Effect of environmentally relevant concentrations of potentially toxic microplastic on coastal copepods

Marja Koski a,*, Jens Søndergaard b, Anette Maria Christensen a,1, Torkel Gissel Nielsen a

a National Institute for Aquatic Resources (DTU Aqua), Technical University of Denmark, Kemitorvet, Building 202, DK-2800, Kgs. Lyngby, Denmark
b Department of Bioscience, Aarhus University, Frederiksborgvej 399, DK-4000, Roskilde, Denmark

ARTICLE INFO
Keywords:
Microplastic
Tire wear particles
Acartia tonsa
Temora longicornis
Feeding
Reproduction

ABSTRACT
Tire wear particles (TWP) are both abundant and potentially toxic types of microplastic (MP) in the coastal ocean. We tested the effects of TWP type (new tires, old tires, rubber granules from artificial turfs) and concentration (10–10,000 TWP L⁻¹) on feeding, reproduction and fecal pellet production of two common coastal copepods at high (400 μg C L⁻¹) and low (40 μg C L⁻¹) food concentration consisting of a cryptophyte Rhodomonas sp. We did not observe any effect of TWP on copepods at environmentally relevant concentrations of <10 TWP L⁻¹. At TWP concentrations that were >100 times higher than the MP concentrations measured in coastal waters, food concentration, copepod feeding mode, TWP concentration and TWP type interacted to influence copepod feeding and pellet production, while reproduction was unaffected. Our results suggest that TWP at the current measured concentrations in the ocean environment is not likely to be a threat to the common coastal copepods.

1. Introduction

Microplastic (MP < 5 mm) has been identified as a threat to the marine environment (GESAMP, 2015), because it is small and abundant (Enders et al., 2015) and more bioavailable than macroplastics (Desforges et al., 2015; Sun et al., 2017, 2018). Many zooplankton species have been shown to ingest MP (Botterell et al., 2019, and references therein), which overlaps in size with the plankton prey of dominant zooplankton species (Enders et al., 2015). Nevertheless, the effects of MP ingestion on the vital rates of zooplankton seem to be limited (Botterell et al., 2019). Most studies on the effects of MP have been made using clean or coated plastic spheres (Phuong et al., 2016). However, there is a concern that environmental MPs might be more harmful than the plastic spheres used in the laboratory, either due to the numerous chemicals that the original plastic products contain, or due to the chemicals that the particles can scavenge from the environment (Andrady, 2017). Leachates from plastic have been demonstrated to cause acute toxicity for several aquatic organisms including phytoplankton (Wik et al., 2009; Capolupo et al., 2020), cladocerans (Wik et al., 2009; Lithner et al., 2009, 2012), copepods (Bejgarn et al., 2015) and mussels (Capolupo et al., 2020).

Tire wear particles (TWP) and rubber granules (RG) from artificial turfs are both abundant (Magnusson et al., 2016; Kole et al., 2017) and potentially toxic (https://echa.europa.eu/hot-topics/microplastics) types of MP in the coastal zone. The estimates of the production of TWPs on the roads within the European Union is approximately 1.3 × 10⁶ tons year⁻¹, of which 10 % could reach the surface waters (Wagner et al., 2018). In addition, a recent analysis suggests that 34 % of the emitted TWPs will reach the world oceans through atmospheric deposit (Evangelou et al., 2020). The estimates of the loss of RGs from artificial turfs in Denmark and Sweden suggest that, respectively, between 380 (Lassen et al., 2015) and 3900 (Magnusson et al., 2016) tons year⁻¹ could be lost. TWPs contain a wide variety of chemical substances including synthetic and natural rubber polymers, fillers such as black carbon, and different types of softeners, as well as metals from brake-abrasion (Baumann and Ismeier, 1998). The most common type of RGs are made from scrap tires, and thus contain most of the same chemical substances as TWPs (https://echa.europa.eu/hot-topics/microplastics), including high amounts of bio-accessible heavy metals such as zinc (Zn), copper (Cu) and cobalt (Co) (Canepari et al., 2018; Halsband et al., 2020).

