Plastic ingestion and trophic transfer between Easter Island flying fish (*Cheilopogon rapanouiensis*) and yellowfin tuna (*Thunnus albacares*) from Rapa Nui (Easter Island)\(^*\)

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**A B S T R A C T**

Millimetre-sized fragments have been documented in many fish species, but their transfer through food webs is still poorly understood. Here we quantified and described plastic fragments in the digestive tracts of 43 Easter Island flying fish (*Cheilopogon rapanouiensis*) and 50 yellowfin tunas (*Thunnus albacares*) from coastal waters around Rapa Nui (Easter Island) in the South Pacific subtropical gyre, and of fish preyed upon by *T. albacares*. Overall, seven *C. rapanouiensis* (16%) individuals had ingested microplastics, most of which resembled the common planktonic prey of the fish. One microplastic was found in the gut of a fish ingested by a tuna, which indicates that trophic transfer may occur between tuna and prey. A single *T. albacares* (2%) had ingested five mesoplastics (15.2–26.3 mm) that were probably not mistaken for prey items, but rather accidentally ingested during foraging on fish prey. The absence of microplastics in *T. albacares* suggests that such small particles, if transferred from the prey, do not accumulate in the relatively large digestive tract of large predators. On the other hand, larger plastic items may accumulate in the gut of tunas, to which they may induce deleterious effects that still need to be examined. However, only a small portion of the fish had ingested mesoplastics. The results of this study suggest that microplastic contamination is not an immediate threat to large predatory fish, such as *T. albacares*, along the coast of Easter Island within the South Pacific subtropical gyre.

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1. **Introduction**

Small plastic particles (<5 mm) known as microplastics (Arthur et al., 2009) have accumulated in the ocean at a global scale. First reported by Carpenter et al. (1972), these particles are now common in the water column (Lusher, 2015), at the sea surface (Erikson et al., 2014; Thompson, 2015), and on the shore (Browne et al., 2011) worldwide. Microplastics are manufactured as pellets used in plastic industry, or scrubbers in cosmetics, and can be directly released into the ocean through sewage. Microplastics can also originate from the fragmentation of larger plastic debris through photo-degradation, physical alteration, or by organisms accidental biting (Andrady, 2015). Whereas the hazards of large plastic debris on marine organisms have been well documented (Derraik, 2002), the impacts of millimeter-sized plastic fragments on marine biota are still poorly understood.

Microplastics have been found in fish from most marine environments (reviewed by Lusher, 2015), but the proportion of individuals containing microplastics varies greatly among the different studies. For example, microplastics were found in only...
0.3% of the examined individuals of 21 fish species from Australian coastal waters (Cannon et al., 2016), whereas >97% of the individuals from 27 species in Chinese fresh and coastal waters had small pieces of plastics in their guts (Jabeen et al., 2017). Planktivorous fish are likely to capture microplastics that are similar in size, shape and colour to their prey (e.g. Hipfner et al., 2018; Ory et al., 2017; Vendel et al., 2017), whereas large predatory fish are unlikely to target microplastics, which are substantially smaller than their natural prey. However, microplastics have been found in the digestive tracts of piscivorous fish in the North Sea (Foekema et al., 2013) and in the Mediterranean Sea (Güven et al., 2017), as well as in top predators from the Mediterranean Sea (Roméo et al., 2015), suggesting that the microplastics were transferred from the prey of these large predators.

Few studies have focused on the transfer of microplastics to higher trophic levels, even though this hypothesis is frequently mentioned (Hipfner et al., 2018; Lusher, 2015; Lusher et al., 2017; Tosetto et al., 2017; Wright et al., 2013). In a laboratory study, Murray and Cowie (2011) identified microplastics in the gut of Norway lobsters (Nephrops norvegicus) fed with fish that had previously ingested microplastics, suggesting vertical transfer of the plastic particles. Farrell and Nelson (2013) showed that digestive tracts and hemolymph of shore crabs (Carcinus maenas) contained microplastics initially ingested by blue mussels (Mytilus edulis) on which the crabs preyed upon. Recently, microplastics were found in fish-feeding cetaceans (Lusher et al., 2018), and in captive grey seals (Halichoerus grypus) that probably got microplastics through their fish food, the Atlantic mackerel (Scomber scombrus), providing evidence for trophic transfer of microplastics to marine top predators (Nelms et al., 2018).

