France). These plastics were produced within the framework of the EU Horizon 2020 project 'Bio-Plastics Europe' and are based on polyhydroxybutyrate-co-valerate (PHBV), and a blend of polylactic acid (PLA) with polybutylene adipate terephthalate (PBAT) in the ratio of 30:1. Low-density polyethylene (LDPE) powder, provided by D+B Handel GmbH (Tostedt, Germany), was used as conventional, non-degradable compound (Table 1).

Granules of the biodegradable plastics were ground in a cryogenic mill (SPEX SamplePrep, 6775 Freezer/Mill) as described by Miksch et al. (2022). Briefly, granules were first cooled in liquid nitrogen to make them brittle and then ground in the mill during repeating intervals of grinding and cooling. LDPE was purchased as powder. The ground material and the LDPE powder were sieved to obtain the particle fraction smaller than 200 μm, which finally was used for the feeding experiments.

2.2. Microplastic staining

Microparticles of the different plastic compounds were stained with Nile Red after Shim et al. (2016). Briefly, Nile Red (Sigma 72485) was dissolved in absolute ethanol (5 $\mu g \cdot mL^{-1}$). Fifteen mg of microplastics (< 200 μm) were added to 10 mL of the Nile Red solution and incubated in reaction tubes on an orbital shaker at 160 rpm in darkness. After incubation overnight, the ethanol was evaporated with a gentle stream of compressed air under a fume hood. The stained particles were then rinsed three times with Milli-Q water and subsequently centrifuged at 7000 g for 15 min. The supernatant was discarded and the stained microplastics were dried. Final microplastic suspensions of 50 $mg \cdot mL^{-1}$ were prepared in Milli-Q water.

2.3. Animal maintenance

Adult specimens of the rockpool shrimp *Palaemon elegans* (37–55 mm length) were collected in August and September 2022 in a shallow bay of the Gullmars Fjord at Kristineberg Marine Research Station (Sweden, 8°14'56.792" N, 11°26'47.569" E). The surface water temperature in the bay was around 18.5 °C. Shrimp were collected with hand nets and transported in thermos-containers to a temperature-controlled room, which was set to 15 °C and 15:9 hours light:dark cycle. The shrimp were kept in the thermos-containers for 24 hours to gradually acclimatize to the new temperature. Subsequently, shrimp were transferred into two 60-L glass aquaria with a continuous flow-through of temperature-controlled filtered surface seawater (14.9 ± 0.1 °C, 22.5 – 29.8 PSU). The water was pumped from 7 m depth from the Gullmars Fjord and provided through the seawater system of Kristineberg Marine Research Station. A maximum of 40 shrimp were kept in each tank. After further 48 hours, randomly selected shrimp were transferred to the experimental setups, consisting of individual glass jars (volume of 400 mL for experiment I, 500 mL for experiment II-V). The jars were filled with 300 or 400 mL fjord water (see above) and continuously aerated. Shrimp were acclimatized in the jars for 24 h before the feeding experiment started.

Table 1
Specification of the plastics used in this study.

Polymer	Designation	Producer	Application
PHBV	T-PHBV	Natureplast	Toys
PLA/PBAT	AMF-PLA	Natureplast	Agricultural mulch films
LDPE	NewLine 234	D+B Handel	Packaging

2.4. Feeding experiments

Rockpool shrimp, *P. elegans*, were fed with 5 mg of food flakes (NovoVert, JBL, Neuhofen, Germany) spiked with 2.5 mg of microplastic particles (PLA, PHBV, and LPDE, respectively). The microplastic particles were either native or stained with fluorescent dye. The food flakes were coated with microplastics one day prior to feeding and agglutinated by adding 100 μL of Milli-Q water. The processed food was dried over night at room temperature. Five separate experiments (I-V) were carried out in a temperature-controlled room at 15 °C and a light:dark cycle of 15:9 hours. In each experiment, food flakes without microplastics were used as control. The acceptance of food by the shrimp was inspected each hour throughout the whole experiment I; shortly before sampling in experiment II-V; and 24 hours after each feeding event in experiment V. Shrimp that did not accept the food at all were not considered for subsequent anatomical and biochemical investigations.

2.4.1. Experiment I: uptake and tracking of microplastics in shrimp

For each treatment, five shrimp were maintained individually in 400-mL jars. They were fed once with food flakes that were coated with fluorescent-dyed microplastics and inspected alive 2 and 6 h after food acceptance for the presence of fluorescent microplastics in intestine and stomach. Individuals were carefully removed from the jars and photographed with an Axiocam 705 Color (ZEISS, Jena, Germany) under a fluorescence stereomicroscope (Leica MZ FLIII with filter ET DSR) at 1.25x magnification. Every two hours, fecal pellets were collected from the jar with a pipette and frozen at −20 °C for further inspection of dyed microparticles. Eight hours after acceptance of the food, the shrimp were removed from the jars. The stomach and intestine of the shrimp were dissected and immediately inspected under a fluorescence microscope for the presence of microplastics. Thereafter, the organs were frozen at −20 °C for further analysis.

To extract microplastics from the stomach and feces, the samples were homogenized with a micro-pestle in a 1.5-mL reaction tube and a density separation was conducted, using sodium iodide solution (1.8 $kg \cdot L^{-1}$). The upper fraction from the density separation vials was filtered through a Polycarbonate Track-Etched black disk membrane (PCTE) with 0.2 μm pore size and 25 mm diameter (GVS, Maine, USA) and rinsed thoroughly with Milli-Q water prior to investigation under a fluorescence microscope. Intestine samples were treated with a pancreatic enzyme solution to remove organic matter as described by von Friesen et al. (2019). Briefly, 1.4 g pancreatic enzyme (Creon® 25000 pankreatin, BGP Products AB, Stockholm) was dissolved in Tris-HCl buffer (1 $mol \cdot L^{-1}$, pH 8.0) at room temperature with a magnetic stirrer to create a 14% (w:v) enzyme solution. Five mL of the enzyme solution were applied to each intestine sample in a scintillation vial and incubated in a shaker overnight at 126 rpm and 37.5 °C. The extracts of stomach, intestine, and feces were then filtered through a Polycarbonate Track-Etched black disk membrane (PCTE) with 0.2 μm pore size and 25 mm diameter (GVS, Maine, USA). The retained microplastics on the filter were counted under a fluorescence microscope (LEITZ DMRBE, 301–371.011, Leica, Wetzlar, Germany). Because of the high numbers of microplastic particles in the stomach samples, ten random photographs of the filter at 5x magnification were taken with the microscope camera (Axiocam 705 Color, ZEISS, Jena, Germany). The average count of microplastics on the image areas (1.8 mm^2) was then extrapolated to the entire filter area of 314 mm^2.

2.4.2. Experiments II-IV: biochemical short-term effects

For each treatment, seven shrimp were maintained individually in 500-mL aerated glass jars. They were fed once with food flakes covered with native non-dyed biodegradable and conventional microplastics, respectively. Food flakes without microplastics were used as control. After 4, 24 and 48 h, shrimp were sampled. The weight and body length of each shrimp were determined during sampling. The midgut glands were dissected, weighed, and transferred into cryovials to be immediately shock-frozen in liquid nitrogen and stored at −80 °C. The samples were transported from Kristineberg Marine Research Station (Sweden) to the Alfred Wegener Institute in Bremerhaven (Germany) in cooled dry shippers (CX100, Taylor Wharton) and further on stored at −80 °C until further analysis.