* Corresponding author.
E-mail addresses: mak@aqua.dtu.dk (M. Koski), js@bios.au.dk (J. Søndergaard), anemc@mst.dk (A.M. Christensen), tgin@aqua.dtu.dk (T.G. Nielsen).
1 Present address: Danish Environmental Agency, Tolderlundsvej 5, DK-5000, Odense C, Denmark.

https://doi.org/10.1016/j.aquatox.2020.105713
Received 3 April 2020; Received in revised form 20 November 2020; Accepted 28 November 2020
Available online 3 December 2020
0166-445X/© 2020 Elsevier B.V. All rights reserved.
Marine zooplankton is dominated by copepods - the link between phytoplankton and larval fish in the pelagic food web (Steele, 1974), and an essential component of biogeochemical cycles (Steinberg and Landry, 2017). Copepods’ ingestion of potentially toxic MP may therefore have adverse effects on the marine ecosystem if chemicals from the plastic particles are assimilated during the gut passage, or if they have direct negative effects on copepods’ vital rates. The first option can result in a bioaccumulation of harmful substances through the food web, while the second option can ultimately influence the functioning of the marine ecosystem, including food web productivity, nutrient cycles or carbon sequestration through the biological pump.

In this study, we investigated the effects of TWP (including RG) on copepod feeding, fecal pellet production, egg production and egg hatching success. These ecophysiological rates determine the gross growth and assimilation efficiencies, and therefore influence food web transfer efficiency, nutrient cycling and particle flux. We used two common coastal copepods, *Acartia tonsa* and *Temora longicornis*, which have similar food size preferences (Berggreen et al., 1988; van Someren Grève, 2013), but differ in their dominant feeding mode. We hypothesized that the response of copepods will be influenced by 1) specific toxicity and concentration of TWP, 2) feeding strategy of the zooplankton species and 3) food concentration. Further, we hypothesized that 4) the observed effects can be both due to the ingestion of particles or due to their toxic leachates. The two copepod species were exposed to TWP concentrations that included environmentally realistic numbers of MP observed in the European coastal waters.

2. Methods

2.1. Copepods, plastic and algae

Copepods were collected using a WP2 net with a non-filtering cod end from the surface layer at the Øresund Coastal, coastal waters of Denmark (56°N, 12°E) 1–2 days before the start of the experiments. Surface water for the experiments was collected at the same time as the copepods, and filtered through 0.2 μm filters. The salinity of the water in all experiments was ca. 10 ppm (ppm). New unused tire was bought from a dealer in May 2018, while the old tire had been used in a person car for >5 years. Plastic particles from an artificial turf were collected from a football field at Nykøbing Falster (Guldborgsund Municipality, Denmark). These rubber granules were made of styrene-butadiene rubber (SBR), thus of old car tires.

The experimental TWP were produced by grinding a submerged piece of tire or a handful of rubber granules in seawater using sand paper. The resulting seawater suspension containing the TWP particles was first filtered through a 20 μm net, and then onto an 8 μm net. Finally, the particles captured onto the 8 μm net were re-suspended into seawater. The TWP offered to copepods therefore consisted primarily of the particles in the size range of 8–20 μm, although smaller particles could have been present due to their adhesion to larger particles during the filtration. The concentrations of TWP particles in these stock solutions were counted using the Coulter Counter (Particle Data Inc.) that confirmed the size distribution of the particles. Particles in the size class of 8–20 μm are readily ingested by both *Acartia tonsa* and *Temora longicornis* (Berggreen et al., 1988; van Someren Grève, 2013, respectively). Also, the size distribution was similar to the average size of the copepod food, the cryptophyte *Rhodomonas salina* (average diameter of 6–7 μm), a typical good quality food source for copepod growth and reproduction (Koski et al., 1998). *R. salina* was offered to copepods at the average food concentrations of 480 ± 55 μg C L⁻¹ (high food; HF) and 43 ± 5 μg C L⁻¹ (low food; LF).

Samples of rubber granules and new and old tire particles were analysed for chemical composition at the trace metal laboratory at Department of Bioscience, Aarhus University in Roskilde. Subsamples of 0.3 g granulated material were digested in 8 mL half-concentrated Merck Suprapure nitric acid in an Anton Paar Multiwave 7000 microoven according to the Danish standard DS 259. A pre-defined program for inorganic samples using high temperature and long destruction time was used to obtain the highest efficiently (15 ± 60 min at 280 °C). For each sample type, 4–5 replicates were used and blanks and Certified Reference Material (MESS-4, marine sediment) were digested along with the samples to evaluate digestion efficiency, measurement accuracy and precision. After digestion, solutions were diluted with MilliQ water and analysed for 60+ elements using an Agilent 7900 ICP-MS. Detection limits were determined as 3 standard deviations on the blank samples and blank values subtracted from the results.

