



Microplastics uptake and accumulation in the digestive system of the mud crab *Rhithropanopeus harrisi*

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Abstract. The ingestion of microplastics has been recorded in hundreds of species. The ingestion rate and degree of impact is species-specific and depends on food preferences, foraging behaviour, and plastic pollution of the area. Currently there is a large knowledge gap regarding ingestion of marine litter by invertebrates in brackish water bodies. Therefore, experiments were conducted to investigate microplastics uptake and potential accumulation in the digestive system of the Harris mud crab *Rhithropanopeus harrisi*. Effects of microplastics on the growth of crabs were also tested. The results show that *R. harrisi* consume microplastics together with food, only plastic fragments too large for the digestion system were removed by crabs. The effect and duration of passage of plastic are not consistent and depend on the size and type of plastic. Microplastic fragments (<0.25 mm) ingested by crab were continuously egested while knotted fibres may accumulate in the stomach. The crabs fed with plastic supplements lost weight or had lower weight increase compared to control group after two-month treatment. However, the differences between treatments were not statistically significant. For the first time, occurrence of plastic in the digestive system of crabs collected from the natural habitats of Pärnu Bay (NE Baltic Sea, Estonia) was studied. Among all the crabs examined, 5% of specimens were found with fibres in their stomach assimilated prior to their capture from nature.

Key words: plastic ingestion, stomach contents, invertebrate, marine litter, plastic fibres, fragments, the Baltic Sea.

1. INTRODUCTION

The global production of plastics shows exponential growth in production, increasing by 1/3 during the last 10 years (Geyer et al., 2017; Ritchie and Roser, 2019). Although, recycling of plastic is also increasing (PlasticsEurope, 2017), millions of tonnes of plastics still reach the marine environment each year (Jambeck et al., 2015).

Particles smaller than 5 mm are generally defined as microplastics (GESAMP, 2015). Microplastics may originate from large pieces fragmented under exposure of UV radiation, mechanically broken down through wave action, sand grinding, and other processes; or they may originate from industrial raw material (Barnes et al., 2009; GESAMP, 2015). Manufactured microplastics are widely

used as particles for industrial cleaning of surfaces, cosmetic products (e.g. peelers, makeup glitter) or packing material (e.g. polystyrene granules) (GESAMP, 2015). Microplastics occur in different shapes: fibres, spheres (beads), film, pellets, and fragments of irregular shape (Chubarenko et al., 2016; de Sá et al., 2018).

Microplastics have been found throughout the water column in most marine habitats of the world (Lusher, 2015). Microplastics can affect marine organisms, food webs and community structure in many ways: transport of epifauna and microbes to a new area, accumulation and transport of toxic substances, or ingestion and accumulation of particles in tissues (Derraik, 2002; Solomon and Palanisami, 2016). The ingestion of microplastics have been recorded in hundreds of species (GESAMP, 2015; Wang et al., 2016).

Uptake of plastic may be accidental, caused by confusion between prey and plastic waste, or intentional, caused by feeding on lower trophic organisms that have themselves consumed microplastics (Derraik, 2002; Solomon and Palanisami, 2016). Ingestion of microplastics can cause physical blocking of the intestinal tract or affect negatively feeding activity, oxygen consumption, reproductive ability, survival, and larval development (Cole et al., 2015; Green, 2016; Solomon and Palanisami, 2016; Sussarellu et al., 2016; Watts et al., 2016; Green et al., 2017).

The ingestion rate and degree of impact is species-specific and depends on food preference, foraging behaviour, and plastic pollution of the area (Setälä et al., 2016; Wójcik-Fudalewska et al., 2016; Railo et al., 2018; Qu et al., 2018). For example, bivalves are directly exposed to microplastics in the water column because of their extensive filter-feeding activity (Bråte et al., 2018; Li et al., 2019). Previous research suggests that crabs may be at high risk for accumulation of microplastics due to consumption of bivalves (Farrell and Nelson, 2013; Watts et al., 2014).

