Queen Conch as Indicator of Pollution by Microplastics in the Caribbean

El Caracol Rosa como Indicador de Contaminación por Microplasticos en el Caribe

Le Lambi Comment Indicateur de Pollution par Microplastiques dans la Caribbe

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ABSTRACT

The occurrence of microplastics in the marine environment is increasing worldwide. These particles are now present in marine sediments and in the water column where they can be ingested by marine organisms. This study was carried out to quantify and analyse microplastics in the wider Caribbean using the mollusc, queen conch (*Strombus gigas*), as an indicator species, and a non-destructive method of sampling. Between three and seven conchs were sampled in each of four sites: Alacranes Reef (Mexico), Florida Keys (USA), Guadeloupe (FWI) and Barbados. Feces from each live conch were collected for analysis. Microplastics were extracted by degradation of organic matter, re-suspended and analysed by stereomicroscope and scanning electron microscope. The protocol used in this study was successful and showed the presence of microplastics in all the conchs sampled. Various forms of plastic particles were found including fragments (the dominant form), fibres and sheets (the least abundant form). The shape of spheres was observed, but in very low quantities, which is why they were not considered in the abundance results. Conchs from Alacranes Reef and Florida had a higher abundance of microplastics than conch from the Eastern Caribbean sites.

KEYWORDS: Microplastics, conch, *Strombus*

INTRODUCTION

Humans produce hundreds of millions of tons of plastic every year. In fact, every day about one megaton of plastic is produced, enough to make almost 22 trillion water bottles and more than 90 percent of that will never be recycled. As much as 12.7 million metric tons of it ends up in the oceans. Today, plastic is the most prevalent type of marine debris, representing more than 60% of the debris swirling through the oceans. Plastics are not all the same. They are made of different chemical building blocks and have different densities. For example, plastic bags are made of polyethylene, margarine tubs are made of polypropylene. Both are lighter than seawater, consequently those plastics float. Other plastics are heavier; they can descend to the bottom. Most plastics in the ocean, however, break up into very small particles called microplastics (particles smaller than 5 mm) and these belong to a group considered as emerging pollutants (Andrady 2011, Baini et al. 2018, Bosker et al. 2018,). Some plastics are intentionally designed to be small. They are called microbeads and are used in many health and beauty products, such as some cleansers and toothpastes.

Microplastics can be ingested by marine animals and pass up the food chain (Isensee and Valdes 2015, Barrows et al. 2018, Wang et al. 2018). These microplastics can have negative effects on organisms, such as a decrease in reproduction, survival and respiration rates. They can also affect the immune system of organisms. Perhaps the most serious, yet least documented, is the role of microplastics as accumulators and bio-magnifiers of persistent organic pollutants (Andersson 2014, Auta et al. 2017, Hurley et al. 2018) such as insecticides (DDT), aromatic hydrocarbons and heavy metals. By ingesting microplastics, animals are exposed to these contaminants and they can accumulate in their tissues (Andersson 2014).

The risk of microplastics to human populations is still not known, but they have a potential risk due to their pervasive and persistent nature. The presence of microplastics in marine foods consumed by humans, such as oysters and mussels has already been documented and considered to have a negative effect on human health (Van Cauwenberghe and Janssen 2014, Waite et al. 2018).

In the Caribbean region, few studies have been carried out to quantify microplastics (Bosker et al. 2018), but information on the concentration of these particles and their effects is important. Investigating contamination by microplastics in marine organisms usually involves the sacrifice of organisms. This is not optimal for species at risk or for bioethical issues. The analysis of feces allows resolution of this issue since it does not involve harming the organism being investigated (Nelms et al. 2018). The use of feces has increased in the study of terrestrial organisms (Castillo et al. 2005, Wasser et al. 2010), as well as aquatic organisms; e.g., for determining stress levels in fish (Turner et al. 2003, Lupica and Turner 2009), and assessing reproductive function in whales and dolphins (Rolland et al. 2005). The present study was carried out using feces samples to determine the relative levels of pollution by microplastics across the wider Caribbean using the mollusc, queen conch (*Strombus gigas*) as an indicator without sacrificing any conchs.

METHODS

Sampling Sites

Queen conch feces were sampled from four locations across the wider Caribbean including the Florida Keys (USA), Alacranes Reef on Campeche Bank in the Gulf of Mexico (Mexico), and two sites in the eastern Caribbean: Guadeloupe (French West Indies) and Barbados (Table 1).

