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Lethal effect of leachates from tyre wear particles on marine copepods

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ABSTRACT

With thousands of tons of Tyre Wear Particles (TWP) entering the aquatic environment every year, TWP are considered a major contributor to microplastic pollution. TWP leach organic compounds and metals in water, potentially affecting the marine food web. However, little is known about the toxicity of TWP leachates on marine copepods, a major food web constituent, and a key group to determine the environmental risk of pollution in marine ecosystems. In this study, we determined the lethal effect of TWP leachates on marine copepods after 24, 48, and 72-h of exposure to 0.05–100% leachate solutions prepared using a concentration of 5 g TWP L^{-1}. The calanoids *Acartia tonsa*, *Temora longicornis* and *Centropages hamatus*, the cyclopoid *Oithona davisae* and the harpacticoid *Amonardia normanni* were used as experimental species. TWP leachates were toxic to all the studied species, with toxicity increasing as leachate solution and exposure time increased. Median lethal concentration (LC_{50}, 72-h) ranged from 0.22 to 3.43 g L^{-1} and calanoid copepods were more sensitive to TWP leachates than the cyclopoid *O. davisae* and the harpacticoid *A. normanni*. Toxicity of TWP leachates was not related to the copepod body size, which suggests that other traits such as foraging behaviour or adaptation to contaminants could explain the higher tolerance of cyclopoid and harpacticoid to TWP leachates compared to calanoid copepods. Although field data on the concentration of TWP and their chemical additives are still limited, our results suggest that TWP leachates can negatively impact planktonic food webs in coastal areas after road runoff events.

1. Introduction

Over the last two decades, plastic pollution has become a major environmental and societal concern. Often overlooked, tire wear particles (TWP), formed by the mechanical abrasion of tires with road surfaces, are now recognized as a significant source of microplastic pollution (Friot and Boucher 2017; Kole et al., 2017; Baensch-Baltruschat et al., 2020; Rødland et al., 2022). TWP can reach concentrations exceeding 28,000 particles L^{-1} in street sweeping wash water (Järlskog et al., 2021), up to 150 mg TWP g^{-1} in gully pot sediment (Mengistu et al., 2021) and 0.056 g L^{-1} in surface waters (Wik and Dave 2009). Depending on their size, TWP can become airbone or be transported by runoff and wastewater effluents into water bodies, soils and sewers (Siegfried et al., 2017; Tamis et al., 2021; Werbowski et al., 2021; Jeong et al., 2022). There is increasing evidence that TWP are polluting all environmental compartments (Baensch-Baltruschat et al., 2020) with ecological impacts in aquatic systems e.g., after road runoff events (Tian et al., 2021). Evangeliou et al. (2020) estimated that the deposition of airborne TWP is likely the major source of microplastics in the ocean. TWP contains a variety of chemical substances such as acetophenone, benzothiazole, n-cyclohexylforamide, phthalide and bisphenol A and metals such as zinc (Zn), manganese (Mn), cobalt (Co), antimony (Sb) and lead (Pb) in TWP leachates (Capolupo et al., 2020). Moreover, Capolupo et al. (2020) identified a few polycyclic aromatic hydrocarbons (PAHs) along with the abovementioned organic and inorganic compounds. In contact with water, TWP can leach these chemicals and additives.

Although the potential impacts of leachates from TWP on freshwater biota was pointed out in the early 90's (Day et al., 1993), the occurrence and environmental effects of TWP pollution have only recently received more attention (Wagner et al., 2018; Halsband et al., 2020). Acute toxicity tests with TWP leachates have been performed for various marine organisms, including phytoplankton (Capolupo et al., 2020; Page et al., 2022) and a few copepod species (Halsband et al., 2020; Yang

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et al., 2022). However, little is still known about the toxicity and potential impacts of TWP leachates on marine plankton food webs. Marine zooplankton constitutes an important link in marine food webs as they inhabit in multiple trophic levels in marine pelagic food webs (Steinberg and Landry 2017). Furthermore, copepods are key contributors in the recycling of nutrients, food web productivity and carbon sequestration via the biological pump (Steinberg and Landry 2017). Effects of pollution on copepods may therefore result in changes to marine ecosystem function underlining the importance to understand the effects of TWP leachates on marine copepods.

The main objective of this study was to investigate the lethal effects of TWP leachates on different marine copepod species, using a commonly used tire found in the Danish market. We hypothesized that copepod's response to TWP leachates will be influenced by 1) leachate concentration, 2) the exposure time and 3) copepod size and feeding behaviour. Acute toxicity experiments were carried out in the laboratory by exposing adults of five copepod species (the cyclopoid *Oithona davisae,* the harpacticoid *Amonardia normanni* and the calanoids *Temora longicornis, Centropages hamatus* and *Acartia tonsa*) to different leachate concentrations to estimate the median lethal concentrations (LC_{50}). These five copepod species were selected because they are abundant species in coastal waters and differ in taxonomy, size, feeding behaviour, and habitats (Steinberg and Landry 2017; Razouls et al., 2022). Description of the swimming/feeding behaviours of the experimental copepods species can be found in (van Someren Gréve et al., 2017). Moreover, the selected experimental copepod species/genera are ecologically relevant (Lampitt and Gamble 1982). The genus *Oithona* is found all through global oceans and is often numerically dominant amongst the metazooplankton (Böttger-Schnack et al., 1989; Hay et al., 1991; Nielsen et al., 1993) *O. davisae* is a coastal and estuarine species originally from Indo West-Pacific that it has been spread to other oceans in the last decades as an invasive species. The three calanoids are common and very abundant in coastal waters (Fransz et al., 1998; Nielsen and Munk 1998; Martynova et al., 2009; Razouls et al. 2005–2023) and the harpacticoid is a widely distributed semibenthic species commonly found in European coastal waters, lagoons and subtidal areas (Castel 1979; Koski et al., 2005).

