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Study on the Presence of Microplastics in Zooplankton Collected from Ennore Estuary, Chennai, Tamil Nadu, India

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Abstract: Microplastic contamination has been considered as a global environmental problem in marine ecosystem. Due to small size (< 5 mm) in overlapping with that of microalgae, microplastics can easily be ingested by a wide range of marine copepods. Microplastics are mistakenly ingested by zooplankton as food, subsequently disrupting the biological process of zooplankton, a crucial food source for many secondary consumers. As copepods dominate zooplankton biomass and provide an essential trophic link in marine ecosystem, copepods are at an increased risk of microplastic ingestion. The seasonal change in microplastics in copepods and the key environmental factors influencing the retention of microplastics in copepods are largely unknown. In the present study we collected copepods from Ennore estuary and identified them up to the species level. The samples were digested and visually examined by using microscope for presence of microplastic. Our results showed that the copepods collected from Ennore estuary contained microplastics in their body.

Keywords: Copepods, Microplastics, Zooplanktons, Pollutants, Ecosystem

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Introduction

Over the past century there has been an exponential increase in plastic demand and production. Continuous improper disposal, accidental loss, and fragmentation of plastic materials, have led to an increase in tiny plastic particles and fibres. Due to human activities, a huge mass of plastic waste is annually produced and finally imported into the global marine environment. For this reason, there has been more than 5 trillion plastic debris with over 250,000 tons floating in the global oceans (Eriksen *et al*., 2014). In the marine environment, because of several abiotic processes such as ultraviolet (UV) radiation and mechanical wear, plastics eventually become small fragments, fibres or granules with particle size <5 mm, that is, "microplastics" (Hidalgo-Ruz *et al*., 2012; Lusher *et al*., 2013).

For the past 50 years plastic litter has become an increasingly conspicuous presence within marine ecosystems (Thompson *et al*., 2004). While the risks that larger plastic debris pose to marine life are well documented (Derraik, 2004), we are only just beginning to understand how microscopic plastic debris, termed “microplastics”, may be impacting upon aquatic organisms (Cole *et al*., 2011; Wright *et al*., 2013). Microplastics describe plastic granules, beads, fragments, and fibres <1 mm in diameter, either manufactured to be microscopic in size or derived from the fragmentation of larger plastic debris following prolonged degradation (Andrady, 2011). Microplastic litter has been identified in aquatic environments across the globe (Hidalgo *et al*., 2012) prompting increasing levels of regulation (Zampoukas *et al.,* 2012). Almost 700 aquatic species in the world were adversely affected by the introduction of microplastics, including sea turtles, penguins, and other crustaceans (Marn *et al*., 2020).

The primary risk associated with microplastics are their bioavailability to marine organisms, a range of marine biota, including fish (Lusher *et al*., 2012), seabirds (Van *et al*., 2011), benthic polychaetes (Wright *et al*., 2013) and zooplankton (Cole *et al*., 2013; Setala *et al*., 2014) have the capacity to ingest microplastics. Consumption of microplastics can result in adverse health impacts including reduced feeding (Cole *et al*., 2013), loss of energetic reserves (Wright *et al*., 2013), hepatic stress (Rochman *et al*., 2013) and reduced fecundity and survival (Lee *et al*., 2013). Microplastics also act as a carrier that adsorbs and bioaccumulates environmental pollutants within living organisms, thereby increasing the bioavailability of pollutants that are eventually biomagnified in the tissues of organisms at higher levels of the food chain, such as humans. The global environmental concern regarding plastic waste has diverted attention towards the development of bio-based biodegradable plastics (Tan *et al*., 2020).

Despite the attention being received for microplastic pollution impacts on zooplankton by the scientific community (Botterell *et al*., 2019), there are limited studies on ingestion of microplastics in natural plankton communities (Sun *et al*., 2018). In particular, copepods, noted as being overwhelmingly abundant and having a pivotal position in marine food webs (Turner, 2004) and highlighted for their susceptibility to microplastic ingestion due to their typical distribution near the surface, are considered an integral link between microplastic transfer and predator and prey interactions (Setala *et al*., 2016). Copepods are among the most studied organisms related to the environmental impacts of microplastics (Cole *et al.,* 2013; Cole and Galloway, 2015).

Previous reports have revealed microplastic ingestion by zooplankton, whereby they are capable of varied microplastic uptake depending on the size of the microplastics, the life stage of the copepod and the species of the copepod. Microplastics are ingested by zooplankton through filter-feeding and egested in faecal pellets, generally within several hours (Barnes *et al.,* 2009; Cole *et al.,* 2013). It is imperative to study the ingestion and egestion of microplastics by planktonic organisms as they are at the base of the food web, hence, they could bio-magnify chemical contaminants associated with microplastics to subsequent trophic levels (Turner, 2004). The types of zooplankton reported to ingest microplastics include holoplankton (Copepoda, Tunicata, and Euphausiacea), meroplankton (Mollusca and Decapoda), and microzooplankton (Dinoflagellata) (Cole *et al.*, 2013).

Studies have demonstrated that microplastics have been ingested by a wide variety of marine organisms (de Sa *et al*., 2018), including zooplankton (Cole *et al*., 2013; Setala *et al*., 2014). Bioaccumulation and retention of microplastics in laboratory trials have been shown to reduce nutritional state, increase mortality and decrease fecundity following experimental exposure (Welden and Cowie, 2016). In contrast, field-based studies have suggested that ingestion of microplastics in the natural environment may be transient and have no long-term effect upon organisms (Hamer *et al*., 2014; Bruck and Ford, 2018). In the case of copepods, experimental exposure to microplastic beads has been shown to result in ingestion via indiscriminate feeding, with *Temoralongi cornis* showing clumping of ingested microplastic beads in the posterior midgut, as well as adherence to external surfaces (Cole *et al*., 2013). In the present study we investigated the presence of microplastics in copepod, a zooplankton collected from Ennore estuary, Chennai, Tamil Nadu, India.