2.2. Treatments

Three types of tire wear particles (TWP) were used in experiments: TWP made from new tire (NT), old tire (OT) and rubber granules (RG) from an artificial turf. NT, OT and RG were used in experiments with the copepod *Acartia tonsa*, and NT and OT were used in experiments with the copepod *Temora longicornis*. Experiments with RG could not be conducted with *T. longicornis*, since this species was not present in the coastal waters in sufficient numbers at the time of the RG experiments. All experiments (3–4 replicates) were conducted with TWP concentrations of 0 (control), 10, 100, 1000 and 10,000 particles L⁻¹, using both high (ca. 400 μg C L⁻¹) and low (ca. 40 μg C L⁻¹) food (*Rhodomonas salina*) concentrations (Table 1). In addition, every experiment included a leachate control, which consisted of water from the treatment with 10,000 TWP L⁻¹, but from where the particles had been filtered out using an 8 μm net. In a few experiments, an extra treatment was added as control for the effect of sand originating from sand paper (see below); the sand paper control consisted of 10,000 sand particles L⁻¹. In the experiments with *A. tonsa* we measured feeding (clearance and ingestion rates), fecal pellet production, egg production and hatching. In the experiments with *T. longicornis*, hatching was not measured due to the low number of produced eggs. Also, since the egg production of *T. longicornis* in the control of the experiments with new tires was unexplainably low, this data was not used. All experiments consisted of a 24-h acclimatization to the experimental food, a 24-h experiment, and additional 48-h incubation for the hatching success. The experiments were conducted between May and September 2018 (Table 1); a control incubation (no TWP) was conducted in every series of experiments to control for a potential effect of the changing season.

2.3. Experimental set up

The acclimatization was conducted in 1 L bottles, each containing

| Date | Species | Treatments | Plastic concentrations (TWP L⁻¹) | Measurements |
|------|---------|------------|---------------------------------|-------------|
| 16.5–18.5 | *T. longicornis* | OT HF + LF | 0, 10, 100, 1000, 10,000 | Mortality, feeding, pellet and egg production |
| 23.5–25.5 | *T. longicornis* | NT HF + LF | 0, 10, 100, 10,000 (HF only) | |
| 6.8–8.8 | *A. tonsa* | RG HF | 1000, 10,000, 10,000LC, sand | Mortality, feeding, pellet and egg production |
| 13.8–16.8 | *A. tonsa* | OT HF + LF | 0, 10, 100, 1000, 10,000LC | |
| 21.8–23.8 | *A. tonsa* | NT HF + LF | 0, 10, 100, 1000, 10,000LC | |
| 20.9–22.9 | *A. tonsa* | RG LF | 0, 10, 100, 1000, 10,000LC, sand | |
the experimental food suspension and ca. 75 adult females and males. After 24 h, 10–14 actively swimming *Acartia tonsa* females and 1–2 males, or 5–8 *Temora longicornis* females, were added to four replicate 0.6 L bottles, while two similar bottles functioned as grazing controls (no copepods), to estimate the growth of *Rhodomonas* sp. in the absence of grazing. The bottles were closed air-tight and mounted on a plankton wheel rotating at ca. 1 round per minute for 24 h at the *in situ* temperature and in darkness, which kept the TWP suspended throughout the incubation. *Rhodomonas* concentrations were measured using a Coulter Counter. The numbers of eggs and pellets were counted and the condition (dead / alive and active) of the females was checked at the end of the experiment, after carefully filtering the contents of the bottles onto, respectively, 180 and 20 μm filters, and pouring into Petri-dishes. The Petri-dishes containing eggs were left in *in situ* temperature for a minimum of 48 h, after which the numbers of eggs and hatched nauplii were counted. The prosome length of females and ca. 30 eggs and fecal pellets were measured for diameter (eggs) or length and width (pellets) for each experiment using an inverted microscope with a precision of 25 μm for females and 5 μm for eggs and pellets.

### 2.4. Calculations and statistics

Clearance and ingestion rates were calculated according to Frost (1972). The gross growth efficiency was estimated by dividing the weight-specific egg production with the weight-specific ingestion (both relative to carbon content). The ratio of the weight-specific pellet