2. Methods

2.1. Experimental copepod species

All species were reared in the laboratory of the National Institute of Aquatic Resources in the Technical University of Denmark for over 100 generations. The cultures were kept in 30 L buckets filled with filtered sea water (FSW), with constant aeration, 28‰ salinity and temperature of 16 °C. The adult copepods used in experiments were maximum 6 weeks old. *A. tonsa, T. longicornis* and *C. hamatus* were fed with a mix of the cryptophyte *Rhodomonas salina*, the diatom *Thalassiosira weissflogii* and the dinoflagellate *Heterocapsa steinii, O. davisae* was fed with the dinoflagellate *Oxyrrhis marina* and *A. normanni* was fed with *T. weissflogii*. All cultures were fed in excess (ca. 400 μg C L^{-1}) 2–3 times weekly. The prosome length (PL) of 30 adult females of each species was measured before the start of the experiments, except for *A. normanni* where the total length (TL) was measured. The carbon weight was calculated according to length-weight equations from the literature (Table 1). All phytoplankton cultures were grown at 18 °C and approximately 28‰ salinity, in B1 media (Hansen 1989) with an illuminance of ca. 75 μE m^{-2} s^{-1} and 14:10 h day:night light exposure.

2.2. Leachate extraction

A new car tire (Imperial 145/70-13 71T- Snowdragon HP-Vinterdæk) was bought from a commercial store (thansen.dk) in August 2021. To generate leachates for the acute toxicity tests, we followed the protocol proposed by (Almeda et al., 2023) with some minor modifications

Table 1
Body length (μm; mean ± SD) and carbon weight (μg C $ind.^{-1}$) of *A. tonsa, T. longicornis*, *C. hamatus*, *O. davisae* and *A. normanni* as well as the length-weight regression employed to estimate carbon weight for each species.

Species	Mean length (μm)	Mean carbon weight (μg C)	Length-weight equations	References
A. tonsa	728 ± 58	2.53 ± 0.6	$W_c = 1.11 \times 10^{-5}\ PL^{2.92}$	(Berggreen et al., 1988)
T. longicornis	647 ± 43	3.34 ± 0.7	LogW = 3.064 log PL -7.696	(Breteler et al., 1982)
C. hamatus	853 ± 74	4.87 ± 1.1	LogW = 2.4492 log PL - 6.0984	(Breteler et al., 1982)
O. davisae	326 ± 51	0.36 ± 0.1	$Wc = 0.0318\ PL^{1.61}$	(Almeda et al., 2010)
A. normanni	672 ± 54	2.2	–	(Tanskanen 1994)[a]

[a] Carbon weight was calculated from length measurements and length-carbon regressions of Tanskanen (1994).

(Fig. 1). Firstly, the tire's outer layer was cut into strips. Subsequently, micronization of tires was conducted by grinding the strips using a stainless-steel pneumatic milling cutter, instead of mill grinder (Almeda et al., 2023). The obtained TWP were collected in a stainless-steel tray where we added liquid nitrogen for easier particle collection. Collected TWP were sieved through a standard steel sieve with a certified 250 μm steel mesh by hand and using a paint brush, instead of using sieve shaker as proposed by Almeda et al. (2023). The size distribution of TWP is shown in Page et al. (2022). The measurements revealed that 98% of the TWP had an equivalent spherical diameter (ESD) of less than 250 μm. It is worth noting that the present study used the same tire and lixiviation protocol as Page et al. (2022).

After size fractionation, 5 g of TWP were added to 1 L glass bottles with screw caps with a polytetrafluoroethylene (PTFE) protected seal along with autoclaved FSW with a salinity of 28‰ and pH of 8.17. The bottles were closed without air, headspace and covered with aluminium foil to ensure dark conditions. The bottles were placed in a rotating plankton wheel (1 rpm) for 72-h in a 20 °C room to allow for lixiviation. After 72-h, the leachates were filtered in a glass vacuum filtration system using glass-fibre filters (Whatman GF/F filters 0.8 μm). The stock solution (100%) was stored refrigerated in 1 L glass bottles for less than 24-h before use in toxicity testing. The chemical analysis of TWP and leachates was conducted as described in Page et al. (2022). The concentrations of organic additives and metals found in both TWP and leachates can be found in Supp. Table S1. The full chemical analysis can be found in Page et al. (2022).

2.3. Experimental setup

Acute toxicity tests were executed by exposing copepods to different leachate solutions (0.1, 1, 10, 25, 50 and 100%) and to a control treatment with only FSW. For *T. longicornis*, an extra test solution of 0.05% was added. All treatments were conducted in triplicates. The test solutions were prepared by serial dilution of the stock leachate solution (100%, 5 g TWP L^{-1}) in autoclaved FSW. We used a solid-to-liquid ratio of 5 g L^{-1} in the stock solution for maximizing the sensitivity and to have dilutions covering the range from no effect to greater than 50% effect to allow precise calculation of LC_{50}. This allows a quantitative comparison of the toxicity among different copepods. The dilutions cover concentrations ranging from 0.005 g L^{-1} to 2.5 g L^{-1}, which increases the environmental relevance and enables a more accurate assessment of the substance's impact. The salinity of the test solutions was 28‰ and the pH of the stock leachate and FSW was 8.07 ± 0.01. Twenty adult copepods were placed in 34 mL bottles containing the test solutions. All the experiments were conducted in the absence of food. The exposure took place in an 18 ± 0.5 °C temperature-controlled room in total darkness.

