Effects of Microplastic on the Population Dynamics of a Marine Copepod: Insights from a Laboratory Experiment and a Mechanistic Model

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Abstract: Microplastic is ubiquitously and persistently present in the marine environment, but knowledge of its population-level effects is limited. In the present study, to quantify the potential theoretical population effect of microplastic, a two-step approach was followed. First, the impact of microplastic (polyethylene, 0.995 g cm$^{-3}$, diameter 10–45 µm) on the filtration rate of the pelagic copepod Temora longicornis was investigated under laboratory conditions. It was found that the filtration rate decreased at increasing microplastic concentrations and followed a concentration–response relationship but that at microplastic concentrations <100 particles L$^{-1}$ the filtration rate was not affected. From the concentration–response relationship between the microplastic concentrations and the individual filtration rate a median effect concentration of the individual filtration rate (48 h) of 1956 ± 311 particles L$^{-1}$ was found. In a second step, the dynamics of a T. longicornis population were simulated for realistic environmental conditions, and the effects of microplastics on the population density equilibrium were assessed. The empirical filtration rate data were incorporated in an individual-based model implementation of the dynamic energy budget theory to deduct potential theoretical population-level effects. The yearly averaged concentration at which the population equilibrium density would decrease by 50% was 593 ± 376 particles L$^{-1}$. The theoretical effect concentrations at the population level were 4–fold lower than effect concentrations at the individual level. However, the theoretical effect concentrations at the population level remain 3–5 orders of magnitude higher than ambient microplastic concentrations. Because the present experiment was short-term laboratory-based and the results were only indirectly validated with field data, the in situ implications of microplastic pollution for the dynamics of zooplankton field populations remain to be further investigated. Environ Toxicol Chem 2022;41:1663–1674. © 2022 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Polyethylene; Population effects; Copepod; Marine environment

INTRODUCTION

The growing plastic production volumes, the wide plastic distribution, and the persistence of plastic in the marine environment are matters of environmental concern; but the impact of plastic on marine organisms, populations, and ecosystems remains poorly understood. The demonstrated effects of microplastic at the species level are increasing (Science Advice for Policy by European Academies, 2019), but often these effects have been observed under unrealistic laboratory conditions at exposure concentrations that are orders of magnitude higher than ambient microplastic concentrations. Because the present experiment was short-term laboratory-based and the results were only indirectly validated with field data, the in situ implications of microplastic pollution for the dynamics of zooplankton field populations remain to be further investigated. Environ Toxicol Chem 2022;41:1663–1674. © 2022 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

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effect mechanisms of plastics is challenging (Marn et al., 2020). So far, most studies have focused on a correlative linkage between the amount of microplastic ingested and a selected endpoint (e.g., mortality [Bucci et al., 2020]), whereas sublethal effects have often been put aside as an area of considerable uncertainty and concern (Gall & Thompson, 2015).

For marine zooplankton, an obvious and demonstrated example of a sublethal effect mechanism after microplastic ingestion is inhibited food assimilation and decreased nutritional value (Cole et al., 2015). The theoretical principle behind this is that not only does the gastrointestinal tract get blocked, resulting in a lower capacity for ingesting food, but also the affected organism wastes energy reserves by trying to digest indigestible microplastic. Under this so-called toxic anorexia, the digestible carbon intake is lower and the feeding rate decreases (Scholten et al., 2005). To date, few studies can support this theoretical principle and present quantitative links between ingestion of plastics and sublethal physiological effects. For example, Cole et al. (2015) found that filter-feeding marine copepods ingested 11% fewer algal cells and 40% less carbon biomass when exposed to 20-µm polystyrene particles (7 particles ml⁻¹). In the same study, a conceptual energetic (carbon) budget suggested that microplastic-exposed copepods could suffer energetic depletion over time (Cole et al., 2015). Using a laboratory mesocosm, Wright et al. (2013) identified that the total available lipid reserves in a marine lugworm were reduced by 30% compared to control animals when chronically exposed to 1% chemically inert polyvinyl chloride (130µm) by weight. Watts et al. (2015) and Sussarellu et al. (2016) reported and quantified the energetic and reproductive impact of microplastic ingestion in crabs and in oysters, respectively. Watts et al. (2015) found, in a 4-week experiment, that ingestion of polypropylene microfibers (1–5 mm in length) reduced the energy available for growth and that this was mainly driven by a reduction in food consumption. Sussarellu et al. (2016) provided evidence of feeding modifications and reproductive disruption in oysters after ingesting polystyrene microspheres (2 and 6 µm). Based on a dynamic energy budget (DEB) model, they found a significant shift of energy allocation from reproduction to structural growth and elevated maintenance costs in the exposed oyster. More recently, for a vertebrate species and based on a DEB model, Marn et al. (2020) found that ingestion of plastic debris by sea turtles disturbs their ontogeny and energy budgeting. A sea turtle with an average plastic load of 3%–25% in its food experiences a reduction of 2.5%–20% in total available energy, respectively. The ingested plastic substantially reduced the physiological condition of individuals but was not likely to cause population-threatening shifts in population sizes (Marn et al., 2020). Finally, there are also first indications about reproductive impairment and transgenerational effects of microplastic ingestion in vinegar flies (see Jimenez-Guri et al., 2021). Although the life cycles, life-history traits, and ecological niches of the tested organisms (i.e., zooplankton [Cole et al., 2015; Shore et al., 2021], lugworm [Wright et al., 2013], crab [Watts et al., 2015], oyster [Sussarellu et al., 2016], sea turtle [Marn et al., 2020], and vinegar fly [Jimenez-Guri et al., 2021]) are dissimilar, these are the first quantified indications that the ingestion of microplastic affects the energy budgeting of the recipient organisms. However, so far, causal relationships at higher levels of organization such as populations remain largely unclear. As such, further research about potential population-level impacts of microplastic is required because when, for example, marine zooplankton population dynamics are hampered by microplastic pollution, this could have cascading implications for entire marine ecosystems (Heneghan et al., 2016). Microplastic population effect data are slowly accumulating, and quantified results about sublethal effects of microplastic ingestion are expected to become increasingly available in coming years. In this context, mechanistic models such as individual-based models (IBMs) of the DEB can provide new insights into how individual sublethal effects that are regularly observed in microplastic exposures can add up to population-level impacts.