The Harris mud crab *Rhithropanopeus harrisi* (Gould, 1841) is the only reproducing crab species in northern Baltic Sea (Ojaveer et al., 2007; Kotta and Ojaveer, 2012). *R. harrisi* is one of the most widely distributed brachyuran crab species globally (Roche and Torchin, 2007). Species has been invaded to the Baltic Sea in the middle of the last century and expanded distribution remarkably during the last decade (Kotta and Ojaveer, 2012). The species is a small (adult maximum carapace width 25 mm) and it tolerates a wide range of environmental variables (Aarnio et al., 2015). The species alters the local food webs because it is both a predator and prey for fish species (Fowler et al., 2013). *R. harrisi* is an opportunistic omnivore that feeds on a mixture of animals, vegetation, and detritus (Czerniejewski and Rybczyk, 2008). It is a predator of littoral grazers such as mussels, isopods and gammarids (Forsström et al., 2015; Nurkse et al., 2018).

In order to better understand and determine the impact of microplastics on marine biota, an increased number of studies covering the various habitats and species from a wide geographical range is needed. Large knowledge gaps exist regarding ingestion by invertebrates in the Baltic Sea and northern marine environments (Bråte et al., 2017). Currently, only a few studies with limited number of taxa involved exists on the presence of microplastics in marine invertebrates from the Baltic Sea (Beer et al., 2018; Näkki et al., 2019).

Current study expands current knowledge of effects of microplastics by evaluating various aspects of coping with ingested plastics by crabs. The objectives of this study were to: (1) describe microplastics uptake and potential accumulation in the digestive system of *R. harrisi*; (2) detect the effect of microplastics on the growth of crabs;

and (3) describe accumulation of plastics in the digestive system of crabs collected from natural habitats. The finding provides information about the duration and extent of the impact of plastics ingestion by invertebrates.

2. MATERIAL AND METHODS

2.1. Collection and maintenance of crabs

Crabs were collected from Pärnu Bay in the north-eastern Baltic Sea, Estonia in July 2018. Only males with carapace width between 11.23 and 19.90 mm (mean width with SE: 15.89 ± 0.2 mm) were used in experiments. In the laboratory crabs were kept in tanks filled with aerated water at temperature 16 °C and salinity 3.7. Crabs were fed with fish fillet (*Theragra chalcogramma*) during the two weeks acclimation period. Crabs were kept without food one week before the experiment started to clear their stomach and intestine.

To prevent the possible contamination in the laboratory crabs were kept in a room where the access of personnel was limited, crabs were fed by fish fillet, and containers were covered by mesh (mesh size 0.5 mm). All plastics used in the experiments were in predefined colour depending on the size of particle.

2.2. Experimental setup

Each specimen was maintained individually in a 2 L container covered by mesh under the same condition as during acclimation period with the exception of absence of aeration in the containers. The container was provided with a shelter for the crabs and water was exchanged weekly. During all experiments the crabs were fed with fish fillet of approx. portion size 0.03 g wet weight. Food was supplemented with a high concentration of microplastics by fully covering with it. Plastic shapes refer to the three most common types of microplastics: fibre, fragments, and beads. A red stranded rope (material polypropylene) was used to create microfibrils by cutting the original rope. The width of the fibre was 0.03 mm, length varied between 0.2 and 0.5 mm. Two sizes of glitter products (material polyethylene terephthalate), for eye makeup (particle size 0.09–0.12 mm) and nail glitter (particle size 0.013–0.25 mm), were used as fragments. Additionally, to detect the maximum size of ingested particles by crab, a gradual variation of beads in size between 0.2 and 1 mm were used. These beads were washed out from peeling shower gel.

At the end of the experiment each crab was dissected and checked for the presence of plastic. For this, crabs were dissected along the abdominal carapace. Then, the hepatopancreas, stomach and intestine were carefully removed by use of forceps and placed in a petri dish.

Plastic particles were estimated visually in each organ under microscope Leica MZ6 at 80× magnification. All plastics were in bright colour and easily distinguishable under microscope.

