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Impacts of Microplastics on the Swimming Behavior of the Copepod *Temora turbinata* (Dana, 1849)

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Abstract: Zooplankton are prone to the ingestion of microplastics by mistaking them for prey. However, there is a lack of knowledge about the impacts of microplastic availability on zooplankton behavior. In this study, we investigated the effects of polystyrene microbeads on swimming patterns of the calanoid copepod *Temora turbinata* under laboratory conditions. We acquired high-resolution video sequences using an optical system containing a telecentric lens and a digital camera with an acquisition rate of 20 frames per second. We estimated the mean speed, NGDR (Net-to-Gross Displacement Ratio, a dimensionless single-valued measure of straightness) and turning angle to describe the swimming behavior in three different treatments (control, low and high concentration of microplastics). Our results revealed that swimming speeds decreased up to 40% (instantaneous speed) compared to controls. The NGDR and turning angle distribution of the organisms also changed in the presence of polystyrene microbeads, both at low (100 beads mL^{-1}) and high microplastic concentration (1000 beads mL^{-1}). These results suggest that the swimming behavior of *Temora turbinata* is affected by microbeads.

Keywords: microplastics; zooplankton; swimming behavior; imaging; *Temora turbinata*

1. Introduction

Microplastic pollution is now a global concern. Disposal and fragmentation of a wide variety of polymers, followed by their dispersion within large-scale circulation systems, have spread microplastics across the oceans, even to the most remote locations [1–3]. Microplastics (MPs) are particles smaller than 5 mm and can be classified as primary or secondary depending on their origin [4]. Primary MPs include fibers [5], pellets [6] and microspheres from cosmetics and other applications [7]. Secondary MPs are the result of the fragmentation of large plastic objects by a myriad of processes such as UV radiation, mechanical abrasion, and biological degradation by microorganisms [8–10].

Primary and secondary MPs have been recorded in the digestive tract of several marine organisms, including fish [11], annelids [12] and mollusks [13]. Neurological problems [14], hormonal impairment [15], false sensation of satiation, loss of body mass [16] and even death [17] are among the negative impacts of MPs reported to date in marine animals. Because of their size, marine zooplankton have been described as potential MP consumers [8,18]. Copepods are dominant organisms in the marine zooplankton, constituting an average of 80% of the mesozooplankton abundance and representing an efficient link in energy transfer as primary consumers [19]. Copepods participate in essential ecological processes including the biological pump, the microbial food web and nutrient recycling [20,21] and respond quickly to changes in the marine environment such as the introduction of contaminants and man-induced variations in pH, salinity and temperature [22,23]. Although at large spatial scales zooplankton displacement in the water column is strongly affected by currents, their individual swimming behavior is a key factor in achieving high fitness and survival as it controls prey detection, predator avoidance and mating [24].

In the early days of zooplankton feeding studies, MP beads were frequently used as tracers of phytoplankton-sized food to investigate copepod ingestion rates [25] but exposure of copepods to MPs was not treated as a matter of concern in terms of pollution. In this study, we asked the question whether copepod motility would be impaired by the presence of MPs. Changes in the swimming behavior of the calanoid copepod *T. turbinata* were analyzed using high resolution imaging techniques under the influence of varying concentrations of primary MPs.

