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Microplastic consumption and physiological response in *Acartia clausi* and *Centropages typicus*: Possible roles of feeding mechanisms

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ABSTRACT

Multi-day experiments were carried out with two Marmara Sea calanoid copepod species: *Acartia clausi* and *Centropages typicus*, to assess the possible role of the type of feeding on the consumption of microplastics and its influence on the rate of energy metabolism of these species. In a mixture of microplastic beads (6 μm diameter) and algae *Rhodomonas salina* (5–10 μm size range) with equal concentrations of about 5000 cells/beads mL $^{-1}$ the ambush feeder *A. clausi* consumed almost 5 times less microplastic 858.8 ± 294.1 beads ind^{-1} day^{-1} than the cruising feeder *C. typicus* and halved its consumption of microplastics alone, while *C. typicus*, on the contrary, increased its consumption rate of pure microplastics to 20237.4 ± 7020.41 beads ind^{-1} day^{-1}. Both types of reaction to microplastics lead to a decrease in the respiratory rates of the copepods. During the 5 days of maintenance on a solely microplastic diet, the respiration rates of *A. clausi* and *C. typicus* decreased 2.2 and 3.4 times, respectively, due to a decrease in the energy spent on motor activity, whilst maintaining basal metabolic energy. It has been shown that in *A. clausi*, consuming microplastics, a decrease in respiration rate occurs in the same way as in individuals starving in filtered water. A more rapid respiration rate decrease in *Centropages typicus* consuming microplastics may be due to the greater energy expenditure on microplastic beads capture and egestion via fecal pellets. *Acartia clausi* seems to exhibit a better strategy in dealing with the adverse consequences of microplastics consumption in comparison to *Centropages typicus*.

1. Introduction

Microplastics are tiny plastic particles ranging from 20–5000 μm (Hanke et al., 2013) or 1–1000 μm (Hartmann et al., 2019), which are widely distributed in the seas and oceans and that result from both the development of a commercial product and the destruction of larger plastics in the natural environment. The lower size range (approximately <50 μm) corresponds to the size of the algae that the copepods usually feed on (Helenius and Saiz, 2017). Marine copepods can potentially capture any particle, both organic (Paffenhöfer and Strickland, 1970; Poulet, 1974, 1983) and non-organic matter (Donaghay and Small, 1979), which are of suitable size. This is probably the reason why many copepod species have been reported to consume microplastic particles (Huntley et al., 1983; Paffenhöfer and Van Sant, 1985; Ayukai, 1987; Cole et al., 2013, 2015, 2016; Lee et al., 2013; Wright et al., 2013; Desforges et al., 2015; Ogonowski et al., 2016; Frydkjær et al., 2017; Scherer et al., 2017; Vroom et al., 2017; Gorokhova et al., 2018; Botterell et al., 2019; Coppock et al., 2019).

Acartia clausi and *Centropages typicus* usually classify as phytophagous, but in fact can actually be omnivores, consuming both ciliates and algae (see Wiadnyana and Rassoulzadegan, 1989). However, within a close range of food objects, they differ in the mechanisms of search and capture of food. *Acartia clausi* is an ambush feeder capturing food items during a 'surprise' ambush type jump, while *Centropages typicus* combines the methods adopted by cruising feeders or current feeders to consume food particles from the uniform flow of water created by the cephalic appendages (Paffenhöfer et al., 1982). *Acartia clausi* has demonstrated complex grazing behavior which includes the ability to optimize capturing food particles whilst avoiding non-food particles and to reject food post-capture (Donaghay and Small, 1979). Food selection patterns of *C. typicus* have been seldom

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studied. Calbet et al. (2007) established the ability of this species to actively select food objects. However, Cole et al. (2013) in 24-hour experiments showed that *Centropages typicus* could not differentiate between algal cells and 7.3 μm microplastic beads. An increase in the concentration of microplastics in a mixture with microalgae of a similar size led to a decrease in the rate of microalgae consumption. This relationship reached saturation at concentrations of >5000 beads mL $^{-1}$.

In the present study, the possible differences in the consumption rate of microplastics beads (of 6 μm diameter at a concentration of 5000 beads mL $^{-1}$) alone or in a mixture with an equal amount of algae *Rhodomonas salina* (5–10 μm size range) and its effect on the total and basal levels of energy metabolism of copepods *Acartia clausi* and *Centropages typicus* were investigated in multi-day experiments. We hypothesize that differences in the feeding strategies of these two species should cause altered behavioral and physiological responses to microplastics.

2. Materials and methods

2.1. Copepod sampling

Zooplankton samples were collected during the first half of April 2019 with a closing Nansen net (opening diameter: 50 cm, mesh size: 200 μm) by oblique hauls from the bottom (about 50 m depth) to the surface at a station located (40°57,52N–28°57,55E) in the Marmara Sea (salinity 18–20 psu, temperature 10–15 °C) near the Bosphorus Strait. Two hours after sampling, healthy adult females of *Acartia clausi* and *Centropages typicus* were individually sorted at the laboratory, from the diluted subsamples using a wide pipette. Selected copepods were placed in 1liter volume vessels containing filtered sea water (20 psu salinity and 17 °C water temperature) and fed on the cryptomonad algae *Rhodomonas salina* sp. at a concentration of approx. 5000 cells ml^{-1} for preliminary acclimation to the experimental conditions.