In a short-term experiment the translocation, retention and excretion of plastics in organs was investigated. The specimens were divided into four groups. A control group was maintained under identical conditions and fed by fish only. Fish were stained with food colour to allow accurate monitoring of the progression of food in the digestive system. Other groups were fed with fish contaminated by different size (fragments <0.12 or <0.25 mm) or type (fibre) of plastics. All individuals were fed once at the same time. Only specimens who fully ingested the food were chosen for the experiment. For each group 24 specimens were selected, three individuals from each group were analysed after a certain time period (3, 6, 9, 12, 24, 36, 48, and 72 h) from the beginning of the experiment.

The long-term presence of fibres in crab intestines was studied during the long-term experiment (duration 4 weeks). Food supplemented with fibres was offered twice a week during two weeks. Four replicate specimens were analysed after a certain time period (2–6, 8, 10, and 12 days) since the last feeding.

The third experiment was conducted to assess potential effects of ingested fragments and fibres on the vitality and growth rate of the crabs. The crabs were divided into three groups with six individuals each. A control group was maintained under identical conditions and fed with fish only. The second group was fed with fish contaminated with fragments (<0.12 mm) and the third with fish together with fibres. Food supplemented with

plastic was offered twice a week during the two months. For the next two weeks, crabs were fed with unlimited amount of pure fish for two weeks. Then crabs were kept without food for five days prior weighing. Individual body weight (g wet weight) were measured by Mettler Toledo AB104 at the beginning and at the end of the experiment. Growth rate was standardized with regard to the specimens' initial weight. One-way ANOVA was used to test for significant differences in weight change of crabs. The assumptions of homogeneity of variance were tested using Levene's Test (StatSoft Inc., 2013).

In the fourth experiment, variable sizes of beads were fed to detect the size limit of ingested bead by crabs. In this experiment, six crabs larger than average (carapace width between 15.60 and 16.72 mm) were used. Crabs were fed once with fish contaminated with different sizes of beads (between 0.2 and 1 mm). For three individuals the experiment lasted 0.5 h, and for a second group 9 h. The maximum size of the beads within the crab intestine was measured at the end of the experiment.

3. RESULTS

Based on the short-term experiment, food material started to exit into the midgut 3–6 h after feeding (Fig. 1). The whole digestive system was cleared of food between 9 h and 12 h after feeding. There were no fragments of plastics in digestive tract 36 h the latest after feeding. The plastic particles were eventually excreted with faecal pellets. The larger the size of the particles, the longer it took to move forward through the tract. After 72 h, the stomach contained fibres in 45% of the specimens.

Time / treatment	0 h	3 h	6 h	9 h	12 h	24 h	36 h	48 h	72 h
Fish	Black	Black	Grey	Grey	Grey	Grey	Grey	Grey	Grey
Fragments (<0.12 mm)	Black	Black	Black	Black	Black	Black	Black	Black	Black
Fragments (<0.25 mm)	Black	Black	Black	Black	Black	Black	Black	Black	Black
Fibres (0.03 × 0.5 mm)	Black	Black	Black	Black	Black	Black	Black	Black	Black

Fig. 1. Localization of food and particles in stomach (marked in black) and intestine (marked in grey).

In the long-term experiment, fibres were found in stomachs even 12 days after the last diet contaminated with fibres (Fig. 2). However, intestines of all specimens were clear at day 5.

In the growth rate assessment experiment, the crabs fed with plastic supplements lost weight or had lower weight increase compared to the control group (Fig. 3). The variability of weight change was higher among specimens treated with plastics. However, the differences between treatments were not statistically significant.

Crabs consumed beads in diameter up to 0.7 mm, larger beads were segregated from food. The 90% of consumed beads did not exceed the size 0.5 mm.

During the study altogether 152 crabs were examined. Among all the crabs, 5% of specimens were found with fibres in their stomach assimilated prior to capture from the nature. Both solitary fibres and fibres knotted together into balls were observed.

4. DISCUSSION

The results reveal that Harris mud crabs *R. harrisii* are opportunistic feeders and able to consume food together with plastic contamination. Specimens did not reject the food or segregate the plastics even when food was covered with a very high concentration of fragments. Many crustaceans are characterized as unable to recognize and discard plastic associated with their food (Egbeocha et al., 2018). Nonselective ingestion of microplastics smaller than 100 µm has been reported for marine species with different feeding modes (Hämer et al., 2014). Experiment of this study showed that only supplements too large for the digestion system were segregated from food by crabs.