Plastic research has the tendency to be highly mediated, and despite current evidence that microplastic presents a low risk to biota at environmentally realistic concentrations (Everaert et al., 2018, 2020), there is a public perception that microplastic is an important environmental health risk (Catarino et al., 2021). To stimulate a more correct communication of scientific findings to the wider public and policymakers, it is important to put scientific results in the right context and to be able to relate them back to real-world conditions. For example, it is instrumental to compare and contextualize the microplastic exposures applied in experimental and/or model-based approaches to the actual environmental microplastic concentrations (Catarino et al., 2021).

In the present study, based on Kooijman (2010) and as suggested by Paul-Pont et al. (2018), an IBM implementation of the DEB theory (DEB-IBM) was used to provide new insights about potential population-level effects of microplastic. The energy uptake and expenditure of the marine copepod Temora longicornis (Müller, 1785) for its essential life functions such as maintenance, growth, maturation, and reproduction were quantified; and the impact of microplastic on this balance was assessed. To do so, a two-step approach was followed. In a first step, a feeding experiment was performed to evaluate the impact of microplastic presence on the filtration rate of T. longicornis and to obtain a concentration–response relation. In a second step, the empirical filtration rate data (from Step 1) were incorporated in the DEB-IBM to simulate theoretical T. longicornis population dynamics. By doing so, the potential theoretical effects of microplastics on the T. longicornis population dynamics have been quantified. Artificial, but realistic, environmental conditions mimicking the abiotic conditions for the southern part of the North Sea were used in both experimental setups as in the model framework.

**MATERIALS AND METHODS**

**Zooplankton**

Temora longicornis (Müller, 1785) is a marine calanoid copepod. Living in the epipelagic zone of the northeast Atlantic ocean, the North Sea, and the Baltic Sea (https://obis.org/taxon/104878) and being a selective filter-feeding organism,
the species is hypothesized to encounter microplastics. Together with Acartia clausi (Giesbrecht, 1889), T. longicornis is one of the most abundant pelagic copepods within the Belgian Continental Shelf (together comprising up to 66% of total zooplankton densities [Van Ginderdeuren et al., 2014]).

**Experimental work followed by ecological modeling**

To quantify the potential population effect of microplastic, a two-step approach was followed. First, experimental work was performed to investigate the impact of microplastic on the filtration rate of T. longicornis individuals. Next, these data were used to parameterize a DEB-IBM to theoretically extrapolate the individual-level effect data to the population level. The second step provided theoretical insight into the potential population effects of microplastics. Although the DEB-IBM was run under realistic ambient environmental conditions, the aim of the model was not to exactly reproduce the dynamics of T. longicornis field populations. The outcome of the model is briefly compared with in situ densities of T. longicornis, but this step cannot be regarded as a true model validation in a strict ecological modeling sense. Because the microplastic vector effect for chemicals is considered unlikely (Koelmans et al., 2022), the focus of the present study was on the direct effects of microplastic.

**Zooplankton collection**

Zooplankton was collected using a WP2-net (diameter 0.70 m, mesh size 50 µm) deployed from the RV Simon Stevin on October 28, 2015, on the Belgian Continental Shelf at station 230 (51°18′31″N, 2°51′01″W) and station 330 (51°26′02″N, 2°48′32″W). After collection, the zooplankton samples were transferred to the Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University. Adult T. longicornis individuals were selected from the zooplankton samples and further cultured in 40-L aquaria filled with 30 L filtered natural seawater (mesh size 0.2 µm) sampled from the same location. The T. longicornis culture was maintained in a temperature-controlled room at 15°C (Halsband-Lenk et al., 2002) and kept in the dark (Mauchline, 1998). The culture was fed a mixture of three algae (75% Rhodomonas salina in L1-30 medium, 12.5% Prorocentrum cordatum in L1-32 medium, and 12.5% Thalassiosira weissflogi in F2 medium) at a concentration of 10 ml L⁻¹, three times a week. Every 2 weeks, the water was replaced with fresh filtered natural seawater. To obtain T. longicornis individuals at the start of the experiment, the content of the aquaria was filtered using a stainless-steel sieve with a mesh size of 20 µm. The copepods were recovered from the sieve. The population density was quantified every 2 weeks, and a density of >30 copepodites L⁻¹ was indicative of favorable growth conditions.