2.2. Mono-algal culture

The original strain of the cryptomonad algae *Rhodomonas salina* (5-10 μm size range) was provided by the Culture Collection of Algae and Protozoa (CCAP), Scotland, UK. Initial microalgae cultures were inoculated in test tubes (30 ml) containing f/2+Si medium previously sterilized at 121 °C for 15 min. All sub-cultures were maintained at 23 °C at a salinity of 32 psu under a 12L:12D photoperiod. The *R. salina* culture volumes were continuously up-scaled from 30-ml test tubes to 250-ml Erlenmeyer flasks, followed by 1-L, 5-L, and 30-L culture containers in photobioreactors, continuously. Illumination was maintained at 200 μmol/m^2/s at the culture surface. Population growth was determined daily by cell counting using Neubauer chambers under Leica DM100 microscope.

2.3. Microplastics emulsion

Polystrene microplastics (Fluoro-Max Red Dry Fluorescent Particles) of 6 μm (Cat No 36-2) were purchased from Thermo ScientificTM. Emulsion of microplastic beads and sea water (22 psu) filtered through a 0.2 μm filter was prepared at a concentration of approx. 1 million beads ml^{-1} using ultrasonic crushing.

2.4. Experimental design

2.4.1. Feeding experiment

To determine the rate of microplastic consumption, selected 10 females of *Acartia clausi* (prosome length of 1.01 ± 0.02 mm) and 5 females of *Centropages typicus* females (prosome length of 1.15 ± 0.024 mm) in prime condition were individually placed in 10 ml cups with four replicates for further experiments.

Four multi-day (8 days) feeding (FEED) treatments with adult *Acartia clausi* were performed in experiments at 20 °C set up as follows:

(**FEEDAI**) control with an exclusive diet of microalgae *Rhodomonas salina* (∼5000 cells ml^{-1});

(**FEEDAII**) a mixture of *Rhodomonas salina* (∼5000 cells ml^{-1}) and microplastic beads (6 μm) at a concentration of ∼5000 beads ml^{-1};

(**FEEDAIII**) exclusive diet of microplastic beads (6 μm) at a concentration of ∼5000 beads ml^{-1};

(**FEEDAIV**) incubation in clean 0.45 μm filtered sea water without algae and microplastics.

Three multi-day (5 days) feeding treatments with adult *Centropages typicus* were performed in experiments at 20 °C set up as follows:

(**FEEDCI**) control with an exclusive diet of microalgae *Rhodomonas salina* (∼5000 cells ml^{-1});

(**FEEDCII**) a mixture of Rhodomonas salina (∼5000 cells ml^{-1}) and microplastic beads (6 μm) at a concentration of ∼5000 beads ml^{-1};

(**FEEDCIII**) exclusive diet of microplastic beads (6 μm) at a concentration of ∼5000 beads ml^{-1};

At the end of each day of experiments with *A. clausi* and *C. typicus*, the following were performed: counting the number of dead and living individuals, the number of fecal pellets they excreted, analyzing the contents of the pellets, updating the concentration of microplastics, algae or their mixture, and replacing seawater with clean aerated water. The concentration of microalgae *Rhodomonas salina* (∼5000 cells ml^{-1}) was chosen as typical for many copepod feeding experiments (Meyer et al., 2002; Carotenuto et al., 2012; Helenius et al., 2019), while the same concentration of microplastics (∼5000 beads ml^{-1}) corresponded to the maximum in the range of particle concentrations of 7.3 μm, affecting the copepod species *Acartia clausi* and *Centropages typicus* in experiments by Cole et al. (2013).

To maintain the particles of microalgae and microplastics in suspension, the water in 10 ml cells was periodically purged with air using a microcapillary pipette. Stirring of the suspension was also facilitated by the fact that copepods often sank to the bottom of the vessel and fed there, shaking the settling particles with frequent movements of the limbs. However, it can be assumed that the settling of particles and copepods led to their consumption of a more concentrated suspension.

The microplastics consumption rate was calculated in accordance with female's daily excretion of fecal pellets and the amount of microplastic beads they contained, as described in (İşinibilir Okyar et al., 2020). The number and size of pellets were counted under a dissection microscope (Zeiss Opton) at magnification of x16, and microplastic content in pellets were determined under a microscope with an increased magnification of x60-150.

2.4.2. Respiration experiments with active and anesthetized copepods

For all type of respiration rate experiments (RESP), 200 individuals of *Acartia clausi and* 100 individuals *of Centropages typicus* adult females, respectively, were constantly kept in several 1 liter vessels at a salinity of 20 psu and 20 °C temperature

under experimental treatments as described below. As the number of individuals gradually decreased, the animals were taken sequentially from the first to the last vessel.

Acartia clausi females were incubated for 6 days in seawater with:

(**RESPAI**) *Rhodomonas salina* only (~5000 cells ml^{-1}), measurements were carried on Day 1 (8 and 4 replicates for respiration rate of active (RAC) and anesthetized (RAN) individuals) and Day 5 (7 and 4 replicates for RAC and RAN, respectively) of the experiment;

(**RESPAII**) microplastics beads of 6 μm diameter (~5000 beads ml^{-1}), measurements were carried out on Day 3 (9 and 6 replicates for RAC and RAN, respectively), Day 5 (on 4 replicates for both RAC and RAN) and Day 6 (on 5 replicates for both RAC and RAN) of the experiment;

(**RESPAIII**) filtered water alone (starvation), measurements were carried out on Day 3 (9 and 5 replicates for RAC and RAN, respectively) and Day 5 (12 and 4 replicates for RAC and RAN, respectively) of the experiment;

Centropages typicus females were incubated for 5 days in seawater with:

(**RESPCI**) *R. salina* only (~5000 cells ml^{-1}), measurements were carried out on Day 1 (8 and 5 replicates for RAC and RAN, respectively) and Day 5 (7 and 3 replicates for RAC and RAN, respectively) of the experiment;

(**RESPCII**) Microplastic beads of 6 μm diameter (~5000 beads ml^{-1}), measurements were carried out on Day 1 (on 3 replicates for both RAC and RAN), Day 2 (on 3 replicates for both RAC and RAN) and Day 5 (8 and 4 for RAC and RAN, respectively) of the experiment.