The duration of passage of food through the crustacean gut system is highly variable depending on the species and

environmental conditions (McGaw, 2006; Hegele-Drywa and Normant, 2009). During current experiments the whole digestive system of *R. harrisii* was cleared of food 9–12 h after feeding. The cardiac shape foregut houses the gastric mill apparatus which functions in mastication of the ingested food (McGaw and Curtis, 2013; Castejón et al., 2015). Contraction of gastric mill and foregut muscles caused agglomeration of ingested plastic fragments in the stomach. The majority of ingested plastic was rejected after the digestion of food in the stomach. The clearance of stomach and intestine of plastics was reported 24–36 h after feeding. Surprisingly, plastic fragments were not found in hepatopancreas during this study. Based on literature, the fragments 0.18–0.25 mm were found in hepatopancreas of crab *Ulva rapax* (carapace width 21–30 mm) (Brennecke et al., 2015). In current study carapace width was 11–20 mm and fragment size 0.09–0.25 mm, so potentially the used particles could move to hepatopancreas. The mechanism of retention of plastic in the gills and hepatopancreas is still unclear since natural particles, such as sand grains and detritus, do not accumulate within these organs in a similar manner (Brennecke et al., 2015).

Contrary to the short-term passage of the fragments in the intestine of crabs, the fibres were found until the end of the experiment. Such fibres may be particularly hazardous as they may clump and knot due to peristaltic stomach movements which potentially prevent egestion. The presence of balls of fibres has been reported widely for several invertebrate species (Murray and Cowie, 2011; Watts et al., 2015; Wójcik-Fudalewska et al., 2016).

Results of this study indicate that the ingestion of microplastics during a two-month period has no statistically significant negative impact on the growth rate of the crab *R. harrisii*. However, the weight change showed lower increases or negative values among crabs

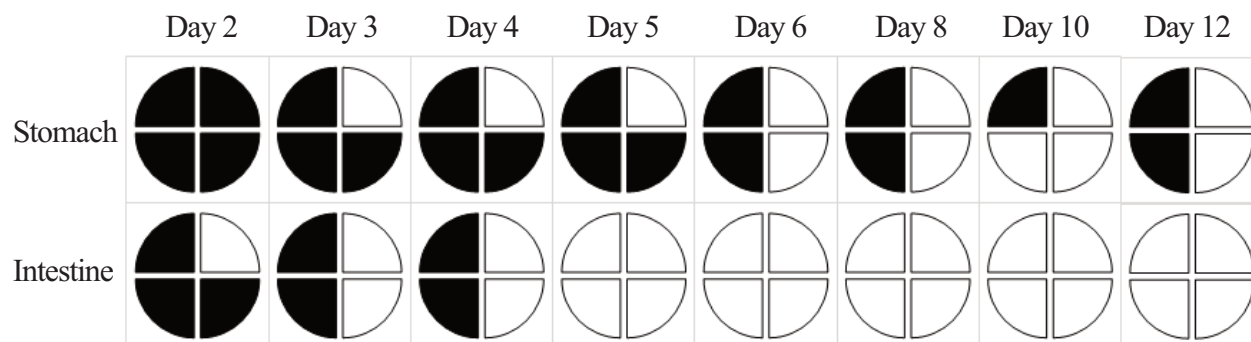


Fig. 2. Content of fibres in stomachs and intestines of crabs 2 to 12 days since the last feeding with fish contaminated by fibres. Each circle represents four replicates; each quarter represents one specimen. Black quarters designate the presence of fibres, white quarter the lack of fibres.