---

**Fig. 1.** A) Egg production (eggs f⁻¹ d⁻¹), egg hatching success (%) and b) fecal pellet production (pellets ind⁻¹ d⁻¹) of *Acartia tonsa* at the high food concentration, as a function of the concentration of particles originating from rubber granules, old tires and new tires (mean ± SE). (Solid symbols) treatments with microplastic particles, (open symbols) leachate controls, (light grey symbols) sand controls. In a) solid circles and solid line indicate egg production, solid diamonds and dashed line hatching success. Controls (0 particles L⁻¹) are missing from the old tire experiments since these experiments were conducted one day after the new tire experiments and one control was assumed to be sufficient (see Table 1). Note different scales of the y-axis.
production to the weight-specific ingestion was used as an indication of assimilation efficiency. Female carbon weights were estimated from the measured prosome lengths and carbon to length regressions of Berggreen et al. (1988) for *Acartia tonsa* and Klein Breteler et al. (1982) for *Temora longicornis*. To estimate the carbon contents of eggs and pellets, their volumes were first estimated assuming, respectively, spherical and cylindrical shapes, and then converted to carbon using the volume to carbon regressions from Kierboe et al. (1985) for eggs and from Urban-Rich et al. (1998) for pellets. The differences in clearance, ingestion, pellet and egg production rates and hatching success between the plastic concentrations, plastic types, food levels and copepod species, were tested using analysis of variance (ANOVA) and Holm Sidak post hoc tests (Appendix Table 1). If the conditions for normality and equal variance were not met, Kruskal-Wallis ANOVA on rank was used.

3. Results

3.1. The effect of TWP particles on reproduction, feeding and pellet production

Copepod egg production and hatching were unaffected by the TWP, irrespective of the plastic type and plastic concentration, copepod species or food concentration (Figs. 1a and 2a). Note that although the egg production appeared to be significantly different between the TWP types...
caused by the treatments (Figs. 1a and 2a). The average egg production and hatching rates of *Acartia tonsa* in the high food concentration were, respectively, 10–41 eggs \( \text{f}^{-1} \text{d}^{-1} \) and > 90% (Fig. 1a), and at the low food concentration 2–6 eggs \( \text{f}^{-1} \text{d}^{-1} \) and > 86% (Fig. 2a), irrespective of the TWP type or concentration. The average egg production of *Temora longicornis* was always low (2–7 eggs \( \text{f}^{-1} \text{d}^{-1} \)), but similarly unrelated to treatment (data not shown).

In contrast to reproduction, TWP had diverse effects on copepod feeding and fecal pellet production, depending on the interactions between food concentration, copepod species, and TWP concentration and type (Figs. 1b, 2b, 3, Table A1). First, abundant food seemed to mitigate the effects of TWP for *Acartia tonsa*, so that no effects of TWP particles on the feeding and pellet production were observed at the high food concentration, although the rates differed between the experiments (Figs. 1b, A1a). The average clearance and pellet production rates in different experiments ranged, respectively, from 0.18 to 0.62 mL ind. \(^{-1} \text{h}^{-1} \) and 33–79 pellets ind. \(^{-1} \text{d}^{-1} \), and were not significantly different between the TWP concentrations (2-way ANOVA; \( p > 0.05 \)).

Temora longicornis pellet production appeared more sensitive to TWP exposure than the pellet production of *A. tonsa*, since a response to increasing TWP concentration was observed both at the high and at the low food concentrations (Fig. 3). Third, high concentrations of new tire particles generally resulted in a decrease, while rubber granules and old tire particles tended to result in an increase of feeding and pellet production, for *A. tonsa* at the low food concentration, and for *T. longicornis* at both low and high food concentrations (Figs. 1b, 2b, 3, A1). Fourth, effects were mostly observed at the concentrations >1000 TWP L\(^{-1}\) (Figs. 1b, 2b, 3, A1) with an exception of the pellet production of *A. tonsa* exposed to old tire particles at the low food concentration, where the pellet production was significantly higher than in the control already at the concentration of 100 OT particles L\(^{-1}\) (Fig. 2b).

Gross growth efficiency (GGE) of *Acartia tonsa* was significantly lower at the high concentration of RG (0.07 ± 0.01) than in the control (0.17 ± 0.03; Table 2; Tukey HSD; \( p < 0.05 \)), likely due to increased feeding rates in this treatment (Fig. A1a). A similarly low GGE was observed in the leachate control of OT (0.09 ± 0.02), whereas the GGE at the leachate control of NT was significantly higher than in the control (0.27 ± 0.03). In general, the GGE was considerably higher at the low food concentration (0.14–0.69) than at the high food concentration (0.07–0.23), with the lowest rates in both food concentrations observed with 10,000 RG L\(^{-1}\) (Table 2). The proportion of the pellet production from ingestion was relatively stable in the high food concentration, irrespective of the TWP treatment (0.04–0.08), but more variable in the low food concentration (0.06–0.30), with significantly higher pellet production to ingestion ratio in the treatments with 10–1000 NT particles L\(^{-1}\) and in NT leachate control (Table 2).