Respiration rate of copepods were determined using the closed, sealed chamber method, using glass experimental and control syringes of 2.0 ml filled up with filtered sea water to 1 ml. The most active females *of Acartia clausi* and *Centropages typicus*, respectively, were gently transferred by a pipette into respirometers supplied by a protective sieve disc (mesh size 100 μm) at the confluent outlet. In order to obtain identical initial oxygen and casual seston content, the control and experimental syringes were connected to a plastic tube with water gently pumped through back and forth several times. The syringes were then separated, closed by stoppers and placed into the chamber at a constant temperature of 20 °C. Incubation periods were about 1.5–2 and 3 h for active and anesthetized females, respectively. At the end of exposure, water samples from experimental or control syringes were transferred to the small measuring flow chamber with a variable volume (up to 0.3 ml) joined to the luminescent dissolved oxygen sensor Hach LDO™ in order to obtain the concentration of dissolved oxygen. Oxygen consumption rates in copepods were calculated by the difference between the final oxygen content in the experimental and control syringes and expressed as amount of oxygen consumed per body wet weight (μg O_2 mg^{-1} h^{-1}). The oxygen concentration in syringes with animals at the end of incubation was at least 80% of the oxygen content in control syringes and about 70% of dissolved oxygen at 100% saturation of pure sea water at 20 °C. Taking into account that in epiplanktonic copepods, even a decrease in oxygen concentration to 25% of saturation does not change the respiration rate in a short-term experiments (Svetlichny et al., 2012b), this made it possible with a margin to exclude the effect of a decrease in oxygen concentration on the physiological state of copepods in our experiments.

Body wet weights (WW_b, mg) of both species were calculated by the equation $WW_b = V_b\ \rho_b$, where V_b is body volume, $V_b = 0.47\ L_{tot}^{0.21} l_{pr}^{0.93} d_{pr}^{1.86}$, L_{tot} is total length, l_{pr} is prosome length and d_{pr} is prosome width (mm) (Svetlichny et al., 2012a) and ρ_b is body mass density equal to 1.05 g cm^{-3}. To determine basal respiration rates, the same individuals were immobilized using magnesium chloride (Svetlichny et al., 2010). For immobilization, water in the syringes containing active females at the end of treatment exposure was initially replaced by a solution of magnesium chloride of isosmotic salinity (20 psu), and following immobilization of copepods, the solution was replaced with oxygen-saturated sea water containing half the initial concentration of magnesium chloride. During incubation, syringes containing anesthetized individuals were rotated every 10 min to avoid development of O_2 gradients within the volume of water. After incubation, individuals were carefully transferred to fresh seawater followed by measurement of length and width parameters. Results of anesthesia experiments were analyzed only when the copepods did not awaken during incubation but restored their activity after being placed in clean water. Individuals which underwent anesthesia were not used in further experiments.

2.5. Survival, pellet production and microplastic consumption rates

At the end of each experimental day, before renewal of water and contents, numbers of dead individuals and amount of ejected pellets were determined in the chambers. Pellet sizes and numbers of microplastics beads were then determined under the microscope. Copepod mortality (m, %) was calculated as: $m = 100\ d\ /(d + s)$, where d is the total cumulative number of dead individuals and s is the number of survivors. The mean daily microplastic consumption rate was calculated in accordance with mean numbers of beads in fecal pellets and pellets per individual.

2.6. Statistical analysis

All data were tested for normality with the Shapiro–Wilk test and homogeneity of variances with Levene's test and treated using one-way ANOVA. Means were compared by the two-tailed Student's t-test ($p < 0.05$) using SPSS software (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA). Linear correlation was used to determine the relationship between proportion of the initial consumption rate and day, microplastic consumption rate and microplastic concentration in water. Correlation coefficient (R_2) and significance ($P < 0.05$) values were then calculated based on regression analysis. All values presented as percentages were arc cosine transformed before performing any statistical test. Values presented in the figures and tables are means ± standard deviations SD.

3. Results

3.1. Copepod mortality in feeding experiments

The mortality of *Acartia clausi* females contained in 10 ml cells fed with algae *Rhodomonas salina* or starved (treatments FEEDAI and FEEDAIV) equally increased from 5 ± 5.8% at the beginning of the experiment to 50 ± 24 and 48 ± 19%, respectively, by Day 8, whereas for individuals fed on a mixture of algae with microplastics or microplastics only (treatments FEEDAII and FEEDAIII), mortality significantly ($p < 0.05$) increased by Day 8 to 88 ± 9.6 and 94 ± 6%, respectively (Fig. 1A). In *Centropages typicus* contained in the algae *R. salina* (FEEDCI) with microplastics (FEEDCIII), 50% mortality occurred between Day 2 and 3 and on Day 4, respectively (Fig. 1B). All *C. typicus* individuals died on Day 5 of microplastics only maintenance (treatment FEEDCIII).