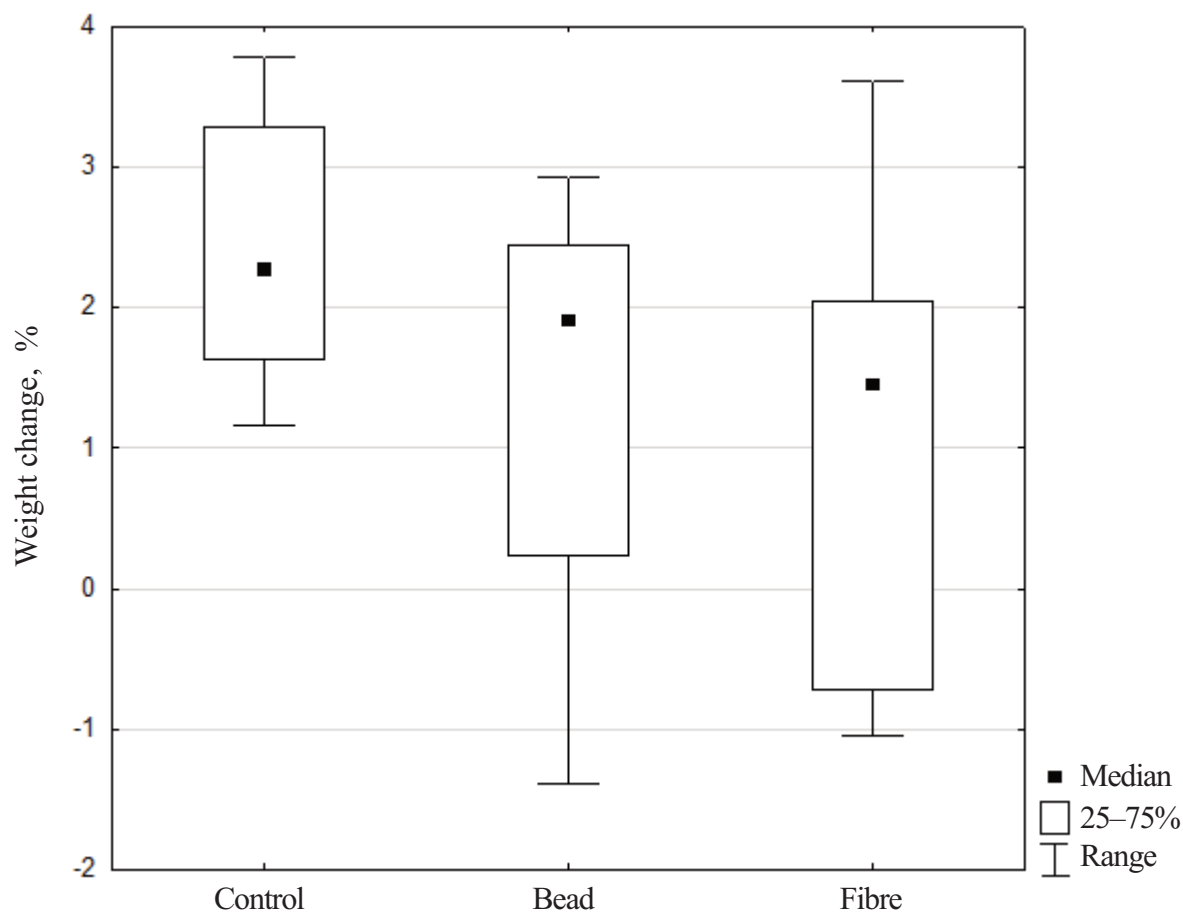


Fig. 3. Weight change of crabs after two-month treatment with contaminated food (except control) followed by unlimited feeding of pure fish for two weeks. One-way ANOVA, $N = 6$, $p > 0.05$.

fed with plastic supplements compared to the control group fed with fish only. High resilience to environmental change (Hegele-Drywa and Normant, 2014a) and ability to survive with limited food (Hegele-Drywa and Normant, 2014b) of the species might shadow the clear outcome of this growth change experiment. The published information about effects of plastic ingestion on growth of different taxa is controversial. For example, negative effects on condition of the shore crab *Carcinus maenas* caused by ingestion of plastic were reported (Watts et al., 2015), while no effects on condition of brown shrimp *Crangon crangon* (L.) (Foekema et al., 2013) and North Sea fishes were found (Devriese et al., 2015).

