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Microplastics reduce net population growth and fecal pellet sinking rates for the marine copepod, *Acartia tonsa*☆,☆☆

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ABSTRACT

Microplastics (<5 mm) are ubiquitous in the global environment and are increasingly recognized as a biological hazard, particularly in the oceans. Zooplankton, at the base of the marine food web, have been known to consume microplastics. However, we know little about the impacts of microplastics across life history stages and on carbon settling. Here, we investigated the effects of ingestion of neutrally buoyant polystyrene beads (6.68 μm) by the copepod *Acartia tonsa* on (1) growth and survival across life history stages, (2) fecundity and egg quality, (3) and fecal characteristics. We found that microplastic exposure reduced body length and survival for nauplii and resulted in smaller eggs when copepods were exposed during oogenesis. Combining these life history impacts, our models estimate a 15% decrease in population growth leading to a projected 30-fold decrease in abundance over 1 year or 20 generations with microplastic exposure. In addition, microplastic-contaminated fecal pellets were 2.29-fold smaller and sinking rates were calculated to be 1.76-fold slower, resulting in an estimated 4.03-fold reduction in fecal volume settling to the benthos per day. Taken together, declines in population sizes and fecal sinking rates suggest that microplastic consumption by zooplankton could have cascading ecosystem impacts via reduced trophic energy transfer and slower carbon settling.

1. Introduction

Human activity has put immense stress on global marine ecosystems, with plastic debris as the source for many environmental problems. An estimated 8 million metric tons of plastic, including 236,000 metric tons of microplastics, make their way to the oceans annually (Jambeck et al., 2015) where these microplastics persist (Hopewell et al., 2009; le Quéré et al., 2010) and break down into smaller pieces (Lambert and Wagner, 2016). While large macroplastics such as packaging materials, grocery bags, and straws (Andrady, 2011; Cressey, 2016) have been shown to negatively impact large marine biota such as birds (Fry et al., 1987; Provencher et al., 2014), whales (de Stephanis et al., 2013; Unger et al., 2016), and economically important fish species (Miranda and de Carvalho-Souza, 2016; Rochman et al., 2015), the impacts of microplastics have only recently been identified. This is in part due to the challenges of knowing environmental concentrations (Andrady, 2011) and serious underestimates of the amount of microplastics in the ocean (Barnes et al., 2009; Eriksen et al., 2014).

Microplastics have been shown to be ingested and negatively affect marine organisms such as brown shrimp (Devriese et al., 2015), barnacles (Goldstein and Goodwin, 2013), corals (Hall et al., 2015), oysters (Green, 2016), and zooplankton (Botterell et al., 2019; Cole et al., 2013, 2015, 2016, 2019; Coppock et al., 2019; Lee et al., 2008, 2013; Rodríguez-Torres et al., 2020; Svetlichny et al., 2021; Wieczorek et al., 2019; Yu et al., 2020). Zooplankton are ubiquitous in the world's oceans (McManus and Woodson, 2012) and provide the critical link between primary producers and secondary consumers in the oceanic food web (Longhurst and Glen Harrison, 1989). Among zooplankton, copepods are known to be vectors for aquatic pollutants (Raisuddin et al., 2007) and are commonly used for ecotoxicological studies (Hussain et al., 2020). Multiple separate studies have shown that microplastics reduce copepod survival (Lee et al., 2013; Svetlichny et al., 2021; Yu et al., 2020; Zhang et al., 2019), fecundity (Heindler et al., 2017; Jeong et al., 2017; Zhang et al., 2019), growth and development (Cole et al., 2019;

Abbreviations: Artificial sea water, (ASW); particulate organic matter, (POM); particulate organic carbon, (POC).

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Jeong et al., 2017), and egg hatching success (Cole et al., 2015), which may all affect overall population fitness. However, no study has synthesized the data from microplastic effects on multiple life history traits to evaluate impacts on net reproductive rates for copepods. Because copepods represent critical links to carbon cycling in ocean systems (Turner, 2002, 2015), any modifications to population growth that would otherwise be unaffected in the absence of microplastics could have severe impacts on the transport of ocean nutrients across marine trophic levels. Such information is critical for understanding the potential long-lasting effects of microplastics on population growth and fitness for organisms at the base of the marine food web.

Copepod fecal material substantially contributes to the settling and eventual storage of atmospheric carbon dioxide to the deep ocean via the biological pump (Longhurst and Glen Harrison, 1989), a process by which dissolved inorganic carbon is transformed into organic biomass via photosynthesis in phytoplankton, consumed by primary consumers, such as zooplankton, then transported to the benthos for eventual storage (Cavan et al., 2017; Ducklow et al., 2001; Roman and McCarthy, 2010; Turner, 2015). Thus, the biological pump is vital to slowing down the rate of climate change as it absorbs and removes CO_2 from the atmosphere, taking up about 30% of anthropogenic carbon emissions (Bopp et al., 2020). The consumption of microplastics, however, can slow carbon transport to the benthos because contaminated fecal pellets have been shown to sink slower (Cole et al., 2016; Coppock et al., 2019; Wieczorek et al., 2019) and have lower densities (Cole et al., 2016; Wieczorek et al., 2019). Microplastics have also affected fecal pellet production rate (Rodríguez-Torres et al., 2020; Yu et al., 2020) and size (Svetlichny et al., 2021), which also has the potential to slow the rate of carbon settling. However, comprehensive modeling of microplastic-laden fecal pellet effects on the biological pump and carbon settling has not been attempted.

The combined effects of microplastics on copepod life-history performance and carbon cycling from fecal pellets have never been considered in the same study, despite how microplastics can impact both areas individually. To understand the potential impact of microplastics on zooplankton life-history and fitness, as well as carbon settling, we reared the common coastal/estuarine calanoid copepod *Acartia tonsa,* in the presence and absence of microplastics with equal availability of algal food. Specifically, we test the hypotheses that 1) exposure to polystyrene microplastic (6.68-μm) particles will result in microplastic consumption, reduced naupliar growth and survival, and reduced adult reproductive output, 2) the combined effects on the above life history traits will reduce net population growth rates, 3) microplastic consumption will reduce fecal pellet size, volume, and estimated sinking rates, and 4) the combined effects on fecal pellets will reduce carbon settling via the biological pump.

2. Materials and methods

2.1. Copepod sampling

Zooplankton were sampled from the coastal areas of Cedar Beach, Long Island (40°57′51.98″ N 73°02′33.06″ W) and Peirce Island, New Hampshire (43°4′17.64″ N, 70°45′44.08″ W) in the months of June and July 2018, respectively. Individuals were collected with a horizontally submerged 200-μm mesh plankton net. The samples were then transferred to 5-L buckets inside an insulated cooler with ice and transported to the University of Vermont (UVM) within the same day. Upon arrival, adult *Acartia tonsa* were manually sorted with a 3-mL transfer pipette from the rest of the zooplankton species under a Leica M80 inverted light microscope. After sorting, *A. tonsa* were transferred to a 3-L container of aerated 30 ppt sea water (ASW) made with Instant Ocean salt (Blacksburg, VA). Individuals were stored in a temperature-controlled laboratory for a minimum of three generations, in a 12:12 h light:dark cycle, at 18 °C.