Mortality was used as an endpoint. Copepod survival was checked

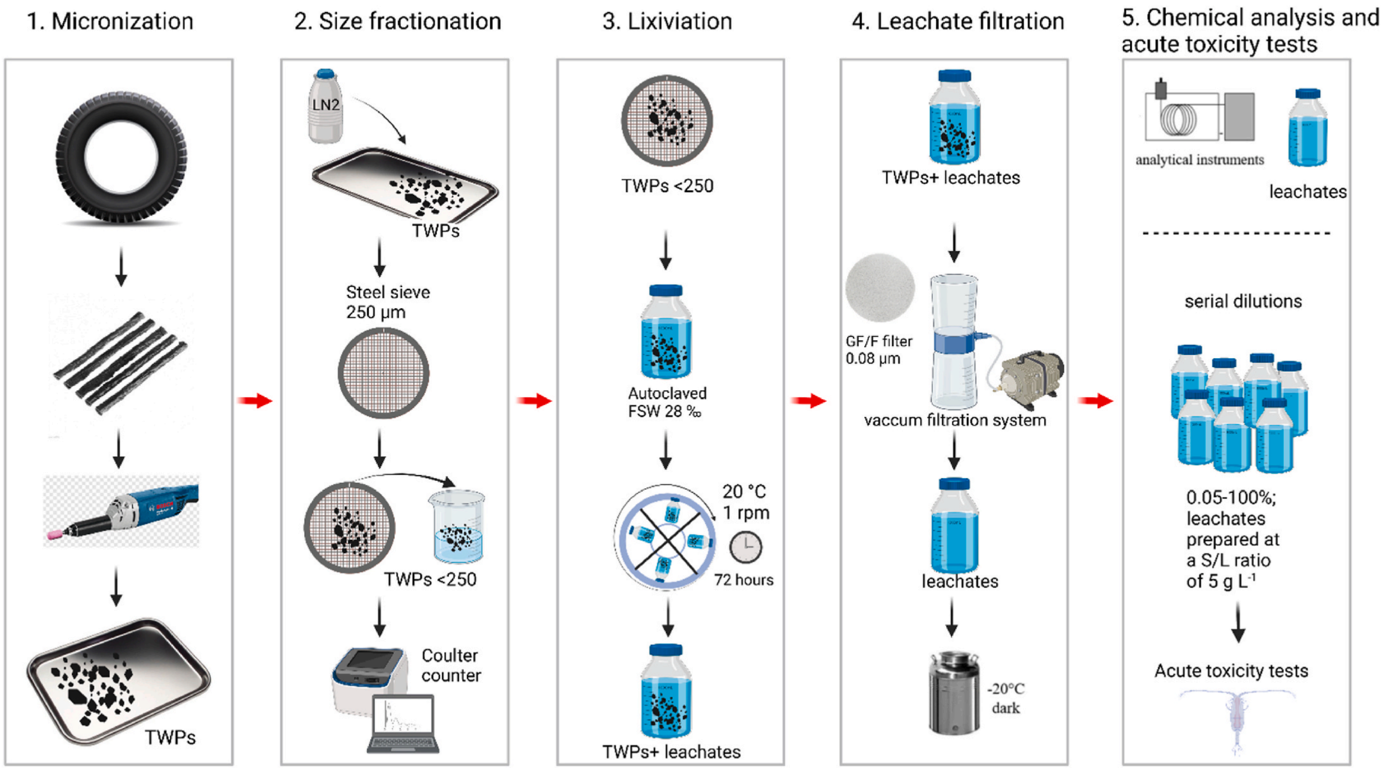

Fig. 1. Overview of leachate generation, adapted from (Almeda et al., 2023). Created with BioRender.com.

every 24-h over the course of 72-h by pouring the bottle content into a Pyrex Glass evaporating dish and inspecting the copepods using a stereomicroscope. A copepod was considered dead if it did not move for over 30 s after poking it with a glass pipette. Copepod mortality was calculated as the proportion of dead individuals from the total amount of copepods in the bottle.

2.4. Calculations and statistics

Data on the mortality of each copepod species versus the TWP leachate solution was fitted to the following sigmoid model for each exposure period (24, 48 and 72-h):

$$M = 100 \big/ \left(1 + e^{-(C - LS_{50})/b}\right) \quad \text{Eq.1}$$

where M is the mortality (%), C is the TWP leachate solution (%), LS_{50} is the median lethal solution (%) and b is the slope. Although the toxicity of plastic leachates does not increase linearly with the plastic load or S/L ratio (Beiras et al., 2019), the estimated LS_{50} values (%) were expressed in their equivalent median lethal concentration (LC_{50}) in g TWP L^{-1} for comparison with other toxicity studies and environmental concentrations of TWP. Furthermore, LS_{50} data for each time point were fitted to the Reduced Life Expectancy model (Verma et al., 2013):

$$LS_{50} = a\mathrm{Ln}LT_{50} + b \quad \text{Eq. 2}$$

where LS_{50} is the median lethal solution (%), a is equal to $-1/d$, where d is constant, LT_{50} is the exposure time and b is ln (NLT)/b, where NLT is the normal life expectancy, specifically is the time until 50% of the organisms die without exposure.