2.4.3. Experiment V: biochemical medium-term effects

For each treatment, seven shrimp were maintained individually in 500-mL aerated glass jars. Shrimp were fed with food flakes covered with native non-dyed microplastics every other day over the course of 12 days. Half of the water in the glass jars was exchanged 24 hours after each feeding. After 12 days, tissue samples were taken and treated as described for experiment II-IV (2.4.2).

2.5. Enzyme assays

2.5.1. Sample preparation

The frozen midgut gland samples were slowly thawed on ice immediately before use in enzyme assays. The midgut glands were transferred individually into 2-mL reaction tubes and homogenized with a micro-pestle in Tris-HCl buffer (20 $mmol \cdot L^{-1}$, pH 7.6) using a ratio of 1:10 (w:v). The homogenized samples were then centrifuged for 10 min at 14,000 g and 4 °C. The supernatants were split and transferred into each of two new 1.5-mL reaction tubes. One half of each supernatant was mixed at a 1:1 ratio (v:v) with Tris-HCl buffer (20 $mmol \cdot L^{-1}$, pH 7.6) supplemented with 2 $mmol \cdot L^{-1}$ EDTA as recommended as extraction buffer for the assay of superoxide dismutase. The other half of the supernatant was frozen at −80 °C for later analysis of digestive esterase activities.

2.5.2. Digestive carboxylesterases

Activities of digestive enzymes were measured with fluorogenic 4-methylumbelliferone (MUF) derivatives of different chain lengths. The hydrolysis of fatty acid esters and the resulting fluorescence was interpreted as esterase activity (Knotz et al., 2006). MUF-butyrate, MUF--heptanoate, and MUF-oleate were dissolved in dimethyl sulfoxide (DMSO, Sigma 276855) to obtain a 5 $mmol \cdot L^{-1}$ substrate solution. One mL of the substrate solution was diluted with 49 mL of 50 $mmol \cdot L^{-1}$ Tris-HCl buffer (pH 7.0), resulting in stock solutions of 0.1 $mmol \cdot L^{-1}$ of fluorogenic substrate and 2% DMSO. Extracts of the midgut glands were diluted with Tris-HCl buffer (50 $mmol \cdot L^{-1}$, pH 7.0) in a ratio of 1:10 (v:v). 290 μL of the stock solution was then pipetted into the wells of a 96-well microplate and 10 μL of sample was added to each well. The plate was shaken for 10 seconds in a microplate reader (Fluoroskan Ascent FL, Thermo Fisher Scientific Corporation, USA) before measurement of the fluorescence at λ_{ex}: 355 nm and λ_{em}: 460 nm. The increase in fluorescence was measured for 5 minutes in 10 intervals of 30 seconds at room temperature. The results were recorded by Ascent Software for Fluoroskan Ascent FL and normalized for tissue mass. Standard curves were prepared with 0–35 $\mu mol \cdot L^{-1}$ 4-methylumbelliferone (MUF, Sigma M1381).

2.5.3. Superoxide dismutase (SOD)

The superoxide dismutase activity was measured after Livingstone et al. (1992), modified by Saborowski et al. (2022). In the first step, xanthine oxidase (XOD) was adjusted prior to routine analyses to obtain an increase in absorption of 0.02 per minute. 882 μL of SOD assay buffer (100 mL K_2HPO_4 (43 $mmol \cdot L^{-1}$) and 50 mL EDTA (0.1 $mmol \cdot L^{-1}$, pH 7.68)), 100 μL of cytochrome c, 10 μL of xanthine and 8 μL of XOD were mixed in a cuvette. The absorbance was measured in a photometer (Specord 200 Plus, Analytik Jena, Jena, Germany) at 550 nm for three minutes at room temperature. Milli-Q water was used as blank.

In the second step, 872 μL of SOD assay buffer, 100 μL of cytochrome c, 10 μL of xanthine, 8 μL of XOD and 10 μL of the sample were mixed in a cuvette. The absorbance was recorded photometrically (Specord 200 Plus, Analytik Jena, Jena, Germany) at 550 nm for three minutes at

room temperature. The amount of sample was adjusted to obtain 50% inhibition of cytochrome c, which corresponds to one unit of SOD activity. Milli-Q water was used as a blank. The SOD activity was then calculated from the slopes of the XOD and SOD reaction rates as reported in the Supporting Information of Saborowski et al. (2022).

2.6. Statistics

The significance level of all statistical analyses was $\alpha = 0.05$. The standard deviation for microplastic numbers in single individuals were calculated from the different counts on the photographs used to extrapolate the numbers to the whole stomach. The SOD and esterase activities of the short-term experiments (II-IV) were compared by a 2-factorial analysis of variance (ANOVA) with polymer type (PLA, PHBV, LDPE and control without plastic particles) and time (4, 24 and 48 hours after feeding) as the two main factors. The SOD and esterase activities of the medium-term experiment (V) were compared by a 1-factorial analysis of variance (ANOVA). Pairwise comparisons were performed using Tukey's HSD (honestly significant difference) test. Prior to the ANOVA, the data were tested for heteroscedasticity by a Brown-Forsythe test.

3. Results

3.1. Uptake of microparticles

3.1.1. Live observations

Only individuals that accepted the offered food in experiment I were used for subsequent observation of their organs under the microscope. Out of five replicates per treatment, three shrimp each from the PHBV- and the PLA- treatments, and four from the LDPE-treatment and control group accepted the food.

Live observations of the stomachs of the shrimp fed with PHBV showed fluorescent particles in all three shrimp at 2 h and 6 h after feeding. Shrimp fed with PLA showed fluorescent particles in their stomachs in two out of three individuals at 2 h after feeding and in all three individuals at 6 h after feeding. In the LDPE treatment, fluorescent particles were found in three out of four individuals at 2 h after feeding and in all four individuals at 6 h after food acceptance.

Live observations of the intestines of fed shrimp showed that two out of three shrimp from the PHBV treatment contained fluorescent particles at 2 h after food acceptance, and only one out of three shrimp after 6 h. Shrimp fed with PLA showed fluorescent particles in one out of three individuals at 2 h and at 6 h after food acceptance. Fluorescent LDPE particles were present in the shrimp intestine in two out of four individuals at 2 h and in only one out of four individuals at 6 h after feeding. (Table 2).

Inspection of the dissected organs at 8 h after feeding showed that all shrimp, which ingested food with PHBV and PLA, contained fluorescent particles in their stomachs. Only two out of four shrimp from the LDPE treatment had fluorescent particles left in their stomach at 8 h after food acceptance and one of these showed only a very faint fluorescent signal. No fluorescent particles were detected in the dissected intestines in any of the individuals.

Table 2
Presence of fluorescent microplastics in the stomach and intestine of individual shrimp fed with different polymers during live observations at 2 h and 6 h after food acceptance and in organ inspections at 8 h after feeding (experiment I). (●) and (○) indicate the presence or absence of fluorescent microparticles, respectively.

Treatment	Stomach			Intestine		
	2 h	6 h	8 h	2 h	6 h	8 h
PHBV #1	●	●	●	●	●	○
PHBV #2	●	●	●	●	○	○
PHBV #3	●	●	●	○	○	○
PLA #1	●	●	●	●	○	○
PLA #2	○	●	●	○	○	○
PLA #3	●	●	●	○	●	○
LDPE #1	○	●	○	●	○	○
LDPE #2	●	●	○	○	●	○
LDPE #3	●	●	●	○	○	○
LDPE #4	●	●	●	●	○	○

3.1.2. Numbers of microplastics in organs and feces

The numbers of microplastics in the stomach varied strongly between individual shrimp but also between the different photographs taken for each stomach to estimate the total number of particles per stomach (Fig. 1). Stomachs of shrimp fed with PHBV particles showed the highest numbers varying from 2440 ± 1399–73012 ± 23298 particles, while those fed with PLA contained between 281 ± 377 and 2299 ± 1069 particles. Animals fed with conventional LDPE microplastics showed the lowest numbers, ranging from 0 to 228 ± 194 particles per stomach.