2. Materials and Methods

2.1. Sampling, Sorting and Exposure of Copepods to MPs

Zooplankton samples were collected in December 2018 from Flamengo inlet, Ubatuba, Brazil (23°31′23″ S, 45°06′13″ W) by means of short oblique tows with a 200-µm mesh-sized plankton net. Animals were transferred to an insulated container and immediately transported to the laboratory, where they were kept in a temperature-controlled room set to match the seawater temperature at the time of sampling (25 °C). Zooplankton organisms were fed for approximately two hours with a saturated concentration of *Isochrysis galbana* under low-intensity aeration. Adult females of the calanoid copepod *Temora turbinata* (Dana, 1849), a dominant species in coastal subtropical waters of the Southwest Atlantic [26], were carefully sorted and 45 healthy individuals were kept in separate 250-mL containers filled with filtered seawater (Whatman® GF/F) for 2 h to acclimatize. Subsequently, 15 females were transferred to each of three 500-mL Nalgene® polycarbonate bottles containing 2×10^4 cells mL^{-1} of *Isochrysis galbana*, set as control (no microspheres) and MP treatments with low (100 mL^{-1}) and high (1000 mL^{-1}) concentrations of surfactant-free polystyrene (PS) latex microspheres (20 µm diameter; Beckman Coulter Inc.). The bottles were sealed with Parafilm M® to avoid bubble formation and transferred to a plankton wheel (1 rpm) for 24 h, at 25 °C. After incubation, 5 individual females belonging to either control or microsphere treatments were retrieved at random from each bottle, inspected for apparent morphological integrity (i.e., only intact and undamaged animals were used), and transferred to a cubic glass vessel containing 500 mL of filtered seawater, where they remained for 15 min to acclimatize before filming with a digital camera system (see below). This time interval was deemed sufficient for complete gut evacuation under the incubation temperature [27]. The sampling and experimental procedures were repeated five times, during four consecutive days.

2.2. Acquisition of Trajectory Data and Analysis of Swimming Behavior

Copepod swimming behavior was recorded in the 5-female batches using a 2D optical system setup consisting of (i) a light-emitting diode (LED) source (660 nm), (ii) a Telecentric lens (0.268X, C-Mount TitanTL, Edmund Optics), and (iii) a 9 MP Basler camera (acA4096-30um with a Sony IMX267 CMOS sensor), operated with an acquisition rate of 20 frames per second (fps). We used a 660 nm LED because most marine organisms, including copepods, are less sensitive to red light, as it is absorbed quickly in the water column [28,29]. Each video observation lasted for 15 min. Image acquisition, spatial coordinate extraction and tracking analysis were performed using software developed by the Laboratory of Planktonic Systems (LAPS/IOUSP). The experimental design is depicted in Figure 1.

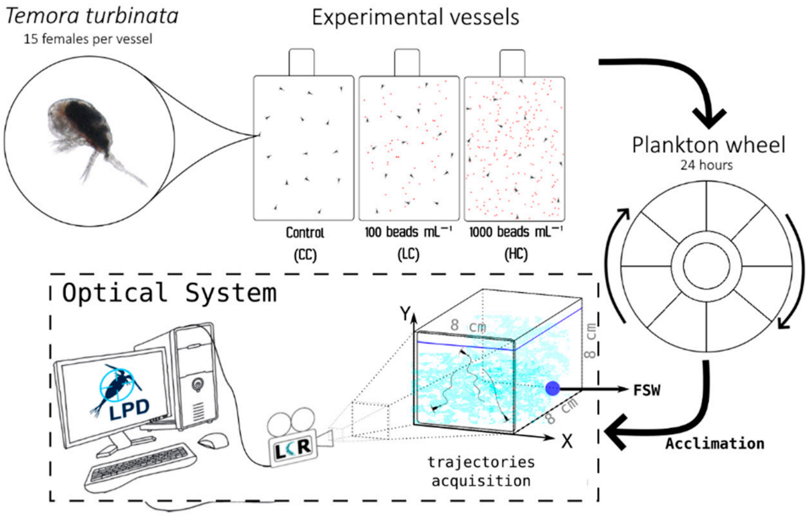


Figure 1. Schematic representation of the experimental setup. CC = Control (with *I. galbana* only); LC = low concentration (*I. galbana* plus 100 beads mL^{-1}); HC = high concentration (*I. galbana* plus 1000 beads mL^{-1}). FSW refers to filtered seawater. LCR (LAPS Camera Recorder) and LPD (LAPS Plankton Detector) are in-house software designed for image acquisition and trajectory extraction, respectively. Image frames were acquired as bidimensional projections (X, Y).