Plastic can be transported at the surface of the ocean over long distances by winds and currents before finally accumulating as microplastic fragments within the subtropical gyres (Eriksen et al., 2014). Microplastics are abundant at the surface of the coastal waters around Rapa Nui (Easter Island), near the centre of the South Pacific Subtropical Gyre (Eriksen et al., 2013; Ory et al., 2017). Risk of microplastic ingestion by planktivorous fish and their potential transfer to higher trophic level fish are therefore enhanced in this area. Herein, we quantified and described plastics in the digestive tracts of yellowfin tuna, Thunnus albacares (Scombroids), and its common prey, the Easter Island flying fish, Chelidonoptera rapanouiensis (Exocoetidae), captured along the coast of Rapa Nui. When possible, microplastics were also examined in the digestive tracts of prey found in T. albacares’ gut. We compared microplastics found at both trophic levels to evaluate the possibility of trophic transfer of microplastics from the prey to the predator.

2. Material and methods

2.1. Sampling of fish

A total of 43 C. rapanouiensis individuals with undamaged digestive tracts were captured at night by angling with a transparent monofilament nylon line within 2 miles of the Rapa Nui coast by local fishermen, in April 2015 (n = 6 fish individuals), November 2015 (n = 18), and April 2016 (n = 19). Entire fish were frozen for further laboratory analyses. Fish were weighed (nearest 0.1 g), measured (fork length, FL to the nearest cm), and their digestive tract (oesophagus, stomach and intestine, termed “gut” throughout the document) was extracted at the Universidad Católica del Norte in Coquimbo in Chile. A total of 50 T. albacares with undamaged guts were caught by local fishermen from small vessels in August 2016 (n = 26) and April 2017 (n = 24) by angling with a transparent monofilament line. Entire C. rapanouiensis were used as bait; all were recovered by the fishermen after having captured a tuna to be used again. The flying fish found in the tunas’ stomach and used to examine the presence of microplastics herein had all started to be digested, meaning that they had been eaten some time before the tuna were captured. Pictures of T. albacares were taken at the harbour, just after landing, in order to determine their fork length (to the nearest 5 cm) with ImageJ. Digestive tracts (oesophagus, stomach and intestine) or stomachs were removed in the harbour by the fishermen, placed in individual sealed Ziploc bags, and transported to the laboratory in Rapa Nui within 2 h. There, the outside part of the gut was thoroughly rinsed with water sieved through a 100-μm sieve to remove potential microplastics coming from the tools of the fishermen that were not sterilized. Guts were then stored in plastic jars filled with 70% ethanol to be analysed at the Universidad Católica del Norte in Coquimbo in Chile.

2.2. Analysis of gut contents

The digestive tracts of C. rapanouiensis (n = 43) and T. albacares (50 stomachs and 32 intestines) individuals were analysed following the method adapted from Ory et al. (2017); all organs were cut open longitudinally with dissecting scissors and their fullness was visually evaluated using five categories: 0 = empty guts or with only trace of digested matter without any identifiable prey, 1 = gut 1–25% full, 2 = gut 26–50% full, 3 = 51–75% full, and 4 = gut >75% full. Stomach and intestine contents were carefully removed from the digestive tract using a wash bottle with water filtered through a 100 μm sieve, and placed in two separate Petri dishes. Work surfaces, dissecting tools and Petri dishes were carefully washed before and between the analyses of fish guts. Nitrile gloves and white cotton coats were worn during the entire lab work. Petri dishes filled with distilled water filtered through a 50 μm sieve were placed next to the sample being analysed to check for potential local contamination by airborne fibres or other particles. No clean airflow chamber was available during collection of fish guts at Easter Island, and dissection of the guts and the analysis of their content in Coquimbo. We consider that these measures are essential to prevent the contamination of the samples by airborne fibres (Foekema et al., 2013; Hermsen et al., 2017), and thus did not count fibres herein. Stomachs and intestines were separated at the pylorus junction, weighed (nearest 0.1 g), photographed and measured to the nearest mm with ImageJ. When visible on the picture, the external diameter of the pylorus was measured to the nearest mm.