2.2. Algal cultures

Three algal species, chain-forming diatom *Thalassiosira weissflogii* (2–32 μm), red microalgae *Rhodomonas lens* (max 12 μm), and chlorophyte *Tetraselmis* sp. (5–14 μm) were selected for experimental use; they are natural prey for *A. tonsa* and have a similar size to the microplastic particles used in this study. All algal species were cultured at UVM in 3 L carboys using F/2 media (Kent Marine, Pro-Culture), with additional silicates (sodium metasilicate nonahydrate, 30 g L^{-1} in reverse osmosis water) for *T. weissflogii* under a 10:14 h light:dark cycle at ambient temperature. For experiments, algal cells were counted on a Sedgewick Rafter cell slide using a Nikon SMZ-800 dissecting scope for particle concentrations, and 167 μg C L^{-1} of each species was combined to add 500 μg C L^{-1} to each experimental replicate for optimal egg production (Feinberg and Dam, 1998). 500 μg C L^{-1} of algae also ensures ingestion is saturated throughout the duration of the experiment and is standard for *ad libitum* feeding for *A. tonsa* (Feinberg and Dam, 1998).

2.3. Microplastics

We used SPHERO red polystyrene particles purchased from Spherotech Inc (Lake Forest, IL), in the size range of 6.0–8.0 μm with a mean particle size of 6.68 μm, encompassing particle sizes from all three algal species. Polystyrene (1.05 g cm^{-3}) is neutrally buoyant in seawater (1.03 g cm^{-3}) and constitutes one of the largest forms of plastic pollution in the environment (Eriksen et al., 2016), and can deteriorate into small, undetectable nano-fragments (Lehner et al., 2019). Microplastic particle concentrations were calculated to equal a 1:1 ratio of microplastics to the number of algal particles, resulting in a microplastic concentration of 1197 particles mL^{-1}, similar to that used in previous studies (Cole et al., 2016). Desired experimental microplastic concentrations were determined by calculating the total weight of beads required for each replicate to yield the 1:1 ratio of algal particles to microplastic beads. The weight of each bead was determined by first calculating the volume of each bead utilizing the average microplastic bead diameter (provided by Spherotech) and the volume of a sphere equation, then converting the result of that equation (volume in m^3) to volume in mL to match the units further in the calculations. We then utilized the density of each bead (provided by Spherotech), multiplied by the volume of each bead, to get the weight of each bead. For example, in an experimental replicate vessel of 600 mL, we wanted a 1:1 ratio of algal particles to microplastic beads (500 μg C L^{-1} of algae is 720,000 algal particles); we multiplied 720,000 (number of microplastic beads we needed) by the weight of each bead, to get total weight of beads required for the replicate. Finally, to solve for the amount of microplastic solution required for each replicate, we used the microplastic bead concentration (provided by Spherotech) in an equation to solve for x (mL beads required) and setting this equation to the total weight of beads required to equal 720,000 particles. This approach resulted in double the particle density in the treatment, but the same density of algal particles in both the control and the treatment replicates to ensure an equal encounter rate of both microplastics and algal particles. Although consumption experiments were not conducted here, equal encounter rates of algal to microplastic particles were used to allow an "equal choice" for the copepods without reducing the amount of algal cells available. This concentration may be considered higher than the average coastal environment concentration (Díaz-Mendoza et al., 2020; Hantoro et al., 2019), however it is reflective of hotspot concentrations due to coastal wastewater and ocean current conveyance (Kang et al., 2015). Although the microplastic concentrations used in this study are reflective of hotspots or regional areas, the spatial distributions of marine organisms, habitats, nutrients and resources are similarly patchy, thus it is important to consider the impacts of microplastic consumption at local, potential hotspot scales rather than average open ocean concentrations. Before using, microplastics were resuspended by vortexing and vigorous shaking. The particle suspension matrix also included 0.02% sodium azide which, when

tested alone, did not cause any effects on growth or survival.

2.4. Experimental setup

Three experiments were conducted to measure the impacts of microplastics on nauplii body size and survival, fecundity, and fecal properties. Experiments were conducted sequentially to have sufficient numbers of individuals for each assay. Treatments included a control (strictly algal exposure) and a 1:1 ratio of algal particles to microplastic particles and were carried out in 1-L pre-leached plastic beakers. Fifty percent water changes were done every 48–72 h by reverse syphoning beaker media through a 30-μm sieve to prevent the loss of any copepods. Afterwards, fresh ASW with algae mixed evenly to a final concentration of 500 μg C L^{-1}, and corresponding microplastic suspension matrix equal to that of the algal concentration were added to the treatment replicates. We acknowledge that, due to only removing 50% of media per water change, microplastic and algae particle densities may have slightly increased over time. However, this increase occurred in all replicates of both treatments, so the 1:1 ratio of algae to microplastic particles was conserved.

2.5. Nauplii Survival and Body Size

Two hundred and forty *A. tonsa* nauplii were introduced to each beaker (3 beakers per treatment, 40 individuals in each) containing 400 mL of ASW. Microplastic exposure occurred for 5 days at 18 °C in a 12:12 h light:dark cycle. After 5 days of exposure, the contents of each beaker were poured over a 30-μm sieve to collect nauplii, which were then carefully rinsed down into Petri dishes. Surviving nauplii were counted and noted as alive or dead based on no movement for ~10 s. Surviving nauplii were then anaesthetized and stained using Acid Lugol's iodine. Five nauplii from each replicate (15 control and 15 plastics) were imaged and body size was measured using Lecia SPOT measuring software under an Olympus iX71 inverted light microscope.

2.6. Copepodite to Adult Survival and Fecundity

One hundred thirty-six juvenile *A. tonsa* (Stage 5 copepodite) were sorted and incubated in 1-L beakers filled with 600 mL of ASW (4 beakers per treatment, 17 individuals in each). Exposure to microplastics occurred for 7 days at 18 °C in a 12:12 h light:dark cycle while the copepodites developed into adults. After 7 days of exposure, from each replicate beaker, adults were carefully poured over a 30-μm sieve. Surviving adults were counted under a Leica M80 inverted light microscope and noted for 'survival' either as alive or dead based on no movement for ~10 s. Surviving adults were then sexed and breeding pairs of adults were created, conserving treatment and replicates. A single breeding pair was placed in a Petri dish with 25 mL of ASW, conserving treatments and replicates. Breeding pairs from each replicate were added to a new Petri dish with new media prepared as described above yielding 10 pairs per treatment. Individuals exposed to microplastics as copepodites were also treated with microplastics during the breeding period. Petri dishes were incubated at 18 °C in a 12:12 h light:dark cycle for 48 h to allow adults to lay eggs. Post exposure, Petri dish contents were anaesthetized and stained using Acid Lugol's iodine. Laid eggs were imaged, counted, and measured across the diameter using Lecia SPOT measuring software under an Olympus iX71 inverted light microscope.

2.7. Net reproduction growth estimates

Values for survival at all life stages (naupliar, copepodite, and adult) and adult fecundity values were separated by treatment (i.e., control or plastic). Net reproductive rate was calculated as the dominant eigenvalue of a combined stage-structured Leslie matrix (Caswell, 2001). Eigenvalues were calculated using the popbio (Stubben and Milligan, 2007) package in R (v 4.0.2). For simplicity, lambda can be considered by the following formula:

$$\lambda = e^{\sum l_x m_x}$$

where l_x represents the number of survived individuals at any stage x, and m_x represents the fecundity at any stage x. More explicitly, a 3 × 3 matrix is constructed where each column represents a particular life-stage (nauplius, copepodite, adult). The header of each column represents the per capita fecundity rates (m_x) for the respective stage x. Values of zero in the header indicate that no reproductive efforts take place at that stage (i.e., naupliar and copepodite stages). Probabilities of within-stage survival (l_x) for any stage x are listed beneath the header in the stage-appropriate row for each column. For example, survival probability in the first stage, naupliar, was placed in the first row beneath the header (the second row) of the first column. Naupliar probabilities of survival are taken from each nauplius body size experiment beaker (see methods *Nauplii Survival and Body Size*) while copepodite probabilities of survival are taken from each copepodite beaker in fecundity experiments (see methods *Copepodite to Adult Survival and Fecundity*). Each beaker was used to generate a single probability estimate for each respective stage based on the proportion of individuals surviving (e.g., if 28 of the original 40 nauplii in one beaker survived, the probability of survival was estimated as 0.7). Survival probabilities of adults were taken from egg laying experiments and assumed to be 1 since no mortality was observed during egg laying. Lastly, the survival probability of the third stage, adults, was included in the third row of the third column (Caswell, 2001). To incorporate error into the calculations of net reproductive rate (fitness, λ), we utilized the replicate estimates of survival and fecundity. Specifically, for each treatment, we matched the naupliar-stage (stage 1) survival probability of each replicate with the copepodite-stage (stage 2) survival probability of each replicate with the fecundity value for each breeding pair to yield the maximum 90 possible calculated fitness values (3 naupliar survival probabilities, 3 copepodite survival probabilities, and 10 fecundity values).