### 3.2. The effect of the leachates

The TWP leachates had different and sometimes contrasting effects on the feeding and reproduction of copepods, depending on the TWP type. In most experiments with *Acartia tonsa* and old tire particles, feeding, pellet production or egg production were lower in the leachate control than in the corresponding concentration of particles (10,000 TWP L\(^{-1}\)) and similar to the control with no TWP addition (Figs. 1, 2, A1b), suggesting that the increase in pellet production rates was due to the physical effect of the TWP rather than the leachate. With new tire particles, the effect of leachate control tended to be similar to the effect of the corresponding concentration of particles (Figs. 1b, 2b, A1), suggesting that the decrease in pellet production and feeding rates at the high concentration of new tire particles could as well be due to the leachates as the particles themselves. The leachate control from the rubber granules in most cases had a similar effect to the leachate from new tires, with feeding and pellet production rates that were similar to the rates with corresponding concentration of particles (Figs. 1, A1b).

However, there were several exceptions to these trends. First, leachate from old tire particles appeared to stimulate the feeding rates of *Acartia tonsa* (Fig. A1a) and the pellet production rates of *Temora longicornis* (Fig. 3), and leachate from the new tires and rubber granules appeared to stimulate the egg production of *A. tonsa* (Figs. 1a and 2a). Second, the pellet production of *T. longicornis* in the leachate from new tires was similar to the control while the pellet production on the corresponding concentration of TWP was reduced (Fig. 3), indicating that the reduction of pellet production was due to the (ingestion) of TWP rather than due to the leachates. Third, negative feeding rates were measured in sand and leachate controls at the high food concentration (Fig. A1a). However, although no experimental error could be identified, these rates could not represent the real ingestion rates as high numbers of fecal pellets were produced in the same incubations (Fig. 2b) and as the gut passage time of copepods at the experimental temperatures are minutes rather than hours (Risbøe et al., 1982). It thus appeared that whereas new tire particles and / or their leachates at high concentrations mainly appeared to induce a reduction in feeding and pellet production rates of copepods, old tire particles induced an increase that was independent of the leachates, and rubber granule particles and / or leachates induced an increase.

### 3.3. Heavy metals in TWP

Zinc (Zn) was by far the most abundant metal in all TWP
Table 2

A) Gross growth efficiencies (GGE) and B) proportions of fecal pellet production from ingestion in the experiments with *Acartia tonsa* (mean ± SD). All calculations are based on the carbon specific rates. The GGE and PP: I ratios in the plastic concentrations from 10 to 1000 particles L⁻¹ were pooled as no significant differences in these were detected. The capital letters indicate values that were not significantly different from each other (Tukey HSD; p < 0.05). Abbreviations as in Table 1.

| Plastic type | Concentration | HF | LF |
|--------------|---------------|----|----|
| A) GGE | | | |
| C | 0 | 0.18 ± 0.03ᵃ | 0.44 ± 0.16ᵃ |
| RG | 10–1000 | 0.15 ± 0.05ᵃ | 0.28 ± 0.07ᵃ |
| OT | 10–1000 | 0.14 ± 0.03ᵃ | 0.69 ± 0.58ᵃ |
| NT | 10–1000 | 0.23 ± 0.04ᵃ | 0.57 ± 0.14ᵃ |
| RG | 10000 (LC) | 0.07 ± 0.01ᵇ | 0.14 ± 0.11ᵇ (0.57 ± 0.22ᵃᵇ) |
| OT | 10000 (LC) | 0.16 ± 0.05ᵇ (0.09 ± 0.02ᵇ) | 0.47 ± 0.22ᵃᵇ (0.95 ± 0.38ᵇ) |
| NT | 10000 (LC) | 0.17 ± 0.03ᵇ (0.27 ± 0.03ᵇ) | |
| B) PP: I | | | |
| C | 0 | 0.08 ± 0.01 | 0.06 ± 0.04ᵃ |
| RG | 10–1000 | 0.10 ± 0.03 | 0.08 ± 0.03ᵃ |
| OT | 10–1000 | 0.05 ± 0.01 | 0.17 ± 0.09ᵃᵇ |
| NT | 10–1000 | 0.07 ± 0.01 | 0.30 ± 0.18ᵇ |
| RG | 10,000 (LC) | 0.04 ± 0.02 | |
| OT | 10,000 (LC) | 0.04 ± 0.01 (0.03 ± 0.001) | 0.14 ± 0.03ᵃᵇ (0.12 ± 0.06ᵃᵇ) |
| NT | 10,000 (LC) | 0.07 ± 0.01 (0.08 ± 0.01) | 0.09 ± 0.01ᵇ (0.24 ± 0.11ᵇ) |