**Experimental design**

Virgin chemically inert polyethylene microplastic spheres (product UVPMS-BR-0.995, density 0.995 g cc⁻¹, diameter 10–45 µm) were purchased from Cospheric. The selected microplastic had a diameter of 10–45 µm, which is similar to the size range of food particles ingested by T. longicornis (Gonçalves et al., 2014) and can thus be considered bioavailable (Cole et al., 2013; Koelmans et al., 2020). A concentration series of microplastic in 10% Tween-80 (i.e., a nonionic surfactant to facilitate suspension in filtered natural seawater) was prepared from a stock solution. The stock solution had a concentration of 10,000 particles L⁻¹ and was obtained by adding 108.3 µg of polyethylene spheres to 0.9 L of fresh filtered natural seawater and 0.1 L of Tween-80. To estimate the required mass, spherical particles were assumed with a mean diameter of 27.5 µm and a density 0.995 g cc⁻¹. The actual concentration of the stock solution was verified by using a Sediwick-Rafter counting chamber, a counting technique that is often used to measure algal cell densities. Temora longicornis was exposed to microplastic concentrations ranging from 0 to 10,000 particles L⁻¹ (i.e., 0, 32, 100, 320, 1000, 3200, 10,000 particles L⁻¹). The exposure with 0 particles L⁻¹ served as a reference (control) for comparison. Each test vial of 40 ml contained exactly one T. longicornis individual, and eight individuals were exposed for 48 h to each of the selected microplastic concentrations. Blank and Tween-80 control exposures were run concurrently.

Filtration rate, which quantifies the capacity of the copepods to filter food from seawater, was used as the test endpoint. The filtration rate was expressed in volume per time unit (microliters per minute) and quantified the volume of seawater filtered to ingest a certain amount of food items. The filtration rate was calculated using the formula of Rigler (Equation 1) as described in Peters (1984):

\[
F = \frac{\ln(C_1) - \ln(C_2)}{N \times \Delta t} \times V
\]

In Equation 1, F (microliters per minute) is the filtration rate, C₁ and C₂ are the algal cell densities at the start (C₁) and the end (C₂) of the exposure duration, V (microliters) is the volume in which the copepods are grazing, N is the number of copepods, and Δt (min) is the exposure duration (≈ 2880 min = 48 h). Algal densities were counted with a Coulter counter (Beckman Coulter model Z1S) with a detection limit of 10³ algal cells ml⁻¹. Prior to use, the Coulter counter electrolyte was filtered over a 0.2-µm mesh size filter to avoid particle contamination. All glassware used in the filtration rate experiment was autoclaved at 121 °C for 40 min. Initial algal densities were 10⁵ cells ml⁻¹, which is sufficiently high to allow quantification of C₁ and C₂ (Equation 1).

**Ecological model for quantifying population effects**

Population models provide a link between the individual and the population based on mathematical equations (Hanson & Stark, 2012). To convert microplastic effects on individuals to potential theoretical effects on the population level, a DEB-IBM was used per Martin et al. (2012). These mechanistic population models have gained considerable interest in
ecotoxicology because they are able to describe and facilitate the interpretation of sublethal effects of a stressor (Vlaeminck et al., 2021). In general, in IBMs the modeled species of a population are simulated individually; as such, they allow for individual variability. In IBMs, functions and processes are often implemented with probabilities. For example, the probability that a copepod will reproduce is dependent on the available energy reserves and environmental conditions (Huntley & Lopez, 1992). On the other hand, DEB models are a series of mechanistic rules that describe and quantify the uptake and use of energy of an individual organism throughout its life cycle. The output of a DEB is typically expressed in biomass or carbon energy. Such models quantify the flow of energy through individual organisms and enable calculations for different developmental stages (Kooijman, 2010). The conceptual schemes of the IBM for *T. longicornis* and the energy fluxes in the DEB model are available in Figures 1 and 2, respectively. Overall, the basic framework of the model contains two entities: the individuals and the environment. The two entities have unique state variables that interact (Figures 1 and 2). The individuals have four primary state variables: (1) structure, determining the actual size of each individual; (2) energy reserves, comprising intermediary energy reserves that are used for both feeding and mobilization processes; (3) maturity, determining the transitions between development stages; and (4) energy buffer, after becoming adults, copepods convert some of the ingested energy into a buffer. Eventually, the buffer is converted to eggs during reproductive events. Note that in the adapted model, no development stages are present, so maturity relates to the moment when copepods start reproducing. The environment has one state variable that is related to the feeding conditions, to be interpreted as that the environment defines the food density. To make this generic DEB-IBM applicable to *T. longicornis*, three adaptations were made.

The first adaptation related to life stage–dependent energy investments of *T. longicornis*. Once mature, *T. longicornis* puts all of its energy in reproduction (Koch & De Schamphelaere, 2019). Therefore, *T. longicornis* follows the standard DEB model from birth to puberty with growth ceasing at puberty (sbp; Kooijman, 2010; Koch & De Schamphelaere, 2019) using the parameterization in Table 1.