Up to seven conch adults (shell lip thickness ≥ 6 mm) were collected at each of the sampling sites. Specimens of queen conch were collected by free diving, picking up the animal by the shell, and bringing it to the water surface. Each conch was then placed in a separate aquarium (approx. 40 l volume) filled with sea water from the collection site. The animals were left in the aquaria for 4 to 6 hours, during which time feces from each animal were collected periodically by syringe and placed in a test tube. The feces samples were then sent to the Center for Research and Advanced Studies (CINVESTAV), Merida, Yucatan, Mexico, where they were frozen at -20 °C until processing. After sample collection, all conchs were returned to their habitat.

Plastic Extraction

Frozen samples were thawed at room temperature. In the laboratory, the methods recommended by Hidalgo-Ruz et al. (2013) and Masura et al. (2015) were followed. To avoid contamination of the samples, all the equipment and glassware was rinsed three times with 96 percent alcohol, and only glassware and cotton coveralls were used. One gram of feces from each conch sample was subsampled and mixed with peroxide (30%) and then left for 48 h to eliminate organic matter. This mixture was then stirred manually and allowed to settle. Subsequently, with the use of a glass Pasteur pipette, the peroxide was removed and replaced. Particles were separated from the sediment via floatation, using a solution of sodium chloride at a concentration of 1.12 g cm^{-3} to re-suspend the microplastics (Hidalgo-Ruz et al. 2013, Masura et al. 2015). Five milliliters of the solution were then taken and filtered through a 0.22 um membrane (Millipore durapore). To remove excess NaCl from the filter, 5ml of distilled water was then passed through the filter.

After filtration, the filters were checked to quantify the abundance and diversity of the microplastics using a Leica Zoom stereoscopic microscope at 40 X magnification. At this magnification it is possible to detect pieces up to 0.001 mm (Waite 2018). The microplastics found were classified into fragments, fibers, sheets and spheres particles following the identification criteria of Hidalgo-Ruz et al. (2013).

RESULTS

All conch analyzed from the four sites across the wider Caribbean had microplastics in their feces. These microplastic particles were different shapes and sizes. Figure 1 shows the various shapes of microplastics observed with optical microscopy and scanning electronic microscopy. In the samples, after digestion of the organic matter it is possible to observe microplastic particles as well as other particles like the calcareous fragments of molluscs, corals or silicon in the diatoms. Thus it is important to use scanning electron microscopy to discriminate between these particles correctly. Conchs from the northwestern sites in the wider Caribbean (Florida Keys and Alacranes Reef) had the highest overall abundance of microplastics in their feces compared with those from the eastern Caribbean sites (Barbados and Guadeloupe) (Figure 2). However the most frequent microplastic particle types were the same across all sites, with fragments being the most abundant and sheets being the least abundant (Figure 2). The size of the fibers varied between 300 and 4500 µm and fragments between 100 and 700 µm.

Table 1. Geographic position of sampling sites of conch, Strombus gigas in the Caribbean used from microplastics analysis.

Site and GPS position	Number organisms	Depth	Substratum
Alacranes reef 22° 22’58’’ N 89° 40’ 58’’ O	7	3 m	Sandy plain with patches of coral, coarse sand with fragments of coral
Barbados 13° 06’ N 59° 38’ O	5	-	-
Florida / 24° 39’ 33.4074’’ N -81° 0’ 24.23’’ O	5	7 m	Seagrass area (Thalassia sp.) with sand and rocks.
Guadalupe, FWI / 16° 19’ N 61° 06’ O	3	-	Seagrass area (Thalassia sp.) with sand

CONCLUSION

Feces have been used as a biological matrix to study hormone levels in aquatic organisms, to provide valuable data on reproductive biology and social behavior, as well as to monitor the health and stress of captive organisms and thus improve the management of their care (Amaral, 2010). Feces have also been used in conch feeding studies (Serviere, et al. 2009). In the current study, feces were found to be highly suitable for measuring comparative levels of contamination by microplastics, without sacrifice of the indicator organisms. Thus, this method is a very useful alternative to tissue or gut analysis, especially when working with protected species

All conchs analyzed in this investigation exhibited contamination by microplastics. Furthermore, a gradient in the concentration of microplastics was observed with the