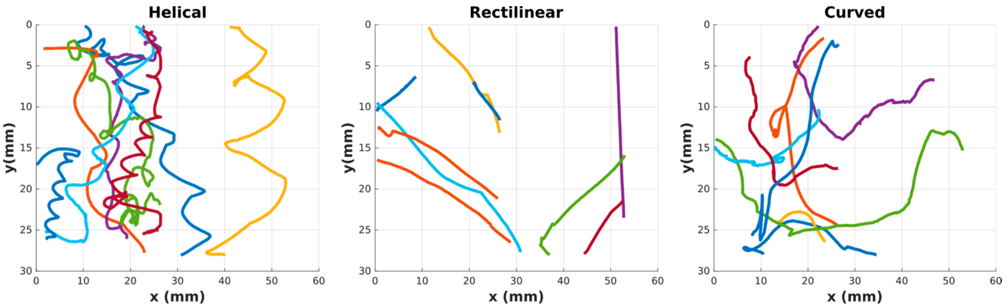
The trajectory extraction process consisted of four main phases: (i) detection of the regions of interest (ROIs) in each frame; (ii) identification of the organisms using a size and contrast range filter; (iii) calculation of the boundary region and centroid position (x, y) of each detected particle in the frame and (iv) generation of 2D trajectories from centroids, having as main guide the closest distance between them in consecutive frames. Additional ROI characteristics, such as area, rotation angle and a time tag, were also obtained.

Trajectories with fewer than 30 frames were discarded to compute behaviorally significant measurements, following Chen and Hwang [30]. We calculated the instantaneous speed, average speed, turning angle and net-to-gross displacement ratio (NGDR). Instantaneous speeds were estimated based on subsequent data points for each trajectory. Average speeds refer to mean speeds for each identified trajectory. The NGDR was calculated from the ratio of the shortest linear distance between start and end points to the total distance traveled. A maximum value of 1 indicates a completely straight path and a value close to 0 indicates a more complex, sinuous path [31]. In this study, we estimated NGDR from segments of fixed size (120 positions) of each track to minimize the effect of spatial scale. Individual turning angles were estimated for every change in movement direction performed by each copepod. Turning angles are reported here as means of individual turning angles after 120 subsequent positions (or image frames) in the bidimensional plane.

Significant differences between controls and treatments were investigated with the Kruskal-Wallis test followed by Dunn's post-hoc test on MATLAB® software R2017a. The significance level was set at $p < 0.05$. Outliers were assigned to data points greater than $q3 + w \times (q3 - q1)$ or less than $q1 - w \times (q3 - q1)$, where w is the maximum whisker length, and q1 and q3 are the 25th and 75th percentiles of the sample data, respectively.

3. Results

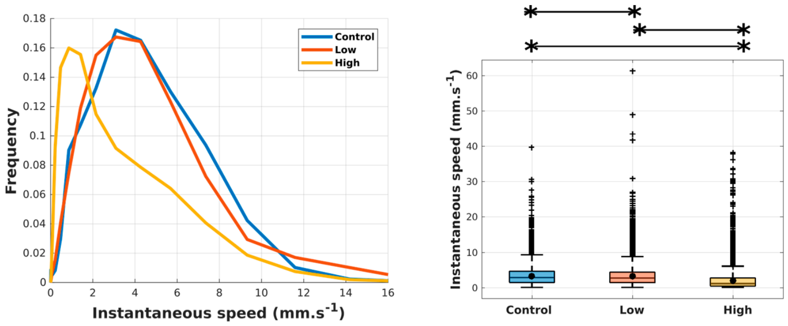
Fifteen videos were acquired containing 883 trajectories, which were visually identified as belonging to three basic types: (i) helical trajectories, when organisms moved forward around an imaginary axis; (ii) rectilinear trajectories, i.e., comparable to a near-straight line; and (iii) curved trajectories, showing intermediate features between the former types (Figure 2). Trajectories with more than 30 points (valid trajectories) represented 74% of the total. No copepod mortality was observed during the experiments.



(**a**) (**b**) (**c**)

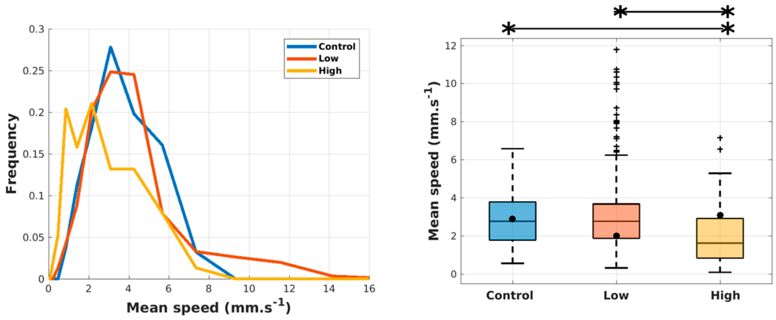
Figure 2. Examples of trajectory types observed in *Temora turbinata* under experimental conditions, including controls and treatments: (**a**) helical-like (n = 158), (**b**) rectilinear (n = 13) and (**c**) curved (n = 482).