The second adaptation considered the temperature dependency of the metabolic rates and life cycle of *T. longicornis* (Kooijman, 2010). To account for this, an Arrhenius correction factor for all metabolic rates was integrated per Pereira et al. (2019):

$$k(T) = k_1 \times \exp\left(\frac{T_A - T}{T}ight)$$

(2)

![Figure 2: General scheme of the energy fluxes considered in the standard dynamic energy budget (DEB) model.](image)

**TABLE 1:** Parameterization of the dynamic energy budget model for *Temora longicornis*

| Parameter                                      | Symbol | Value     | Unit       |
|-----------------------------------------------|--------|-----------|------------|
| Maximum surface-specific assimilation rate    | $P_{Am}$ | 125.328   | J day$^{-1}$ cm$^{-2}$ |
| Maximum surface-specific searching rate       | $F_m$   | 6.5       | L day$^{-1}$ cm$^{-2}$ |
| Digestion efficiency from energy food to reserve | $\kappa_d$ | 0.13616 | –          |
| Energy conductance for structural growth and maintenance | $\nu$ | 0.022927 | cm day$^{-1}$ |
| Allocation fraction for structural growth and maintenance | $\kappa$ | 0.8 | – |
| Reproduction efficiency                      | $\kappa_r$ | 0.95 | – |
| Volume-specific somatic maintenance rate      | $\phi_M$ | 16.6937   | J day$^{-1}$ cm$^{-3}$ |
| Specific costs for structure                  | $E_S$   | 4400      | J cm$^{-3}$ |
| Maturity maintenance rate                     | $k_j$   | 0.002     | day$^{-1}$ |
| Maturity at birth                             | $E^b$   | 0.0003344 | J        |
| Maturity at puberty                           | $E^p$   | 0.08769   | J        |
| Weibull aging acceleration                    | $h_a$   | 5.341 × 10$^{-5}$ | day$^{-2}$ |
| Gompertz stress coefficient                   | $s_G$   | 0.0001    | –        |
| Arrhenius temperature                         | $T_A$   | 10,380    | K        |

*Parameters taken from the Add-my-Pet database (Marques et al., 2018).
In Equation 2, \(k(T)\) is the parameter value at temperature \(T(K)\), \(k_1\) is the parameter value at the reference temperature \(T_1(K)\), and \(T_A\) is the species-specific Arrhenius temperature parameter (Table 1; Marques et al., 2018).

The third adaptation related to the effects of plastic ingestion on the filtration rate of \(T.\ longicornis\). Based on the toxic anorexia theory (Scholten et al., 2005), a decrease of food ingestion with increasing microplastic concentrations was expected. A log-logistic relationship between the surface-specific ingestion rate, \(J_{XAm}\) (joules per day per square centimeter) and the microplastic concentration was assumed:

\[
J_{XAm,\text{micro}} = J_{XAm} \times \frac{1}{\left(1 + \exp(-\beta \times (\ln C - \ln EC50))\right)}
\] (3)

In Equation 3, \(C\) (particles per liter) is the environmental microplastic concentration, \(EC50\) (particles per liter) is the observed 50% effect concentration, and \(\beta\) is the slope of the observed relationship between the microplastic concentrations and the surface-specific ingestion rate. What happens in Equation 3 is the incorporation of the experimentally inferred EC50 (Step 1) into the DEB model (Step 2) to determine the population impacts. As such, this equation can be regarded as crucial in the present research because the linkage is made between the experiment that was performed (Step 1) and the DEB-IBM model (Step 2). The surface-specific ingestion rate, \(J_{XAm}\), is calculated from the literature-based surface-specific assimilation rate, \(\rho_{Am}\) (joules per day per square centimeter), and digestion efficiency, \(\kappa_X\) (Marques et al., 2018; Table 1):

\[
\rho_{Am} = \kappa_X \times J_{XAm}
\] (4)

A DEB-IBM that was adapted in three ways was used to understand the potential theoretical effects of microplastics on \(T.\ longicornis\). The aim was to simulate a theoretical dynamic cycle of a \(T.\ longicornis\) population and to assess the effects of microplastics on the population density equilibrium inferred from this theoretical cycle. To optimize the ecological relevance of the outcome of the model, realistic environmental conditions that represent the abiotic environment of specific months were embedded in the model. To do so, the \(T.\ longicornis\) population dynamics were simulated under monthly variable microplastic concentrations, seawater temperature conditions, and food levels that are relevant and realistic for the southern part of the North Sea (Supporting Information, Table S1). To develop relevant seawater temperature scenarios (degrees Celsius) and food density scenarios (milligrams of chlorophyll a [Chl a] per cubic meter), in situ monthly average data were obtained for the Belgian Continental Shelf for the year 2016 from the Belgian Marine Data Centre. The average monthly food level available for the copepods was inferred from Chl a concentrations (milligrams of Chl a per cubic meter) and converted to energy content (joules per liter) based on an average carbon-to-Chl ratio of 80 g C g Chl a\(^{-1}\) (Alvarez-Fernandez & Riegman, 2014) and an energy density of 550 kJ mol C\(^{-1}\) (Kooijman, 2010). Based on the monthly realistic temperature and food scenarios (Supporting Information, Table S1), \(T.\ longicornis\) population dynamics in the corresponding month were predicted. Sea surface temperature was assumed to be constant during the entire month, and food was assumed to be constant each day; that is, all individuals in the population could maximally consume the density listed in Supporting Information, Table S1. The initial population density was 100 individuals m\(^{-3}\) and comprised five adults and 95 juveniles. Assuming steady-state conditions, \(T.\ longicornis\) population development (number of individuals per cubic meter) was simulated for 2000 days. The average population density from Days 1000 to 2000 was considered the equilibrium population density of the population, as visually determined from the initial runs of the fitted logistic growth model (Supporting Information, Figures S1 and S2).