Statistically significant differences ($\alpha \leq 0.05$) in the average mortality of copepods among experimental treatments were evaluated using one way analysis of variance (ANOVA) and post hoc Dunnett's test for comparison between the control and the experimental treatments. When data did not follow a normal distribution, the non-parametric test, Kruskal-Wallis was used, followed by Dunn's test for the comparison between the control and the experimental treatments. Normality was tested using Shapiro-Wilk test and the homogeneity of variances was tested using Brown-Forsythe test.

3. Results

Acute exposure to TWP leachates induced elevated mortality in all studied species, with increasing mortality with increased leachate solution over time (Fig. 2). The calanoids *A. tonsa* and *T. longicornis* had a mortality greater than 88% at TWP leachate solutions of >25%, and a mortality of 34–64% at leachate solutions of 0.05–10% (Fig. 2A and B). *C. hamatus* had a 100% mortality only in the highest leachate solution, while for the lower solutions, the mortality ranged between 31 and 68% (Fig. 2C). *O. davisae* exhibited 100% mortality at the TWP leachate solutions of 50 and 100%, whilst in solutions ≤25% the mortality was lower than 31% (Fig. 2D). *A. normanni* showed a high tolerance to TWP leachates at solutions ≤50% but had close-to 100% mortality at the highest TWP leachate solution (Fig. 2E). Mortality of *T. longicornis* at all exposure solutions was significantly different than the mortality in the control, while the mortality of *C. hamatus* was significantly different from the control in solutions >1% (Dunnett's test; $p < 0.05$; Table 2), and the mortality of *A. tonsa* in solutions >25% (Dunn's test; $p < 0.05$; Table 2). The mortalities of *O. davisae* and *A. normanni* were significantly different from the control at leachate solutions of >50% and 100%, respectively (Dunnett's test; $p < 0.05$; Table 2) (see Fig. 3).

The relationship between copepod mortality and TWP leachate solution was well described by the sigmoidal model for all exposure times and species (Fig. 2; Table 3). However, LS_{50} and slope (b) varied up to one order of magnitude between the species and exposure times (Table 3), with decreasing LS_{50} with increasing exposure time (Fig. 3). The calanoids *A. tonsa* and *T. longicornis* were the least tolerant species with LS_{50} of 72-h of 4.46 ± 1.22 and 8.76 ± 1.45% respectively (Table 3), whereas *C. hamatus* had a 72-h LS_{50} of 23.56 ± 4.91%, *O. davisae* a 72-h LS_{50} of 29.51 ± 2.43% and *A. normanni* a 72-h LS_{50} of 68.63 ± 4.22% (Table 3). The calanoid species were thus the most

