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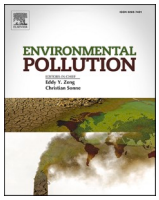

Acute and partial life-cycle toxicity of a tri-polymer blend of microplastics in the copepod *Acartia tonsa*☆

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ABSTRACT

Microplastics are a prolific environmental contaminant that pose a risk to marine organisms. Ecotoxicological studies have identified microplastics can cause sub-lethal harm to aquatic biota. However, prior studies often lack comparability and environmental relevance, for example focussing upon monodisperse beads at extremely high concentrations. Copepods are keystone marine taxa that play vital roles in the marine food web and biogeochemical cycling. In this study, we adapted ISO methods to conduct acute and partial life-cycle toxicity tests exposing adult and juvenile life stages of the copepod *Acartia tonsa* to a fully characterised tri-polymer microplastic blend comprising cryoground polyethylene, polypropylene, and nylon particles (5–100 μm) at concentrations ranging 0–1000 μg L^{-1}. The tests considered the toxicity of microplastics on a wide number of endpoints including adult survival, algal ingestion rates, egg production and size, larval development ratio and juvenile survival. Mortality, egg size and larval development ratio proved to be the most sensitive endpoints. The tri-polymer blend had an $LC50_{72h}$ value of 182 μg L^{-1} providing a baseline for future toxicity testing using this method.

1. Introduction

Microplastics are ubiquitous environmental contaminants found in natural ecosystems across the globe, including sediments, soils, ground-, fresh- and marine water and sea ice (Rochman, 2018). They are now considered an irreversible and planetary boundary threat (Morasae et al., 2024; Villarrubia-Gómez et al., 2018). These solid, insoluble polymeric particles and fibres, with any dimension ranging 1–1000 μm (ISO, 2023), are either manufactured to a microscopic size (primary microplastics) or derive from the fragmentation of macroplastics (secondary microplastics) (Cole et al., 2011; Hartmann et al., 2019). Secondary microplastics stem from a wide variety of domestic, commercial, industrial, agricultural and maritime materials, including single-use plastics, packaging, textiles, tyres and paints (Knight et al., 2020; Muller-Karanassos et al., 2021; Wang et al., 2021). Aeolian deposition, wind, run-off, rivers and wastewater systems facilitate the transport of macro- and microplastics into the natural environment where they are subject to photooxidative, physical and biological degradation (Auta et al., 2017; Zhang et al., 2021). As such, microplastics in environmental settings are diverse in polymer, size, shape, chemical composition, colour and state of degradation (Kooi et al., 2021; Rochman et al., 2019; Thompson et al., 2024).

Owing to their prevalence and small size, microplastics are readily adhered to, inhaled or ingested by a vast array of terrestrial, freshwater, and marine species (Chang et al., 2022). Ecotoxicological studies have evidenced microplastic exposure can cause genotoxicity (Sun et al., 2021), cytotoxicity (Fleury and Baulin, 2021), heightened immune response (Détrée and Gallardo-Escárate, 2018), physiological effects (Sussarellu et al., 2016) and altered behaviour (McCormick et al., 2020). While a lack of methodological harmonisation makes broader

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comparative analyses challenging (de Ruijter et al., 2020; Horn et al., 2020; Thornton Hampton et al., 2022a; Thornton Hampton et al., 2022b), a recent meta-analysis concluded that microplastics are hazardous to aquatic life, posing a greater toxicological risk than natural particulates (Ogonowski et al., 2023). As small microplastics are diverse and multifaceted, with different physiochemical properties and associated matters such as chemicals and microorganisms, it is crucial for future research to consider the multidimensional attributes of microplastics (Li et al., 2024). A major limitation of many previous microplastic toxicity studies is that they lack environmental relevance; for example, using a singular type of particle (e.g. spherical beads which are rarely identified in environmental samples) with a narrow size distribution (i.e. monodisperse) dosed at unrealistically high particle concentrations (Connors et al., 2017; Gouin et al., 2019; Gouin et al., 2024; Gouin et al., 2022; Pencik et al., 2023). Identifying the harm microplastics pose to reproduction, growth and survival of keystone organisms is crucial in driving targeted interventions, including regulatory and legislative change (e.g. Global Plastics Treaty) (Aanesen et al., 2024). Improving the environmental relevance of toxicological studies and incorporating dose-response metrics will facilitate the development of more environmentally relevant risk assessments and thresholds (Chowdhury et al., 2024; Mehinto et al., 2022; Thornton Hampton et al., 2022a).

Copepods are among one of the most abundant and diverse groups of marine zooplankton, with habitats ranging from coastal waters to the open ocean, and from polar regions to tropical seas. Marine copepods play a critical role in the global ocean, supporting food webs, and contributing to fisheries productivity, nutrient flux, and carbon sequestration (Botterell et al., 2023; Turner, 2015). Given their ecological importance, global distribution and high abundance, sensitivity to environmental stressors, and ease of culturing, copepods have been recommended as valuable model organisms for toxicological testing (Nilsson et al., 2018; Raisuddin et al., 2007). In this study, we investigate the toxicity of a tri-polymer blend of microplastics, comprising irregularly shaped cryo-milled particles of low-density polyethylene (LDPE), polypropylene (PP) and nylon (PA-6) ranging 5–100 μm diameter, on the marine copepod *Acartia tonsa*. These polymers were selected as they are commonly found within environmental water samples and are representative of microplastics found throughout the water column due to their different densities (Erni-Cassola et al., 2019). *A. tonsa,* is a globally distributed calanoid copepod, prevalent in estuaries, coastal waters, and upwelling zones, with a short life-cycle with distinct morphological differences between life stages (Fig. 1A) (Besiktepe and Dam, 2020). Prior studies have observed 6–31 μm microplastics can be readily ingested by *A. tonsa* (Cole et al., 2013; Shore et al., 2021; Svetlichny et al., 2021), while 0.4–3.8 μm microplastics can adhere to the carapace and appendages of calanoid copepods (Cole et al., 2013). To facilitate methodological harmonisation and promote comparative analyses between studies (de Ruijter et al., 2020; Horn et al., 2020; Thornton Hampton et al., 2022a; Thornton Hampton et al., 2022b), our methodology comprises a 72-h acute toxicity test with adult *A. tonsa* adapted from ISO14669 "Determination of acute lethal toxicity to marine copepods" (ISO, 1999), and a partial life-cycle test recommended in ISO16778 "Calanoid copepod early-life stage test with Acartia tonsa" (ISO, 2015). The microplastics constituting the tri-polymer blend were chemically characterised with thermal desorption and pyrolysis GC-MC as well as GCxGC-MS to elucidate and document the chemicals present in the polymers which could potentially contribute to observed toxicity. The study aims to elucidate whether acute and chronic exposure to a blend of microplastics poses a lethal or sublethal risk to a globally relevant marine species, with dose-response endpoints supporting the development of risk assessments and thresholds.

2. Materials & methods

2.1. Seawater, microalgae, and copepods

Filtered seawater (FSW) was prepared by filtering natural seawater, collected from station L4 in the western English Channel (McEvoy et al., 2023), through a 0.22 μm filter (Whatman) and diluting with ultrapure water (Milli-Q) to achieve the required salinity (i.e. 20–30 ‰). Temperature, salinity, pH, and oxygen saturation of FSW and experimental media was routinely monitored (Hach HQ4300 multiprobe), with all parameters meeting the ISO16778 validity criteria (ISO, 2015).