For each month-specific temperature and food scenario (Supporting Information, Table S1), the \(T.\ longicornis\) population was virtually exposed to a concentration series of microplastic between 0 and 2000 particles L\(^{-1}\), and the corresponding equilibrium population density was quantified. It was hypothesized that the equilibrium population density of \(T.\ longicornis\) would decrease as a result of microplastic exposure. This equilibrium population density is closely related to the carrying capacity of a population, but the latter is regarded as an intrinsic population characteristic that does not change if the population is impacted by a stressor (Hixon, 2008). Following Martin et al. (2012), intraspecific variation in the DEB-IBM parameters was allowed, to account for the natural heterogeneity in a population and to prevent synchronization of life histories. Based on this inherent stochasticity of the DEB-IBM and by running 10 iterations, insight was obtained about the potential population-level effects of microplastics on a \(T.\ longicornis\) population under relevant and realistic environmental conditions. The DEB-IBM implementation and the outcome from this modeling step have to be regarded as merely theoretical; that is, the dynamics of the \(T. longicornis\) population as inferred from the DEB-IBM were not validated against a population experiment or in situ observations. As such, the effects of microplastics on the population density equilibrium can only be compared relative to each other. However, because the environmental conditions used to run the model were relevant and realistic for the Belgian Continental Shelf (Supporting Information, Table S1), the obtained equilibrium population density was compared with some recently observed \(T.\ longicornis\) population densities at the Belgian Continental Shelf (Deschutter et al., 2019). Still, this comparison may not be regarded as a validation step in its strict ecological modeling interpretation.

**Statistical analyses**

From the experimental design, a quantified link between the algal filtration rates of \(T.\ longicornis\) and microplastic exposure (i.e., 0–10,000 particles L\(^{-1}\)) has been inferred. Subsequently, the 48-h EC50 (particles per liter), the 48-h EC10 (particles per liter), and the slope of the concentration–response relationship were
quantiﬁed using the package drc in R (Ver 3.5.2; R Foundation for Statistical Computing, 2018). The DEB‐IBM was developed in NetLogo 5.3.1 (Wilensky, 1999). Using the drc package in R (Ritz et al., 2015), a two‐parameter log‐logistic curve was ﬁtted through the equilibrium population density as a function of the microplastic concentrations. By doing so, the corresponding EC50 and EC10 at the population level was obtained.

RESULTS

Individual effects of microplastic through ingestion

Under laboratory‐controlled conditions and after exposure to a concentration series of microplastic, it was found that microplastic reduces the ﬁltration rate of the marine copepod T. longicornis (Figure 3). The ﬁltration rate of the control group (0 particles L−1) was 14.3 ± 1.6 µl min−1, whereas at 10,000 particles L−1 it had decreased to 1.6 ± 1.3 µl min−1. After unit conversion, the ﬁltration rate in the control group and at low microplastic exposure (≥100 particles L−1) had an average ﬁltration rate of approximately 1 ml h−1, which is realistic rate for a small copepod like T. longicornis (Poulsen & Kierboe, 2005). From the microplastic concentration‐response relationship of individual effects, the 48‐h EC50 and 48‐h EC10 were estimated to be 1956 ± 311 and 232 ± 88 particles L−1, respectively (Figure 3; Supporting Information, Data S1).

Population dynamics and impact of microplastic

A DEB‐IBM was used to simulate T. longicornis population dynamics in the Belgian Continental Shelf. Although the model was not validated based on ﬁeld data and thus not intended to exactly reproduce T. longicornis densities and its dynamics, the equilibrium population densities obtained are of the same order of magnitude as recent in situ observations. For example, the DEB‐IBM predicts the highest equilibrium population density of the population in April (i.e., 2350 ± 40 individuals m−3; Figure 4; Supporting Information, S1; Data S2). This predicted density is in concordance with the measured T. longicornis density in April 2015 of 2449 ± 622 individuals m−3 (Deschutter et al., 2019; Supporting Information, Figure S3). Also, in other months, the DEB‐IBM‐predicted equilibrium population densities remain in a realistic range and of the same order of magnitude as what has been observed in the ﬁeld (R² = 0.63; Supporting Information, Figure S3; Deschutter et al., 2019). As such, the use of relevant and realistic environmental conditions as input to the model contributes to the achievement of ecologically relevant equilibrium population density estimations. Microplastic adversely affects the ﬁltration rate of T. longicornis individuals (Figure 3), and the DEB‐IBM simulations indicate that microplastic also negatively impacts the T. longicornis population dynamics (albeit at microplastic concentrations that are orders of magnitude higher than realistic in situ concentrations—see Discussion). In general, it was found that the equilibrium population density is inversely related to microplastic concentrations (Figure 4; Supporting Information, Figure S1). For example, in the theoretical simulation for the environmental conditions mimicking the month of April (i.e., 8.9 °C and 43.4 J L−1; Supporting Information, Table S1), the equilibrium population density decreases from 2350 ± 10 to 0 individuals m−3 if microplastic concentrations increase from 0 to 1200 particles L−1 (Figure 4; Supporting Information, S1). At very high microplastic concentrations (>1200 particles L−1),
the population is not able to survive and becomes extinct (Figure 4). The DEB-IBM simulations indicate that the equilibrium population density decreases if microplastic concentrations increase, and this for all the theoretical, but relevant and realistic, combinations of seawater temperature and food densities imposed (Figure 4).