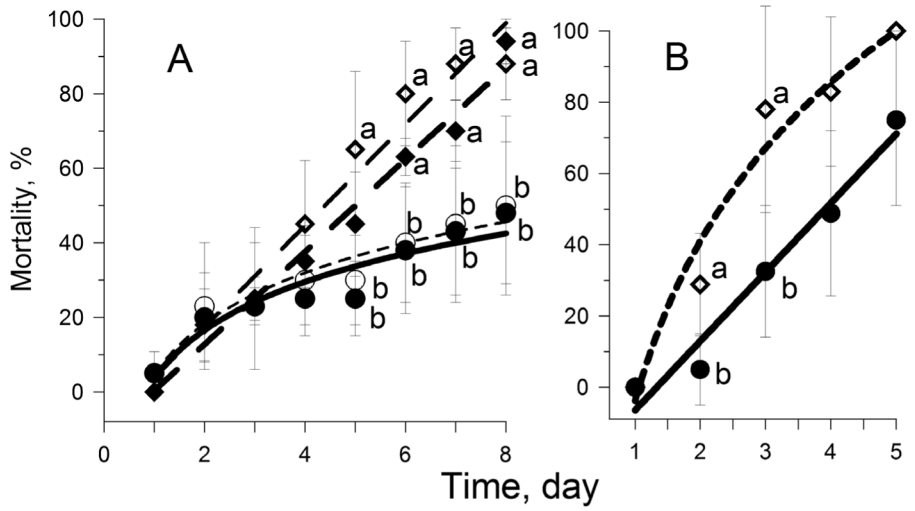

Fig. 1. Mortality of adult *Acartia clausi* (A) fed only on algae *Rhodomonas salina* (Treatment **FEEDAI**, ●, ——), a mixture of algae and microplastics (Treatment **FEEDAII**, ◊, — — —), pure microplastics (Treatment **FEEDAIII**, ♦, - - -) and during starvation (Treatment **FEEDAIV**, ○, - - - -), and adult *Centropages typicus* (B) during 5 day maintenance on algae *Rhodomonas salina* (Treatment **FEEDCI**, ●, ——), and pure microplastics (Treatment **FEEDCIII**, ◊, - - -). Low-case letters (a,b) are the significant variable differences from Duncan's Multiple Range test (DMRT), $P < 0.05$. Note, data for Treatment **FEEDCII** are not provided.

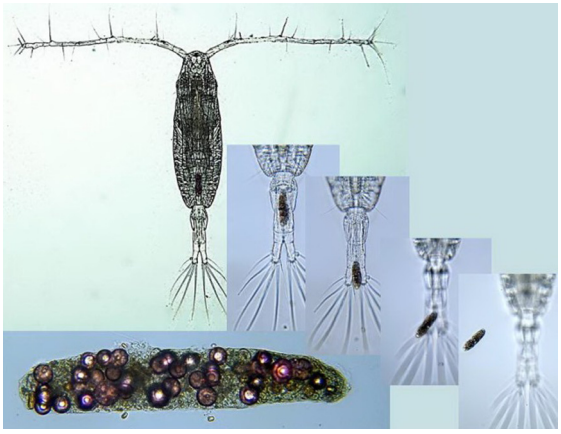

Fig. 2. *Acartia clausi* female. Production of fecal pellets containing microplastic beads. Close-up photo of adult female and excreted pellet containing 6 μm beads.

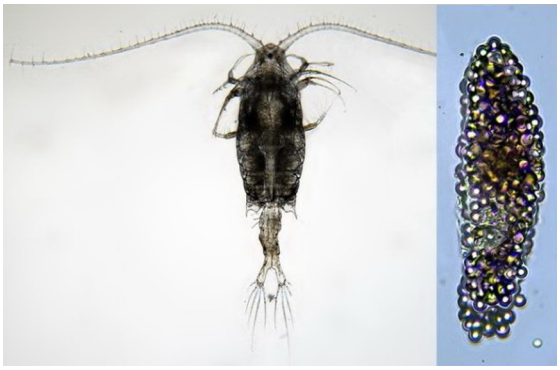

Fig. 3. *Centropages typicus* female and example of fecal pellet with microplastic beads of 6 μm.

3.2. Pellet size, production and microplastic consumption rate

Both *Acartia clausi* and *Centropages typicus* showed the ability to consume microplastics of 6 μm size, due to their presence in the fecal pellets (Figs. 2 and 3).

During our multi-day experiments (8 and 5 days for *Acartia clausi* and *Centropages typicus*, respectively), no time specific trends were found for pellet production rates, quantities of microplastic beads within pellets or microplastic bead consumption rates for all experiments (Fig. 4 which shows the results of microplastic consumption experiments only). However, significant interspecific variation and differences between treatment types were obvious.

Table 1 shows the cumulative average values of the studied parameters for 4 days of observation periods. As indicated, the average pellet sizes produced by *Acartia clausi* exposed only to microplastics were 15% larger (significant at ($p < 0.001$)) than pellets excreted by specimens exclusively fed on algae, while fecal pellets of *Centropages typicus* females that consumed only microplastics were almost twice as large as those produced by copepods which consumed algae. Pellet production rates for *A. clausi* were 2.2 times higher (significant at $p < 0.001$) in individuals fed only on algae compared to the microplastic diet. In contrast, for *C. typicus*, pellet production rates were 4.6 times greater following microplastic exposure than for specimens consuming the algae *Rhodomonas salina*.