The instantaneous swimming speeds (Figure 3) were significantly lower in copepods exposed to plastic microbeads compared to the control, the same trend being observed for mean swimming speeds (Figure 4). The effect of the high MP concentration on the swimming performance of *T. turbinata* was more clearly discerned in the mean speeds (Figure 4; Table 1).



(**a**) (**b**)

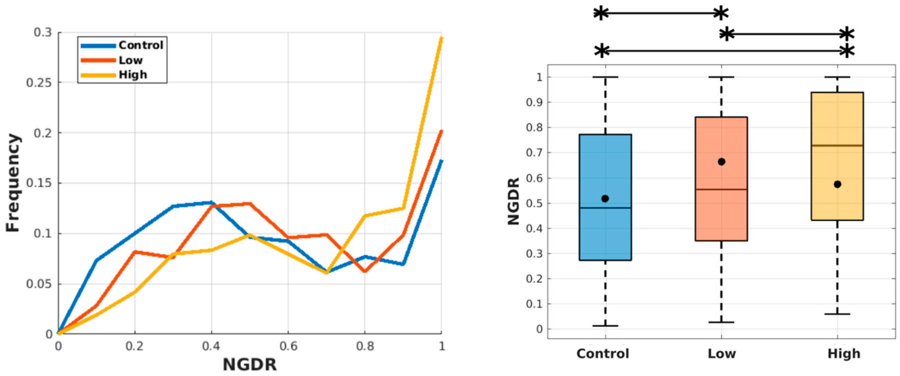
Figure 3. Instantaneous swimming speeds of *T. turbinata* in the control group (with *I. galbana* only) and under MP addition at 100 (Low) and 1000 beads mL^{-1} (High). MP treatments contained *I. galbana* at the same concentration as in the control group. Copepods were observed after being transferred to filtered seawater. (**a**): frequency distribution. (**b**): mean instantaneous speeds, lower and upper limits, and outliers. Horizontal bars on the top of the right panel represent statistical differences between groups.



(**a**) (**b**)

Figure 4. Trajectory-based mean swimming speeds of *T. turbinata* in the control group (with *I. galbana* only) and under MP addition at 100 (Low) and 1000 beads mL^{-1} (High). MP treatments contained *I. galbana* at the same concentration as in the control group. Copepods were observed after being transferred to filtered seawater. (**a**): frequency distribution. (**b**): trajectory mean speeds, lower and upper limits, and outliers. Horizontal bars on the top of the right panel represent statistical differences between groups.

The NGDR also changed under the influence of microbeads, with a decreasing trend of sinuosity in the short-term paths (120 steps) as MP concentration increased (Figure 5). In addition, NGDR segments denoting near-rectilinear steps (i.e., NGDR ~ 1) were more frequent with the increase in microbead concentration.



(**a**) (**b**)

Figure 5. NGDR of *T. turbinata* in the control group (with *I. galbana* only) and under MP addition at 100 (Low) and 1000 beads mL^{-1} (High). MP treatments contained *I. galbana* at the same concentration as in the control group. Copepods were observed after being transferred to filtered seawater. (**a**): frequency distribution. (**b**): mean NGDR, and lower and upper limits. Horizontal bars on the top of the right panel represent statistical differences between groups.

Turning angles displayed by the *T. turbinata* control group had a normal distribution (μ = 19.33, σ = 12.02), whereas for the LC group a bias existed towards smaller turning angles (Table 1). A more irregular turning angle distribution was noticed for the HC group, which translated into a higher standard deviation compared to the control and the LC treatment (Figure 6; Table 1).