Enzymatic extraction of the intestines yielded low numbers of microplastics. Microplastics were only found in the intestines of one shrimp fed with PHBV (6 particles) and in one shrimp fed with LDPE (1 particle). No microplastics were detected in the intestines of shrimp fed with PLA.

The numbers of microplastic particles in the feces varied strongly between individuals. The feces of shrimp fed with PHBV-coated food contained microplastics in all three individuals. In shrimp fed with PLA-coated food, microplastics could be detected in the feces of two out of three shrimp. Only one out of four shrimp fed with LDPE-coated food yielded feces that contained microplastics. (Table 3).

3.2. Digestive carboxylesterases

When using the short-chained MUF-butyrate as substrate (experiment II-IV), carboxylesterase activities in the midgut glands of *P. elegans* varied significantly over time ($F_{(2, 36)} = 6.37$, $p < 0.01$) and between the different food treatments ($F_{(3, 36)} = 18.39$, $p < 0.01$). Carboxylesterase activities were highest at 24 h after feeding for all treatments, showing a significant difference to activities at 4 h after feeding. Maximum activities of the different treatments were 229.9 ± 58.7 $U{\cdot}g^{-1}$ for shrimp fed with PHBV, 300.1 ± 29.8 $U{\cdot}g^{-1}$ for PLA, 228.1 ± 20.9 $U{\cdot}g^{-1}$ for LDPE and 182.9 ± 14.3 $U{\cdot}g^{-1}$ in the control shrimp (means ± SD). Shrimp fed with microplastics always showed a significantly higher carboxylesterase activity in their midgut glands than the control shrimp fed only with algae food flakes. Furthermore, shrimp fed with PLA showed also significantly higher carboxylesterase activities than shrimp fed with PHBV or LDPE (Fig. 2). No statistical interaction could be detected between time and food treatment ($F_{(6, 36)} = 0.57$, $p = 0.75$).

When using the medium-chain carboxylester MUF-heptanoate as substrate (experiment II-IV), carboxylesterase activities in the midgut glands of *P. elegans* again varied significantly over time ($F_{(2, 36)} = 55.51$, $p < 0.01$) and between the different food treatments ($F_{(3, 36)} = 4.94$, $p < 0.01$). Carboxylesterase activities at all times were significantly different from each other time point. When comparing the food treatments, only activities in midgut glands of shrimp fed with PLA were significantly higher than activities in the control. Carboxylesterase activities were highest after 48 h, with 325.2 ± 9.2 $U{\cdot}g^{-1}$ (mean ± SD) for shrimp fed with PLA and 238.8 ± 47.8 $U{\cdot}g^{-1}$ (mean ± SD) for control shrimp. No significant effect on carboxylesterase activity was observed in shrimp fed with PHBV or LDPE when compared to the control, or when comparing the microplastic treatments with each other (Fig. 3). Again, the main factors time and food treatment did not interact with each other ($F_{(6, 36)} = 1.04$, $p = 0.42$).

When using the long-chain carboxylester MUF-oleate as substrate (experiment II-IV), carboxylesterase activities in the midgut glands of *P. elegans* varied significantly over time ($F_{(2, 36)} = 39.71$, $p < 0.01$). Carboxylesterase activities were highest after 48 h, being significantly higher than activities after 4 and 24 h. No significant effect on

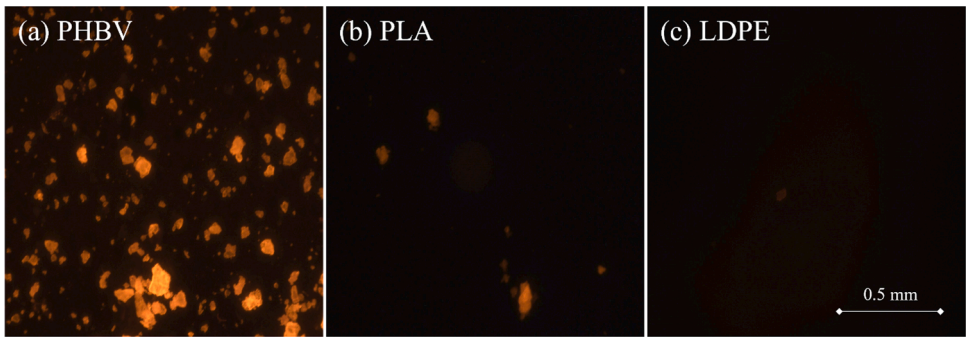


Fig. 1. Sample pictures (image area of 1.79 mm^2) of stomach contents extracted from shrimp 8 h after being fed with fluorescent-stained microplastics. Stomach contents of (a) PHBV #1, (b) PLA #1 and (c) LDPE #4. The scale (0.5 mm) applies to all photographs.

Table 3
Number of microplastics found in the feces of the individual shrimp from the different microplastic treatments.

Treatment	After 4 h	After 6 h	After 8 h	Total
PHBV #1	2756	34	0	2790
PHBV #2	21	0	0	21
PHBV #3	0	0	193	193
PLA #1	1	3	4	8
PLA #2	0	0	0	0
PLA #3	8	0	0	8
LDPE #1	0	0	0	0
LDPE #2	0	0	0	0
LDPE #3	0	0	0	0
LDPE #4	0	0	1	1

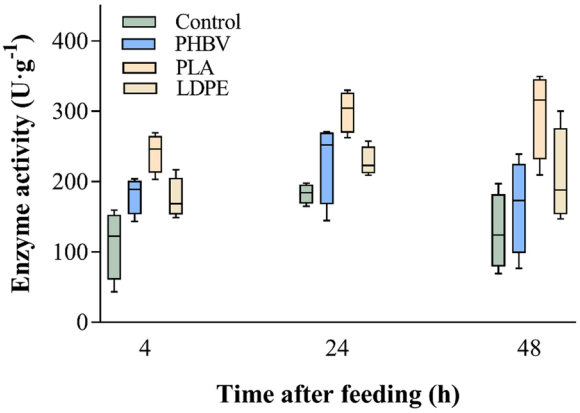


Fig. 2. Carboxylesterase activities in the midgut glands of *Palaemon elegans* after 4, 24 and 48 h of feeding, measured with MUF-butyrate as substrate (median, 25% and 75% percentile, minimum and maximum, n =12).

carboxylesterase activity was observed among the different food treatments ($F_{(3, 36)} = 0.12$, $p = 0.95$), neither between control shrimp fed with algae food flakes and shrimp fed with either PHBV, PLA or LDPE, nor when comparing the microplastic treatments with each other (Fig. 4). No statistical interaction could be detected between time and food treatment ($F_{(6, 36)} = 0.49$, $p = 0.81$).