Microalgal prey comprised *Tetraselmis suecica* (4–10 μm) and *Tisochrysis lutea* (3–8 μm), purchased from Reefshotz® (Swansea, UK), and maintained in autoclaved FSW with Guillard's F/2 algal nutrient media. Microalgal concentrations (cells mL^{-1}) were ascertained using a Sedgewick-Rafter chamber and fed to copepods at a concentration of 50,000 cells mL^{-1} in a ratio of 10 % *T. suecica* and 90 % *T. lutea* by cell count. This blend of microalgal prey is suitable for maintaining the health of the copepods throughout culturing and experimental work.

Cultures of *A. tonsa* were procured (ReefShotz®) and maintained in controlled-temperature laboratories at Plymouth Marine Laboratory (UK). Copepods were acclimated for a minimum of 48 h in 5 L beakers containing lightly aerated FSW with microalgal prey. Prior to experimental use, copepod stocks were carefully poured through a 200 μm sieve suspended in a basin of FSW to isolate adults, and then adult female *A. tonsa* selected based upon morphological features. A wide-bore liquid pipette was used to transfer adult life-stages to an evaporating dish to confirm copepod identification and condition (i.e. motile with no damage to antennules). Following this, copepods were subsequently maintained in a 5 L beaker of FSW for 24 h prior to experimental use. Adult male and female *A. tonsa* were used for the acute toxicity test, and only female *A. tonsa* used for the partial-life cycle test.

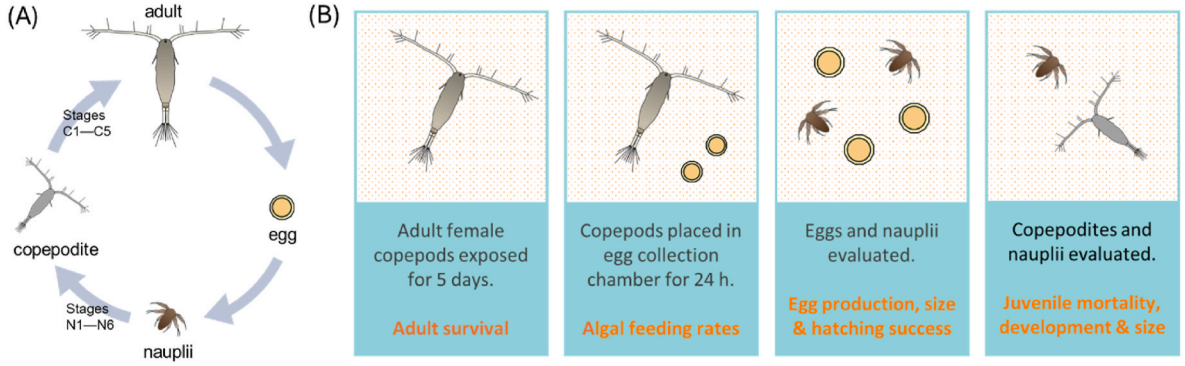

Fig. 1. (A) *Acartia tonsa* life cycle, whereby fertilised eggs hatch into nauplii (six stages), which subsequently develop into copepodites (five stages), before maturing into adults. (B) The partial life-cycle toxicity test involves exposing adult female copepods and their progeny to particulates, with evaluations of a number of endpoints (orange text). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Microplastic preparation and characterisation

Microplastics comprised low density polyethylene (LDPE), polypropylene (PP) and nylon (PA-6) microplastics. These buoyant (PP ~0.9 g cm^{-3}; LDPE ~0.9 g cm^{-3}) and neutrally buoyant (PA ~1.14g cm^{-3}) polymers are ubiquitous in marine surface waters where zooplankton feed (Cui et al., 2022; Lindeque et al., 2020; Ramírez-Álvarez et al., 2020). Cryomilled microplastics (<200 μm; 18,000 RPM high-speed rotor mill) were prepared from virgin pellets by Carat GmbH. In the laboratory, microplastic stocks were dry-sieved and particles >100 μm were further cryoground by-hand using liquid nitrogen and a ceramic pestle and mortar. A 5–100 μm size fraction of each microplastic stock was attained by suspending the cryoground microplastics in ultrapure water and sequentially vacuum filtering the particles through 100 μm meshes and 5 μm Nucleopore membrane filters (Whatman). Particles of this size fall within the optimal prey size of *A. tonsa* (~7–250 μm depending on life-stage) (Berggreen et al., 1988). Microplastics were carefully transferred into glass Petri dishes using a stainless-steel spatula, and then covered with aluminium foil and dried at 60 °C for 48 h. A 1 g L^{-1} tri-polymer microplastic stock (5–100 μm; 1:1:1 ratio of polymers by mass) was created by adding 115 mg d.*w.* (Sartorius R200D mass balance) of each polymer to a 500 mL glass bottle containing 345 mL of FSW. The tri-polymer blend consisted of equal ratios by mass to maintain a balanced and controlled experimental design.

Based on the relationship between density, mass and volume, assuming particles are spherical with a continuous uniform distribution of particles by size, 1 μg L^{-1} of microplastics would be in the order of 1 microplastic mL^{-1}. However, given cryogenic milling can produce extremely small particulates that can form aggregates (Gardon et al., 2022), it was imperative to undertake particulate analyses. Particle size distribution and morphology of the microplastics were assessed by Morphology G3, an automated static image analysis providing morphological information and dimensions (volume = Dv and number = Dn) of the dispersed particles. The analyses were performed on a 2.5 mL seawater microplastic suspension (as used in exposure) using a wet cell (Malvern Panalytical, UK). Five areas were analysed using two microscopic lenses (x5 and x20), with a total of 2.4 cm^2 per aliquot. Analyses were carried out on 2 aliquots for each sample, representing between 20,000 and 40,000 particles per sample.

The chemical composition of the microplastic stocks procured from Carat GmbH were investigated by i) double-shot thermal desorption and pyrolysis GC-MS as well as ii) chemical extraction (dichloromethane/ethyl acetate) of particles followed by screening analysis with GC × GC-MS. All analysis was performed in triplicate with triplicate laboratory/procedural blank samples. Details of the analytical methods and data processing are provided in the Supplementary Materials.

2.3. Experimental set-up

Pilot experiments were conducted to compare adult survival and juvenile development times across a range of salinities (20–35 ‰) and temperatures (15–20 °C), with optimal conditions being 20 ‰ FSW at ~20 °C. As such, all exposures were conducted with 20 ‰ FSW maintained at 19.5 ± 0.5 °C in a controlled-temperature laboratory with a 16:8 light:dark photoperiod (1800–2100 lux light intensity, SpectroSense 2+ Skye light sensor). Experimental media, comprising aerated FSW with microalgal prey (50,000 cells mL^{-1}), was used to partially-fill experimental chambers (720 mL glass jars). The tri-polymer microplastic stock was inverted ten times to resuspend the microplastics, and then a micropipette used to dispense the necessary volume to achieve final microplastic concentrations of 0–10 mg L^{-1}. Next, cohorts of adult *Acartia tonsa* were carefully added to each experimental chamber using a wide bore liquid pipette; cohorts comprised 8 copepods (male and female) for the acute toxicity test and 5 copepods (female only) for the partial life-cycle toxicity test. Jars were topped up to the brim with experimental media (i.e. FSW with microalgae), and lids carefully applied to minimise any air bubbles. The ISO14669 and ISO16778 protocols (ISO, 1999; 2015) use static exposure systems that are suitable for testing soluble chemicals. However, microplastics tend to float or sink in static systems, resulting in uneven dispersion and reduced interaction between the stressor and copepods. As such, experimental chambers were secured onto a plankton wheel and rotated continuously (<2 rpm) to promote dispersion and interaction between particles and the copepods.