(9116–15,377 mg (kg DW)⁻¹), followed by iron (Fe; 255–459 mg (kg DW)⁻¹), cobalt (Co; 0.73–112 mg (kg DW)⁻¹), copper (Cu; 12.2–27.8 mg (kg DW)⁻¹) and lead (Pb; 4.7–28.7 mg (kg DW)⁻¹); Table 3). Chromium (Cr) and nickel (Ni) were present in all TWP at the concentrations < 10 mg (kg DW)⁻¹, whereas the concentrations of vanadium (V) and manganese (Mn) were always <5 mg (kg DW)⁻¹, and the concentrations of arsenic (As), cadmium (Cd) and mercury (Hg) did not exceed 1 mg (kg DW)⁻¹. The concentrations of all metals were lowest in OT particles, and in most cases less than one third of the concentrations in NT or RG particles. The concentrations of V, Cr, Ni and Hg were higher in NT particles than in RG particles, whereas the concentrations of Co, Cu and Cd were higher in RG particles than in NT particles. Particularly, the concentration of Co in RG particles at 112 mg (kg DW)⁻¹ was ca. 8 times higher than in NT particles (14.2 mg (kg DW)⁻¹), and >150 times higher than in OT particles (0.73 mg (kg DW)⁻¹; Table 3).

Copepod pellet production was not related to the metal concentrations when food was abundant. However, at the low food concentration, the pellet production at the 10,000 TWP L⁻¹ appeared somewhat negatively related to the concentrations of Co, Cu, Zn and Cd, with the highest pellet production rates occurring at the lowest concentrations of these metals (App. Fig. 2). This relationship was not detectable at the lower concentrations of TWP, nor if ingestion rate, egg production or hatching success was used to represent copepod responses (data not shown). Also, since the concentrations of Co, Cu and Cd varied in the similar way in the different TWP, it was not clear which of these metals induced the negative effect.

Table 3

Concentrations of heavy metals in the TWP (mg (kg DW)⁻¹; mean ± SD of 4-5 replicates). The detection limit is indicated in the parenthesis after the metal. (V) Vanadium, (Cr) chromium, (Mn) manganese, (Fe) iron, (Co) cobalt, (Ni) nickel, (Cu) copper, (Zn) zinc, (As) arsenic, (Cd) cadmium, (Hg) mercury, (Pb) lead. Other abbreviations as in Table 1.

| Metal | RG | NT | OT |
|-------|----|----|----|
| V (0.05) | 1.71 ± 0.35 | 4.52 ± 0.15 | 0.94 ± 0.05 |
| Cr (0.28) | 1.58 ± 0.22 | 9.49 ± 0.58 | 2.10 ± 0.24 |
| Mn (0.02) | 4.61 ± 1.17 | 3.47 ± 0.08 | 3.71 ± 0.20 |
| Fe (0) | 303 ± 63 | 459 ± 7 | 255 ± 30 |
| Co (0.00) | 112 ± 42 | 14.2 ± 0.4 | 0.73 ± 0.05 |
| Ni (0.04) | 3.34 ± 0.72 | 7.56 ± 0.49 | 0.84 ± 0.05 |
| Cu (0.0) | 27.8 ± 2.4 | 18.4 ± 0.3 | 12.2 ± 1.7 |
| Zn (0.01) | 15377 ± 750 | 11274 ± 233 | 916 ± 568 |
| As (0.139) | 0.849 ± 0.407 | 0.897 ± 0.118 | 0.297 ± 0.055 |
| Cd (0.002) | 0.966 ± 0.273 | 0.649 ± 0.019 | 0.108 ± 0.043 |
| Hg (0.004) | 0.014 ± 0.005 | 0.081 ± 0.010 | 0.017 ± 0.004 |
| Pb (0.00) | 21.8 ± 3.5 | 28.7 ± 0.9 | 4.7 ± 1.5 |

4. Discussion

4.1. No effect of TWP on copepod reproduction

Our result suggested that a short exposure to TWP at environmentally relevant concentrations does not affect the reproduction of *Acartia tonsa* or *Temora longicornis*, even though the particles would be potentially toxic. The average egg production and hatching rates of *A. tonsa* in the high food concentration corresponded to typical rates previously observed for this species in the laboratory, under similar food conditions (Kiorboe et al., 1985).