Similar to the acute feeding experiments, feeding *P. elegans* with microplastic-coated food continuously over 12 days (experiment V) showed significantly higher carboxylesterase activities of 366.3 ± 16.4 $U{\cdot}g^{-1}$ (mean ± SD) for shrimp fed with PLA particles compared to 240.2 ± 30.2 $U{\cdot}g^{-1}$ (mean ± SD) for the control shrimp, when using MUF-butyrate as substrate (Fig. 5a, Tukey's HSD: $q = 5.22$, $p < 0.01$). Shrimp fed with LDPE also had significantly higher carboxylesterase activities in the midgut gland compared to the control animals (Fig. 5a, Tukey's HSD: $q = 5.41$, $p = 0.01$). No significant effect on

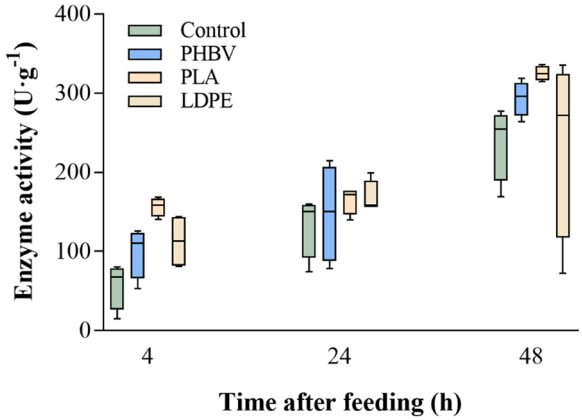


Fig. 3. Carboxylesterase activities in the midgut glands of *Palaemon elegans* after 4, 24 and 48 h of feeding, measured with MUF-heptanoate as substrate (median, 25% and 75% percentile, minimum and maximum, n =12).

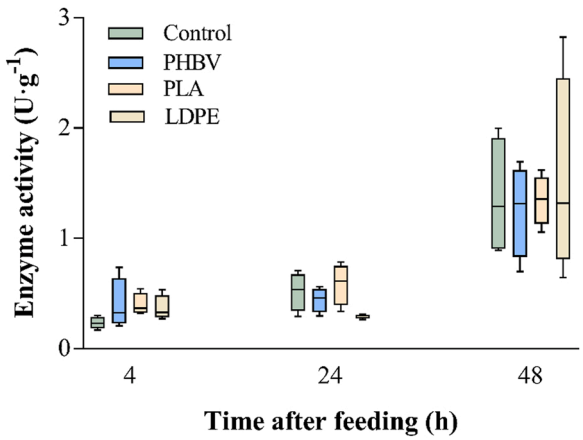


Fig. 4. Carboxylesterase activities in the midgut glands of *Palaemon elegans* after 4, 24 and 48 h of feeding, measured with MUF-oleate as substrate (median, 25% and 75% percentile, minimum and maximum, n =12).

carboxylesterase activity was found in any of the microplastic treatments compared to the control, when using MUF-heptanoate or MUF-oleate as substrate (Fig. 5b, c).

3.3. Oxidative stress response

No significant differences between the SOD activities were observed in the midgut glands of the shrimp when fed a single time (experiment II-IV) with PHBV, PLA, LDPE or only algae food flakes as control ($F_{(3, 36)} =$

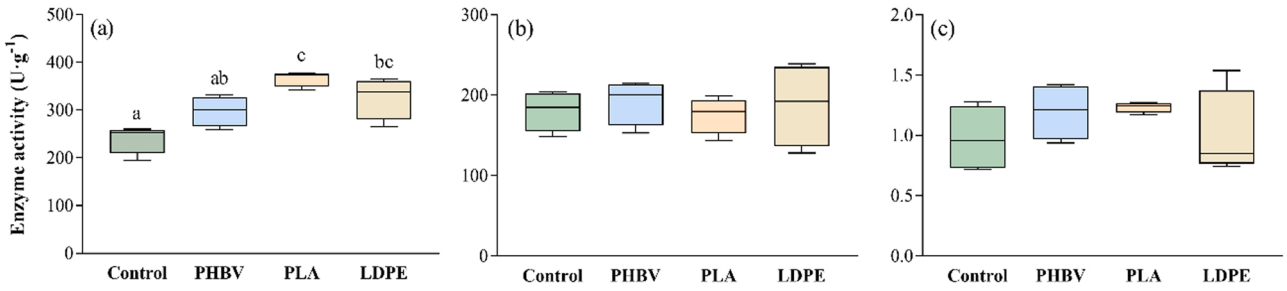


Fig. 5. Carboxylesterase activities in the midgut glands of *Palaemon elegans* fed with PHBV, PLA and LDPE particles, and food flakes only as control, measured after 12 days of continuous feeding with (a) MUF-butyrate, (b) MUF-heptanoate, and (c) MUF-oleate as substrate. Different letters indicate significant differences (median, 25% and 75% percentile, minimum and maximum, n = 4).

0.09, p = 0.45). However, carboxylesterase activities varied significantly over time ($F_{(2, 36)} = 8.52$, $p < 0.01$). *Palaemon elegans* showed the highest SOD activities 48 h after feeding, with 70.5 ± 9.4 U·g^{-1} in shrimp fed with PHBV, 63.6 ± 6.7 U·g^{-1} in shrimp fed with PLA, 56.3 ± 31.2 U·g^{-1} in shrimp fed with LDPE, and 52.5 ± 15.3 U·g^{-1} in control shrimp (means ± SD), being significantly higher than activities after 4 and 24 h (Fig. 6). The main factors time and food treatment did not interact with each other ($F_{(6, 36)} = 1.64$, p = 0.16).

Prolonged feeding of *P. elegans* over 12 days (experiment V) showed no significant differences in SOD activities in the midgut glands between any of the treatments ($F_{(3, 12)} = 1.23$, p = 0.34). The SOD activities varied between 35.2 ± 8.4 U·g^{-1} for shrimp that had been feeding on PLA and 50.1 ± 15.6 U·g^{-1} for individuals feeding on PHBV (means ± SD, Fig. 7).

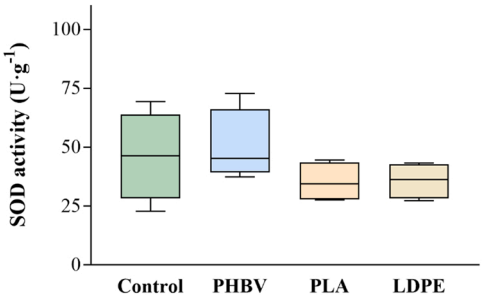


Fig. 7. Superoxide dismutase (SOD) activities in the midgut glands of *Palaemon elegans* after 12 days of continuous feeding (median, 25% and 75% percentile, minimum and maximum, n = 4).

4. Discussion

The rockpool shrimp *Palaemon elegans* is an euryhaline species in coastal waters around Europe, where it inhabits primarily vegetated areas such as seagrass meadows or rocky habitats overgrown with algae (Dalla, 1985; Grabowski, 2006). Its occurrence in the shallow littoral zone makes it vulnerable to anthropogenic pollution, including microplastics that enter the ocean mostly via terrestrial runoffs (Auta et al., 2017). Especially at the Swedish west coast, from where the shrimp were taken, plastic litter is highly abundant (OSPAR, 2017). Seagrass meadows have been shown to trap microplastics that are transported along with currents, potentially acting as sinks for microplastic particles (de Los Santos et al., 2021; Li et al., 2023).

In this study, we investigated the uptake of microparticles of biodegradable and conventional plastics during feeding on algae food flakes mimicking the natural diet of the shrimp. We used higher concentrations of plastics than expected in the environment but resembling those of natural particles, such as sand grains or diatom frustules, which are readily ingested by various species and may induce similar biochemical reactions as anthropogenic particles (e.g. Korez et al., 2020, 2022; Ogonowski et al., 2018; Schmidt et al., 2021). The aim was to better track the particles in the organisms and to induce potential effects to compare them between biodegradable and conventional plastics. However, some shrimp did not accept food flakes offered to them in the experiments. This might be due to behavioral changes during pro-ecdysial or anecdysial stages of moulting, when crustaceans cease feeding (Drach and Tchernigovtzeff, 1967; Lee and Fielder, 1982), or other physical conditions affecting the metabolism, such as diseases (Johnson, 1989). Accordingly, only healthy shrimp that accepted the food were used for further investigations.