Gut contents were placed in 10-cm glass Petri dishes and visually examined under a Carl Zeiss Stemi 2000-C dissection microscope with a magnification ranging from 6.5X to 50.0X to check for the presence of microplastics. Petri dishes were covered whenever they were not being examined to prevent contamination from airborne particles. Gut contents were not chemically digested so that natural food items consumed by the fish could be analysed. Prey were identified to general taxonomic groups: fish, crustaceans, gastropods, cephalopods, bivalves, nematodes, and conserved in 95% ethanol. Fish prey found in the gut of T. albacares were identified to the lowest taxonomic level possible. When the gut of these fish prey was still intact, their content was removed following the same method as described above, and analysed for the presence of microplastics. In total, the entire guts of three fish found in the stomach of three different T. albacares were examined: one Emmelichthys karnellai (fork length = 29 cm), and two unidentified fishes (fork length = 20 and 23 cm).

2.3. Description of microplastics

Microplastics were characterized using the features described in
Ory et al. (2017): type (hard or soft fragment, thin film, thread [diameter >0.1 mm], pellets), hardness (hard, soft but firm, brittle), dominant colour (>50% of the particle surface), and edge sharpness (smooth or angular). A picture of each particle was taken using a Canon Powershot SX210 IS with a 35-mm adaptor fixed to the microscopic binoculars. Particle size was measured to the nearest 0.1 mm with the program ImageJ. Microplastics were stored in 5 mL Eppendorf tubes filled with 95% ethanol to be analysed. Fibres (diameter ≤ 50 μm) were not recorded in this study due to the high risk of contamination from airborne fibres during gut collection on Easter Island, and gut dissection and analysis in Coquimbo. Indeed, one to five fibres were repeatedly found in the control Petri dishes.

2.4. Polymer analysis

Selected particles found in the digestive tract of the fish were analysed by infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR) for particles ≥ 200 μm and in transmittance mode (µ-FTIR) for samples < 200 μm. The spectra in reflectance mode (ATR) were acquired using an Agilent Handheld 4300 FTIR Spectrometer with a DTGS detector, with controlled temperature, and a diamond ATR sample interface; the analyses were performed at the sample surface. Transmittance spectra were obtained with a Nicolet Nexus spectrophotometer equipped with a Continuum microscope and a MCT-A detector cooled by liquid nitrogen; the analyses were performed in micro-samples previously compressed with a Thermo diamond anvil cell (DAC). All spectra were obtained with a resolution of 4 cm⁻¹ and 32 scans (FTIR-ATR) or 128 scans (µ-FTIR). Spectra are shown as acquired, without any further manipulation except for the baseline correction and removal of the CO₂ absorption at approximately 2300–2400 cm⁻¹ (µ-FTIR). Identification of all polymers were based on the presence of specific absorption bands (see Table S1). The identification of the samples was not only relying on the match between the sample and the library, which does not include all the polymer mixtures neither degraded polymers or copolymers, but was based on best expert judgment from the presence of specific absorption bands.

2.5. Statistical analysis

Data for which measures of central tendency are reported were tested for normality using the Shapiro-Wilk normality test. As most data were not normally distributed, we reported non-parametric median ± median absolute deviation (MAD) for all data to facilitate interpretation. All statistical analyses were conducted in R version 3.2.4 (www.cran.r-project.org/an).

3. Results

3.1. Plastic in C. rapanouiensis

Fifteen potential microplastics were visually isolated from C. rapanouiensis guts, 11 of which were confirmed to be plastic by FTIR spectroscopy. Three of the remaining four particles were inorganic and one was organic; all four were not considered for calculations (frequency of occurrence and average numbers) of plastic contamination in the fish. One to three microplastics were found in seven (16%) out of the 43 C. rapanouiensis examined (Table 1 and Fig. 1a and b), with a median number of 1.5 ± MAD 0.7 microplastics per fish that had ingested at least one fragment. Five C. rapanouiensis had ingested only one microplastic, and two fish had ingested three microplastics each. Eight (73%) of all 11 microplastics were found in the stomach of five fish, and three in the intestine of three fish. No microplastics were found in fish with empty guts (n = 4).