2.4. Acute toxicity test

Acute toxicity tests were conducted using mortality as an endpoint. Cohorts of adult *A. tonsa* (N ≤ 5) were exposed for 72 h to microplastic concentrations of: 0, 40, 80, 120, 160, 200, 400, 600, 800 or 1000 μg L^{-1}. After the exposure period, each bottle was carefully poured through a 200 μm sieve and the adult copepods backwashed into glass evaporating dishes using FSW. Copepods were visualised under a stereomicroscope (Olympus SZX16), with mortality evidenced by complete loss of motility and movement for 10 s after prodding. To calculate the LC50, representing the concentration at which a given stressor is expected to cause mortality in 50 % of a population, a model function was performed (see '*Statistical analyses*').

2.5. Partial life-cycle toxicity test

The ISO16778 protocol (ISO, 2015) describes an early-life stage toxicity test focussing upon hatching success, mortality and the proportion of juveniles that reach the morphologically distinct copepodite stage, enabling the calculation of a Larval Development Ratio (LDR). As microplastics cannot pass the egg membrane and early nauplii stages do not feed, we opted to incorporate the early-life stage protocol into a partial life-cycle test. The partial life-cycle test (described below; Fig. 1B) was devised to ascertain whether chronic exposure to microplastics (0, 10, 100 or 1000 μg L^{-1}; N = 5) causes adverse health effects in adult female copepods and their progeny, with an array of endpoints considered:

Adult survival. Adult copepods were exposed (see Section 2.3 '*Experimental set-up*' for details) for five days, with a 100 % water change conducted halfway. To facilitate water changes and following the exposure period, experimental media was poured through a 200 μm sieve, slightly submerged in water, to retain adults without causing physical damage. Adult mortality was determined by observing the sieves under a stereomicroscope (Olympus SZX16), with mortality evidenced by complete loss of motility and movement. Copepods were then gently backwashed into fresh experimental media.

Algal feeding rates. Adult copepods were transferred to egg production chambers, which separate copepod eggs from the adult females, for a 24 h exposure period. Each chamber was inserted into a 1 L beaker containing 700 mL of the relevant experimental media and 700 μL of Guillard's F/2 to prevent nutrient limitation. As the system could not be housed on a rotating plankton wheel, a glass pipette airline was placed between the chamber and the beaker to enhance the microplastic distribution and homogenisation in the water column. Water samples (1 mL) were taken before (t0) and after the exposure period (t24), and preserved with 0.1 mL Lugol's solution. Algal concentrations were determined using a Sedgewick Rafter chamber, and microalgal clearance and ingestion rates calculated using the equation of Frost (1972).

Egg production rate, egg size and hatching success. Copepod eggs and nauplii (juveniles) were collected by removing the egg production chambers, and then carefully pouring experimental media through a 50 μm sieve. Sieves were analysed under a stereomicroscope to quantify the numbers of eggs and nauplii present (T_0), taking care to scan through all focal planes given the nauplii are motile. Egg production rates were calculated by determining the number of eggs produced by adult female copepods over the 24 h exposure period. Additionally, ≤10 copepod eggs were visualised and measured in two dimensions to determine

mean egg size (Olympus SZX16; Olympus CellSens software). Copepod eggs and nauplii were transferred into a six-well plate containing 12 mL of experimental media (~75 % of well volume). After 48 h, the well plates were visualised under an Olympus SZX16 microscope and the number of nauplii enumerated (T_2). Hatching success was calculated as the number of nauplii (T_2) divided by the total number of eggs and nauplii at the start of the experiment (T_0).

Early life stage development. Early life stages were maintained under experimental conditions until ~50 % of the nauplii in the controls had developed into copepodites (14 days following egg counts). Water changes were conducted every 2–3 days by using a serological pipette equipped with a 20 μm sheath (to prevent removal of nauplii) to carefully extract and replace ~50 % of the experimental media. The study was terminated by adding a few drops of Lugols to each well. Juvenile copepods were visualised under an Olympus SZX16 microscope, quantifying the number of nauplii and copepodites present in each cohort, and measuring the prosome length of copepodites (Olympus CellSens software). The larval development ratio was calculated as the number of copepodites divided by the total number of juveniles (nauplii and copepodites) present. Juvenile mortality was calculated as the number of juveniles (T_{14}) divided by the total number of eggs and nauplii at the start of the experiment (T_0).

2.6. Statistical analysis

Data were collated in Microsoft Excel (365) and analysed using R statistical software (version 3.4.1). Data were tested for normality using a Shapiro–Wilk test and homogeneity of variance was visually inspected to satisfy parametric requisites. A one-way analysis of variance (one-way ANOVA) and Tukey's post hoc tests were used to compare the number of eggs laid per female, the clearance rate, and the ingestion rate. A Kruskal-Wallis test, with pairwise comparisons usings Dunn's test, was used to compare the larval developmental ratio (LDR), prosome length, and egg size. To evaluate dose response data (LC50 tests), a binomial general linear model was conducted with "probit" link function and prediction responses calculated for 50 % population mortality using the Mass package (Ripley et al., 2013). The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Characterisation of the microplastics

The tri-polymer blend of microplastics displayed an irregular morphology and wide size distribution (SI, Fig. S1). Particle size distribution morphology indicated a particle size distribution of 45–150 μm (Dv 10–90 % of the particles) based on particle volume (SI, Fig. S2A), and 0.6–3.0 μm Dn 10–90 % of the particles) based on particle count (SI, Fig. S2B). Differences in reported size distribution likely stem from the irregular shape of the particles and non-binomial distribution of particles by size, which is reflective of microplastic size distributions in the natural environment (Lindeque et al., 2020).

Pyrograms obtained of the individual microplastics confirmed the polymer composition of the particles to be isotactic PP, PE and PA-6 (polycaproamide), respectively (see further details in SI). Plastic chemicals present in the particles were investigated by both thermal desorption (TD) analysis and by chemical extraction of particles followed by GC × GC-MS. The number of peaks with identified mass spectral matches >85 % in the replicates of the samples from TD analysis were 10–22 (PP replicates), 4 (all PA-6 replicates), and 11–17 (LDPE replicates). Three compounds were identified in blanks. Most of these compounds (including the blanks) were assigned to alkanes or alkane-like structures, most likely polymer chain related for PP and LDPE. From GC × GC-MS analysis, 167 compounds were tentatively identified in LDPE, 108 in PP and 13 in PA-6 (SI, Table S2). Five compounds were detected in all the materials. 25 compounds were detected in both PP and LDPE. As for the TD analysis, most of the peaks were also here assigned as alkanes, either straight chain (mainly LDPE) or branched (mainly PP).

3.2. Acute toxicity test

At microplastic concentrations of 0–600 μg L^{-1} adult mortality was observed to increase with increasing microplastic concentration, with 100 % mortality observed at concentrations of 400–600 μg L^{-1} (Fig. 2A). However, adult mortality was reduced to 55 % and 37 % when exposed to microplastic concentrations of 800 μg L^{-1} and 1000 μg L^{-1} respectively. Notably, treatments containing 800–1000 μg L^{-1} of microplastics showed evidence of particle flocculation, comprising agglomerations of microalgae and microplastics (Fig. 2B), that was not evident in bottles containing lower concentrations of microplastics. Discounting the 800–1000 μg L^{-1} data, a dose-response regression analyses was performed, enabling the calculation of a $LC20_{72h}$ value of 66 μg L^{-1} and $LC50_{72h}$ value of 182 μg L^{-1} (Fig. 2C).

3.3. Partial life-cycle toxicity test

Adult survival. Adult survival was significantly affected by exposure to high microplastic concentrations (Kruskal Wallis, $P < 0.01$), with 100 % mortality observed in the 1000 μg L^{-1} treatment (Dunn's test: $P < 0.01$). In the 0–100 μg L^{-1} treatments, adult copepods had an average survival of 87–93 % after 72 h (Fig. 3A) and 77–87 % after 144 h exposure (Fig. 3B). There was no significant difference in adult survival between controls and the 10 μg L^{-1} (Dunn's test: $P = 0.29$, Day 3; $P = 0.42$, Day 6) and 100 μg L^{-1} treatments (Dunn's test: $P = 0.43$, Day 3; $P = 0.28$, Day 6).