Mean bead concentrations in *A. clausi* pellets (445–522 beads pellet^{-1}) were much lower than for *C. typicus* and not dependent on the presence or absence of algae, while for *C. typicus* bead concentrations were highest (3056.5 ± 1181.1 beads pellet^{-1}) when consuming pure microplastics. The microplastic consumption rate for *A. clausi* was 1.7 times higher when fed a mixture of algae and microplastics (858.8 ± 294.1 beads ind^{-1} day^{-1}) compared to a diet of pure microplastics (512.9 ± 337.4 beads ind^{-1} day^{-1}), however for *C. typicus*, the microplastic consumption rate was significantly ($p < 0.001$) higher (4.9 times) when exposed to microplastics only (20237.4 ± 7020.4 beads ind^{-1} day^{-1}) compared to a mixture of algae and microplastics (4128.2 ± 2335.8 beads ind^{-1} day^{-1}).

3.3. Effect of diet on the respiration rate of Acartia clausi

Total respiration rate in active females, consuming *Rhodomonas salina* algae on Day 1 and 5 of the experiment (1.55 ± 0.22 and 1.52 ± 0.38 μg O_2 mg^{-1} h^{-1}, respectively) and basal respiration

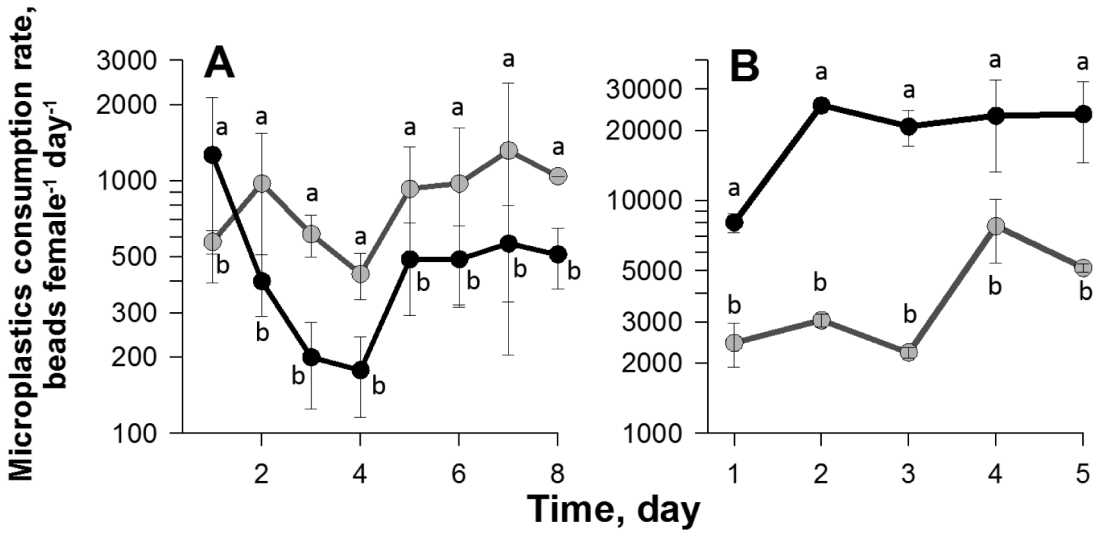

Fig. 4. Microplastic consumption rates during 8 and 5 day exposure periods of *Acartia clausi* (A) and *Centropages typicus* (B), respectively, to diets of a mixture of algae *Rhodomonas salina* and microplastic beads (Treatments **FEEDAII** and **FEEDCII**, respectively, ○) and microplastics only (Treatments **FEEDAIII** and **FEEDCIII**, respectively, ●). Low-case letters (a,b) are the significant variable differences from Duncan's Multiple Range test (DMRT), $P < 0.05$.

Table 1
Mean pellet size, pellet production rate and microplastic consumption during 5–8 days of the experiment with *Acartia clausi* and 1–4 days of the experiment with *Centropages typicus* for 3 treatments: algae *Rhodomonas salina* (~5000 cells ml^{-1}); mixture of algae and microplastics at concentrations of 5000 + 5000 cells/beads ml^{-1}; microplastics only (~5000 beads ml^{-1}). Values are given as Average ±SD.

Diets	Length of pellets (mm)	Pellet production rate (pellets ind^{-1}day^{-1})	Mean bead concentration in pellets (beads pellet^{-1})	Microplastic consumption rate (beads ind^{-1} day^{-1})
Acartia clausi				
Algae	0.129 ± 0.015	2.44 ± 0.8	–	–
Algae + microplastics	0.142 ± 0.011	2.1 ± 1.0	522.1 ± 175.8	858.8 ± 294.1
Microplastics	0.151 ± 0.012	1.09 ± 0.3	445.3 ± 158.3	512.9 ± 337.4
Centropages typicus				
Algae	0.31 ± 0.02	1.6 ± 1.3	–	–
Algae + microplastics	0.35 ± 0.05	3.2 ± 1.2	1325.5 ± 517.7	4128.2 ± 2335.8
Microplastics	0.61 ± 0.11	7.3 ± 5.0	3056.5 ± 1181.1	20237.4 ± 7020.4

rate of anesthetized females (0.52 ± 0.11 and 0.52 ± 0.04 μg O_2 mg^{-1} h^{-1}) remained at approximately the same level. Total respiration rates in females consuming microplastics and starving females, in the 5-day experiment decreased significantly by 2.2 and 2.4 times (compared with those who consumed algae) to 0.678 ± 0.059 and 0.608 ±0.077 μg O_2 mg^{-1} h^{-1}, respectively, however, the basal respiration rates decreased only 16 and 30%, respectively (Fig. 5).