The first marine microplastic population-level EC50 and EC10 have been quantified in the present study (Figure 5; Table 2). To do so, a concentration–response curve was fitted through the microplastic-dependent equilibrium population density of the *T. longicornis* population in each month with a viable population according to the DEB-IBM. For example, when fitting a concentration–response curve through the April simulations, an EC50 of $719 \pm 10$ particles $L^{-1}$ was inferred for the environmental conditions mimicking the abiotic situation in April (Table 2). In Figure 5, a detailed example of such a concentration–response curve for the environmental conditions in June is shown, that is, the month with the lowest EC50 ($311 \pm 8$ particles $L^{-1}$) and EC10 ($195 \pm 12$ particles $L^{-1}$). All month-specific EC50 and EC10 data for the *T. longicornis* population are summarized in Table 2. As a yearly average, an EC50 of $593 \pm 376$ particles $L^{-1}$ and an EC10 of $389 \pm 292$ particles $L^{-1}$ were found.

**DISCUSSION**

The pelagic copepod *T. longicornis* was exposed to a concentration series of microplastic, and the filtration rate decreased by 50% at a microplastic concentration of $1956 \pm 311$ particles $L^{-1}$. Compared with the scientific literature on microplastic ecotoxicology, the individual effects of microplastic through ingestion found in the present study (Figure 3) are in the lower spectrum of reported effect concentrations (Everaert et al., 2020; Koelmans et al., 2022). For the marine copepod *Centropages typicus*, Cole et al. (2013) also found that microplastic (polystyrene, 7.3 $\mu$m) had a significant logarithmically shaped impact on the ingestion rate. *Centropages typicus* had a feeding rate of 34 algal cells individual$^{-1}$ h$^{-1}$ in the absence of microplastic, but when exposed to $25,000,000$ particles $L^{-1}$ the feeding rate decreased to $<10$ algal cells individual$^{-1}$ h$^{-1}$. Important to note is that in the study of Cole et al. (2013) the microplastic concentration series was not representative of in situ conditions (i.e., ranged from 4,000,000 to $25,000,000$ particles $L^{-1}$ [polystyrene, 7.3 $\mu$m]) and that no effect concentrations were inferred from the data. Moreover, when exposed to polystyrene beads sized 20.6 $\mu$m
at identical microplastic concentrations, no changes in the ingestion rate have been reported. In line with the present study, two other studies also report low effect concentrations for marine species (nonzooplanktonic). First is the study of Reichert et al. (2018), who exposed the coral Acropora spp. to a single concentration of microplastic (polyethylene, 37–163 μm) of \(3799 \pm 546 \text{ particles L}^{-1}\) and reported bleaching and tissue necrosis compared to nonexposed control organisms. Second is the study of Gardon et al. (2018), who found, based on a concentration series experiment with polystyrene pellets, adverse growth and reproduction effects for the bivalve Pinctada margaritifera at 320 particles L\(^{-1}\). However, in both studies, different endpoints were used to assess the effect of microplastic, and different species were exposed. In a freshwater environment, Bosker et al. (2019) investigated the impact of primary microplastic (1–5 μm) on a population of Daphnia magna and found a significant 21% reduction in the total biomass compared to control conditions after 3 weeks. The exposure concentrations, however, ranged from \(10^5\) to \(10^8\) particles L\(^{-1}\) and cannot be considered ecologically relevant, and no concentration response has been observed (Bosker et al., 2019). The examples given above illustrate that interstudy comparison remains difficult because it is hard to disentangle species-specific differences from methodological differences given the different approaches used across studies (Koelmans et al., 2022). Therefore, alignment methods such as those recently proposed for freshwater (Koelmans et al., 2020) would ease interstudy comparison. In the present study, one of the first effect concentrations of a zooplanktonic species is reported based on a microplastic concentration series in which organisms were exposed to a concentration series of microplastic concentrations that included relevant and realistic microplastic concentrations (i.e., 0, 32, 100, 320, 1000, 3200, 10,000 particles L\(^{-1}\)), and this resulted in a 48-h EC50 of \(1956 \pm 311 \text{ particles L}^{-1}\). The marine copepod Temora longicornis followed a concentration response when exposed to a concentration series of microplastic (Figure 3). This concentration–response curve complements the limited knowledge that is currently available about the effects of microplastic at the individual level. Important to note is also that at microplastic concentrations \(\leq 100\text{ particles L}^{-1}\), no adverse effects were observed (Figure 3) for individual organisms. The concentration–response curves indicate no impact of microplastic particles at the individual and population levels as long as microplastic concentrations remain in the 0–100 particles L\(^{-1}\) range (Figures 3–5). This means that, on average, environmental concentrations (~0.01–10 particles m\(^{-3}\)) of microplastic are at least 3–5 orders of magnitude lower than the effect concentrations reported in the present research (see Doyle et al., 2011; Goldstein et al., 2012; Lusher et al., 2014). In this context, it is sometimes argued that most environmental microplastic concentration data have been collected using 330-μm mesh size, thus likely resulting in an underestimation of the actual microplastic concentration (Kang et al., 2015; Lindque et al., 2020). However, even if the size frequency distribution of environmental microplastic is taken into account and considering an exponent of \(a = 1.6\) (Kooi & Koelmans, 2019) to extrapolate toward lower microplastic sizes, the environmental concentrations are still 2–3 orders of magnitude lower than the effect concentrations found in the present results. Indeed, there are occasional pollution hotspots where the in situ microplastic concentrations already exceed the effect concentrations (Law & Thompson, 2014; Norén, 2007), but these are often local situations and do not represent overall pollution levels. Note, however, that as the human population continues to grow and our dependence on plastic is not expected to change under a business-as-usual approach and circular economy initiatives fail (Lau et al., 2020), a steady and substantial increase in marine microplastic concentrations and the probability of causing adverse effects are expected.