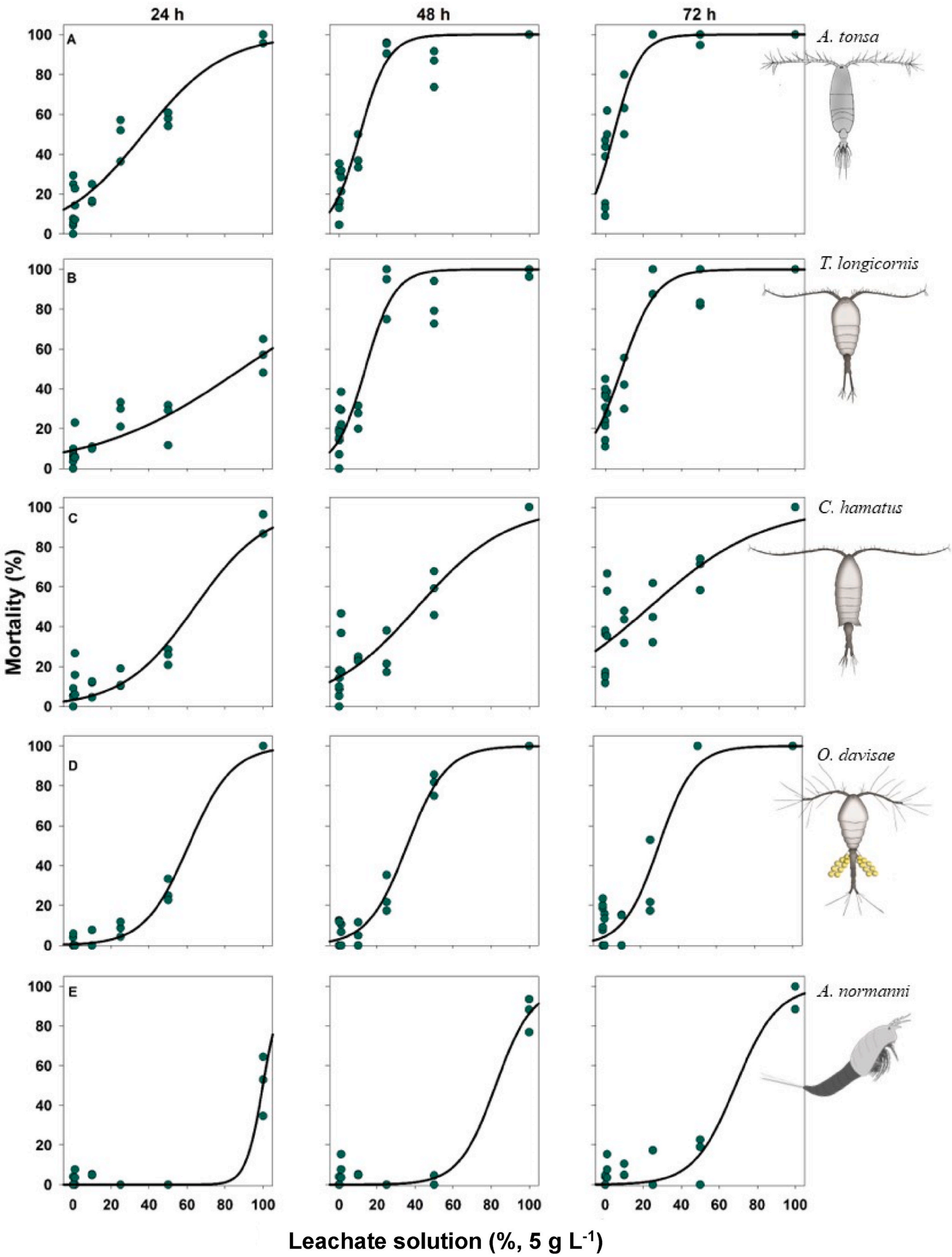

Fig. 2. Mortality (%) of *A. tonsa* (A), *T. longicornis* (B), *C. hamatus* (C), *O. davisae* (D) and *A. normanni* (E) after 24, 48 and 72-h of exposure to a range of leachate solutions (% from 5 g L^{-1}). Regression lines based on Eq. (1). Parameters from the regression model are indicated in Table 3.

Table 2
Mean mortality (±SD) of all copepods after 72-h for each TWP leachate solution (%). (*) indicates mortality rates that were significantly different from the control (Dunn's test; $p < 0.05$).

Species	Mortality % ± SD							
	0	0.05	0.1	1	10	25	50	100
A. tonsa	12.5 ± 3.2	–	43.2 ± 4.1	54.0 ± 6.9	64.4 ± 15.0	100*	98.2 ± 3.0*	100*
T. longicornis	15.6 ± 5.3	34.9 ± 3.6*	36.3 ± 11.1*	33.8 ± 5.5	42.6 ± 12.8*	95.8 ± 7.2*	88.4 ± 10.1*	100*
C. hamatus	14.2 ± 2.1	–	30.6 ± 11.5	53.3 ± 16.2*	41.2 ± 8.4*	46.3 ± 14.9*	67.9 ± 8.4*	100*
O. davisae	15.5 ± 6.8	–	10.9 ± 11.9	9.7 ± 8.5	10.1 ± 8.8	30.7 ± 19.4	100*	100*
A. normanni	1.3 ± 2.3	–	1.6 ± 2.7	8.9 ± 6.0	6.8 ± 3.3	5.8 ± 10.0	13.9 ± 12.2	96.2 ± 6.7*

Table 3
Parameters from the sigmoidal model relating copepod mortality to leachate solution (Eq. (1)) after 24, 48 and 72-h of exposure. LS_{50}: median lethal solution (%). *LC_{50}: estimated median lethal concentration expressed in g TWP L^{-1}.

Species		LS_{50} ± SE (%)	*Slope* ± *SE*	r^2	*p*	*LC_{50} ± SE (g L^{-1})
A. tonsa	24 h	36.98 ± 3.35	21.27 ± 2.90	0.91	<0.0001	1.85 ± 0.17
	48 h	10.84 ± 1.40	7.54 ± 1.23	0.91	<0.0001	0.54 ± 0.07
	72 h	4.46 ± 1.22	6.95 ± 1.72	0.85	0.0017	0.22 ± 0.06
T. longicornis	24 h	88.62 ± 5.92	38.74 ± 4.38	0.82	<0.0001	4.43 ± 4.6
	48 h	13.70 ± 1.80	7.71 ± 1.34	0.88	<0.0001	0.68 ± 0.09
	72 h	8.76 ± 1.45	9.10 ± 1.62	0.88	<0.0001	3.80 ± 0.07
C. hamatus	24 h	64.08 ± 3.61	18.87 ± 2.40	0.93	<0.0001	3.00 ± 0.18
	48 h	41.31 ± 4.47	23.51 ± 3.83	0.86	<0.0001	2.06 ± 0.22
	72 h	23.56 ± 4.91	30.03 ± 6.84	0.74	0.0003	1.18 ± 0.24
O. davisae	24 h	60.69 ± 1.77	11.59 ± 1.43	0.99	<0.0001	3.03 ± 0.09
	48 h	35.74 ± 1.37	10.35 ± 0.93	0.98	<0.0001	1.79 ± 0.07
	72 h	29.51 ± 2.43	9.25 ± 1.81	0.92	<0.0001	1.47 ± 0.12
A. normanni	24 h	99.88 ± 24.50	4.49 ± 886.13	0.91	0.0006	4.99 ± 1.22
	48 h	82.36 ± 3.64	9.63 ± 1.84	0.97	<0.0001	4.12 ± 0.18
	72 h	68.63 ± 4.22	11.00 ± 2.16	0.95	<0.0001	3.43 ± 0.21

sensitive, followed by the cyclopoid *O. davisae* and the harpacticoid *A. normanni*. The equivalent LC_{50} in g TWP L^{-1} ranged from 0.22 to 3.43 g L^{-1} (Table 3).