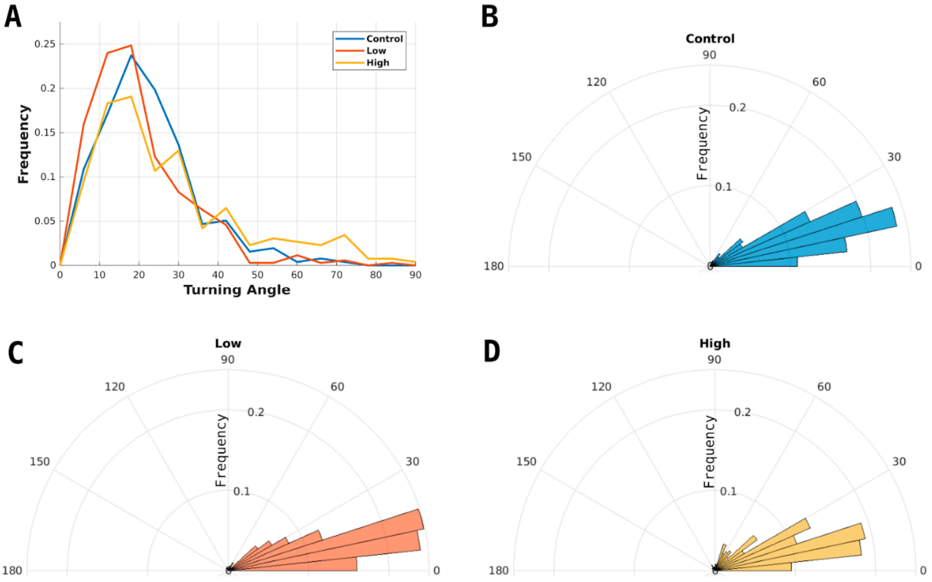


Figure 6. Frequency distribution of the turning angles (in degrees) of *T. turbinata*. (**A**) comparative histogram for the control and treatments, (**B**–**D**) turning angle frequencies for the control, low and high MP concentrations, respectively. The experimental conditions were the same as indicated in Figures 3–5.

The metrics analyzed here to describe the swimming patterns of *T. turbinata* in the absence and in the presence of MPs are presented in Table 1 as means and standard deviations. Significant differences are depicted by comparing each treatment to the control.

Table 1. Effects of microplastics in the swimming activity of *T. turbinata*. CC = Control; LC = Low Concentration (100 beads mL^{-1}) and HC = High Concentration (1000 beads mL^{-1}). Results are presented as means ± standard deviations; significant differences are indicated in bold (Kruskal Wallis test followed by Dunn's post-hoc test, $p < 0.05$).

Treatment	Mean Speed (mm s^{-1})	Mean Instantaneous Speed (mm s^{-1})	NGDR	Turning Angle (°)
CC (n = 187)	2.88 ± 1.35	3.23 ± 2.34	0.52 ± 0.3	19.33 ± 12.05
LC (n = 309)	3.09 ± 1.92	**3.19 ± 2.55**	**0.57 ± 0.28**	**17.69 ± 15.42**
HC (n = 157)	**1.99 ± 1.41**	**1.94 ± 2.29**	**0.66 ± 0.28**	27.22 ± 24.27

4. Discussion

Swimming is crucial to find prey, encounter mates and avoid predators, affecting zooplankton survival and fitness [32]. However, the impact of MPs on zooplankton swimming behavior is still largely unknown. Here, we found that MPs modify basic patterns of copepod swimming. Our results are consistent with previous findings that MPs cause a decrease in swimming speed of *Artemia* nauplii, *Daphnia* and barnacle larvae at certain concentrations [33–35]. We also observed a reduction in trajectory complexity, as NGDR increased under both MP levels.