3.4. Effect of microplastic beads on energy metabolism of Centropages typicus

For *Centropages typicus* females consuming algae *Rhodomonas salina*, the weight specific respiration rates of active individuals during the 5 days of exposure decreased significantly from 1.19 ± 0.34 to 0.73 ± 0.14 μg O_2 mg^{-1} h^{-1}, while respiration rates of anesthetized individuals remained at the same level of about 0.33 μg O_2 mg^{-1} h^{-1}. For females consuming microplastics over the same time period, total respiration rate decreased 3.4 times from 1.0 ± 0.18 to 0.29 ± 0.14 μg O_2 mg^{-1} h^{-1}, while estimated respiration rates of anesthetized females were unreliable ($p > 0.05$) ranging from 0.26–0.33 μg O_2 mg^{-1} h^{-1}, displaying no differences from the respiration rates of anesthetized females consuming algae (Fig. 6).

3.5. Effect of microplastic beads on the metabolic scope of activity of Acartia clausi and Centropages typicus

The metabolic scope of activity (SA) or the amount of energy spent by active organisms in water and various manipulation activities by the limbs is determined from the difference between total respiratory rates of active and anesthetized individuals (see Hochachka and Somero, 2002). To assess the dynamics of SA, we used data obtained on days 1, 3, 5, and 6 of *Acartia clausi*, experiments which consumed only microplastics or were starved, as well as data for *Centropages typicus* on days 1, 2, and 5 which were exposed to microplastics (RESPAII, RESPAIII and RRESPCII, respectively).

As can be seen in (Fig. 7), in *A. clausi* that consumed only microplastics for 6 days, the SA decreased by about 8.6 times in accordance with the power equation $y = 1.09x - 1.08$, while for *C. typicus* the SA, after 5 days on the same diet, decreased by almost 7 times in accordance with the power equation $y = 0.65x - 1.15$. The dynamics of SA in *A. clausi* individuals, which were exposed to starvation or pure microplastics was identical. In *Acartia clausi* exposed to microplastics, the SA was 1.5 and 2.3 times greater than for *C. typicus* on the first and fifth days, respectively.

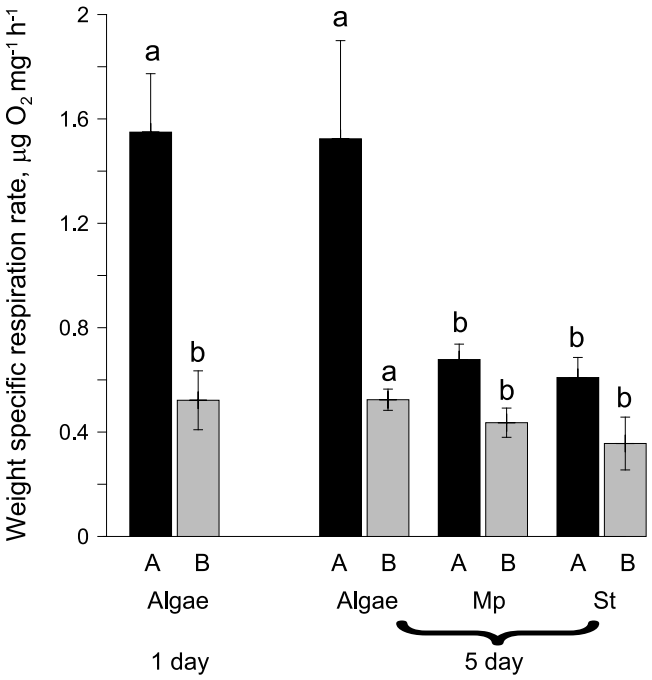

Fig. 5. Respiration rates of active and anesthetized *Acartia clausi* females following 1 day exposure to *Rhodomonas salina*, 5 day exposure to a mixture of algae and 6 µm microplastics (Treatment **RESPAI**) and starvation (Treatment **RESPAIII**). Low-case letters (a,b) are the significant variable differences from Duncan's Multiple Range test (DMRT), $P < 0.05$.