The sensitivity of copepods to TWP leachates was not related to their body size. The smaller species *O. davisae* (0.36 μg C; Table 1) had a higher LS_{50} than the calanoids (*A. tonsa,* 2.53 μg C; *T. longicornis,* 3.34 μg C; *C. hamatus,* 4.87 μg C; Table 1). Furthermore, the harpacticoid *A. normanni* (2.2 μg C; Table 1) had 10 times higher LS_{50} than the similar-sized calanoids *A. tonsa* and *T. longicornis* (Fig. 4; Table 1). However, there was a positive relationship between body carbon weight and tolerance to TWP leachates among calanoid species, where the LS_{50} increased with calanoid carbon content (Fig. 4).

4. Discussion

Chemical composition and concentration of organic chemicals and metals differed notably between the particles and the leachates (Supp. Table S1). Naphthalene was the organic compound found in the leachates at the highest concentration (4.32 ± 0.23 ng mL^{-1} at 100%; Supp. Table S2). Saiz et al. (2009) studied the effect of naphthalene, one of the most common PAH found in seawater, on adult *O. davisae*, demonstrating no significant mortality at concentrations of ≤10 μg L^{-1} after 24-h exposure. This suggests that naphthalene was not the main compound responsible for toxicity in our experiments. The organophosphate flame retardant (OPFR), tris (2-chloroisopropyl)phosphate was also detected in the leachates but the toxicity of this organic plastic additive on marine copepods is unknown. Strontium (Sr; 4351.20 ± 36.14 ng mL^{-1}, Supp. Table S1) and Zn (1053.26 ± 74.04 ng mL^{-1}, Supp. Table S1) were the most abundant metals in our leachates. Studies have shown that metals, if in excess, can be toxic to marine organisms (Jeong et al., 2019; Zidour et al., 2019). Recently, Yang et al. (2022) identified Zn as the dominant toxicant in TWP leachates affecting the harpacticoid copepod *Tigriopus japonicus* (96-h LC_{50} 7.03 mg L^{-1}, Supp. Table S2). Moreover, Cherkashin (2020), tested Zn^{2+} on *Calanus glacialis* and *Neocalanus plumchrus* copepodites and showed 48-h LC_{50} of 962 μg L^{-1} and 4381 μg L^{-1}, respectively (Supp. Table S2). Furthermore, Okamoto et al. (2015) showed that Zn had high toxicity on the freshwater species, *Daphnia magna* (24-h EC_{50} of 0.72 mg L^{-1}), which is lower than the concentrations measured in our leachates. Capolupo et al. (2020) and Halsband et al. (2020) also highlight that Zn is likely one of the main contributors causing the toxicity of TWP leachate on plankton. The effects of Sr on marine copepods are unknown, but Okamoto et al. (2015) found that Sr had a toxic effect on *D. magna* at a concentration almost 30 times higher (24-h EC_{50} of 120 mg L^{-1}) than the concentration found in our leachates, indicating very low toxicity of Sr on aquatic biota. Our results suggest that either Zn caused the mortality, or that the coexistence of the various chemicals resulted in a cocktail effect or other leached additives that were not measured in this study. For instance, Li et al. (2023) found that antioxidant N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) used as an additive in tyre rubber contributed notably to the TWP toxicity to freshwater zooplankton. Further studies (e.g., chemical fractioning of leachates and directed-effect analyses, DEA) are needed to identify which compounds drive the toxicity to enable a reduction or removal of these compounds from car tires and other rubber products.

TWP leachates can negatively affect a wide range of marine organisms (Halsband et al., 2020; Tian et al., 2021), including phytoplankton (Capolupo et al., 2020; Page et al., 2022) and copepods (Halsband et al., 2020; Yang et al., 2022). Our results show dose-, time- and species-dependent sensitivity to leachate toxicity. Mortality of copepods increased with increased leachate solutions and exposure time, as expected. Yang et al. (2022) found a 96-h LC_{50} of 5.34 g L^{-1} (Supp. Table S2) for *T. japonicus* while Halsband et al. (2020) found 48-h LC_{50} values of 35 g L^{-1} and <5 g L^{-1} for *Calanus* sp. and *Acartia longiremis*, respectively (Supp. Table S2). Our LC_{50} were lower than those reported found in Yang et al. (2022) and Halsband et al. (2020). The differences in LC_{50} among studies with TWP leachates and copepods could be attributed to differences in TWP characteristics (particle size, tire type) and experimental/leaching conditions (solid to liquid ratio, temperature, salinity, incubation time) and/or differential tolerance amongst copepod species. For instance, Yang et al. (2022) incubated 10 g L^{-1} for two months, Capolupo et al. (2020) 80 g L^{-1} at 25 °C for 14 d and Halsband et al. (2020) 10 and 100 g L^{-1} at 20 °C for 14 d, while they all used different types of tires. Wik and Dave (2006) showed a wide range of LC_{50} values when using 25 different types of tires, indicating that