Estimates of population abundance were calculated using population growth models ($N_t = N_0 e^{rt}$) with calculated λ values as the net reproductive rates ($\frac{N_t}{N_0}$), r is the intrinsic growth rate (generation^{-1}), t is time in generations, N_0 is the initial number of individuals, and N_t is the number of final individuals at any generation t. We considered using logistic growth models rather than exponential growth models to estimate population size (e.g. $\frac{dN}{dt} = rN\left(\frac{1-N}{K}\right)$) where r is the intrinsic growth rate (generation^{-1}), N is the population size, K is the carrying capacity, and $\frac{dN}{dt}$ is the change in population size over time. Though logistic growth models represent a more realistic population growth scenario, the use of this model would be inappropriate for the present study because we cannot estimate the population carrying capacity. However, it is worth noting that the estimated intrinsic rate of growth is the same for both models. So, the effects would not be substantially different for a population which experiences logistic growth rather than exponential growth with the same growth rate.

2.8. Fecal properties and carbon settling modeling

Two hundred *A. tonsa* eggs were introduced to each 1-L Tupperware beaker (4 beakers per treatment) containing 600 mL of ASW. Copepods were incubated until the final molt to the adult stage (20 days) at 18 °C in a 12:12 h light:dark cycle. Adults were placed in groups of 2 or 3 in new Petri dishes and were not assayed for survival in this experimental trial as the focus was on fecal pellets. A 1:1 ratio of algae and microplastic particles were added per 25 mL of ASW and left alone for 48 h. Post exposure, all fecal pellets in each Petri dish were counted, imaged, and measured using Lecia SPOT measuring software under an Olympus iX71 inverted light microscope. Fecal volume was calculated using the equation for volume of a cylinder, $V = \pi r^2 h$. To estimate the reduction

in sinking rates of *A. tonsa* fecal pellets contaminated with microplastics compared to pellets strictly containing natural algae, we used a modified Stokes equation (Komar et al., 1981) for sinking cylinders at low Reynolds numbers

$$\omega_s = 0.0790 \frac{1}{\mu}(\rho_s - \rho) g L^2 \left(\frac{L}{D}\right)^{-1.664}$$

where μ is the viscosity of 30 ppt sea water at 18 °C (g cm^{-1} s^{-1}), ρs is fecal pellet density (g cm^{-3}), ρ is the density of 30 ppt sea water at 18 °C (g cm^{-3}), g is the acceleration due to gravity (981 cm s^{-2}), L is the length, and D is the diameter of fecal pellets (cm) from our study. We used the pellet density for *Acartia tonsa* reported by (Feinberg and Dam, 1998), who used the same algal species and equitable algal concentration as used in this study (1.14 g cm^{-3}). Because fecal density was not measured in this study, and no studies have used *A. tonsa* for studying the effects of microplastics onfecal properties, we used the fecal density of 1.14 g cm^{-3} in both our control and microplastic fecal pellet calculations to eliminate any bias. We acknowledge that in using a fecal density not affected by microplastics in this calculation, we likely underestimate the effects of microplastics on fecal sinking rates. However, this calculation provides a foundation for further research on microplastics and fecal sinking rates as well as carbon settling. We also acknowledge that using a fecal density by Feinberg and Dam (1998), and not our own study, may affect our results. However, Feinberg and Dam (1998) used the same copepod from the same geographic region and same algal species and concentrations as the present study.

To further estimate the effects of microplastic consumption by zooplankton on carbon settling, we built upon the estimate from (Cole et al., 2016) that explored the difference in time it took for fecal pellets contaminated with microplastics to sink to the benthos compared to control fecal pellets. Here, we modeled the combined effects of smaller fecal volumes experimentally calculated from this study (Fig. 5A) and the estimated 1.76-fold difference in fecal sinking rates (Fig. 5D) on carbon transport calculated using the modified Stokes equation (Komar et al., 1981). Control: (average depth of the ocean (Charette and Smith, 2010)) 3682 m/20.77 m day^{-1} (average fecal sinking rate) = 177.27 days (time for fecal pellets to sink to the benthos); 247,949 μm^3 (average fecal volume)/177.27 days = 1398.71 μm^3 day^{-1} (rate of total fecal volume sinking per day); plastic: (average depth of the ocean (Charette and Smith, 2010)) 3682 m/11.83 m day^{-1} (average fecal sinking rate) = 311.24 days (time for fecal pellets to sink to the benthos); 108,072 μm^3 (average contaminated fecal volume)/311.24 days = 347.23 μm^3 day^{-1} (rate of total fecal volume sinking per day); 1398.71 μm^3 day^{-1}/347.23 μm^3 day^{-1} = difference in fecal volume sinking per day. The relative impacts of microplastics on the amount of fecal volume sinking per day are consistent regardless of depth, e.g., 600 m or 3000 m, making this model applicable to any ocean depth.

2.9. Statistical analysis

All data analysis was conducted using R statistical software version 3.6.3. To analyze fecal properties, fecal sinking rates, fecundity, and naupliar body size data, the *lme* function from R package *nlme* was utilized to perform a linear mixed-effects analysis to compare the relationship between the two treatments, modeling replicate as a random effect. *P*-values were obtained by running a Type II Wald Chi Square test. Using function *glmer* from the R package *lme4*, a generalized linear mixed-effects model was used for both copepodite-adult and naupliar survival analysis. Utilizing a binomial distribution, survival was entered as either 'alive' (1) or 'dead' (0), with replicate modeled as a random effect. Significant differences were confirmed where $P < 0.05$. To analyze differences in net reproductive rates, we constructed a generalized linear model for fitness with treatment as a fixed effect. A one-way ANOVA was used to test differences in fitness values.

3. Results

3.1. Microplastic uptake

Microplastics were observed in the intestinal tracts of both nauplii and adult *A. tonsa* copepods confirming ingestion (Fig. 1A–B). Microplastics were also observed in adult fecal pellets, confirming consumption and successful egestion of the polystyrene beads (Fig. 1C–D), and adhered to adult *A. tonsa* swimming legs (Fig. 1E).

3.2. Nauplii

After a 5-day exposure period in the presence of microplastics, *A. tonsa* nauplii had a 28.6% decrease in survival compared to individuals reared in algae alone (control: 70 ± 12.5%; plastic: 50 ± 10.9%; Fig. 2A; Generalized linear model, df = 239, z = −2.75, $P < 0.01$). Nauplii experienced a 12.2% decrease in body length compared to the control (control: 172.28 ± 29.32; plastic: 151.28 ± 15.20 μm; Fig. 2B; Linear mixed-effects model, df = (4, 24), $\chi^2 = 4.98$, $P < 0.05$), while no significant effect on body width in individuals exposed to microplastics was observed (76.19 ± 8.32 μm) compared to the control (82.12 ± 15.29 μm); (Fig. 2C; Linear mixed-effects model, df = (4, 24), $\chi^2 = 1.03$, $P = 0.31$).