Algal ingestion rates. Adult copepods consumed an average of 135,000–173,000 algal cells per day, with no significant difference between treatment (Fig. 3B; ANOVA, $P = 0.47$).

Egg production and egg size. Female copepods produced an average of 17.4–24.0 eggs per day, with no significant difference in egg production rate between treatments (Fig. 3D; ANOVA, $P = 0.33$). However, exposure of adult females to microplastics resulted in the production of significantly larger eggs compared with controls (Fig. 3E; Kruskal Wallis, $P < 0.001$). Egg size in the control was 80.1 ± 0.3 μm, which was significantly smaller than observed in the 10 μg L^{-1} treatment (81.8 ± 0.5 μm; Dunn's test, $P < 0.001$) and 100 μg L^{-1} treatment (81.6 ± 0.5 μm; Dunn's test, $P < 0.001$).

Early life stage development. Copepodites had an average prosome length of 320–338 μm, with no significant difference in size between treatments (Fig. 3F; Kruskal Wallis, $P = 0.31$). Larval developmental ratios (LDR) averaged 0.45 ± 0.12 in the control treatment, which was higher than the LDR of 0.28 ± 0.10 in the 10 μg L^{-1} treatment and 0.34 ± 0.12 in the 10 μg L^{-1} treatment; however, values were not significantly different between treatments (Fig. 3G; Kruskal Wallis, $P = 0.57$). Survival rates of juveniles averaged 40–53.5 %, with no significant different in juvenile survival between treatments (Fig. 3H; Kruskal Wallis, $P = 0.69$).

4. Discussion

In this study, established methodologies (ISO, 1999; 2015) were adapted to facilitate the toxicological testing of microplastics using the copepod *A. tonsa*. The 72-h acute toxicity test provided an LC50 of 182 μg L^{-1} for the tri-polymer blend of microplastics, with 100 % mortality observed at concentrations of 400–600 μg L^{-1}. While the use of a rotating plankton wheel facilitated continuous particulate suspension, it resulted in the formation of microalgal-microplastic agglomerations at higher particulate concentrations, adding complexity to the results. Adult survival also proved a sensitive endpoint within the partial life-cycle test, with 100 % mortality in copepods exposed to 1000 μg microplastics L^{-1}. The chronic exposure study showed limited evidence

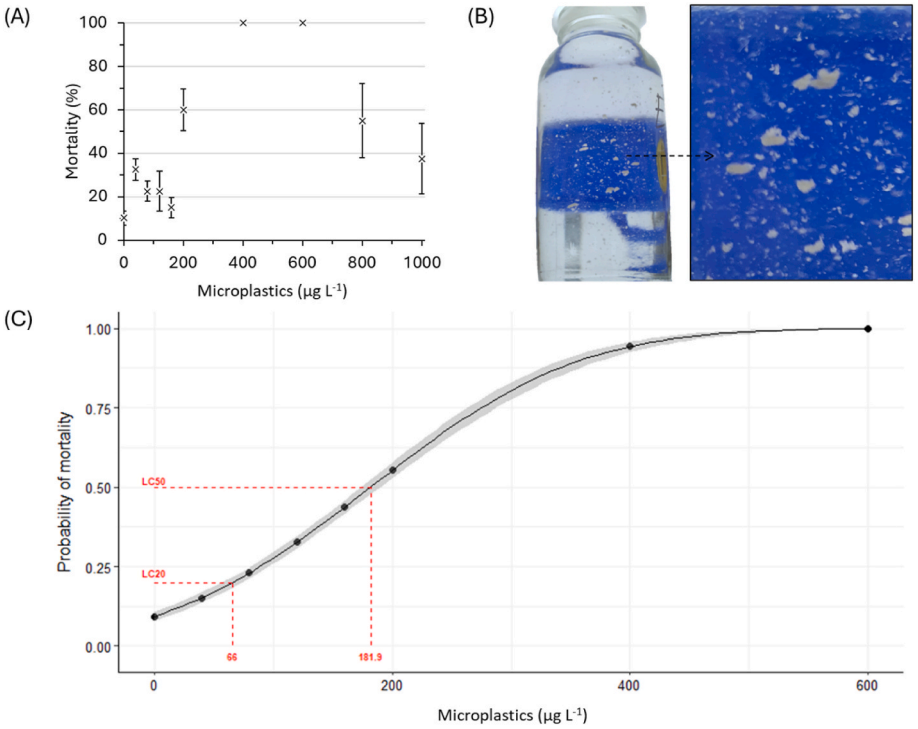

Fig. 2. Acute toxicity of a tri-polymer blend of microplastics with adult *Acartia tonsa*. (A) Adult mortality (%) exposed to microplastic concentrations of 0–1000 µg L^{-1}. (B) Photograph of a bottle containing experimental media with 1000 µg L^{-1} of microplastics; inset depicts a zoomed-in image of the agglomeration of particles within the media. (C) A regression analysis comparing probability of mortality with microplastic concentration (0–600 µg L^{-1}), with a calculated LC50 of 182 µg L^{-1}.

of sub-lethal health effects on juvenile life stages. Additionally, chemical analysis of the tri-polymer blend demonstrated a limited number of chemicals with potential for contributing to toxicity.

In both the acute toxicity and partial life-cycle test, adult mortality proved to be a statistically significant endpoint. This was somewhat surprising given microplastics are typically associated with causing sub-lethal harm (i.e. effects on growth and reproduction) over chronic timescales. Relatively few microplastic studies evidence mortality as a relevant endpoint at environmentally relevant concentrations (Doyle et al., 2022; Foley et al., 2018; Thornton Hampton et al., 2022c; Yu et al., 2020). For example, a meta-analysis of *Daphnia magna* (cladoceran) neonate inhibition studies found >50 % mortality has only been observed with concentrations ≥1000 µg nanoplastics L^{-1} and ≥10,000 µg microplastics L^{-1} (Pikuda et al., 2023). Similarly, a review of copepod ecotoxicity data found Lowest Observed Effect Concentrations (LOEC) for mortality in adult copepods (e.g. *Calanus* spp., *Tigriopsus* spp.) were typically ≥10,000 µg microplastics L^{-1} (Yu et al., 2020). The lethal mortality of particulates in *Acartia* spp. is variable. For example, adult *A. clausi* demonstrated 88–94 % mortality following 8-days exposure to ~6000 µg L^{-1} of 6 µm polystyrene spheres, with mortality becoming significantly different from controls after 5-days exposure (Svetlichny et al., 2021). Further, *A. tonsa* nauplii demonstrated a significant 28.6 % decrease in survival following 5-days exposure to ~200 µg L^{-1} of 6–8 µm polystyrene spheres (Shore et al., 2021), while *A. clausi* nauplii showed a significant ~80 % decrease in survival following 48 h exposure to <20 µm polyvinyl chloride particles at concentrations of 10, 000 µg L^{-1} (Beiras et al., 2019). However, no decrease in survival was observed in adult female *A. tonsa* exposed to ~4–400 µg L^{-1} of 1.4–32 µm polyethylene microplastics for 48 h (Bellas and Gil, 2020).