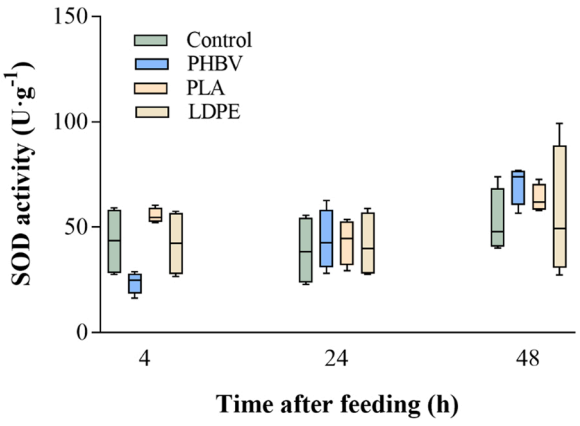


Fig. 6. Superoxide dismutase (SOD) activities in the midgut glands of *Palaemon elegans* at 4, 24, and 48 h after feeding. The two-factorial analyses of variance showed no significant difference between the treatments (median, 25% and 75% percentile, minimum and maximum, n =12).

4.1. Ingestion and retention

Live observations of the shrimp showed that all individuals that accepted the food flakes also ingested microplastics. Particle ingestion was observed for both biodegradable and conventional plastics suggesting that the shrimp take up the particles indiscriminately when feeding on regular food. Concomitant uptake of indigestible particles by various shrimp species during feeding is common and was observed, for example, in North Sea shrimp, *Crangon crangon*, which co-ingested grains from sandy sediments while feeding on tail muscle of conspecifics in laboratory experiments (Schmidt et al., 2021). Korez et al. (2020) reported up to 3000 indigestible particles in individual stomachs of *C. crangon*, which matches the range of particle numbers in the stomachs of *P. elegans* in our study. Other species contained e.g. sand grains, shell fragments of bivalves, and parts of crustacean exoskeletons (Saborowski et al., 2019).

Studies with the amphipod *Gammarus fossarum* suggest that uptake and egestion of biodegradable microplastics does not differ from that of conventional plastics (Straub et al., 2017). However, our results indicate

differences in the uptake and retention of plastics depending on their chemical composition. The inspection of the dissected organs showed that the biodegradable PHBV and PLA particles were retained in high numbers in the stomachs for 8 h after feeding. In contrast, most stomachs of shrimp offered the conventional LDPE plastic particles were void of microplastics 8 h after feeding, although fluorescent microplastics were clearly visible in all shrimp during live observations at two and six hours after food ingestion. Since the particle concentrations in the stomachs were not quantified after two and six hours it cannot be categorically excluded that the shrimp selectively take up less LDPE particles than particles consisting of PLA or PHBV. Alternatively, the shrimp may have eliminated most of the conventional LDPE particles between 6 and 8 hours after ingestion while the biodegradable PHBV and PLA particles were retained inside the stomachs. A longer retention time in the stomach of the shrimp could lead to a higher exposure to associated chemicals potentially leaching from the microplastics. Some plastic particles were egested with the feces. The amount of LDPE particles in the feces were substantially lower than the numbers of microplastics in fecal pellets of animals that had been feeding on food enriched with PLA and PHBV particles. Accordingly, a substantial fraction of the LDPE particles that had been detected visually may not have passed the gut of the animals to be released with the feces. As *P. elegans* is an omnivorous species that also feeds on detritus, the shrimp is likely exposed to considerable loads of indigestible particles with their natural food (Janas and Baranska, 2008). Other species, such as the North Sea shrimp *Crangon crangon*, but also the congener Atlantic ditch shrimp *Palaemon varians*, possess mechanisms to cope with ingested indigestible particles. Both species have been shown to regurgitate indigestible particles including microplastics (Korez et al., 2019; Saborowski et al., 2019; Schmidt et al., 2021). Potentially, *P. elegans* is also capable of regurgitating ingested indigestible particles, such as microplastics. Accordingly, the majority of the ingested LDPE particles might have been evacuated through the esophagus between the last live observation (6 h) and dissection (8 h) resulting in low numbers of LDPE particles in the intestines and in the fecal material.

In contrast to the LDPE particles, the biodegradable PHBV and PLA particles, were found in high numbers in the dissected stomachs. Saborowski et al. (2019) found in *P. varians* that sole fluorescent polyacryl fibers without nutritional value were most rapidly evacuated (50% after about 7 h) by regurgitation. However, when the particles were taken up together with food particles, they remained in the stomach for more than 11 h (50% evacuation). The authors suggested that the shrimp may perceive the nutritional value of ingested material and retain digestible matter longer in the stomach than indigestible particles. Organisms with non-selective feeding habits can have other mechanisms to optimize the net energy intake, e.g. by adjusting the uptake or absorption rate, but also the gut passage times (Taghon, 1981; Cammen, 1989). Accordingly, the faster evacuation of the LDPE particles from the stomach may indicate a lower nutritional value or digestibility of the conventional polymer as compared to the two biodegradable plastics.

4.2. Digestion

Biodegradable materials can be degraded enzymatically by organisms and may potentially provide nutrients that can be assimilated (Wang et al., 2022). The activities of digestive enzymes are adjusted in invertebrates depending on the availability of food, its quality, and the state of starvation of the organism (Johnston and Freeman, 2005; Karasov and Douglas, 2013; Koussoroplis et al., 2017). Accordingly, the ingestion of microplastics has been shown to alter digestive enzyme activities in, e.g., fish, mollusks, and crustaceans (Korez et al., 2019; Romano et al., 2018; Wang et al., 2020). Enzyme activities may also be modulated by polymer type of ingested plastics, as shown for the mussel *Mytilus galloprovincialis* (Trestrail et al., 2021).

In our experiments, *P. elegans* fed with algae flakes with biodegradable PLA and PHBV, and with conventional LDPE microplastics exhibited elevated short-chain carboxylesterase activities in the midgut gland. Esterases are a diverse group of enzymes that are capable of hydrolyzing ester bonds potentially resulting in the release of potential nutrients from ingested food items or macro-molecules. The increase in esterase activity in response to the ingestion of microplastics may indicate a molecular or sensory resemblance to natural food (Luo et al., 2020). This increase in activity may have been induced by chemicals leaching from the plastics, which are added to the material during production to shape their properties. Chemical additives of plastics may exhibit bioactive properties and affect digestive processes (Barrick et al., 2021). Moreover, only shrimp that were fed with PLA microparticles showed elevated medium-chain carboxylesterase activities in the midgut gland. The medium-chain carboxylester MUF-heptanoate is not only a substrate for esterase, but also for lipase activity. Lipases are a subclass of esterases that have also been shown to increase in nauplii of the brine shrimp *Artemia persimilis* when exposed to PLA (Barbir et al., 2023). Interestingly, the ingestion of PLA microplastics also led to the highest increase in short-chain carboxylesterase. Bacterial carboxylesterases, for example, can hydrolyze the polymer PBAT, which is also a component of the PLA used in our study (Wu et al., 2023). A recent study also reports the presence of carboxylesterases in the gastric fluid of the edible crab *Cancer pagurus* (Crustacea, Decapoda), which are capable of hydrolyzing biodegradable plastics made from PLA and PBAT (Miksch et al., 2023). However, it is unclear if the carboxylesterases in *P. elegans* can also hydrolyze the ingested PLA microparticles. The increased biodegradability combined with the longer retention time of PLA microparticles in the shrimp may result in an increased leaching of associated chemicals, which, in turn, may have induced the elevated short- and medium-term carboxylesterase activities.