However, our experiments only lasted for 48 h, whereas it is conceivable that a longer-term exposure to TWP could influence reproduction. This could happen if 1) TWP exposure resulted in a decrease in feeding or assimilation rates, particularly when food is limiting, or 2) the chemicals and additives in the TWP were assimilated and bioaccumulated, and had a detrimental effect for reproduction. Cole et al. (2015) demonstrated a decrease in the hatching success and egg size of the copepod *Calanus helgolandicus* following a prolonged exposure to a high (75,000 MP particles mL⁻¹) concentration of microplastic and suggested that this was due to a food deficiency induced by microplastic ingestion. Assimilation of potentially toxic compounds could have negative effects on reproduction already at lower concentrations. For instance, Zn and PAH compounds that both are present in TWPs and RGs have been shown to reduce the reproductive output of *A. tonsa* at concentrations >0.65 μg Zn L⁻¹ (Sunda et al., 1987) and >60 μg PAH L⁻¹ (depending on the PAH species; Bellas and Thor, 2007). Longer experiments could therefore result in negative effects on reproduction due to chronic exposure to TWP, either through a prolonged reduced food uptake or through an assimilation and bioaccumulation of toxic substances.

Egg production of small copepod species such as *A. tonsa* responds to food intake at a short time lag of <20 h (Tester and Turner, 1990), which could suggest that a 48 h incubation would be sufficiently long for the negative effects to develop. Nevertheless, an inhibition of reproduction could, for instance, also result from a longer-term exposure as demonstrated by Turner et al. (2001), if the toxic compounds bio-accumulate, or if they interfere with the assimilation of essential nutrients. The chronical effects could also be species-specific, if, for instance, the lipid content of copepods influence the bio-accumulation or toxic effects, although the few existing studies suggest that lipid reserves have limited effect on copepods response to toxic substances (Hansen et al., 2016; Jager et al., 2017, but see Halsband et al., 2020).
4.2. The effect of copepod feeding mode and TWP concentration

Many copepod species, including Acartia spp. and Temora longicornis are able to reject unfavorable food items, including plastic spheres (Donaghay and Small, 1979; Cowles et al., 1988; DeMott, 1989) and toxic algae (Teegarden et al., 2008, Xu, 2018). However, food selection can be species-specific (Xu, 2018), and depend, for instance, on pre-conditioning to the food (Donaghay and Small, 1979) or food concentration (DeMott, 1995). Our study indicated that the TWP did not interfere with the feeding of the ambush feeding copepod A. tonsa when the food concentration was high, whereas the feeding of the feeding-current feeding copepod T. longicornis was affected both at the low and high food concentrations. Although the effect of feeding mode on microplastic ingestion remains to be studied, it seems reasonable to assume that the interaction between feeding behavior and food concentration will influence copepods response to microplastic, and that the feeding responses will be further complicated by the feeding history of the copepods and the properties of the plastic particles such as aging (Vroom et al., 2017) or dimethyl sulfide (DMS) exposure (Procter et al., 2019). Our data indicate that feeding-current feeding copepods are more prone to ingest TWP than ambush feeding copepods, which suggest that the feeding mode of the dominant zooplankton needs to be considered when investigating the ecosystem effect of plastic pollution.

The concentrations of TWP used in the present study ranged from high but realistic at 10 particles L\(^{-1}\) to extremely high at 10,000 particles L\(^{-1}\). The typical concentrations of microplastic that have been measured in different coastal waters range from < 1 to ca. 500 particles m\(^{-3}\) (Collignona et al., 2012 and references therein, Frías et al., 2014; Enders et al., 2015), although concentrations of up to 4000 particles m\(^{-3}\) have been measured in the urban estuaries in China (Zhao et al., 2015). Recently, Lorenz et al. (2019) measured microplastic concentrations of up to 245 particles m\(^{-3}\) in the North Sea, with 86 % of the particles being < 100 μm in size, and Enders et al. (2015) found up to 500 MP particles of > 10 μm in size m\(^{-3}\) in European coastal waters. These studies show that the MP concentrations in the smaller size fraction are higher than previously documented, since most of the studies have used Manta nets with mesh sizes of 330 μm. Nevertheless, the concentrations that induced effects on copepod feeding and pellet production in our experiments (≥1000 TWP L\(^{-1}\)) were several orders of magnitude higher than the highest measured concentrations in the North Sea by Lorenz et al. (2019), and 25 times higher than the highest concentrations measured in urban estuaries and river mouths in China by Zhao et al. (2015). It seems therefore likely that the effects of environmentally relevant concentrations of TWP for the two dominant coastal copepod species are limited.