Inconsistencies across studies, including variation in species, life-stage, exposure length, particle polymer, size and shape, and environmental conditions (e.g. media, temperature, salinity), confound unifying hypotheses regarding the drivers of observed toxicity. However, that a 96-h exposure to >400 µg microplastics L^{-1} caused 100 % mortality in *A. tonsa* – not evident in other studies – would suggest the tri-polymer blend is substantially more toxic than the single form of microplastics used in prior studies. Isobe et al. (2019) predicts microplastic concentrations may exceed 1000 µg L^{-1} in East Asian seas and the central North Pacific Ocean by the 2060s, exceeding the lethal concentration observed here.

In the acute toxicity study, microplastic concentrations of 400–600 µg L^{-1} caused 100 % mortality, however at microplastic concentrations of 800–1000 µg L^{-1} mortality ranged 37–55 %. This decreased mortality was concomitant with observations of microplastic-microalgal agglomerations – akin to artificially produced marine snows (Shanks and Edmondson, 1989) – which are too large for adult *A. tonsa* to consume (Berggreen et al., 1988). This would suggest microplastic ingestion is intrinsic to instigating lethal toxicity. Microplastic ingestion may affect energetic uptake (Cole et al., 2015), cause tissue damage and inflammation in the gastrointestinal tract (Ahrendt et al., 2020), incite cytotoxicity or a heightened immune response that can cascade through the biological hierarchy (Li et al., 2022), or facilitate the release of additives from the plastic to the organism (Gewert et al., 2021; Lehtiniemi et al., 2021). Chemicals and metals that leach from plastics can cause cytotoxicity and endocrine disruption (Alijagic et al., 2024; Silva et al., 2016; Lin et al., 2023). For example, exposure to nylon microplastics resulted in premature moulting in juvenile *Calanus finmarchicus* (copepod), which was postulated to have been triggered by oestradiol agonists leaching from the plastic (Cole et al., 2019).

Chemical analysis of the tri-polymer blend of microplastics revealed several leachate compounds, however the number of chemicals with expected hazardous properties was low compared to consumer plastics (Sørensen et al., 2023). Most of the tentatively identified compound structures were assigned as straight-chain and branched alkanes with expected low toxicity. Other noteworthy compounds were mainly identified as non-intentionally added substances (NIAS), such as monomers (e.g. caprolactam and 1,8-diazacyclotetradecane-2,9-dione), solvents (e.g. phenoxybenzene) and possible production by-products and impurities (e.g. aromatic hydrocarbons). The plasticizer diethyl phthalate was detected in PA-6 and PP, while the UV-stabilizer

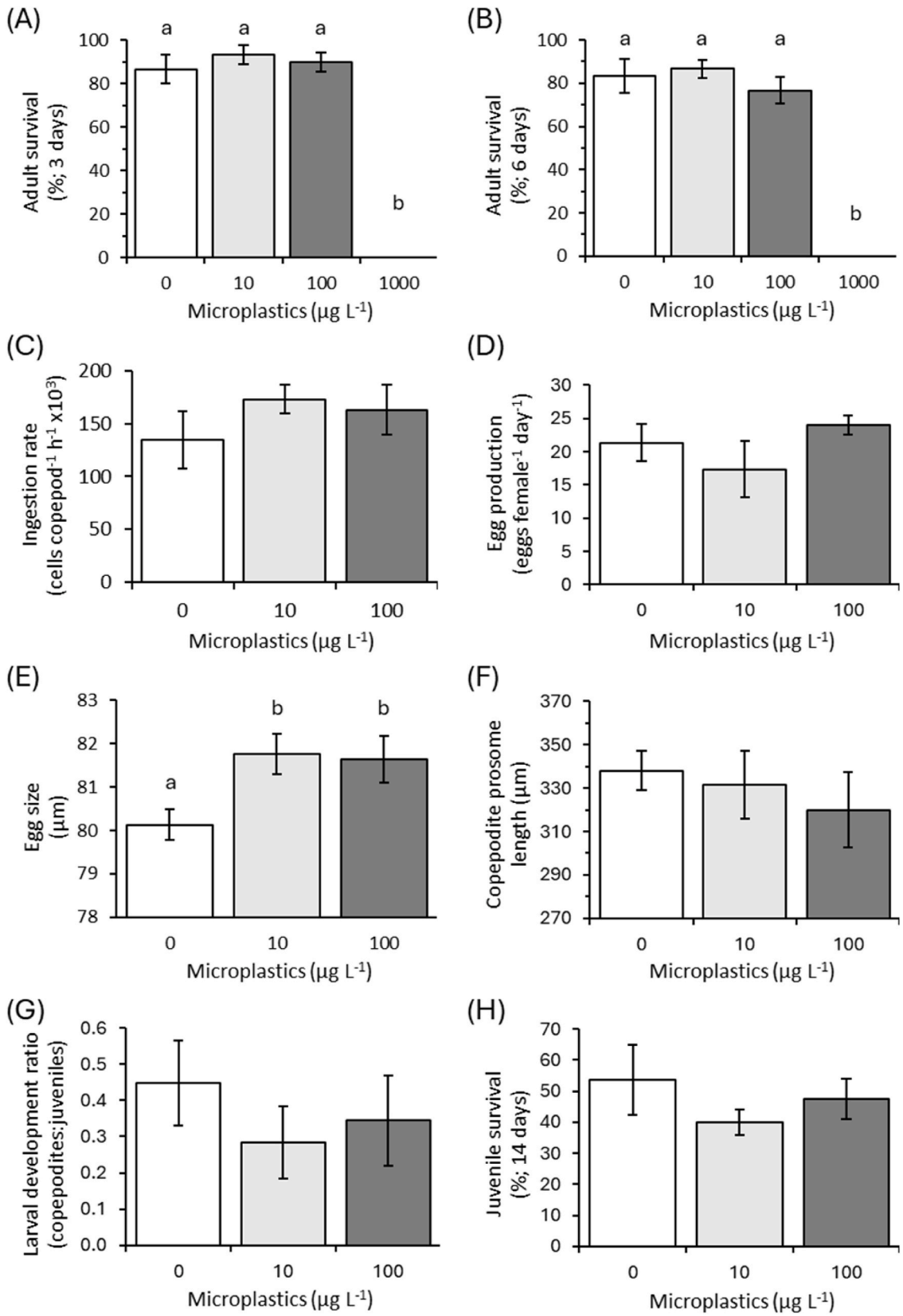

Fig. 3. Results of partial life-cycle toxicity test in which adult and developing *Acartia tonsa* were exposed to a tri-polymer blend of microplastics (0–1000 µg L^{-1}). Endpoints: (A) Adult mortality (%; 3 days); (B) Adult mortality (%; 6 days); (C) Algal ingestion rate (cells $copepod^{-1}$ $hour^{-1}$ $x10^3$); (D) Egg production (eggs $female^{-1}$ day^{-1}); (E) Egg size (µm); (F) Copepodite prosome length (µm); (G) Larval development ratio (copepodites:juveniles); and (H) Juvenile survival (%; 14 days post egg production). Results are presented as mean values and error bars denote standard error. Please note scales do not start at 0 in Panel E and F.

degradation product 2,4-di-*tert*-butylphenol was detected in PP. The latter is commonly detected in polyolefins related to addition of UV-stabilizers, while the former may also originate from contamination processes. In aquatic exposures using ingestible particle-sizes, chemical exposure may occur either directly from leached chemicals in the exposure media, or via transfer of chemicals from ingested particles to biotic fluids or tissues. To fully understand these exposure mechanisms and their role in driving the toxicity of microplastics, further work is necessary. While no hazardous chemicals were identified from the chemical analysis of the microplastics used here, chemical characterization is crucial in validating any microplastic particle toxicity study (Delaeter et al., 2022; Gunaalan et al., 2020; Sørensen et al., 2024).