Similar to acute feeding, continuous feeding on microplastics over 12 days stimulated the short-chain esterase activity for shrimp fed with PLA and LDPE particles. LDPE contains no ester-bonds that could be hydrolyzed by these enzymes, making this increase more likely to be due to leaching additives rather than degradation products of the polymer. Carboxylesterases not only play an important role in physiological processes such as digestion, but are also known for hydrolyzing plastic additives such as phthalates (Ozaki et al., 2017). The PLA and LDPE used in this study were intended to be used for comparable applications as foils or films and may, therefore, share some characteristics, which may induce a similar increase in carboxylesterase activity by the two types of plastics.

4.3. Oxidative stress response

Ingestion of microplastics induces oxidative stress in various crustacean species such as *P. varians*, *Litopenaeus vannmei* or *Procambarus clarkii* (Saborowski et al., 2022; Zeng et al., 2023; Zhang et al., 2022), while other crustaceans such as *C. crangon* did not show an oxidative stress response upon uptake of microplastics (Korez et al., 2022). Oxidative stress responses can be induced by associated pollutants or by the particles themselves (e.g. Ahmad, 1995; Valavanidis et al., 2006; Magesky and Pelletier, 2018; Almeida et al., 2019), or by leaching additives from biodegradable single-use plastics (Savva et al., 2023). As a defense response, cells release reactive oxygen species (ROS). Reactive oxygen species do not only attack pathogens, but can also induce damage to own tissue, which is why cells rely on an antioxidant defense system that removes ROS from the cells (Halliwell and Gutteridge, 2015). This process is initiated by antioxidants such as the enzymes superoxide dismutase (SOD).

In this study, *P. elegans* showed no oxidative stress response after ingestion of microplastics. Neither feeding the shrimp a single time, nor feeding the shrimp continuously over 12 days with microplastics showed an increase in SOD activity. This was evident in midgut glands of shrimp offered algae flakes with conventional LDPE particles, as well as biodegradable PLA and PHBV particles. Similarly, the North Sea shrimp *C. crangon* did not show elevated activities of antioxidant enzymes after

ingestion of biodegradable PLA and conventional polyvinyl chloride (PVC) microparticles (Korez et al., 2022). *Crangon crangon* is adapted to the ingestion of indigestible particles by a fine-meshed filter in the stomach, which prevents larger particles from entering the midgut gland and by regurgitation of particles to void the stomach. The lack of oxidative response of *P. elegans* fed with microplastics may be in line with the assumption that *P. elegans* can evacuate the stomach by regurgitation, which prevents the advancement of particles into the midgut gland, which is the biochemically active organ.

In contrast, *P. varians* displayed increasing SOD activities in midgut gland tissue of individuals that were constantly exposed to polystyrene microbeads of different sizes (Saborowski et al., 2022). In that species, the SOD activity increased over the first eight hours of exposure and remained constant or decreased thereafter. However, microplastics used by Saborowski et al. (2022) were significantly smaller with diameters between 0.1 and 9.9 μm. Smaller particles are more likely to pass the pyloric filter and enter the midgut gland, where cells may perceive them as pathogens and produce superoxide ions to destroy them and increase SOD activity as counter reaction to protect own tissues (Inada et al., 2012). Because of the rather large size (up to 200 μm) of the particles used in the current study, the probability of smaller particles reaching the midgut gland is low.

5. Conclusion

The rockpool shrimp *P. elegans* ingested all offered microplastics, irrespective of the polymer type. The results indicate that ingested biodegradable plastic particles are retained longer in the stomach of shrimp than particles from conventional plastics suggesting a higher nutritional value of the biodegradable plastics. Ingestion of both conventional and biodegradable microplastics can alter digestive enzyme activities, with ingestion of PLA showing the strongest induction. However, ingestion of microplastics did not induce oxidative stress in *P. elegans*. Still, longer retention times and biodegradability might enhance the overall exposure of the animals to the ingested material, which may have long-term effects resulting from, for example, leaching of chemical additives. As a consequence, the image of biodegradable plastics as environmentally friendly alternative to conventional plastics needs to be reconsidered.

Funding

This research has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 860407 (BIO-PLASTICS EUROPE).

CRediT authorship contribution statement

Anna-Sara Krång: Conceptualization, Supervision, Writing – review & editing. **Lukas Miksch:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Maria Granberg:** Conceptualization, Supervision, Writing – review & editing. **Chiau Yu Chen:** Investigation, Methodology, Writing – review & editing. **Reinhard Saborowski:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Lars Gutow:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Kristine Reuter, Agnes Niggenaber, Alejandro Martinez-Marcon and Christian Prantler for being a great help in experimental set up, laboratory analyses, as well as sampling of the shrimp. We acknowledge the support of the staff from Kristineberg Center for Marine Research and Innovation and the Alfred Wegener Institute Bremerhaven.

References

Ahmad, S., 1995. Oxidative stress from environmental pollutants. Arch. Insect Biochem. Physiol. 29, 135–157. https://doi.org/10.1002/arch.940290205.

Almeida, J.C., Cardoso, C.E.D., Pereira, E., Freitas, R., 2019. Toxic effects of metal nanoparticles in marine invertebrates. In: Gonçalves, G., Marques, P. (Eds.), Nanostructured Materials for Treating Aquatic Pollution. Engineering Materials. Springer, Cham. https://doi.org/10.1007/978-3-030-33745-2_7.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environ. Int. 102, 165–176. https://doi.org/10.1016/j.envint.2017.02.013.

Barrick, A., Champeau, O., Chatel, A., Manier, N., Northcott, G., Trambly, L.A., 2021. Plastic additives: challenges in ecotox hazard assessment. PeerJ 9, e11300.

Barbir, J., Arato, E., Yu Chen, C., Granberg, M., Gutow, L., Krang, A.S., Kröger, S.D., Leal, W., Liwarska-Bizukojc, E., Miksch, L., Paetz, K., Prodana, M., Saborowski, R., Rojas, R.S., Witt, G., 2023. Assessing eco-toxicity of an innovative bio-based mulch film: a multienvironmental and multi-bioassay approach. Front. Environ. Sci. 11, 1171261. https://doi.org/10.3389/fenvs.2023.1171261.

Briones-Fourzán, P., Monroy-Velázquez, L.V., Estrada-Olivo, J., Lozano-Álvarez, E., 2020. Diversity of seagrass-associated decapod crustaceans in a tropical reef lagoon prior to large environmental changes: a baseline study. Diversity 12 (5), 205. https://doi.org/10.3390/d12050205.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23 (23), 2388–2392. https://doi.org/10.1016/j.cub.2013.10.012.

Cammen, L.M., 1989. In: Lopez, G., Taghon, J., Levinton (Eds.), Ecology of marine deposit feeders. Lecture Notes on Coastal and Estuarine Studies, 31. Springer, New York, NY. https://doi.org/10.1007/978-1-4684-7671-2_9G.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49 (2), 1130–1137. https://doi.org/10.1021/es504525u.

Dalla, Via J., 1985. Oxygen consumption and temperature change in the shrimp *Palaemon elegans*. Mar. Ecol. Prog. Ser. 26, 199–202.

D'Costa, A.H., 2022. Microplastics in decapod crustaceans: accumulation, toxicity and impacts, a review. Sci. Total Environ. 832, 154963 https://doi.org/10.1016/j.scitotenv.2022.154963.

Drach, P., Tchernigovtzeff, C., 1967. Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. Vie et Milieu 595–610.