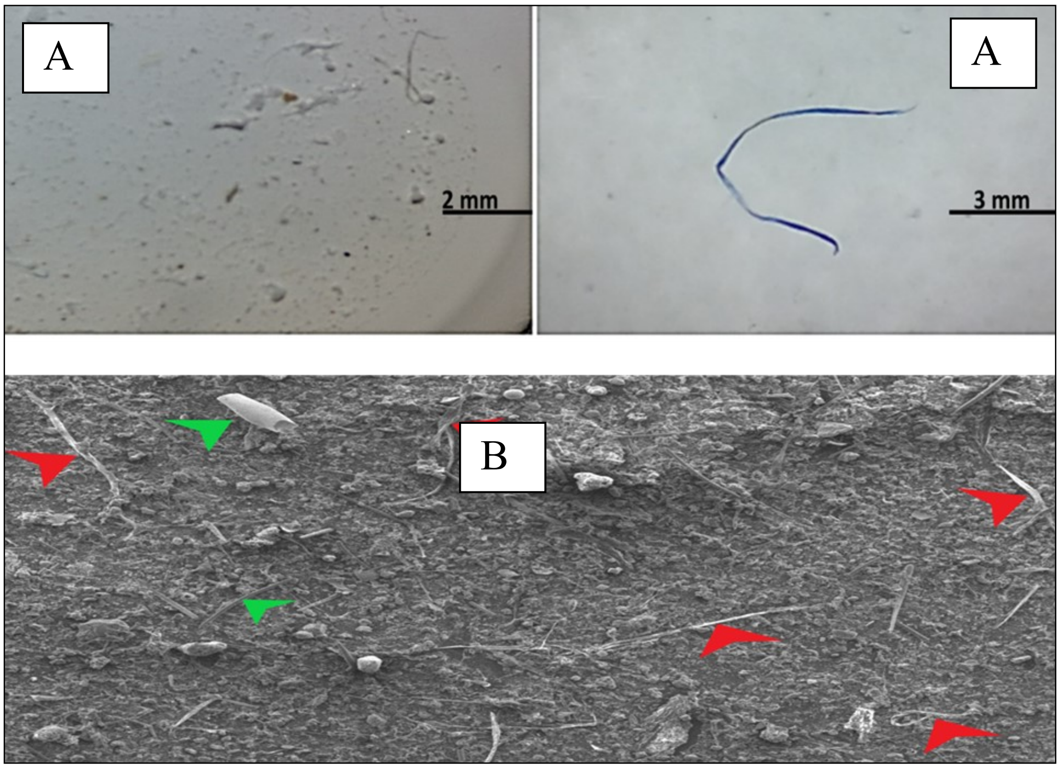

Figure 1. Various shapes of microplastics observed in queen conch feces using A) optical microscopy and B) scanning electronic microscopy. Red arrows show microplastics and green arrows show inorganic structures such as diatoms and calcareous fragments.

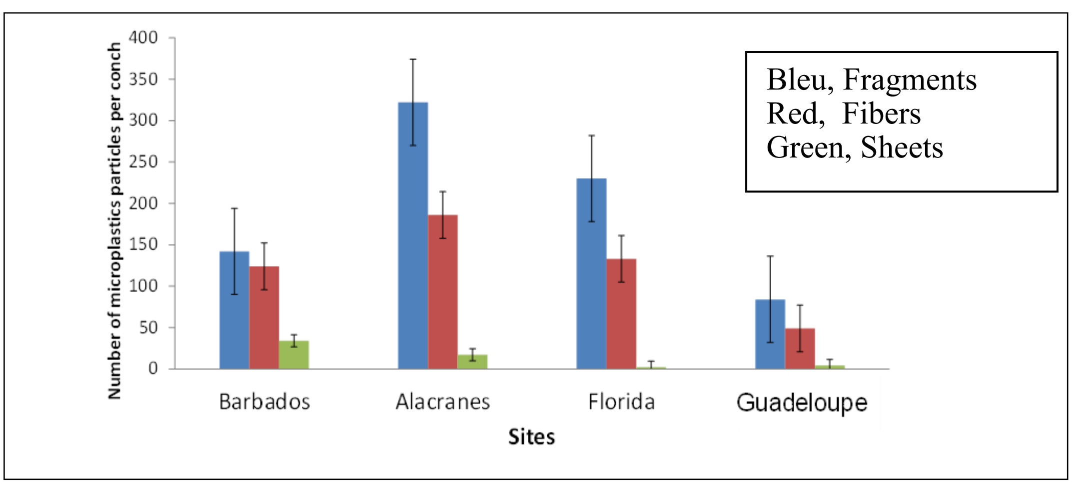

Figure 2. Comparison of the abundance of various shapes of microplastics found in feces of the queen conch, *Strombus gigas* across the five sites sampled in the wider Caribbean.

northwestern Caribbean sites being highest and eastern sites lowest. Similar results are presented by Beckwith et al. (2018). Given that microplastics contain bisphenol A (a known endocrine disrupter, Cole 2011), the higher concentrations seen in the northwestern sites could be contributing to the low reproductive activity of conch from nearshore Florida (Delgado et al 2004). However, the Florida samples in our study came from an offshore spawning aggregation, so further testing nearshore is needed to confirm this assertion. Future research should investigate seasonal variation in microplastics, as well as their abundance in relation to distance from the coast, rivers and other possible sources. The effect of microplastic concentration on reproductive activity and larval development and their distribution should also be considered.

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