In the partial life-cycle study, observed reductions in average copepodite size, larval development ratio and juvenile survival were not statistically significant. Algal ingestion rates were 21–29 % higher in microplastic treatments as compared with the control, but this was not statistically significant. Similarly, no difference was observed in the feeding rates of adult female *A. tonsa* exposed to ~4–400 μg L^{-1} of 1.4–32 μm polyethylene microplastics (Bellas and Gil, 2020). However, at concentrations up to ~30,000 μg L^{-1}, 45 μm latex spheres have been shown to dramatically reduce the clearance rate of *Acartia* spp. copepodites, with this reduced feeding linked to an uptick in their predatory-escape response when the antennules came into contact with the particles (Hansen et al., 1991). In another study, exposure to ~6000 μg L^{-1} of 6 μm polystyrene spheres resulted in decreased respiration rates, attributed to reduced motility stemming from energetic depletion (Svetlichny et al., 2021). Feeding behaviours can also be affected by microplastic exposure, with widespread evidence of rejection and regurgitation of microplastics across a range of copepods including *Acartia* spp. (Ayukai, 1987; Huntley et al., 1983; Xu et al., 2022).

Egg production rate was not affected by microplastic exposure, which corresponds with studies in which *A. tonsa* were exposed to ~4–400 μg L^{-1} of 1.4–32 μm polyethylene microplastics (Bellas and Gil, 2020) and ~17 μg L^{-1} of 8–20 μm tyre wear particles (Koski et al., 2021). Whilst the egg production was higher at 100 μg L^{-1} than 10 μg L^{-1} and the control treatment, this was not significant and further research using increased copepod numbers and treatment replicates would be beneficial. In this study, egg size was significantly increased in the microplastic treatments (81.7 ± 0.5 μm) compared with controls (80.1 ± 0.3 μm). Conversely, Shore et al. (2021) observed *A. tonsa* exposed to ~200 μg L^{-1} of 6–8 μm polystyrene spheres for 9-days produced significantly smaller eggs (73.3 ± 6.3 μm) compared with controls (79.1 ± 3.3 μm). In the copepod *Calanus helgolandicus*, 4–6 days exposure to ~330 μg L^{-1} of 20 μm polystyrene spheres also resulted in significantly smaller egg sizes (Cole et al., 2015). Differences in egg size may result from changes in food availability, nutritional quality and endocrine disruption stemming from chemicals leaching from the microplastics. However, the changes in egg size are small (<2 μm) and measurements may be subject to user bias, so automation of such measurements (e.g. ImageJ macros) is recommended for future studies.

Despite our best efforts, hatching success was not measured in this study for two reasons: (i) nauplii were motile making enumeration prone to inaccuracies without sample preservation; and (ii) enumeration was time-consuming, necessitating leaving well-plates on brightly lit, warm microscope plates for extended periods, which could have adversely affected the developing copepods. Approximately half the juvenile copepods reached copepodite stage 14 days after eggs were initially counted, which is longer than observed in the ISO guidance notes (ISO, 2015), although temperature and salinity are known to cause high degrees of variance. Shore et al. (2021) observed *A. tonsa* copepodites exposed to ~200 μg L^{-1} of 6–8 μm polystyrene spheres for 5–7 days showed a non-significant ~10 % decrease in survival and a significant 12.2 % decrease in body length. The sub-lethal endpoints assessed throughout the partial life-cycle test often indicated different biological responses between treatments, but these differences typically lacked statistical significance given high variability between replicates.

We also observed differences between the acute and chronic tests, with 1000 μg L^{-1} of microplastics causing 100 % mortality in adult A. tonsa in the chronic exposure study and 20–60 % mortality in the acute toxicity test. Such differences may have resulted from differences in copepod numbers (five individuals $bottle^{-1}$ in the acute toxicity test vs eight individuals $bottle^{-1}$ in the partial-life cycle test), copepod sex (males and females in the acute toxicity test vs females only in the partial-life cycle test), the growth phase of the microalgae, or subtle differences in rotational speed of the motor, or inter-batch differences in the copepods. Replicability and repeatability is an issue noted within the ISO16778 protocol notes (ISO, 2015). As such, we would recommend that future testing using this protocol increases copepod numbers and replicate size to reduce uncertainty.

5. Conclusion

Toxicity tests using the tri-polymer microplastic blend on the copepod *Acartia tonsa* provided a $LC50_{72h}$ of 182 μg L^{-1} and identified adult survival as a significantly sensitive endpoint. Given mortality is an uncommon endpoint in microplastic studies, this would indicate that the tri-polymer blend is substantially more toxic that a single polymer exposure. The data provides important data for subsequent risk evaluations and the determination of toxicity thresholds. Despite measuring many endpoints within the partial life cycle test, there was limited evidence of sub-lethal effects on the juvenile life stages using environmentally relevant concentrations. However, we provide several recommendations and suggestions which may aid and improve future toxicity test protocols, including increased replication (individual and treatment numbers), and software automation.

CRediT authorship contribution statement

Zara L.R. Botterell: Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Rachel L. Coppock:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis. **Alessio Gomiero:** Writing – review & editing, Funding acquisition, Conceptualization. **Penelope K. Lindeque:** Writing – review & editing, Resources, Funding acquisition. **Stefania Piarulli:** Writing – review & editing, Resources, Data curation. **Thomas Rees:** Writing – review & editing, Investigation. **Lisbet Sørensen:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Matthew Cole:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126105.

Data availability

Data will be made available on request.

References

Aanesen, M., Ahi, J.C., Abate, T.G., Khan, F.R., de Vries, F.P., Kite-Powell, H., Beaumont, N.J., 2024. Insights from international environmental legislation and protocols for the global plastic treaty. Sci. Rep. 14, 2750.

Ahrendt, C., Perez-Venegas, D.J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., Galbán-Malagón, C., 2020. Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Mar. Pollut. Bull. 151, 110795.

Alijagic, A., Kotlyar, O., Larsson, M., Salihovic, S., Hedbrant, A., Eriksson, U., Karlsson, P., Persson, A., Scherbak, N., Färnlund, K., 2024. Immunotoxic, genotoxic, and endocrine disrupting impacts of polyamide microplastic particles and chemicals. Environ. Int. 183, 108412.

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.

Ayukai, T., 1987. Discriminate feeding of the calanoid copepod *Acartia clausi* in mixtures of phytoplankton and inert particles. Mar. Biol. 94, 579–587.

Beiras, R., Tato, T., López-Ibáñez, S., 2019. A 2-Tier standard method to test the toxicity of microplastics in marine water using Paracentrotus lividus and Acartia clausi larvae. Environ. Toxicol. Chem. 38, 630–637.

Bellas, J., Gil, I., 2020. Polyethylene microplastics increase the toxicity of chlorpyrifos to the marine copepod Acartia tonsa. Environ. Pollut. 260, 114059.

Berggreen, U., Hansen, B., Kiørboe, T., 1988. Food size spectra, ingestion and growth of the copepod Acartia tonsa during development: implications for determination of copepod production. Mar. Biol. 99, 341–352.

Besiktepe, S., Dam, H.G., 2020. Effect of diet on the coupling of ingestion and egg production in the ubiquitous copepod, Acartia tonsa. Prog. Oceanogr. 186, 102346.

Botterell, Z.L., Lindeque, P.K., Thompson, R.C., Beaumont, N.J., 2023. An assessment of the ecosystem services of marine zooplankton and the key threats to their provision. Ecosyst. Serv. 63, 101542.

Chang, X., Fang, Y., Wang, Y., Wang, F., Shang, L., Zhong, R., 2022. Microplastic pollution in soils, plants, and animals: a review of distributions, effects and potential mechanisms. Sci. Total Environ. 850, 157857.