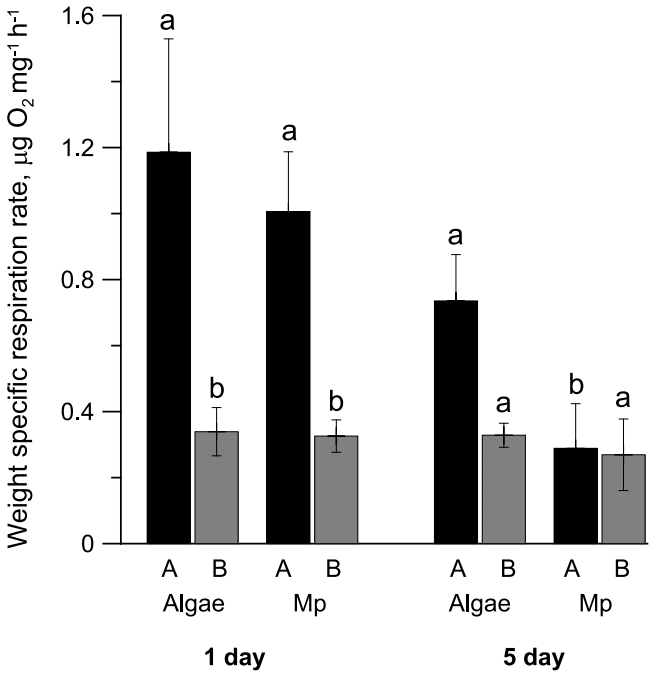

Fig. 6. Respiration rates in active (A, black bars) and anesthetized (B, gray bars) *Centropages typicus* females on first day with algae *Rhodomonas salina* and 5 day of exposure with algae and microplastic of 6 µm. Low-case letters (a,b) are the significant variable differences from Duncan's Multiple Range test (DMRT), $P < 0.05$.

4. Discussion

4.1. Microplastics consumption and mortality

Cole et al. (2013) showed the ability of *Acartia clausi* and *Centropages typicus* to consume microplastics with a preference for a fine fraction of 7.3 µm in diameter. In their 24 h experiments, an increase in microplastic concentration led to a decrease in the rate of algal food intake in these species, which, according to these authors, could be a potential cause of an increase in their mortality. They observed that microplastic consumption rate "reached saturation at concentrations of > 5000 beads mL^{-1}".

In our multi-day experiments with the same high concentration of 6 µm microplastic beads, a high mortality index was found for both species that consumed microplastics. *A. clausi* consuming microplastics mixed with algae or microplastics alone showed a mortality rate of 50% (the proportion of all individuals) on day 5 of the experiment which was significantly ($p < 0.01$) higher than mortality rates in specimens either consuming algae or even starved (Fig. 1A). On Day 8 of the experiment, in individuals consuming a mixture of algae and microplastics or microplastics alone, mortality reached 88%–94%, respectively, whereas mortality rate in the control group averaged 48 ± 19%. It appears evident that microplastics produced a direct effect on the viability of copepods, since in starving individuals the mortality rate also did not exceed 50% on average.

In contrast to *A. clausi,* mortality rates for *C. typicus* females consuming microplastics was significantly higher than for those feeding on algae on Day 2 and on all subsequent days of the experiment (Fig. 1B). In general, *C. typicus* was found to be more sensitive to laboratory conditions. Even in relatively large vessels with volumes of 0.5–1.0 L, *C. typicus* specimens did not survive for more than one week. The stronger mortality effect of microplastics on *C. typicus* compared to *A. clausi* can be explained by

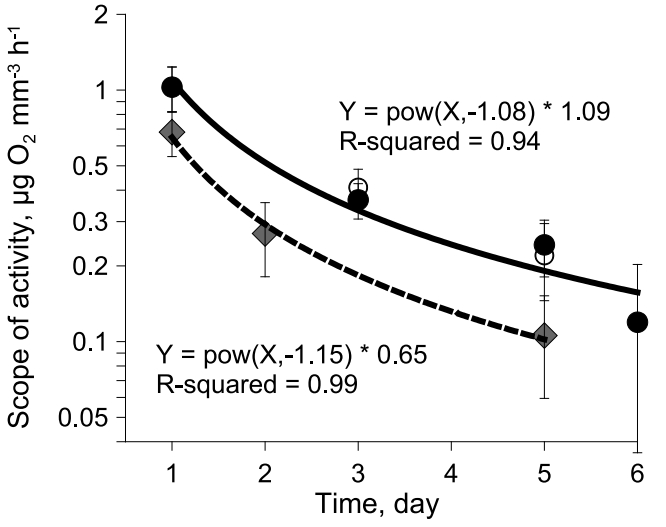

Fig. 7. Timescale dynamics of scope of activity (SA) in *Acartia clausi*: starved (○) and consumed microplastics (●) and *Centropages typicus*: consumed only microplastics (♦).

the different behavioral responses of these species (Table 1). In a microplastic medium, *A. clausi* halved feeding activity, confirming the ability of this species to avoid non-food particles or reject food post-capture (Donaghay and Small, 1979; Ayukai, 1987; Katechakis et al., 2004). We note that the pellet production rates of *A. clausi* in our experiments corresponded to the minimum levels recorded both at sea (Miralto et al., 2002) and in laboratory conditions (Ianora et al., 1996). It can be assumed that for *A. clausi* individuals taken directly from the sea, where diatoms *Sceletonema* spp. predominated in April, the monoculture of the algae *Rhodomonas salina* turned out to be an unusual diet.

During the 24 h experiments performed by Cole et al. (2013), the feeding rate of *C. typicus* females exposed to microplastics was also reduced. However, in our 5 day experiments, *C. typicus* females displayed a clear preference for microplastic beads, evident from fecal pellet production, pellet size, average concentrations of beads in pellets and microplastics consumption rates which were 1.7, 2.3, 2.3 and 4.9 times respectively larger compared to pellets produced by copepods fed a mixture of microplastics and algae (Table 1). Following consumption of pure microplastics, the pellet production rate was on average 4.6 times higher in comparison to a mixed diet of beads and *Rhodomonas salina* algae. Such a selective preference for microplastics over natural food indicates a potentially more active role in the decontamination of water from microplastics, through transferal to bottom ecosystems via sinking fecal pellets for *C. typicus* and possibly all cruising feeder copepods), compared to ambush feeders such as *A. clausi*.