3.3. Copepodite to adult stage

Rearing from copepodite (juvenile *A. tonsa*) to the adult stage in the presence of polystyrene microbeads showed no significant effect on survival (Fig. 3A; Generalized linear model, df = 100, z = −0.50, $P = 0.28$). In addition, the presence of microplastics did not affect total fecundity over a 48-h period of egg laying (Fig. 3B; Linear mixed-effects model, df = (4, 12), $\chi^2 = 1.30$, $P = 0.32$). In contrast, eggs produced by adults reared in the presence of microplastics had a 7.3% reduction in diameter (control: 79.07 ± 3.27 μm; plastic: 73.32 ± 6.32 μm; Fig. 3C; Linear mixed-effects model, df = (4, 80), $\chi^2 = 30.03$, $P < 0.001$).

3.4. Net reproductive rate

The addition of microplastics on copepodite survival and adult fecundity did not exhibit effects individually. However, net reproductive rate (λ) calculated from combined nauplii and copepodite to adult survival probability and fecundity data yielded 15% lower reproductive rates for plastic-exposed copepods compared to non-exposed copepods (control: 1.62 ± 0.08; plastic: 1.37 ± 0.1; Fig. 4; Linear model, df = (160), $P < 0.0001$).

3.5. Egested fecal pellets, sinking rates, and carbon settling modeling

Incorporation of microplastics resulted in a 26.8% decrease in fecal length (control: 158.19 ± 38.04 μm; plastic: 115.74 ± 36.02 μm; Linear mixed-effects model, df = (5, 353), $\chi^2 = 10.72$, $P < 0.001$), and a 24.7% reduction in width (control: 42.62 ± 8.24 μm; plastic: 32.10 ± 7.48 μm; Linear mixed-effects model, df = (5, 353), $\chi^2 = 9.92$, $P < 0.01$) of fecal pellets egested by adults that had developed in the presence of microplastics since the egg stage. In the absence of microplastics, adult fecal pellets had an average volume of 2.48E5 ± 1.33E5 μm^3. Fecal pellets containing microplastics had volumes reduced by 56.4% averaging 1.08E5 ± 7.79E5 μm^3 (Fig. 5A; Linear mixed-effects model, df = (5, 353), $\chi^2 = 12.50$, $P < 0.001$). On average, adults reared strictly with algae produced 38.3% more fecal pellets than adults reared with both microplastics and algae (control: 10.35 ± 4.02 pellets; plastic: 6.39 ± 4.36 pellets; Fig. 5B; Linear mixed-effects model, df = (5, 8), $\chi^2 = 3.91$, $P < 0.05$). Overall, copepods exposed to microplastics produced an estimated 75% less total fecal material (control: 2.76E6 ± 1.22E6 μm^3; plastic: 7.03E5 ± 3.51E5 μm^3; Fig. 5C; Linear mixed-effects model, df = (5, 8), $\chi^2 = 14.98$, $P < 0.001$). Although not quantified, we observed

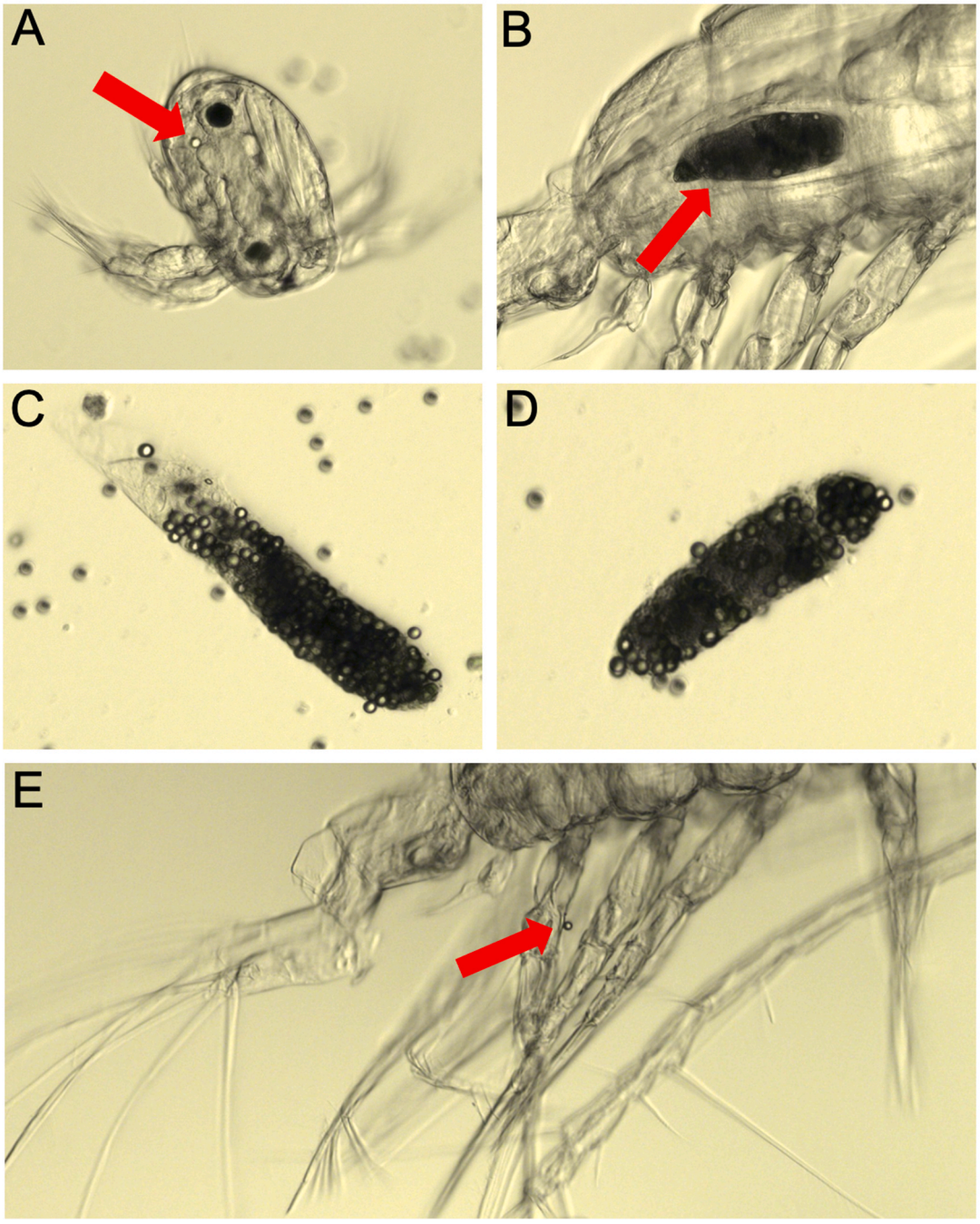

Fig. 1. Images of microplastic contamination in *Acartia tonsa.* (A) Nauplii with microplastic bead in its hindgut; (B) fecal pellet laden with 5-μm polystyrene microplastics inside the hindgut of an adult; (C) egested fecal pellet contaminated with microplastics; (D) fragmented microplastic fecal pellet egested by an adult; (E) microplastic bead trapped in hind swimmers of an adult. All microplastic concentrations were a 1:1 ratio of algae to 5-μm microplastic particles.

more fragmented fecal pellet pieces in the microplastic treatments relative to controls that could have broken off during the process of egestion (Fig. 1D).