Chowdhury, O.S., Schmidt, P.J., Anderson, W.B., Emelko, M.B., 2024. Advancing Evaluation of Microplastics Thresholds to Inform Water Treatment Needs and Risks. Environment & Health.

Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S., Booth, A.M., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and moulting in a coldwater copepod. Environ. Sci. Technol. 53, 7075–7082.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49, 1130–1137.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 12, 6646–6655.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.

Connors, K.A., Dyer, S.D., Belanger, S.E., 2017. Advancing the quality of environmental microplastic research. Environ. Toxicol. Chem. 36, 1697–1703.

Cui, Y., Liu, M., Selvam, S., Ding, Y., Wu, Q., Pitchaimani, V.S., Huang, P., Ke, H., Zheng, H., Liu, F., 2022. Microplastics in the surface waters of the South China sea and the western Pacific Ocean: different size classes reflecting various sources and transport. Chemosphere 299, 134456.

de Ruijter, V.N., Redondo-Hasselerharm, P.E., Gouin, T., Koelmans, A.A., 2020. Quality criteria for microplastic effect studies in the context of risk assessment: a critical review. Environ. Sci. Technol. 54, 11692–11705.

Delaeter, C., Spilmont, N., Bouchet, V.M., Seuront, L., 2022. Plastic leachates: bridging the gap between a conspicuous pollution and its pernicious effects on marine life. Sci. Total Environ. 826, 154091.

Détrée, C., Gallardo-Escárate, C., 2018. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel Mytilus galloprovincialis. Fish Shellfish Immunol. 83, 52–60.

Doyle, D., Sundh, H., Almroth, B.C., 2022. Microplastic exposure in aquatic invertebrates can cause significant negative effects compared to natural particles-A meta-analysis. Environ. Pollut. 315, 120434.

Erni-Cassola, G., Zadjelovic, V., Gibson, M.I., Christie-Oleza, J.A., 2019. Distribution of plastic polymer types in the marine environment; A meta-analysis. J. Hazard Mater. 369, 691–698.

Fleury, J.-B., Baulin, V.A., 2021. Microplastics Destabilize Lipid Membranes by Mechanical Stretching, vol. 118. Proceedings of the National Academy of Sciences, e2104610118.

Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631, 550–559.

Frost, B.W., 1972. Effect of size and concentration of food particles on the feeding behaviour of the marine planktoinic copepod *Calanus pacificus*. Limnol. Oceanogr. 17, 805–815.

Gardon, T., Paul-Pont, I., Le Moullac, G., Soyez, C., Lagarde, F., Huvet, A., 2022. Cryogrinding and sieving techniques as challenges towards producing controlled size range microplastics for relevant ecotoxicological tests. Environ. Pollut. 315, 120383.

Gewert, B., MacLeod, M., Breitholtz, M., 2021. Variability in toxicity of plastic leachates as a function of weathering and polymer type: a screening study with the copepod Nitocra spinipes. Biol. Bull. 240, 191–199.

Gouin, T., Becker, R.A., Collot, A.G., Davis, J.W., Howard, B., Inawaka, K., Lampi, M., Ramon, B.S., Shi, J., Hopp, P.W., 2019. Toward the development and application of an environmental risk assessment framework for microplastic. Environ. Toxicol. Chem. 38, 2087–2100.

Gouin, T., Ellis-Hutchings, R., Pemberton, M., Wilhelmus, B., 2024. Addressing the relevance of polystyrene nano-and microplastic particles used to support exposure, toxicity and risk assessment: implications and recommendations. Part. Fibre Toxicol. 21, 39.

Gouin, T., Ellis-Hutchings, R., Thornton Hampton, L.M., Lemieux, C.L., Wright, S.L., 2022. Screening and prioritization of nano-and microplastic particle toxicity studies for evaluating human health risks–development and application of a toxicity study assessment tool. Microplastics and Nanoplastics 2, 2.

Gunaalan, K., Fabbri, E., Capolupo, M., 2020. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. Water Res. 184, 116170.

Hansen, B., Hansen, P.J., Nielsen, T.G., 1991. Effects of large nongrazable particles on clearance and swimming behaviour of zooplankton. J. Exp. Mar. Biol. Ecol. 152, 257–269.

Hartmann, N.B., Hüffer, T., Thompson, R., Hassellöv, M., Verschoor, A., Daugaard, A., Rist, S., Karlsson, T.M., Brennholt, N., Cole, M., Herrling, M., Heß, M., Ivleva, N., Lusher, A., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ. Sci. Technol. 53, 1039–1047.

Horn, D.A., Granek, E.F., Steele, C.L., 2020. Effects of environmentally relevant concentrations of microplastic fibers on Pacific mole crab (Emerita analoga) mortality and reproduction. Limnol. Oceanogr. Lett. 5, 74–83.

Huntley, M.E., Barthel, K.G., Star, J.L., 1983. Particle rejection by *Calanus pacificus*: discrimination between similarly sized particles. Mar. Biol. 74, 151–160.

ISO, 1999. Determination of Acute Lethal Toxicity to Marine Copepods, Water Quality. International Standard of Organization.

ISO, 2015. Calanoid Copepod Early-Life Stage Test with Acartia Tonsa, Water Quality. International Standard of Organization.

ISO, 2023. ISO Definitions of Key Terms for Plastic Pollution. International Organisation for Standardization, Geneva, Switzerland.

Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 417.

Knight, L.J., Parker-Jurd, F.N., Al-Sid-Cheikh, M., Thompson, R.C., 2020. Tyre wear particles: an abundant yet widely unreported microplastic? Environ. Sci. Pollut. Control Ser. 27, 18345–18354.

Kooi, M., Primpke, S., Mintenig, S.M., Lorenz, C., Gerdts, G., Koelmans, A.A., 2021. Characterizing the multidimensionality of microplastics across environmental compartments. Water Res. 202, 117429.

Koski, M., Søndergaard, J., Christensen, A.M., Nielsen, T.G., 2021. Effect of environmentally relevant concentrations of potentially toxic microplastic on coastal copepods. Aquat. Toxicol. 230, 105713.

Lehtiniemi, M., Hartikainen, S., Turja, R., Lehtonen, K.K., Vepsäläinen, J., Peräniemi, S., Leskinen, J., Setälä, O., 2021. Exposure to leachates from post-consumer plastic and recycled rubber causes stress responses and mortality in a copepod Limnocalanus macrurus. Mar. Pollut. Bull. 173, 113103.

Li, C., Li, X., Bank, M.S., Dong, T., Fang, J.K.-H., Leusch, F.D., Rillig, M.C., Wang, J., Wang, L., Xia, Y., 2024. The "microplastome"–a holistic perspective to capture the real-world ecology of microplastics. Environ. Sci. Technol. 58, 4060–4069.

Li, Z., Chang, X., Hu, M., Fang, J.K.-H., Sokolova, I.M., Huang, W., Xu, E.G., Wang, Y., 2022. Is microplastic an oxidative stressor? Evidence from a meta-analysis on bivalves. J. Hazard Mater. 423, 127211.

Lin, W., Luo, H., Wu, J., Liu, X., Cao, B., Liu, Y., Yang, P., Yang, J., 2023. Polystyrene microplastics enhance the microcystin-LR-induced gonadal damage and reproductive endocrine disruption in zebrafish. Sci. Total Environ. 876, 162664.

Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environ. Pollut., 114721

McCormick, M.I., Chivers, D.P., Ferrari, M.C., Blandford, M.I., Nanninga, G.B., Richardson, C., Fakan, E.P., Vamvounis, G., Gulizia, A.M., Allan, B.J., 2020. Microplastic exposure interacts with habitat degradation to affect behaviour and survival of juvenile fish in the field. Proc. R. Soc. B 287, 20201947.