To scale up from the individual level to the population level, a DEB-IBM was used. The population equilibrium density would decrease by 50% at microplastic concentrations ranging from 311 to 1303 particles L\(^{-1}\), depending on the temperature and food density scenario (Table 2). The latter means that effect concentrations at the population level (i.e., \(593 \pm 376\text{ particles L}^{-1}\)) were, on average, 4-fold lower than effect concentrations at the individual level.

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**TABLE 2: Population-based and month-specific effect concentrations**

| Month | Temperature (°C) | Food level (J L\(^{-1}\)) | EC50 (particles L\(^{-1}\)) | EC10 (particles L\(^{-1}\)) |
|-------|-----------------|--------------------------|-----------------------------|-----------------------------|
| January | 5.3 | 5.1 | NA | NA |
| February | 5.4 | 8.1 | NA | NA |
| March | 6.6 | 18.1 | NA | NA |
| April | 8.9 | 43.4 | 719 (±11) | 484 (±17) |
| May | 11.8 | 22.4 | 360 (±7) | 214 (±10) |
| June | 14.2 | 15.6 | 311 (±8) | 195 (±12) |
| July | 17.0 | 16.7 | 1303 (±11) | 945 (±20) |
| August | 16.8 | 11.8 | 400 (±11) | 228 (±15) |
| September | 16.7 | 12.3 | 466 (±11) | 273 (±16) |
| October | 15.2 | 9.0 | NA | NA |
| November | 10.7 | 10.3 | NA | NA |
| December | 8.6 | 8.0 | NA | NA |

*The sensitivity of Temora longicornis to microplastic under different environmental conditions, and its corresponding median effect concentration and 10% effect concentration.

EC50 = median effect concentration; EC10 = 10% effect concentration; NA = not assessed (in these months the T. longicornis population does not survive under the environmental conditions according to the dynamic energy budget theory).
In heavily polluted locations, in situ microplastic concentrations could (albeit in rare occasions) exceed these concentrations and thus hamper the zooplankton population dynamics. The fact that a population can have a superior sensitivity than individual organisms aligns with a study from Alonzo et al. (2016). However, predictions from simple population models, as in Stark et al. (2014), suggested that individual-level effects might have different consequences for populations depending on which endpoint was affected at the individual level, which endpoint was simulated at the population level, and which species was considered. As such, the relationship between effects at the individual level and effects at the population level cannot easily be described by predefined numbers and patterns (Hanson & Stark, 2012), and comparison with other species can lead to ambivalence.

The present modeling study is more representative of a laboratory T. longicornis population than a population in the Belgian Continental Shelf. Although the monthly scenarios were run based on in situ realistic environmental conditions (Supporting Information, Table S1), the population model in the present study is not representative of field populations. The outcomes of the model simulations are to be regarded as a merely theoretical exercise. Therefore, the variation in the equilibrium population density due to microplastic exposure could only be compared relative to each other. In future studies, it is recommended that an initial population test be performed under laboratory conditions to parameterize the DEB-IBM based on the outcome of this experiment. In a next step, an independent validation step can be performed where the model predictions are compared with in situ observations. A future way to valorize the population effect levels of microplastics is by integrating them as forcing functions in marine food-web models, and hence to be able to integrate plastic pollution in multiple stressors research together with other drivers such as ocean warming, ocean acidification, and ocean deoxygenation. To date, no effect concentrations for other populations or functional groups are available, and this hampers such integration. From field observations and laboratory experiments, it is generally accepted that microplastic is being taken up either passively through filter-feeding activities or actively because organisms mistake plastic for food particles (Browne et al., 2008; Van Cauwenbergh et al., 2015). A reduction of feeding efficiency due to ingestion of microplastics was documented for zooplankton, lugworms, fish, and bivalves exposed to different sizes and types of microplastics (Cole et al., 2015; Farrell & Nelson, 2013; Sussarellu et al., 2016), meaning that sublethal population-level effects are likely to be expected. For some of these species, similar DEB-IBMs as in the present study are available or being constructed. Future research could proceed and follow a similar approach as in the present study or per Sussarellu et al. (2016) or Marn et al. (2020) to quantify the potential population-level effects of microplastics for key species in the marine food web. Integration of this population-level information in a marine food web will then allow us to quantify the effect of microplastic on the functioning of marine ecosystems in the long run and to put plastic pollution in the perspective of the multistressed global ocean.