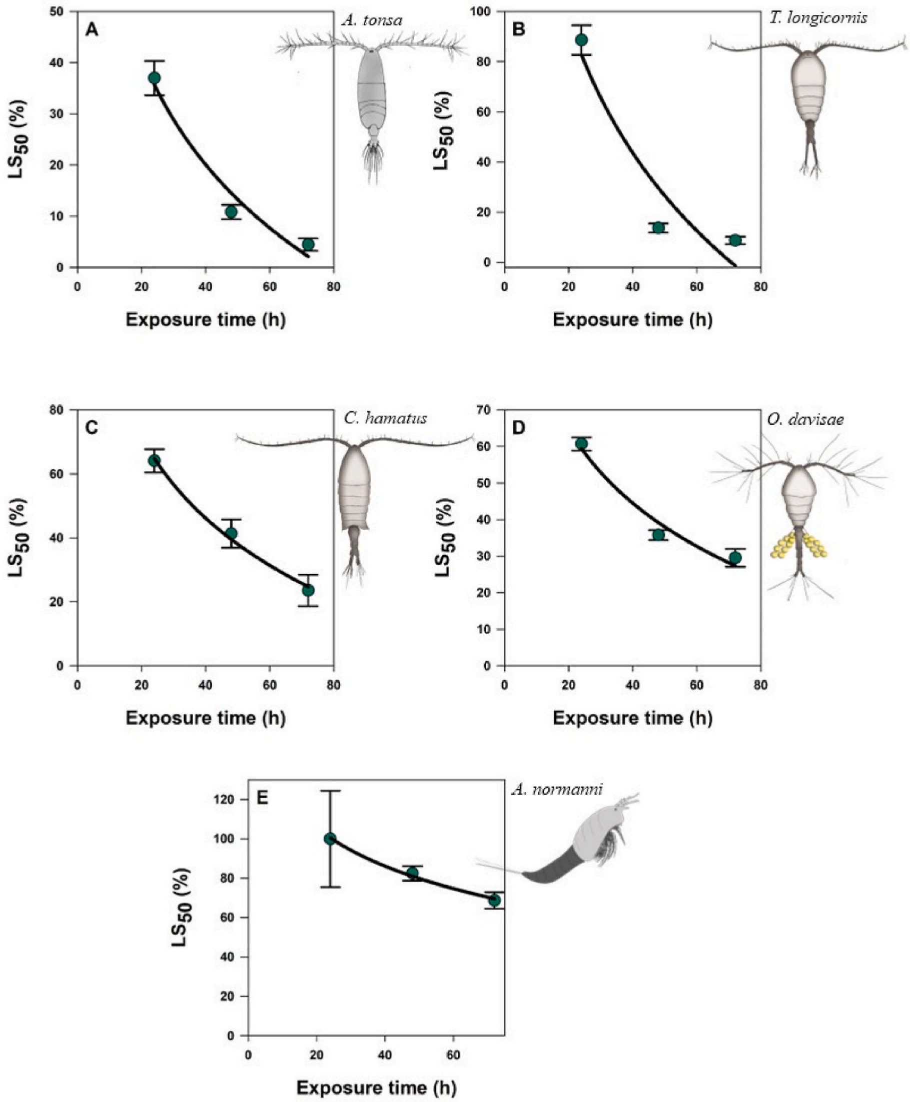

Fig. 3. LS_{50} (%) of *A. tonsa* (A), *T. longicornis* (B), *C. hamatus* (C), *O. davisae* (D) and *A. normanni* (E) as a function of exposure time (h). The model fitted to the data is the Reduced Life Expectancy model (Verma et al., 2013): $LS_{50} = -a\ lnLT_{50} + b$. Note the difference in the y-axis.

differences in the chemical composition of tires can also cause differences in toxicity. Hence, it is important to keep in mind variations in methods and tire types when comparing acute toxicity of TWP leachates among studies. This highlights the importance of establishing standardized methods for leachate production for more conclusive and comparable results among studies (Almeda et al., 2023).

Species-specific sensitivity could be explained by differences in (a) feeding/swimming behaviour, (b) oral intake of water and (c) adaptation to pollution. The three calanoids used in this study are feeding current feeders, meaning that their foraging strategy is "active" compared to the other two taxonomic orders, which are ambush feeders (passive foraging strategy) and therefore less motile (Kiørboe 2011). van Someren Gréve et al. (2017) studied the swimming behaviour of copepods with different foraging techniques, showing that *T. longicornis* adults were swimming constantly, whereas *C. hamatus* spent only half of the time swimming. In contrast, *Oithona nana*, which uses the same feeding strategy as *O. davisae*, had very low swimming activity and spent most of the time sinking slowly through the water column. Based on our observations throughout the experiments, the calanoids were the most active compared to the other two species, especially *A. tonsa* and *T. longicornis*, despite *A. tonsa* being able to switch from active to passive feeding strategy depending on food availability (Kiørboe et al., 1996). *A. normanni and O. davisae* showed low swimming activity. The main pathways that toxic environmental substances can enter the food web are through ingestion (along with the food) or diffusion from the water (Wang and Fisher 1998). Studies have shown that the accumulation of toxic substances, such as heavy metals, in marine organisms is lower through ingestion compared to absorption from water (Wang et al., 1996). However, Kadiene et al. (2019) confirmed the oral intake of water and suggest that the uptake of dissolved metals by this process can influence the bioaccumulation of metals in copepods. Therefore, toxic substances in leachates could also enter an organism's system through oral intake of water and not only through diffusion or together with ingested prey. More research is needed to evaluate the role of oral intake of water oral in the bioaccumulation of metals in copepods considering feeding behaviour.