Materials and Methods

Ennore creek is a backwater located in Chennai, Tamil Nadu, India, latitude**:** 13.2146° N longitude**:** 80.3203° E of Chennai along the Coromandel Coast of the Bay of Bengal (Mathu Mitha *et al*., 2021). Zooplanktons were collected using 200 µm plankton nets. Morphological identification was performed according to International Council for the Exploration of the Sea (ICES) protocols (Roger *et al*., 2000). The samples were treated with 10% KOH solution at 60 C without agitation for 48 h to increase the digestion capacity (Kuhn *et al*., 2017). After that the digested samples were centrifuged at 3000 rpm for 15 min and the supernatant was discarded and the pellet was observed under a light microscope. Not all particles were visible to the naked eye; therefore, the use of a stereomicroscope was necessary for a preliminary visual sorting and the microplastics were photographed.

Results and Discussion

In the present study we identified some copepods, such as *Acartiella keralensis*, *Neodiaptomus strigilipes*, *Pseudodiaptomus binqhami* and *Cyclops* sp. (Figs. 1-4). The interaction between marine organisms and microplastics depends on the possibility of that organism encountering the microplastic particles in the ocean, as well as the susceptibility of that species if their interaction is occurring (Engler, 2012; Setala *et al*., 2014). According to patterns of ocean currents and biological productivity, the overlap of marine organisms and microplastics is most likely to occur in shelf sea regions (Clark *et al*., 2016); in these areas, marine copepods are frequently found in huge abundance and have high probability to ingest the microplastics. Marine copepods (the dominant members of zooplankton community) have a crucial niche in marine ecosystem because they consume primary producers (for example, phytoplankton) and transfer the energy to higher trophic levels. Also, the copepods have potential effects on biological pump because they can excrete faces for carbon export to deeper-seawater or coprophagous biota in marine environment (Cole *et al*., 2016).

Fig. 1: *Acartiella keralensis.*

Fig. 2: *Pseudodiaptomus binqhami.*

Fig. 3: *Neodiaptomus strigilipes.*

Fig. 4: *Cyclops* sp.

As marine copepods can ingest microplastics with a size range similar to their prey (i.e., microalgae), accurate predictions of microplastic effects in these organisms are pivotal to better understand how marine ecosystem will respond to microplastic contamination. Actually, several studies have demonstrated that microplastic exposure can negatively affect the important life traits (e.g., growth and reproduction) in many copepods including *Paracyclopina nana* (Jeong *et al*., 2017), *Calanus finmarchicus*, *Pseudocalanus* spp., *Acartia longiremis* (Vroom *et al*., 2017) and *Tigriopus japonicus* (Zhang *et al*., 2019).

In the present study it was visible to identify the presence of microplastics in the copepod collected from Ennore estuary (Figs. 5, 6). Cole *et al*. (2013) has investigated the effects of microplastics on the feeding behaviour, reproduction, and survival of the pelagic copepod *Calanus helgolandicus*, a small filter-feeding crustacean. Many studies have evidenced the microplastic-induced adverse effects in marine copepods, which range from impede feeding capacity to physiological damages (Lee *et al*., 2013; Cole *et al*., 2019; Choi *et al*., 2020).

The adverse impacts of microplastics on copepods are thought to primarily include physical damage and chemical effect. The former is mainly related to the ingested microplastics being capable to entangle feeding appendages and block the alimentary tract in the stressed individuals hence, causing reduced feeding, malnutrition and even death (Cole *et al*., 2013; Jeong *et al*., 2017; Zhang *et al*., 2019). The other risk from the intake of plastics is attributed to its inherent chemical nature (for example, the plastics normally contain additives and some of them display endocrine disruption activity) and large surface area-to-volume ratio which enables them to accumulate POPs or metals (Wright *et al*., 2013); the consequence is that, after consumption of microplastics, the incorporated or absorbed toxicants can have damaging effects in marine copepods.

(5) (6)

Figs. 5, 6: Microplastics in Copepods.

The problem of microplastic ingestion by zooplankton, however, does not end there. Recent studies have also shown that microplastics egested within copepod faecal pellets result in the pellets having less structural integrity (Cole *et al*., 2016). Additionally, if the egested microplastics were of low density (e.g. polystyrene) then the faecal pellets sank more slowly. It is postulated that this will increase the chances of them being eaten by other marine animals, resulting in the movement of the plastics through the food chain. The problem is two-fold; first moving the plastics through the food chain further disperses their potential to have negative effects, and secondly, this may reduce the organic matter reaching the seabed and increase the amount of particulate matter in the water column, with possible repercussions for wider marine ecological processes, and even the oceans climate control capacity.

Conclusion

Our finding confirmed the presence of microplastic debris in the copepod collected from the Ennore estuary. Better knowledge of the extent of microplastic contamination of oceans waters is now a research imperative. Research in the field of microplastics has been growing a lot in recent years; nevertheless, the small dimensions of items in question involve several complexities regarding the sampling strategies adopted, the analytical methods for their quantification, and implications about their risks in the environment and for human health, which are not yet thoroughly understood.

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