The production of fecal pellets in *C. typicus* was also lower than in the same species that fed on the algae *Hymenomonas elongata* (12 μm) in the experiments of Carlotti et al. (1997), as in our experiments in small vessels (<50 ml). One of the reasons for this may be the uncontrolled loss (underestimation) of fecal pellets as a result of consumption them by copepods. However, as previously shown (Noji et al., 1991; Poulsen and Kiørboe, 2005; Iversen and Poulsen, 2007), many copepods reject fecal pellets to a greater extent, but can fragment them (coprorhexy). A small amount of fragmented granules in our experiments was observed only with C. typicus. Consequently, the results of pellet production by this species may be underestimated, which can only increase the difference in microplastic consumption between *C. typicus* and *A. clausi*.

4.2. Influence on respiration rate

Previously, Cole et al. (2015) first measured respiration rates of *Calanus helgolandicus* in a mixture of phytoplankton and microplastics, showing that low concentrations (75 beads ml^{-1}) did not significantly affect the energy metabolism of these copepods. In our study, we demonstrated the effects of microplastics not only on the respiration rates of active *Acartia clausi* and *Centropages typicus* females, but also on immobilized individuals, in order to understand which components of energy metabolism are more sensitive to microplastic uptake. As is known, in actively swimming copepods, the total respiration rate R_{tot} is due to the expenditure of energy on activity (R_a), associated with maintaining the position in space, searching for and capturing food, the basal energy of maintaining the vital functions of body tissues (R_b), and specific dynamic actions related to food uptake, i.e. energy for renewal or growth of body tissues (R_{SDA}): $R_{tot} = Ra + R_b + R_{SDA}$.

In our experiments at 20 °C, R_{tot} measurements for *A. clausi* and *C. typicus*, consuming the algae *Rhodomonas salina*, were 1.55 ± 0.22 and 1.19 ± 0.34 μg O_2 mg^{-1} h^{-1}, respectively. These values, after conversion to amount of oxygen per unit dry weight (DW) (assuming roughly a coefficient of DW ≈ 0.1 WW), corresponded to the maximum reported respiration rates of these species at 20 °C (see review data by (Gaudy and Thibault-Botha, 2007; Hubareva et al., 2008). In the anesthetized females *A. clausi* and *C. typicus*, R_b values at the beginning of the experiment were 0.5 ± 0.11 and 0.34 ± 0.07 μg O_2 mg^{-1} h^{-1}, respectively, being 3.1 and 3.5 times smaller than R_{tot}. These are very high activity indicators, taking into account the fact that, with the highest motor activity of calanoid copepods, the R_{tot} / R_b ratio varies from 3 to 7 (Pavlova and Minkina, 1987; Svetlichny and Umanskaya, 1991; Buskey, 1998; Svetlichny and Hubareva, 2002).

After a 5-day exposure period to microplastics alone, R_{tot} of *A. clausi* decreased 2.2 times to the same level as starving females (Fig. 6), while R_b in both cases decreased only by 16 and 30%. This allows us to evaluate the R_{SDA}, by comparing with R_b in the control experiment, as 5.8%–11% of R_{tot}, which is typical for R_{SDA} of copepods and other organisms (Svetlichny and Hubareva, 2005). We can therefore conclude that a 2.3-fold decrease in R_{tot} occurred for this species only in connection with a decrease in motor activity, due to a lack of energy resources. R_a of *A. clausi* females, calculated as $R_{tot} - R_b$ decreased by 4.3 times over 5 days with the same trend as in starving females.

In *C. typicus* with 5 days exposure to microplastics alone, R_{tot} decreased significantly more than for *A. clausi* by 3.5 times to the level of R_b(Fig. 7). It is likely that the greater consumption of microplastics depleted the energy resources of *C. typicus*. At the same time, we did not find a significant change in the more conservative R_b. In general, R_a in females of both species decreased in very close time dependence. But the absolute difference in their R_a values may reflect diversity in the ecological specialization of the studied species, i.e. an energetically more costly jumping type of routing movement in *A. clausi* in comparison with the relatively uniform swimming action of *C. typicus*. It can also be assumed that the consumption of microplastics without compensation for the subsequent energy expenditure will cause additional energy depletion and change in respiration rates of such copepods will occur according to an accelerated decrease in energy metabolism compared to natural starvation.

5. Conclusion

Thus, the data obtained allows us to conclude that if the microplastic effect is strong enough to lead to a decrease in the consumption rate of algae, as shown previously (Ayukai, 1987; Hansen et al., 1991; Cole et al., 2013), crustaceans can respond to this, by reducing energy metabolism through reduced motor activity. For further studies, it will be beneficial to determine to what extent different concentrations of microplastics with respect to algae will affect the energy metabolism of these two copepod species under different nutritional schemes.

CRediT authorship contribution statement

Leonid Svetlichny: Performed lab work, Writing, Analyzed data. **Melek Isinibilir:** Performed Lab work, Writing paper. **Taras Mykitchak:** Performed lab work, analyzed data. **Kamil Mert Eryalçın:** Performed Microalgae Culture, Writing and building tables and figures in paper, Analyzed statistical analysis. **Ezgi E. Türkeri:** Performed fieldwork. **Esin Yuksel:** Performed fieldwork. **Ahmet Erkan Kideys:** Writing, Analyzed data.

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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