Endres, H.-J., 2017. Bioplastics. In: Wagemann, Tippkötter (Eds.) Biorefineries. Advances in Biochemical Engineering/Biotechnology, Volume 166. Springer International Publishing, Cham, Switzerland, pp. 427–468, 10.1007/10_2016_75K.

Farady, S.E., 2019. Microplastics as a new, ubiquitous pollutant: strategies to anticipate management and advise seafood consumers. Mar. Policy 104, 103–107. https://doi.org/10.1016/j.marpol.2019.02.020.

von Friesen, L.W., Granberg, M.E., Hassellöv, M., Gabrielsen, G.W., Magnusson, K., 2019. An efficient and gentle enzymatic digestion protocol for the extraction of microplastics from bivalve tissue. Mar. Pollut. Bull. 142, 129–134. https://doi.org/10.1016/j.marpolbul.2019.03.016.

Grabowski, M., 2006. Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. Aquat. Invasions 1 (3), 116–123.

Gray, A.D., Weinstein, J.E., 2017. Size-and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (*Palaemonetes pugio*). Environ. Toxicol. Chem. 36 (11), 3074–3080. https://doi.org/10.1002/etc.3881.

Hakkarainen, M., 2002. Aliphatic Polyesters: Abiotic and biotic degradation and degradation products. In: Degradable aliphatic polyesters. Advances in Polymer Science, vol 157. Springer, Berlin, Heidelberg. https://doi.org/10.1007/3-540-45734-8_4.

Halliwell, B., Gutteridge, J.M.C., 2015. Free radicals in biology and medicine, 5th Edition. Oxford University Press, USA.

Inada, M., Sudhakaran, R., Kihara, K., Nishi, J., Yoshimine, M., Mekata, T., Kono, T., Sakai, M., Yoshida, T., Itami, T., 2012. Molecular cloning and characterization of the NADPH oxidase from the kuruma shrimp, Marsupenaeus japonicus: early gene up-regulation after Vibrio penaeicida and poly(I:C) stimulations in vitro. Mol. Cell. Probes. 26 (1), 29–41. https://doi.org/10.1016/j.mcp.2011.11.002.

Isobe, A., Azuma, T., Cordova, M.R., Cózar, A., Galgani, F., Hagita, R., Kanhai, L.D., Imai, K., Iwasaki, S., Kako, S., Kozlovskii, N., Lusher, A.L., Mason, S.A., Michida, Y., Mituhasi, T., Morii, Y., Mukai, T., Popova, A., Shimizu, K., Tokai, T., Uchida, K., Yagi, M., Zhang, W., 2021. A multilevel dataset of microplastic abundance in the world's upper ocean and the Laurentian Great Lakes. Micro Nanoplastics 1 (1), 14. https://doi.org/10.1186/s43591-021-00013-z.

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

Janas, U., Baranska, A., 2008. What is the diet of *Palaemon elegans* Rathke, 1837 (Crustacea, Decapoda), a non-indigenous species in the Gulf of Gdańsk (southern Baltic Sea)? Oceanologia 50 (2), 221–237.

Johnson, S.K., 1989. Handbook of shrimp diseases. Texas Agricultural and Mechanical University, National Sea Grant Program, TAMU-SG, 90-601. 19 pp.

Johnston, D., Freeman, J., 2005. Dietary preference and digestive enzyme activities as indicators of trophic resource utilization by six species of crab. Biol. Bull. 208 (1), 36–46. https://doi.org/10.2307/3593099.

Karasov, W.H., Douglas, A.E., 2013. Comparative digestive physiology. Compr. Physiol. 3 (2), 741. https://doi.org/10.1002/cphy.c110054.

Knotz, S., Boersma, M., Saborowski, R., 2006. Microassays for a set of enzymes in individual small marine copepods. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 145 (3), 406–411. https://doi.org/10.1016/j.cbpa.2006.07.019.

Korez, Š., Gutow, L., Saborowski, R., 2019. Feeding and digestion of the marine isopod *Idotea emarginata* challenged by poor food quality and microplastics. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 226, 108586 https://doi.org/10.1016/j.cbpc.2019.108586.

Korez, Š., Gutow, L., Saborowski, R., 2020. Coping with the "dirt": brown shrimp and the microplastic threat. Zoology 143, 125848. https://doi.org/10.1016/j.zool.2020.125848.

Korez, Š., Gutow, L., Saborowski, R., 2022. Fishing in troubled waters: limited stress response to natural and synthetic microparticles in brown shrimp (*Crangon crangon*). Environ. Pollut. 302, 119023 https://doi.org/10.1016/j.envpol.2022.119023.

Koussoroplis, A.M., Schwarzenberger, A., Wacker, A., 2017. Diet quality determines lipase gene expression and lipase/esterase activity in *Daphnia pulex*. Biol. Open 6 (2), 210–216. https://doi.org/10.1242/bio.022046.

Lee, C.L., Fielder, D.R., 1982. Maintenance and reproductive behaviour in the freshwater prawn *Macrobrachium australiense* Holthuis (Crustacea: Decapoda: Palaemonidae). Mar. Freshw. Res. 33 (4), 629–646. https://doi.org/10.1071/MF9820629.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103.

Li, C., Zhu, L., Li, W.T., Li, D., 2023. Microplastics in the seagrass ecosystems: a critical review. Sci. Total Environ., 166152 https://doi.org/10.1016/j.scitotenv.2023.166152.

Livingstone, D.R., Lips, F., Martinez, P.G., Pipe, R.K., 1992. Antioxidant enzymes in the digestive gland of the common mussel Mytilus edulis. Mar. Biol. 112, 265–276. https://doi.org/10.1007/BF00702471.

Lo, H.K.A., Chan, K.Y.K., 2018. Negative effects of microplastic exposure on growth and development of *Crepidula onyx*. Environ. Pollut. 233, 588–595. https://doi.org/10.1016/j.envpol.2017.10.095.

de Los Santos, C.B., Krång, A.S., Infantes, E., 2021. Microplastic retention by marine vegetated canopies: simulations with seagrass meadows in a hydraulic flume. Environ. Pollut. 269, 116050 https://doi.org/10.1016/j.envpol.2020.116050.

Luo, H., Zhao, Y., Li, Y., Xiang, Y., He, D., Pan, X., 2020. Aging of microplastics affects their surface properties, thermal decomposition, additives leaching and interactions in simulated fluids. Sci. Total Environ. 714, 136862 https://doi.org/10.1016/j.scitotenv.2020.136862.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9 (9) https://doi.org/1346-1360.10.1039/C6AY02415G.

Magesky, A., Pelletier, É., 2018. In: Saquib, Q., Faisal, M., Al-Khedhairy, A., Alatar, A. (Eds.), Advances in Experimental Medicine and Biology, 1048. Springer, Cham. https://doi.org/10.1007/978-3-319-72041-8_17Q.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zambonino-Infante, J., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Mar. Environ. Res. 112, 78–85. https://doi.org/10.1016/j.marenvres.2015.09.009.

Miksch, L., Koeck, M., Gutow, L., Saborowski, R., 2022. Bioplastics in the sea: rapid in-vitro evaluation of degradability and persistence at natural temperatures. Front. Mar. Sci. 9, 920293 https://doi.org/10.3389/fmars.2022.920293.

Miksch, L., Gutow, L., Saborowski, R., 2023. Gastric carboxylesterases of the edible crab *Cancer pagurus* (Crustacea, Decapoda) can hydrolyze biodegradable plastics. Emerg. Contam., 100275 https://doi.org/10.1016/j.emcon.2023.100275.