Taking into account size, shape, and density of fecal pellets at low Reynolds numbers using a modified Stokes equation (Komar et al., 1981), contaminated fecal pellets were calculated to sink 1.76-fold slower than pellets containing algae (control: 20.77 $\pm$ 7.57 m day^{-1}; plastic: 11.83 $\pm$ 5.44 m day^{-1}; Fig. 5D; Linear mixed-effects model, df = (5, 353), $\chi^2 = 110.84$, $P < 0.001$). Integrating this estimated slower fecal sinking rate with our results of smaller fecal volumes into a carbon settling model, we estimate that the consumption of microplastics results in 4.03-fold less carbon settling to the benthos per day compared to a system devoid of microplastics.

4. Discussion

Our results confirm the hypothesis that microplastics were consumed by all life history stages of *A. tonsa*, and early life stage growth and survival, as well as egg size, were reduced after microplastic exposure. We also confirmed the hypothesis that in the presence of microplastics, the combination of reduced survival across each life history stage and, although not significantly affected, fecundity resulted in significantly reduced net population growth rates. Finally, our results support the hypothesis that the consumption of microplastics reduced fecal pellet

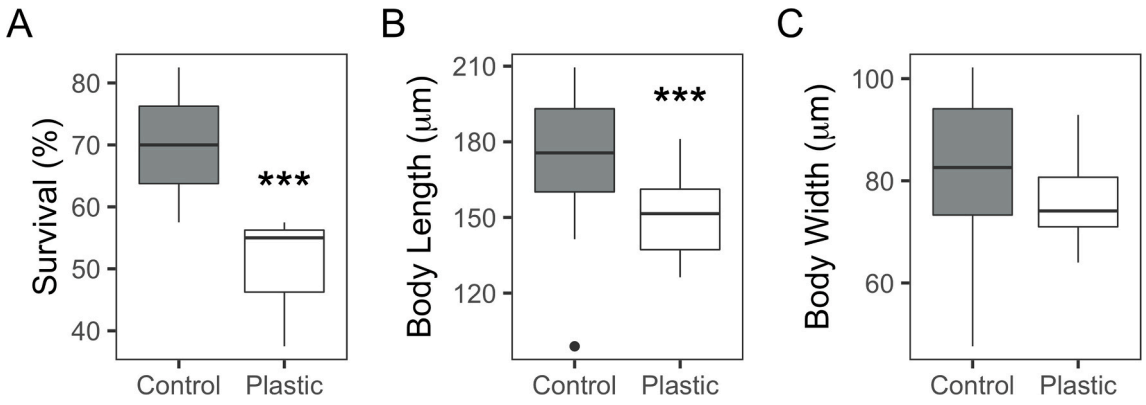

Fig. 2. The impact of microplastics on *Acartia tonsa* nauplii after a 5-day exposure to polystyrene microbeads. (A) Percent survival; (B) body length; (C) body width. Treatments: control (grey) and plastic (white); asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

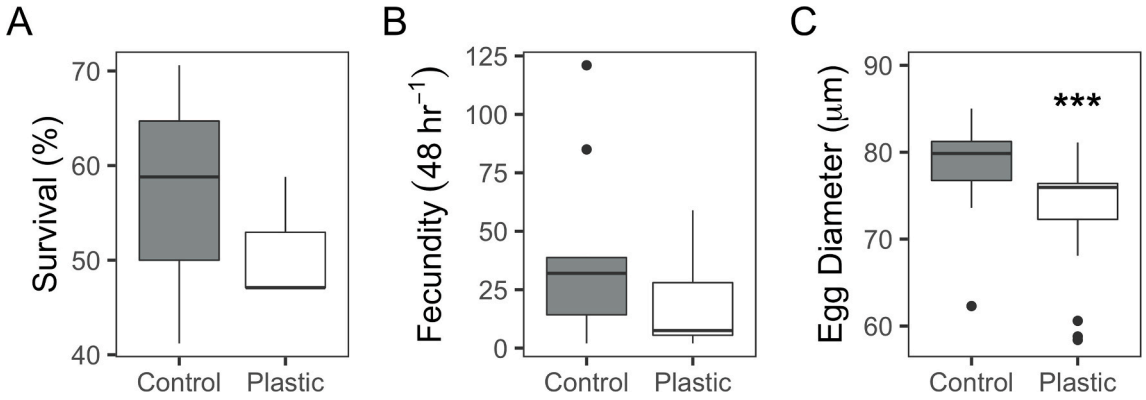

Fig. 3. The impacts of microplastics as *Acartia tonsa* transition from copepodite to the adult stage in a 7-day exposure. (A) Percent survival; (B) total reproductive output during a 48-h laying period; (C) egg diameter. Treatments: control (grey) and plastic (white); asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

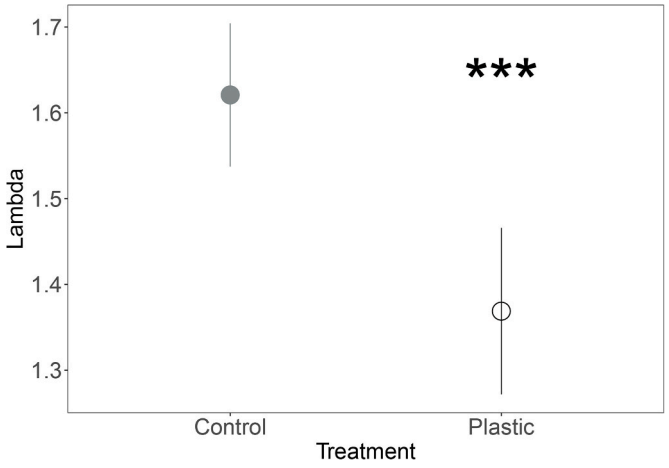

Fig. 4. Net reproductive rate. Total fitness (λ) calculated from combined survival probability and fecundity data for control (grey) and plastic treated (white) copepods; asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

volume and sinking rates which, when extended in a model, suggest that carbon settling to the benthos per day may be reduced.

4.1. Effects of microplastics in early life development and fitness

The results of this study confirm results from previous studies, which suggest that consumption of microplastics negatively affect copepod early life stage development (Jeong et al., 2017; Lee et al., 2013; Yu et al., 2020). Our results expand this understanding by showing reduced naupliar body size in the presence of microplastics. In contrast, Yu et al. (2020) found no significant effect on naupliar body size in the presence of microplastics. This difference could be explained by differences in the timing and duration of exposure; we exposed eggs to microplastics and allowed them to develop in the presence of microplastics until hatching, whereas Yu et al. (2020) started microplastic exposure after nauplii had hatched. Yu et al. (2020) also found a decrease in embryonic development time in the presence of microplastics which they attribute to possible microplastic leachates. Such leaching could have also occurred in our study, though it was not measured. A reduction in body size could also result from less overall carbon biomass consumed due to the consumption of microplastics instead of algal particles or the co-ingestion of microplastics and algae. Maximum algal particle clearance size for nauplii stages (NII-IV) is approximately 7 μm (Berggreen et al., 1988), suggesting that nauplii actively select and consume particles 7 μm and smaller, which encompasses the microplastics used in this study. Newly hatched *A. tonsa* nauplii are approximately 70 μm in length (Marcus and Wilcox, 2007), falling in the size range of microplastics infrequently assayed environmentally (van Sebille, 2015). Taken together, these results suggest that nauplii could be consuming large numbers of small, under-assayed microplastic particles in nature, highlighting an important area for future investigation.