The size of the 11 microplastics identified in C. rapanouiensis varied between 0.1 and 2.1 mm (median = 0.6 ± 0.6 mm). Eight of all microplastics ingested were blue (Fig. 1a and b and Table 2), and three were black (Table 2). All microplastics were hard fragments or flake-like fragments. Infrared spectroscopy of the 11 microplastics revealed that six were flakes composed of polyvinyl acetate (PVAc), commonly used in paints and various coatings, three were polyethylene (PE), widely used in many applications, and two were polyester, known to be used as a fibreglass laminating resin, for instance, in boats and car parts (Table S1 and Fig. S2).

3.2. Plastics in T. albacares and their prey

Five particles visually identified as potential microplastics were isolated from T. albacares guts. Two hard fragments (green and blue; Fig. 1c and d), ranging from 0.4 to 0.6 mm, were in one individual T. albacares; these fragments had spectra similar to those of composites based on polyamide, but could not be fully confirmed to be plastics. In addition, one white paint-like flake (3.0 mm) was also found in the same fish (Fig. 1e), but was lost during manipulations before its chemical composition could be analysed with infrared spectroscopy. For conservative reasons, these three particles were not included in the quantitative analyses. No microplastics were found in fish with empty guts (n = 3). The two remaining particles were discarded from the analysis because one was inorganic (non-plastic) and the other organic.

The digestive tract of the one individual E. karnellai (Emmelichthyidae) preyed upon by a T. albacares did not contain any organisms nor microplastics. Two unidentified fish were found in two other T. albacares. One of these fish had three particles in its stomach: the particles were blue on one side and white on the other side (Fig. 1f), and seemed to have originated from the same, larger piece. The size of the largest fragment was 0.1 mm. They looked similar to paint fragments also found in water samples from the same area (Nicolas Christian Ory, Martin Thiel, personal observation), but could not be analysed by spectroscopy because they were too brittle to be manipulated. The gut of the other unidentified fish was full, but did not contain microplastics.

Five mesoplastics, ranging from 15.2 to 26.3 mm (median = 22.9 ± 1.9 mm), were found in one individual T. albacares (Table 1): two were polypropylene (PP) green threads (Fig. 2d and S2), similar to individual threads from ropes used by local fishermen for mooring, but different to the transparent monofilament lines used to fish tunas. The three other mesoplastics were hard fragments that differed in colour and chemical composition: one black and one dark-green polypropylene fragment (Fig. 2a,c), and one pale-green polyethylene fragment (Fig. 2b). The size of three of these five mesoplastics exceeded the diameter of the pylorus (20 mm) of the individual that ingested them and of the median pylorus diameter of other T. albacares (15 ± 4 mm; n = 15) examined in this study.

3.3. Diet of C. rapanouiensis and T. albacares

The diet of C. rapanouiensis was mainly composed of small zooplankton organisms. Remains of small fish (scales, fin rays, spines and vertebrae) were present in 22 (51%; Table 3) of the 43 fish analysed, parts of crustaceans (shell fragments, appendages, antennae) were found in 21 individuals (48%), and shell fragments of molluscs (mostly pteropods) were identified in 15 individuals (35%). Nematodes, probably parasitic, were also found in the guts of 26 individuals (61%). The stomachs of 21 individuals (48%) contained only digested matter in which no prey could be identified. Fish remains (flesh, scales, fin rays, spines, vertebrae, and eye lenses) were found in 41 (82%; Table 3) of the 50 T. albacares
analysed, which confirms that the tunas captured along Rapa Nui were mostly piscivores. Remains of *C. rapanouiensis* were also found in five (10%) *T. albacares*, which confirms that this species is a common prey of *T. albacares*. Entire individuals and parts (e.g. exoskeleton fragments, appendages, antennae and eyes) of zooplanktonic organisms were present in 26 of all individuals (52%); molluscs (mostly cephalopod soft parts and mandibles) were found in 18 (36%) of the 50 individuals.