4.3. Toxicity of TWP and its leachates

TWP and its leachates are some of the most toxic MP particles, containing a complex mixture of chemicals at high concentrations, such as benzo[b]fluoranthene, n-cyclohexylformamide and different metals, as well as smaller amounts of other compounds such as acenaphthene, phenanthrene, bisphenol A and antimony (Sb). In addition, car tire leachates can have a low pH, which might increase the bioavailability of the compounds (Capolupo et al., 2020). Indeed, the leachates from car tire samples have been demonstrated to have detrimental effects on algae, crustaceans, mussels, zebra fish and copepods, already at relatively low concentrations (Nelson et al., 1994; Wik and Dave, 2006; Wik et al., 2009; Capolupo et al., 2020; Halsband et al., 2020).

The reduction of pellet production and feeding at the high concentrations of NT particles and their leachates suggested that this type of TWP had a toxic effect on copepods, whereas the effect of OT was mainly physical. It was however not clear whether the toxicity of NT particles was due to heavy metals: although the concentrations of several heavy metals were highest in NT particles, concentrations of most of the metals that appeared negatively related to pellet production (Co, Cu, Zn and Cd) were highest in RG particles. The negative effects of NT could have been due to organic compounds not measured here, such as benzo[a]pyrene or diverse PAH. However, it could also have been that the toxicity of RG was underestimated due the lack of experiments with the more sensitive copepod Temora longicornis, and that in reality both RG and NT with their high heavy metal concentrations could have induced negative effects for this copepod.

The complex and sometimes contrasting results of the effects of different TWP and their leachates on copepod feeding or pellet production did not allow for any generalizations or evidence of the effects of particular compounds. However, it appears likely that the toxicity of the TWP and their leachates will influence the response of zooplankton, and the interactions between the chemical content of the environmentally relevant microplastic particles, their leachates and vital rates of copepods will need more attention in future studies.

5. Conclusions

Our results indicated that whereas TWPs had no effect on copepod reproduction, high concentrations of OT and RG in some cases increased feeding and pellet production rates, while high concentrations of NT in some cases decreased the same rates. The increased feeding rate could result in a lower gross growth efficiency (GGE), and changed ingestion to pellet production rate could change the assimilation efficiency and the vertical flux through copepod fecal pellets. This suggests that exposure to high concentrations of microplastic might influence the food web transfer efficiency or vertical flux, although the effects are only measurable at the concentrations far above the concentrations that have been measured in the ocean. In general, our results point towards complex interactions between plastic type (including chemical composition) and concentration, chemical leachates, behavior of organisms and environmental variables that hamper any efforts to generalize the ecosystems responses to microplastic. However, at the environmentally relevant TWP concentrations tested here, we did not observe any impact of TWP on the two dominant coastal copepod species.

Funding

This research was part of the Project ‘Mikroplastik i havmiljøet – viden om skadevirkninger, detektionsmetoder og skæbne’, and MarinePlastic -The Danish center for research in marine plastic pollution funded by the Velux Foundations. This research was furthermore supported by the H2020 CLAIM project (Cleaning Litter by developing and Applying Innovative Methods in European seas; Grant Agreement No. 774586) to TGN.

CRediT authorship contribution statement

Marja Koski: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft. Jens Søndergaard: Investigation, Formal analysis, Writing - original draft. Anette Maria Christensen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We wish to thank Jack Melby for the help with the laboratory cultures, and Søren Nielsen and Guldborgsund Municipality for providing the rubber granules.
Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.aquatox.2020.105713.

References
Andrady, A.L., 2017. The plastics in microplastics: a review. Mar. Pollut. Bull. 119, 12–22.
Baumann, W., Ismeier, M., 1998. Kautschuk und Gummi – Daten und Fakten zum Umweltschutz. Springer Verlag, Berlin.
Bejgarn, S., MacLeod, M., Bogdal, C., Breitholtz, M., 2015. Toxicity of leachate from rubber used in synthetic turf under chemical and physical stress. Environ. Sci. Pollut. Res. 206, 597–604.
Baumann, W., Ismeier, M., 1998. Kautschuk und Gummi
Canepari, S., Castellano, P., Astilfi, M.L., Materazzi, S., Ferrante, R., Fiorini, D., Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lideque, P., 2020. The effects of selected PAHs on reproduction and survival of the calanoid copepod Acartia tonsa. Sci. Total Environ. 749, 139731.
Chowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.
Cowles, T.J., Olson, R.J., Chisholm, S.W., 1988. Food selection by copepods: discrimination on the basis of food quality. Mar. Biol. 100, 41–49.
DeMott, W.R., 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnol. Oceanogr. 34, 140–154.
DeMott, W.R., 1995. Food selection by calanoid copepods in response to between-lake variation in food abundance. Freshw. Biol. 33, 171–180.
Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific ocean. Arch. Environ. Contam. Toxicol. 69, 1130–1137.
Collignona, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864.