One of our initial hypotheses was that copepod's sensitivity to leachates would be size-dependent, expecting smaller species to be the most affected due to larger surface to volume ratio and thus larger uptake of leachates. Instead, we found that the effect of leachates was related to taxonomy rather than size. This contrasts with the observed positive correlation between copepod body size and tolerance to other pollutants such as the water-soluble fraction of crude oil (Jiang et al., 2012). However, the sensitivity to leachates was size-dependent within

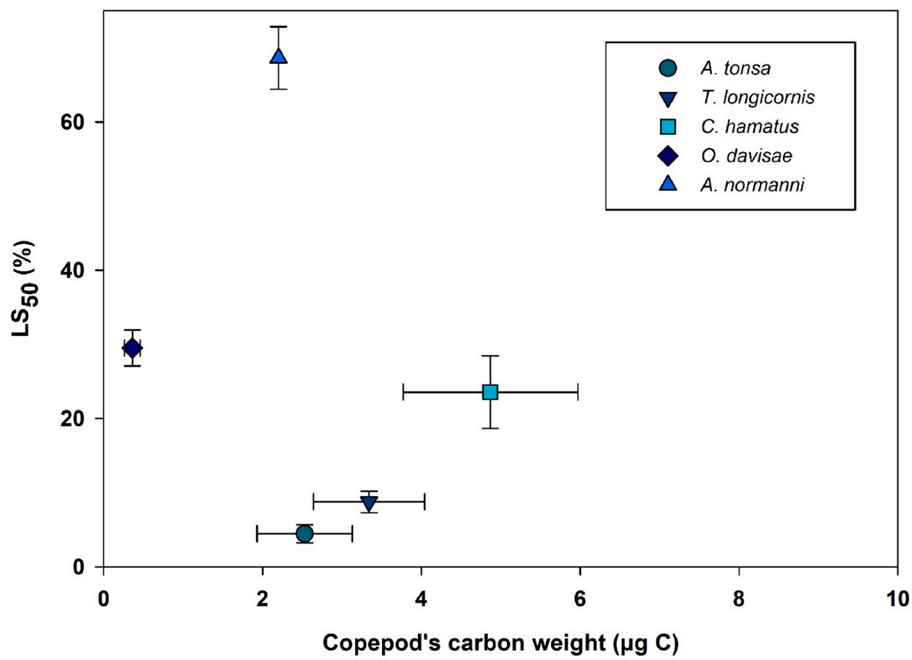

Fig. 4. LS_{50} (%) as a function of copepod carbon weight (µg C; mean ± SE) for all species.

the calanoids as expected, based on surface to volume ratio. Further research on the stage-specific effects of TWP leachates on planktonic copepods are needed to evaluate the intraspecific size-dependence of copepod sensitivity to leachate and their potential impact during development/the life cycle. A study suggests that the copepod *Oithona* spp., specifically *O. nana*, has increased tolerance and adaptability to coastal pollution from maritime, urban and industrial activities (Drira et al., 2018). *O. davisae* is an invasive species found in harbours and polluted bays (Uye and Sano 1995), which suggests certain adaptation to pollution that allows this species to inhibit these anthrophonic affected habitats. The studied harpacticoid inhabits lagoons and subtidal areas, which are more susceptible to chronic pollution. Although data is limited, benthic and semi benthic harpacticoids are adapted to harsher environments (O'Brien et al., 1988), thus their tolerance to pollution might be higher than pelagic calanoids (Medina et al., 2008). Therefore, adaptation to pollution could explain some of the observed differences in sensitivity to TWP leachates among species.

Field data on the concentration of TWP particles and their leachates is still limited. Peter et al. (2020) detected 2-hydroxybenzothiazole, a tire derivative, at concentrations up to 150 mg L^{-1} in stormwater runoff. Although the range of LC_{50} (0.22–3.43 g L^{-1}) in our study was not in the range of the available predicted environmental concentrations in surface waters estimated by Wik and Dave (2009) (0.03–56 mg L^{-1}), we observed mortality greater than 30% for the calanoids in our three lower TWP leachate solutions (0.05%, 0.1% and 1%, equivalent to 2.5, 5 and 50 mg L^{-1}) which fall into the predicted environmental concentrations. Therefore, although environmental data is still scarce, our results indicate that TWP leachates can negatively affect marine copepods, specially calanoids, with potential harmful impacts on marine plankton food webs, particularly in coastal areas after runoff events.

5. Conclusion

TWP leachates caused mortality on the five marine copepod species. Calanoids were the most sensitive copepods to TWP leachates, even at exposure solutions in the range of predicted environmental concentrations. Despite their small size, the cyclopoid *O. davisae* was more tolerant than calanoids to TWP leachates. The harpacticoid was the less sensitive species amongst the studied copepods. Traits such as motile behaviour and adaptation to pollution could influence the tolerance of marine copepods to TWP leachates. We need more empirical data of TWP concentration in sea water and in sediments to better evaluate the environmental risk of this type of pollution. Given the importance of copepods in marine environments, our results suggest that TWP leachates can negatively impact marine plankton food webs and consequently marine coastal ecosystems.

CRediT authorship contribution statement

Evanthia Bournaka: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. Rodrigo Almeda: Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition. Marja Koski: Conceptualization, Methodology, Validation, Writing – review & editing, Supervision. Thomas Suurlan Page: Investigation, Writing – review & editing. Rebecca Elisa Andreani Mejlholm: Investigation, Review & editing. Torkel Gissel Nielsen: Conceptualization, Methodology, Validation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106163.

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