McEvoy, A.J., Atkinson, A., Airs, R.L., Brittain, R., Brown, I., Fileman, E.S., Findlay, H.S., McNeill, C.L., Ostle, C., Smyth, T.J., 2023. The Western Channel Observatory: a century of physical, chemical and biological data compiled from pelagic and benthic habitats in the western English Channel. Earth Syst. Sci. Data 15, 5701–5737.
Mehinto, A.C., Coffin, S., Koelmans, A.A., Brander, S.M., Wagner, M., Thornton Hampton, L.M., Burton, Jr A.G., Miller, E., Gouin, T., Weisberg, S.B., 2022. Risk-based management framework for microplastics in aquatic ecosystems. Microplastics and Nanoplastics 2, 17.
Morasae, E., Botterell, Z., Andrews, S.V., Beaumont, N., Boisseaux, P., Chadwick, H., Cherrington, R., Cole, M., Coppock, R., Deakin, K., 2024. Using systems mapping to understand the constraints and enablers of solutions to plastic pollution. J. Environ. Manag. 371, 122994.
Muller-Karanassos, C., Arundel, W., Lindeque, P.K., Vance, T., Turner, A., Cole, M., 2021. Environmental concentrations of antifouling paint particles are toxic to sediment-dwelling invertebrates. Environ. Pollut. 268, 115754.
Nilsson, B., Jepsen, P.M., Bucklin, A., Hansen, B.W., 2018. Environmental stress responses and experimental handling artifacts of a model organism, the copepod Acartia tonsa (Dana). Front. Mar. Sci. 5, 156.
Ogonowski, M., Wagner, M., Rogell, B., Haave, M., Lusher, A., 2023. Microplastics could be marginally more hazardous than natural suspended solids–A meta-analysis. Ecotoxicol. Environ. Saf. 264, 115406.
Pencik, O., Durdakova, M., Molnarova, K., Kucsera, A., Klofac, D., Kolackova, M., Adam, V., Huska, D., 2023. Microplastics and nanoplastics toxicity assays: a revision towards to environmental-relevance in water environment. J. Hazard Mater. 454, 131476.
Pikuda, O., Dumont, E.R., Chen, Q., Macairan, J.-R., Robinson, S.A., Berk, D., Tufenkji, N., 2023. Toxicity of microplastics and nanoplastics to Daphnia magna: current status, knowledge gaps and future directions. TrAC, Trends Anal. Chem., 117208
Raisuddin, S., Kwok, K.W., Leung, K.M., Schlenk, D., Lee, J.-S., 2007. The copepod Tigriopus: a promising marine model organism for ecotoxicology and environmental genomics. Aquat. Toxicol. 83, 161–173.
Ramírez-Álvarez, N., Mendoza, L.M.R., Macías-Zamora, J.V., Oregel-Vázquez, L., Alvarez-Aguilar, A., Hernández-Guzmán, F.A., Sánchez-Osorio, J.L., Moore, C.J., Silva-Jiménez, H., Navarro-Olache, L.F., 2020. Microplastics: sources and distribution in surface waters and sediments of todos santos bay, Mexico. Sci. Total Environ. 703, 134838.
Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D., Ripley, M.B., 2013. Package 'mass'. Cran r 538 (113–120), 822.2.
Rochman, C.M., 2018. Microplastics research—from sink to source. Science 360, 28–29.
Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H.D., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711.
Shanks, A., Edmondson, E., 1989. Laboratory-made artificial marine snow: a biological model of the real thing. Mar. Biol. 101, 463–470.
Shore, E.A., DeMayo, J.A., Pespeni, M.H., 2021. Microplastics reduce net population growth and fecal pellet sinking rates for the marine copepod, Acartia tonsa. Environ. Pollut. 284, 117379.
Silva, P.P.G., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F., 2016. Leachate from microplastics impairs larval development in brown mussels. Water Res. 106, 364–370.3.
Sørensen, L., Gomes, T., Igartua, A., Lyngstad, I.L., Almeida, A.C., Wagner, M., Booth, A. M., 2023. Organic chemicals associated with rubber are more toxic to marine algae and bacteria than those of thermoplastics. J. Hazard Mater. 458, 131810.
Sørensen, L., Zammite, C., Igartua, A., Christensen, M.M., Haraldsvik, M., Creese, M., Gomes, T., Booth, A.M., 2024. Towards realism in hazard assessment of plastic and rubber leachates–Methodological considerations. J. Hazard Mater. 480, 136383.
Sun, T., Zhan, J., Li, F., Ji, C., Wu, H., 2021. Evidence-based meta-analysis of the genotoxicity induced by microplastics in aquatic organisms at environmentally relevant concentrations. Sci. Total Environ. 783, 147076.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., 2016. Oyster Reproduction Is Affected by Exposure to Polystyrene Microplastics, vol. 113. Proceedings of the National Academy of Sciences, pp. 2430–2435.
Svetlichny, L., Isinibilir, M., Mykitchak, T., Eryalçın, K.M., Türkeri, E.E., Yuksel, E., Kideys, A.E., 2021. Microplastic consumption and physiological response in Acartia clausi and Centropages typicus: possible roles of feeding mechanisms. Reg. Stud. Mar. Sci. 43, 101650.
Thompson, R.C., Courtene-Jones, W., Boucher, J., Pahl, S., Raubenheimer, K., Koelmans, A.A., 2024. Twenty years of microplastics pollution research—what have we learned? Science, eadl2746.
Thornton Hampton, L.M., Bouwmeester, H., Brander, S.M., Coffin, S., Cole, M., Hermabessiere, L., Mehinto, A.C., Miller, E., Rochman, C.M., Weisberg, S.B., 2022a. Research recommendations to better understand the potential health impacts of microplastics to humans and aquatic ecosystems. Microplastics and Nanoplastics 2, 1–13.
Thornton Hampton, L.M., Brander, S.M., Coffin, S., Cole, M., Hermabessiere, L., Koelmans, A.A., Rochman, C.M., 2022b. Characterizing microplastic hazards: which concentration metrics and particle characteristics are most informative for understanding toxicity in aquatic organisms? Microplastics and Nanoplastics 2, 20.
Thornton Hampton, L.M., Lowman, H., Coffin, S., Darin, E., De Frond, H., Hermabessiere, L., Miller, E., de Ruijter, V.N., Faltynkova, A., Kotar, S., 2022c. A living tool for the continued exploration of microplastic toxicity. Microplastics and Nanoplastics 2, 13.
Turner, J.T., 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog. Oceanogr. 130, 205–248.
Villarrubia-Gómez, P., Cornell, S.E., Fabres, J., 2018. Marine plastic pollution as a planetary boundary threat–The drifting piece in the sustainability puzzle. Mar. Pol. 96, 213–220.
Wang, C., Yu, J., Lu, Y., Hua, D., Wang, X., Zou, X., 2021. Biodegradable microplastics (BMPs): a new cause for concern? Environ. Sci. Pollut. Control Ser. 28, 66511–66518.
Xu, J., Rodríguez-Torres, R., Rist, S., Nielsen, T.G., Hartmann, N.B., Brun, P., Li, D., Almeda, R., 2022. Unpalatable plastic: efficient taste discrimination of microplastics in planktonic copepods. Environ. Sci. Technol. 56, 6455–6465.
Yu, S.-P.Y., Cole, M.C., Chan, B.K., 2020. Effects of microplastic on zooplankton survival and sublethal responses. Oceanogr. Mar. Biol.
Zhang, K., Hamidian, A.H., Tubić, A., Zhang, Y., Fang, J.K., Wu, C., Lam, P.K., 2021. Understanding plastic degradation and microplastic formation in the environment: a review. Environ. Pollut. 274, 116554.