Microbeads were often used to mimic phytoplankton cells in grazing experiments and their ingestion by copepods has been recorded [36–40]. Polystyrene spheres are not necessarily ingested by copepods when offered alone, while phytoplankton cells are consumed at rates up to 3 times higher than non-living items when both are available, denoting a clear particle selectivity pattern [41]. However, algal ingestion by copepods decreases when high MP concentrations are offered in combination with natural food [42]. For instance, Ayukai [43] reported that phytoplankton ingestion by the copepod *Acartia clausi* decreased in the presence of MPs of similar size (15.7 µm) and concentration (1140 beads mL^{-1}) used in the present study. The food offered to *T. turbinata* in our experiments (*I. galbana*) had a size spectrum (5–7 µm) within the capture range previously reported for this copepod genus [44] and algal concentration was kept constant in the different MP treatments and control. Thus, it is unlikely that behavioral differences observed in our data resulted from varying levels of "satiation" associated with MP consumption at different concentrations. This is reinforced by the fact that copepod trajectories were recorded for control and MP treatments while animals were swimming in filtered seawater, after full gut evacuation, minimizing potential bias between starved and fed individuals.

MP ingestion has been shown to cause enzyme (cholinesterase and catalase) impairment in microcrustaceans, affecting the cholinergic system and causing oxidative stress [34]. Energy deficit, reduced growth, and low fertility rates have also been reported as negative impacts of MP consumption [45,46]. In addition, MPs may accumulate on the outer surface of copepods and become entangled around the antennae, swimming legs, and feeding appendages [42], probably affecting swimming performance and other functions. Although no observation of either metabolic damage or particle trapping on copepod surfaces is available from our experiments, the reduction in swimming speed and the high NGDRs and turning angles observed after MP exposure may be interpreted as evidence of a direct impact from plastic microbeads. Also, under "normal" experimental conditions (i.e., without MP exposure) *T. turbinata* increases its average swimming speed in the presence of food [47], but we found an opposite trend for copepods exposed to microbeads.

The MP concentrations (100 and 1000 beads mL^{-1}) applied in our experiments were similar or lower than those utilized in previous investigations of MP consumption by copepods [42,43,45]. The extent to which such concentrations are realistic for the natural environment, even under a MP accumulation scenario in the oceans, is a matter of debate because so far most marine studies have targeted MP particles larger than 300 μm [42], although evidence exists that fibrous polymers in the range of ~20 μm are present in coastal sediments [6]. Sampling with an 80-μm mesh-sized plankton net yielded a peak concentration of a mere 0.1 MPs mL^{-1} in the west coast of Sweden [48], but such estimate is probably related to particles larger than 100 μm or more, because of mesh size selectivity [49]. Considering the sampling constraints and the fact that plankton abundance in the size range of 80–100 μm is typically three to four orders of magnitude lower than in the 15–20 μm size range [50], a concentration of 100–1000 mL^{-1} for 20-μm sized spherical MPs would not be surprising for a coastal environment, particularly near densely populated urban areas. Interestingly, a recent study found that MPs smaller than 25 μm accounted for about 70% of the total number of airborne, plastic-derived particles settled in pristine continental areas of the United States [51]. Thus, in case atmospheric transport is shown to represent a relevant MP input to the oceans, it is likely that small-sized particles will account for a major proportion of the total plastic load in surface waters.

As they move up and down the food web, MPs potentially affect diverse ecosystem processes [52,53], both locally and remotely. For instance, Cole and Galloway [45] observed that fecal pellets of the copepod *Calanus helgolandicus* exposed to 20 μm MPs (1000 beads mL^{-1}) had a 2.25-fold reduction in their sinking rate, which translated to an increase of 53 days for pellets to reach the seafloor, considering the average depth of the ocean [54]. In addition, the relative energy cost of locomotion increases as the size of the organism decreases [55], meaning that for small zooplankton such as copepods, changes in swimming behavior due to MP influence likely affect vertical migration and prey-predator interactions, modifying the amount of energy available for the different trophic levels [38,56]. Such potential impact of MPs on copepods may thus cause large-scale alterations in the carbon flow in marine environments. Therefore, it is imperative that the impacts of MP on copepod behavior are elucidated and considered in trophic models and biogeochemical studies in the future.

5. Conclusions

This study shows that the availability of polystyrene microspheres modified the swimming performance of the pelagic copepod *T. turbinata* at both low and high MP concentrations. Changes in copepod swimming behavior as a response to the presence of microplastics may elicit individual-based effects leading to potential impacts on several ecological traits, including bottom-up transference of MP particles to higher trophic levels.

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Conflicts of Interest: The authors declare no conflict of interest.

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