This study documents the presence of microplastics in stomach contents of *R. harrisi* collected from the Estonian coastal waters. Balls of fibres were found in the stomachs of 8 out of 152 crabs. Fibres are considered as the most common type of microplastics in many areas (Browne et al., 2011). Degraded fishing gear and sewage contaminated by fibres from washing clothes are con-

sidered as sources of plastic microfibre in the marine environment (Browne et al., 2011; De Witte et al., 2014). All crabs collected for this study originated from areas where many abandoned fishing nets were observed (author's pers. comm.). The amount of ingested plastics is highly dependent on species' feeding mode (Setälä et al., 2016; Scherer et al., 2017) and the study area, as correlations between concentrations of plastics in the environment and marine animals have been found (Qu et al., 2018). As many as 66% of mussels contained plastic in the Åland Sea area (Baltic Sea) (Railo et al., 2018) and around 80% of specimens in several marine areas around Europe has been reported (Vandermeersch et al., 2015; Nadal et al., 2016). Compared to this, the observed results in this study of 5% of individuals with plastic in their stomach, was very low.

The EU requires from member states that assessment systems are established and indicators are developed to evaluate the environmental status of their national marine waters. Among other criteria, member states are

required to ensure that the amount of micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species (EU 2017/848). The current study provides baseline knowledge and first insight about amounts of micro-litter ingested by invertebrates in Estonian national waters. For integrated assessment of the amount of ingested plastic and for development of indicators, further studies with different taxa are planned.

5. CONCLUSIONS

The present work highlights, for the first time, that the Harris mud crab *R. harrisi* in Estonian coastal waters of the NE Baltic Sea can ingest microplastics. The results indicated *R. harrisi* are opportunistic feeders and are able to consume food together with plastic contamination. The experiment showed that only supplements too large for the digestion system were removed by crabs. The effect and duration of passage of plastics are not consistent and depend on the size and type of the plastic. Microplastic fragments (<0.25 mm) ingested by crabs were continuously egested while knotted fibres may accumulate in the stomach. Among all crabs, 5% of specimens were found with fibres in their stomach assimilated prior to capture from nature.

In order to national and international assessment the amount and effect of ingested micro-litter to fulfil the requirements of EU directive, large scale baseline studies in northern Baltic Sea is needed. For integrated assessment special studies including taxa from different food-web level is essential.

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Mikroplastiku allaneelamine ja esinemine rändkrabi *Rhithropanopeus harrisi* seedekulglas

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EL nõuab liikmesriikidelt mereala keskkonnaseisundi hindamist vastavalt etteantud kriteeriumidele. Muude kriteeriumide hulgas on liikmesriigid kohustatud tagama, et mereloomade poolt neelatud mikroprügi kogus ei kahjustaks liikide tervist. Käesoleva töö tulemused näitavad, et rändkrabi *Rhithropanopeus harrisi* tarbib üldjuhul valimatult plastikuga saastunud toitu. Krabid eemaldasid toidu küljest vaid seedekulglaga jaoks liiga suured tükid. Plastiku seedekulglaga läbimise kiirus sõltus plastiku suurusest ja kujust. Katse käigus tühjenes krabide magu ja soolestik <0,12 mm suurustest osakestest hiljemalt 24 h jooksul ning <0,25 mm suurustest osakestest 36 h jooksul. Plastikkiud kogunesid mõningatel juhtudel kerajateks moodustisteks ja esinesid krabide maos ka eksperimendi lõpul 12 päeva pärast viimast toitmist plastikuga saastunud toiduga. Pärast kahekuulist katseperioodi olid plastiklisandiga toitu saanud krabide juurdekasvu mediaanväärtused kontrollgrupiga võrreldes küll madalamad, kuid erinevus ei olnud statistiliselt oluline. Töö käigus määrati esmakordselt Eesti rannikumere piirkonnas plastiku esinemist looduslikust keskkonnast kogutud põhjelaestikus. Pärnu lahest kogutud 152 krabist sisaldas 5% krabide magu kiulisi moodustisi.