Nauplii that consumed microplastics had reduced survival which can lower the proportion of nauplii that survive to the copepodite stage, and in turn reduce the number of reproducing adults, which can affect net

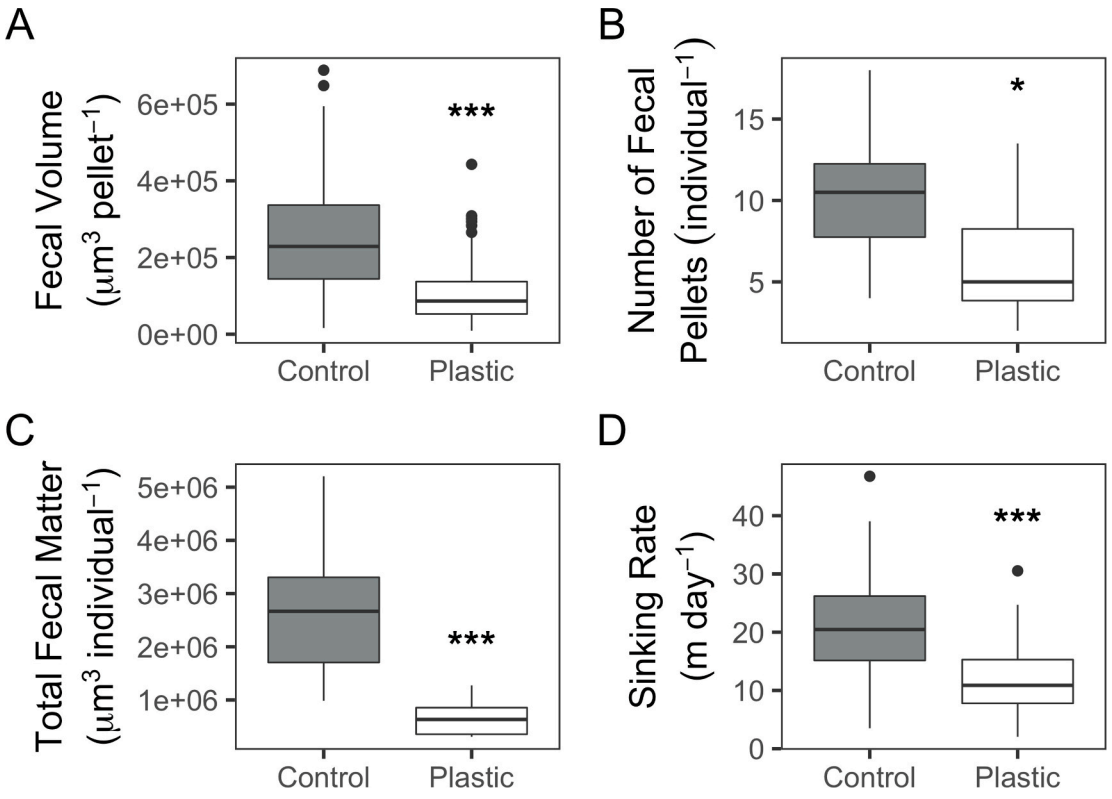

Fig. 5. Impacts of microplastic consumption on egested fecal pellets produced by adult *Acartia tonsa.* (A) Fecal volume per pellet, (B) number of fecal pellets per individual, (C) total fecal material produced per individual, (D) estimated fecal pellet sinking rates by using a modified Stokes equation (Komar et al., 1981) for sinking cylinders at low Reynolds numbers. Treatments: control (grey) and plastic (white); asterisks indicate statistical significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

population growth. These results concur with Yu et al. (2020) in that the survival of nauplii was most significantly affected out of all three copepod stages, suggesting that nauplii are the most sensitive to microplastic exposure. Though previous experiments have used microplastics to evaluate zooplankton ingestion, to our knowledge only one other study has investigated the negative effects of microplastics across copepod life-history stages (Yu et al., 2020). Microplastic exposure caused a decrease in both development time and overall survival as a culture of copepods aged from N1 to the adult stage, which could reduce overall population size or fitness, however this was not modeled in (Bellas and Gil, 2020; Cole et al., 2015; Jeong et al., 2017; Lee et al., 2013; Rodríguez-Torres et al., 2020; Yu et al., 2020). Other studies, however, only consider single traits and do not measure or estimate overall fitness (Bellas and Gil, 2020; Cole et al., 2015; Jeong et al., 2017; Lee et al., 2013; Rodríguez-Torres et al., 2020; Svetlichny et al., 2021; Yu et al., 2020). The net reproductive rate (λ) for a population determines the overall abundance of offspring producing individuals, the calculation of which has been well-documented (Caswell, 2001; Steiner et al., 2014). The present study is the first to estimate λ for an environmentally and ecologically relevant zooplankton species in the presence of microplastics. The reduction of fitness under microplastics relative to the control suggests that, over 20 generations (~one year), the 15% decrease in net reproductive rate, an estimate at the level of individual females per generation, could yield populations of *A. tonsa* which reduce in abundance by over an order of magnitude or 30-fold less ($N_{20\text{-Control}} = 1.5E6$; $N_{20\text{-Plastic}} = 4.9E4$). This reduction in abundance could have profound consequences for marine food webs given the critical link copepods play between primary producers and upper trophic levels, including key fisheries (Beaugrand and Reid, 2003; Möllmann et al., 2008). Moreover, exposure to microplastics is expected to increase susceptibility to other potent environmental stressors or pollutants such as cadmium (Bellas and Gil, 2020). Thus, the effects of microplastics could yield negative synergistic effects on abundance in the presence of additional marine pollutants. Only a handful of studies have attempted to evaluate effects of microplastics in copepods over multiple generations. Two of these studies used the harpacticoid copepod, *Tigriopus japonicus*, and did not evaluate overall fitness for copepods over two generations of chronic particle exposure (Lee et al., 2008, 2013). The other did not track generations or measure changing population growth rate, despite measuring changing population sizes over a period of time that should amount to multiple generations (Heindler et al., 2017).

Coupled with the effects of reduced survival, nauplii that have energetic shortages (i.e. reduced lipid content) or reduced body sizes due to microplastic exposure may also impact higher trophic organisms that prey on early stage zooplankton (Cole et al., 2015). This could cause an energy imbalance at the start of the oceanic food chain, potentially cascading energy shortages into further trophic levels.

4.2. Differences in reproductive quality and output

Our results revealed a 7.3% reduction in egg diameter produced as a result of consuming microplastics, contrasting with previous research which found no effect of microplastics on egg size (Rodríguez-Torres et al., 2020). Differences in egg size could be attributed to less carbon biomass consumed due to the misidentification of microplastics for algal particles, or random co-ingestion of microplastics and algae. In support of reduced egg size due to reduced consumption of algae, we found that fecal volume per individual was reduced in the presence of microplastics (Fig. 5C). These results support the idea that the consumption of microplastics decreased the amount of carbon and nitrogen available for egg production. Consuming algae rich with carbon and nitrogen is essential for copepod growth and egg production (Kuijper et al., 2004). After consumption, up to 85% of algae carbon biomass is assimilated,

specifically contributing to egg production in adult females of *A. tonsa* (Kiørboe, 2008). Additionally, 55% of egg biomass comprises proteins, where nitrogen is essential for protein synthesis (Kiørboe et al., 1985). The quality (i.e., C:N ratios) and quantity of ingested algal biomass and egg production in marine copepods are directly related (Nobili et al., 2013). When this balance is disrupted, this can lead to decreased fecundity and smaller eggs (Nobili et al., 2013). Our results of smaller eggs as a result of microplastic consumption are comparable to observed reductions (2.5%) in egg diameters of *Calanus helgolandicus* in the presence of microplastics (Cole et al., 2015). However, microplastic exposure for *C. helgolandicus* started at the onset of the adult stage, which differs from our study where exposures started in early copepodite stages (Cole et al., 2015) - the stage when females begin egg production (Norrbin, 1994) - which could explain the three-fold more negative impact measured in our study. The first traces of female gonad oogenesis occur during the first of six copepodite stages of *Acartia* sp. (Norrbin, 1994). Oocytes continue to grow in the developing copepodites until the adult stage (Mauchline, 1998). Smaller eggs caused by microplastic consumption could result in stunted growth, high naupliar mortality before the copepodite stage, or could permanently decrease the individual's potential size at adulthood (Sun et al., 2019), though further research is needed to demonstrate these effects (Botterell et al., 2019; Cooney and Gehrs, 1980); we suggest full life exposures to microplastics with body length and lipid content assayed at each stage.