The two-step methodology applied in this research with first a laboratory-based experiment followed by incorporating the results in a mechanistic ecological model had many benefits but also some limitations. The first limitation relates to the environmental representativeness of the experimental setup. In this context, the individual test vials had a small volume (40 ml), meaning that the possible encounter rate of plastic may have been higher than expected in the field. Per Jones-Williams et al. (2020), the possible encounter rate is to be defined as the ratio of the number of plastics to the number of individuals in a cubic meter of water. In the present study, encounter rates range from 1.28% at 32 particles L$^{-1}$ to 400% at 10,000 particles L$^{-1}$. These encounter rates are indeed at the higher end of those recorded in the field, though not exceptional. For example, for copepods, an encounter rate of 2.6% was calculated in the northeast Pacific Ocean (Desforges et al., 2015). The highest encounter rate so far has been recorded by Sun et al. (2017) in the South China Sea, with 120% for zooplankton species. As such, use of the low-volume test vials should be considered because in worst-case conditions the chance that an individual indeed encounters the plastic is higher than what is observed in the field. In addition, the use of Tween may contribute to these worst-case conditions because Tween as a surfactant will minimize the aggregation of the microplastic particles and fibers. In the field, it has been shown that micro- and especially nanoplastics tend to form aggregates (Wang et al., 2021). By using a surfactant in the experimental setup, this process is reduced and may thus lead to higher actual microplastic concentrations than those in the field. The control experiment with Tween indicated that Tween in itself had no effect on the endpoints selected. This means that the conditions in the test vials can be regarded as worst-case conditions with slightly elevated encounter rates and lower aggregation potential than under field conditions. Besides that, the present modeling study is not easily translated to a field population of T. longicornis (see previous paragraph); a final limitation of the experimental work is that only the effect of microplastic on the filtration rate of the copepod has been assessed and that in the mechanistic model no potential elimination or release of microplastic from the individual organisms was assumed (Sherborne et al., 2020). Furthermore, microplastics are able to interact with multiple adverse outcome pathways. For example, future research could focus on the (potential) change in metabolic rate via oxygen consumption because these data are easily incorporated into the DEB model. A final and obvious recommendation relates to the use of “natural” plastic particles and fibers of different shapes, sizes, polymer types, and weathering states in the experimental setup instead of virgin microplastic.

Ecological models have the potential to become instrumental for the sake of a better understanding of potential microplastic-related ecotoxicological effects. Ecological models allow us to perform predictions on effects that cannot be easily tested under field or laboratory conditions, because of technical issues with small and variable plastic particles and
fibers, for example. Performing population or community experiments is often time-consuming and costly; hence, mechanistic models are considered as valuable alternatives (Forbes et al., 2011; Huang et al., 2020). With the present study, researchers can be stimulated to approach microplastic research from a different angle because this will be needed to overcome some of the frequently mentioned drawbacks of current ecotoxicity work (e.g., unrealistic high exposure concentrations). Ecological models allow an endless combination of polymers, sizes, species (sublethal) endpoints, and so forth to be tested. The approach followed in this research enabled us to further the state of the art of microplastic research. Therefore, an important recommendation is to extend the methods presented for other shapes, sizes, and polymer types and for other species. Also, in this method the inclusion of extra stressors such as ocean warming, ocean acidification, ocean deoxygenation, and eutrophication is possible in future research.

CONCLUSIONS

A mechanistic model was used to assess how individual sublethal effects due to microplastic pollution can add up to population-level impacts. In a short-term experiment, the pelagic copepod T. longicornis was exposed to a concentration series of microplastic (0–10,000 particles L\(^{-1}\)), and it was found that the filtration rate decreased by 50% at a microplastic concentration of 1956 ± 311 particles L\(^{-1}\). To scale up from the individual level to the population level, an IBM implementation of the DEB theory was used. It was found that the population equilibrium density would decrease by 50% at microplastic concentrations ranging from 311 to 1303 particles L\(^{-1}\), depending on temperature and food density. Although these population effect concentrations were approximately 4-fold lower than individual effect levels, in general, the environmental microplastic concentrations remain orders of magnitude below the effect concentrations found. Because the present study was short-term and laboratory-based and only indirectly validated with field data, the in situ repercussions of microplastics for zooplankton field populations remain to be further investigated.

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