Nazareth, M., Marques, M.R.C., Leite, M.C.A., Castro, Í.B., 2019. Commercial plastics claiming biodegradable status: is this also accurate for marine environments? J. Hazard. Mater. 366, 714–722. https://doi.org/10.1016/j.jhazmat.2018.12.052.

Ogonowski, M., Gerdes, Z., Gorokhova, E., 2018. What we know and what we think we know about microplastic effects–a critical perspective. Curr. Opin. Environ. Sci. Health. 1, 41–46. https://doi.org/10.1016/j.coesh.2017.09.001.

Okoffo, E.D., Chan, C.M., Rauert, C., Kaserzon, S., Thomas, K.V., 2022. Identification and quantification of micro-bioplastics in environmental samples by pyrolysis–gas chromatography–mass spectrometry. Environ. Sci. Technol. 56 (19), 13774–13785. https://doi.org/10.1021/acs.est.2c04091.

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175.

OSPAR (2017). Beach Litter – Abundance, Composition and Trends. Sheet reference: EIHA17/D1002. https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/pressures-human-activities/marine-litter/beach-litter/ (accessed 07.09.2023).

Ozaki, H., Sugihara, K., Watanabe, Y., Moriguchi, K., Uramaru, N., Sone, T., Ohta, S., Kitamura, S., 2017. Comparative study of hydrolytic metabolism of dimethyl phthalate, dibutyl phthalate and di (2-ethylhexyl) phthalate by microsomes of various rat tissues. Food Chem. Toxicol. 100, 217–224. https://doi.org/10.1016/j.fct.2016.12.019.

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., Gerdts, G., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9 (1), 1505. https://doi.org/10.1038/s41467-018-03825-5.

Romano, N., Ashikin, M., Teh, J.C., Syukri, F., Karami, A., 2018. Effects of pristine polyvinyl chloride fragments on whole body histology and protease activity in silver barb *Barbodes gonionotus* fry. Environ. Pollut. 237, 1106–1111. https://doi.org/10.1016/j.envpol.2017.11.040.

Rosato, A., Romano, A., Totaro, G., Celli, A., Fava, F., Zanaroli, G., Sisti, L., 2022. Enzymatic degradation of the most common aliphatic bio-polyesters and evaluation of the mechanisms involved: an extended study. Polymers 14 (9), 1850. https://doi.org/10.3390/polym14091850.

Saborowski, R., 2015. Nutrition and digestion. In: Chang, E., Thiel, M. (Eds.), Natural History of the Crustacea. Vol IV Physiological regulation. Oxford University Press, New York, pp. 285–319 (pp).

Saborowski, R., Paulischkis, E., Gutow, L., 2019. How to get rid of ingested microplastic fibers? A straightforward approach of the Atlantic ditch shrimp *Palaemon varians*. Environ. Pollut. 254, 113068 https://doi.org/10.1016/j.envpol.2019.113068.

Saborowski, R., Korez, Š., Riesbeck, S., Weidung, M., Bickmeyer, U., Gutow, L., 2022. Shrimp and microplastics: a case study with the Atlantic ditch shrimp *Palaemon varians*. Ecotoxicol. Environ. Saf. 234, 113394 https://doi.org/10.1016/j.ecoenv.2022.113394.

Savva, K., Borrell, X., Moreno, T., Pérez-Pomeda, I., Barata, C., Llorca, M., Farré, M., 2023. Cytotoxicity assessment and suspected screening of plastic additives in bioplastics of single-use household items. Chemosphere 313, 137494. https://doi.org/10.1016/j.chemosphere.2022.137494.

Schmidt, N., Korez, Š., Saborowski, R., Gutow, L., 2021. Sand grains in the stomach of brown shrimp, *Crangon crangon*: crunchy garnish, supportive macerator, or simply dirt? J. Sea Res. 170, 102020 https://doi.org/10.1016/j.seares.2021.102020.

Shim, W.J., Song, Y.K., Hong, S.H., Jang, M., 2016. Identification and quantification of microplastics using Nile Red staining. Mar. Pollut. Bull. 113 (1–2), 469–476. https://doi.org/10.1016/j.marpolbul.2016.10.049.

Straub, S., Hirsch, P.E., Burkhardt-Holm, P., 2017. Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *Gammarus fossarum*. Int. J. Environ. Res. Public Health 14 (7), 774. https://doi.org/10.3390/ijerph14070774.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113 (9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Taghon, G.L., 1981. Beyond selection: optimal ingestion rate as a function of food value. Am. Nat. 118 (2), 202–214.

Trestrail, C., Walpitagama, M., Miranda, A., Nugegoda, D., Shimeta, J., 2021. Microplastics alter digestive enzyme activities in the marine bivalve, *Mytilus galloprovincialis*. Sci. Total Environ. 779, 146418 https://doi.org/10.1016/j.scitotenv.2021.146418.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64 (2), 178–189. https://doi.org/10.1016/j.ecoenv.2005.03.013.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013.

Vogt, G., 2021. Synthesis of digestive enzymes, food processing, and nutrient absorption in decapod crustaceans: a comparison to the mammalian model of digestion. Zoology 147, 125945. https://doi.org/10.1016/j.zool.2021.125945.

Wang, L., Peng, Y., Xu, Y., Zhang, J., Liu, C., Tang, X., Lu, Y., Sun, H., 2022. Earthworms' degradable bioplastic diet of polylactic acid: easy to break down and slow to excrete. Environ. Sci. Technol. 56 (8), 5020–5028. https://doi.org/10.1021/acs.est.1c08066.

Wang, X., Huang, W., Wei, S., Shang, Y., Gu, H., Wu, F., Lan, Z., Hu, M., Shi, H., Wang, Y., 2020. Microplastics impair digestive performance but show little effects on antioxidant activity in mussels under low pH conditions. Environ. Pollut. 258, 113691 https://doi.org/10.1016/j.envpol.2019.113691.

Wei, X.F., Capezza, A.J., Cui, Y., Li, L., Hakonen, A., Liu, B., Hedenqvist, M.S., 2022. Millions of microplastics released from a biodegradable polymer during biodegradation/enzymatic hydrolysis. Water Res. 211, 118068 https://doi.org/10.1016/j.watres.2022.118068.

Welden, N.A., Cowie, P.R., 2016. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. Environ. Pollut. 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020.

Wu, P., Li, Z., Gao, J., Zhao, Y., Wang, H., Qin, H., Gu, Q., Wie, R., Liu, W., Han, X., 2023. Characterization of a PBAT Degradation Carboxylesterase from *Thermobacillus composti* KWC4. Catalysts 13 (2), 340. https://doi.org/10.3390/catal13020340.

Zeng, Y., Deng, B., Kang, Z., Araujo, P., Mjøs, S.A., Liu, R., Lin, J., Yang, T., Qu, Y., 2023. Tissue accumulation of polystyrene microplastics causes oxidative stress, hepatopancreatic injury and metabolome alterations in *Litopenaeus vannamei*. Ecotoxicol. Environ. Saf. 256, 114871 https://doi.org/10.1016/j.ecoenv.2023.114871.

Zhang, X., Jin, Z., Shen, M., Chang, Z., Yu, G., Wang, L., Xia, X., 2022. Accumulation of polyethylene microplastics induces oxidative stress, microbiome dysbiosis and immunoregulation in crayfish. Fish. Shellfish Immunol. 125, 276–284. https://doi.org/10.1016/j.fsi.2022.05.005.

Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to Daphnia magna. Environ. Pollut. 267, 115392. https://doi.org/10.1016/j.envpol.2020.115392.