In adult exposures, we found no difference in fecundity (total eggs produced), hatching rates, or survival in the experimental time frame of 48 hours, which concurs with results from Bellas and Gil (2020) after a 48-hour exposure period to polyethylene microplastics to *A. tonsa.* In contrast, Rodríguez-Torres et al. (2020) found that the presence of microplastics increased egg production rates. The authors suggest that microplastics may have triggered stress-induced egg production, which we did not see in our results. Yu et al. (2020) found that the presence of polyethylene and polyamide 6 microplastic particles decreased egg hatching rates and the proportion of gravid females, as well as increasing the time between females carrying egg sacs. The differences in egg hatching rates could be attributed to the longer observation period of 14 days (Yu et al., 2020) compared to our observation period of 2 days. A decrease in egg production was also observed in the copepod *Parvocalanus crassirostris*, however these differences were attributed to handling stress, as well as extreme microplastic concentrations of 10, 000, 20,000, and 40,000 particles mL^{-1} (Heindler et al., 2017). We did not observe mortality within the 48-hour time frame of our experiment, whereas mortality of adult copepods *Acartia clausi* and *Centropages typicus* increased over an 8-day experimental period when exposed to microplastics (Svetlichny et al., 2021). Along with Bellas and Gil (2020), we suggest that longer, multigenerational microplastic exposures may reveal significant effects on fecundity, hatching rates, and survival of *A. tonsa* in future studies.

It is important to recognize that not all studies show a negative effect of microplastics on marine organisms, highlighting that the effects of microplastic exposure may be taxa-specific. A recent meta-analysis by (Foley et al., 2018) shows that studies in echinoderms, mollusks, and adult fish have found negative effects of microplastics, though studies using adult fish were sparse (Foley et al., 2018). Considering crustaceans, exposure to polystyrene microspheres decreased ion exchange in the lamellae of the shore crab *Carcinus maenas*, though recovery was possible within the acute time frame of exposure (Watts et al., 2016). A meta-analysis by (Bucci et al., 2020) revealed that in studies using crustaceans, some showed no effect on mortality or reproductive output, which is speculated to be driven by the wide range in particle sizes and concentrations used in the studies reviewed. In the marine isopod *Idotea emarginata,* a 6-week microplastic exposure period revealed no significant effects of microplastic consumption on mortality, growth, and intermolt duration (Hämer et al., 2014). The authors hypothesize this could be due to different sizes and distribution patterns of microplastics in the marine environment and the feeding method of *I.emarginata*, which is not a filter feeder, unlike most calanoid copepods (Koehl and Strickler, 1981).

4.3. Fecal property changes occurring with incorporated microplastics

To our knowledge, the present study is the first to incorporate fecal volume and fecal sinking rates to model the potential impacts of microplastic consumption by copepods on the rate of carbon settling, a key factor in the biological pump, estimating a 4.03-fold reduction when microplastics were consumed.

The biological pump modulates fluxes of earth's climate (Honjo et al., 2014) and is responsible for long-term storage of anthropogenically produced carbon (Longhurst and Glen Harrison, 1988). Zooplankton fecal pellets are estimated to contribute, on average, approximately 40% of total particulate organic carbon (POC), transporting carbon and particulate organic matter (POM) to the ocean's interior and benthic sediment (Cavan et al., 2017; Steinberg and Landry, 2016), thus substantially supporting total oceanic carbon flux (Turner, 2015). Fecal pellets remove POC from the euphotic zone, reducing the chances that carbon is released back into the atmosphere as CO_2 (Turner, 2002). Our results reveal that copepods reared with microplastics, on average, egested smaller (Fig. 5A) and fewer fecal pellets (Fig. 5B), thus less fecal material in general (Fig. 5C). Combining the density of *A. tonsa* fecal pellets produced by the consumption of the same algal species from (Feinberg and Dam, 1998) and reduced fecal size due to microplastics measured from this study, we calculated that contaminated fecal pellets sink 1.76-fold slower than uncontaminated pellets (Fig. 5D). A caveat to this result is that contaminated fecal pellets are less densely packed with biomaterial (pers. obs., E. Shore), and prone to fragmentation. Thus, smaller fragments could have been overlooked, resulting in an underestimate of total fecal matter observed in the microplastic treatment and inflation of the difference in total fecal matter between treatment groups (Fig. 5C). Even so, the ultimate consequences will be the same: fragmentation will contribute to the reuptake rather than settling of POC. In addition, less fecal matter produced suggests *A. tonsa* are consuming less biomaterial in the presence of microplastics, which can result in less dense fecal pellets. This has also been observed in other copepods (Cole et al., 2016), affecting the structural integrity and sinking rate of fecal pellets and thus carbon settling.

To understand the potential implications of microplastics on carbon settling, we constructed a model building on (Cole et al., 2016). The authors measured a reduction in contaminated fecal pellet density and sinking rate produced by *Calanus helgolandicus,* and utilized average ocean depth, estimating contaminated fecal pellets would take 53 days longer than control pellets to reach the benthos (Cole et al., 2016). Using our measured 56.45% reduction in fecal volumes, calculated 1.76-fold slower fecal sinking rates, and average ocean depth (Charette and Smith, 2010), we estimate that total carbon settling via contaminated copepod fecal pellets would slow 4.03-fold. Our modeled estimates of the effects of microplastic consumption by copepods on carbon settling are conservative given that we used a single estimate of fecal density for both control and microplastic sinking models even though other studies have demonstrated that contaminated copepod fecal pellets had lower density (Cole et al., 2016), which would further slow carbon settling.

From the present study, as well as previous studies (Cole et al., 2016; Coppock et al., 2019; Rodríguez-Torres et al., 2020; Svetlichny et al., 2021; Wieczorek et al., 2019; Yu et al., 2020) there is substantial evidence for effects of microplastics on copepod and salp fecal pellet properties. Previous work by Coppock et al. (2019) found that, depending on the type of microplastic ingested, fecal pellet volume and fecal sinking rates produced by the copepod *Calanus helgolandicus* were affected by the ingestion of microplastics, however these results were not extended to understand the potential impacts of microplastics on carbon settling. Rodríguez-Torres et al. (2020) found that microplastics did not affect fecal sinking rates, however, there was a decrease in fecal pellet production rate by arctic *Calanus* copepods. In another copepod,

Tigriopus japonicus, microplastic exposure was found to cause a decrease in fecal pellet production rate (Yu et al., 2020), which could also reduce the rate of carbon settling. In contrast to the above studies, recent work by Svetlichny et al. (2021) found no difference in the rate of fecal pellet production rate by copepod *Acartia clausi* and found a slight increase in microplastic-contaminated fecal pellet size compared to the control (Svetlichny et al., 2021). Another study found that fecal pellets of the rotifer *Brachionus plicatilis* were strip shaped only in the presence of microplastic treatments (Sun et al., 2019), which could affect fecal pellet sinking rates differently than calculated in the presented study. The critical difference between these studies and ours is that we exposed *A. tonsa* to microplastics from the egg stage to adulthood and measured fecal pellets over a period of 2 days as adults. The studies described above (Cole et al., 2016; Coppock et al., 2019; Rodríguez-Torres et al., 2020; Svetlichny et al., 2021; Yu et al., 2020) exposed copepods only at the onset of the adult stage. The life-long exposure in the present study could have resulted in more severe negative consequences, such as accumulation of microplastics in the gut compromising fecal pellet size and integrity, than what was seen in previous studies. It is also important to highlight that the incorporation of microplastics in fecal pellets egested by *Salpa fusiformis* reduced density, volume, and size, thus reducing sinking speeds by 1.35-fold and 1.47-fold with the incorporation of polyethylene and polystyrene microplastics respectively (Wieczorek et al., 2019). However, salp fecal pellets are spherical, which greatly differ from ellipsoid copepod fecal pellets, which can explain the differences in fecal sinking rates calculated in the present study.

Microplastics were observed in both the anterior and posterior portions of adult *A. tonsa* intestinal tracts, confirming both ingestion and passage through the body, which is consistent with the findings of previous copepod studies (Cole et al., 2016; Coppock et al., 2019; Lee et al., 2013; Rodríguez-Torres et al., 2020; Svetlichny et al., 2021; Yu et al., 2020). Consumption of microplastics by zooplankton introduces these particles into the base of the marine food system (Fig. 6), creating numerous vector possibilities to other marine organisms (Fig. 6F and H), which holds the potential to affect higher trophic level organisms (Setälä et al., 2018). A reduction in microplastic laden fecal pellets sinking rates (Fig. 6D) also allows for the potential reuptake rate of these pellets by other marine organisms leading to bioaccumulation in the food web. Biomagnification of microplastics has been detected in numerous fish species (Arias et al., 2019; Boerger et al., 2010; Rochman et al., 2015; Wang et al., 2020), some of which are found in the mesopelagic zone, where fecal pellets are a significant portion of their diet (le Mézo and Galbraith, 2021).

The microplastic concentration equal to the algal concentration of 500 μg C L^{-1} used in this study is higher than most microplastic concentrations estimated in nature (Díaz-Mendoza et al., 2020; Hantoro et al., 2019). However, microplastic concentrations comparable to that used in this study have been detected in nature, reflecting a hotspot of microplastics due to anthropogenic coastal wastewater and ocean current conveyance (Kang et al., 2015). Although the concentrations used in this study may be reflective of microplastic hotspots, the spatial distributions of marine organisms, habitats, nutrients and resources are similarly patchy, thus it is important to consider the impacts of microplastic consumption at local, potential hotspot scales rather than average ocean concentrations. In addition, there is uncertainty in quantifying oceanic plastic debris because, as larger macroplastic pieces break down into microplastics, plastic particles increase, challenging the accurate estimation of concentrations in nature (Andrady, 2011). Common microplastic quantification techniques use surface collection nets too large to assay particles smaller than 335 μm (Thompson et al., 2004) and missing microplastics in mid-water and benthic zones, leading to the underestimation of true zooplankton encounter rates with microplastics (Andrady, 2011). Indeed, using a new autofluorescence method for detecting microplastics, a recent study suggests that average plastic concentration is 5–7 orders of magnitude higher in surface water environments than previously estimated (Brandon et al., 2020), indicating that improved technologies yield more accurate and higher estimations of contaminant concentrations. Taken together, these studies justify the use of high microplastic concentrations for experimental work.

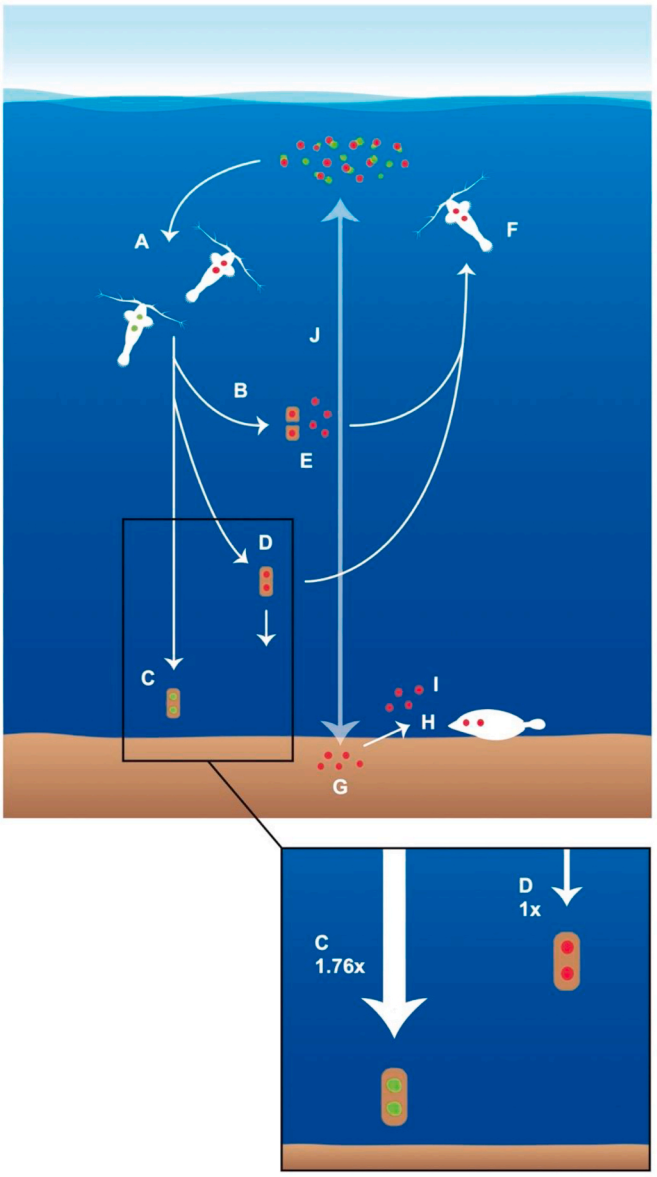

Fig. 6. Schematic of potential transport vectors of low-density microplastics via zooplankton in the marine water column. (A) Zooplankton ingest microplastic particles (red dots), either by co-ingestion with algae (green particles) or misidentification of microplastics for prey; (B) zooplankton egest these microplastics in their fecal pellets; (C) fecal pellets containing natural algal prey are more dense, and sink more quickly, denoted by the numbers shown; (D) fecal pellets contaminated with low-density microplastics will sink significantly slower; (E) fecal pellets containing microplastics are more prone to fragmentation due to the lack of dense organic material, releasing microplastic particles into the water column; (F) zooplankton, in diel vertical migration, may ingest free-floating microplastics or consume contaminated fecal pellets, thus returning the microplastic particles to the surface; (G) benthic sedimentation of microplastics; (H) consumption of microplastics by benthic organisms such as fish; (I) microplastics stirred up by upwelling, ocean currents, or scavenging organisms; (J) sinking of microplastic particles due to gravity or returned to the surface via oceanic flux. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusions and future directions

Our results demonstrate that microplastic exposure can affect the marine environment at the organismal level through reduced nauplii body length, multi-stage survival, and egg diameters. We further highlight that exposure to microplastics at an early life-stage, in combination with reduced survival and fecundity, could result in reduced net reproductive rate and ultimately lower population abundance, stressing the importance of studying fitness consequences at all life history stages. Additionally, we show that copepods exposed to microplastics produce less total fecal matter and that contaminated fecal pellets have smaller volumes and slower sinking rates. Through modeling, we show that microplastics hold the potential to reduce the efficiency of oceanic carbon settling which, in conjunction with current anthropogenic carbon production, could have major implications for oceanic carbon storage globally.

Credit author statement

Emily A. Shore: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Visualization. James A. deMayo: Formal analysis, Visualization writing – Review and Editing. Melissa H. Pespeni: Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. E.A.S designed all experiments, conducted most data analysis, and wrote the manuscript with M.H.P. Net reproduction rate analysis and discussion was conducted by J.A.D.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117379.

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