4. Discussion

4.1. Microplastics in *C. rapanouiensis*

Herein, 16% of the *C. rapanouiensis* captured around Rapa Nui had ingested microplastics, which confirms microplastic ingestion by planktivorous fish species in this area (Ory et al., 2017). This proportion falls within the range reported for other planktivorous species in the North Pacific Central Gyre (NPCG) (35%, Boerger et al.,...
Microplastic ingestion by visually-oriented planktivorous fish has been reported worldwide (Lusher, 2015); such fish might be particularly susceptible to ingest microplastics in the oligotrophic waters of oceanic gyres where small plastic fragments are abundant and can constitute a large part of the nekton (Moore et al., 2001).

A majority of microplastics found in the guts of C. rapanouensis were blue, as also found in juvenile Decapterus muroadsi, another planktivorous fish species from Rapa Nui that preferentially ingested microplastics similar to its common copepod prey (Ory et al., 2017). Flying fish also commonly feed on copepods (Carpenter and Niem, 1999; Van Noord et al., 2013), although, here, no copepods could be identified in C. rapanouensis, probably because they already had been digested when the fish were captured. However, the size of the microplastics (0.1–3.1 mm) ingested by C. rapanouensis was overall similar to that of zooplankton, suggesting that C. rapanouensis has mistakenly ingested microplastics that resemble their prey.

Fibres have often been found to be the most common synthetic particles in fish (e.g. Collard et al., 2018; Jabeen et al., 2017; Mizraji et al., 2017). However, the prevalence of fibres in the samples may often be overestimated when the collection and the analysis of the samples are not conducted under strictly controlled clean air (Hermansen et al., 2017). Fibres were not recorded herein because of the high risk of airborne contamination, which was confirmed by repeated findings of one to five fibres in the blanks placed adjacent to the samples analysed. No other synthetic material was found in the blanks; we are thus confident that all microplastics found in the samples were ingested by the fish.

### Table 3
Frequency of occurrence of different food items in the digestive tracts of C. rapanouensis (n = 43) and T. albacares (n = 50) which had ingested microplastic or not.

<table>
<thead>
<tr>
<th>Food items</th>
<th>Frequency of occurrence (% of total fish)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish with no plastic</td>
</tr>
<tr>
<td>C. rapanouensis (total number of individuals)</td>
<td>36</td>
</tr>
<tr>
<td>Fish parts (spines, scales, eyes, vertebrae)</td>
<td>44.4</td>
</tr>
<tr>
<td>Crustaceans fragments</td>
<td>44.4</td>
</tr>
<tr>
<td>Mollusc</td>
<td>30.6</td>
</tr>
<tr>
<td>Pteropod shells</td>
<td>27.8</td>
</tr>
<tr>
<td>Cephalopod beak</td>
<td>0.0</td>
</tr>
<tr>
<td>Bivalve shells</td>
<td>5.6</td>
</tr>
<tr>
<td>Nematodes</td>
<td>55.6</td>
</tr>
<tr>
<td>Undefined items</td>
<td>11.1</td>
</tr>
<tr>
<td>T. albacares (total number of individuals)</td>
<td>49</td>
</tr>
<tr>
<td>Fish</td>
<td>81.6</td>
</tr>
<tr>
<td>Whole</td>
<td>18.4</td>
</tr>
<tr>
<td>Parts (spines, scales, eyes, vertebrae)</td>
<td>75.5</td>
</tr>
<tr>
<td>C. rapanouensis</td>
<td>10.2</td>
</tr>
<tr>
<td>Crustaceans fragments</td>
<td>51.0</td>
</tr>
<tr>
<td>Mollusc</td>
<td>36.2</td>
</tr>
<tr>
<td>Cephalopods shells</td>
<td>4.1</td>
</tr>
<tr>
<td>Cephalopods parts</td>
<td>34.7</td>
</tr>
<tr>
<td>Undefined items</td>
<td>22.4</td>
</tr>
</tbody>
</table>
studies. Consequently, continuous ingestion and egestion of millimetre-sized plastics may threaten top-predator fish via contaminant transfer (Hermabessiere et al., 2017) rather than by physical blockage of the digestive system.

4.3. Mesoplastics in T. albacares

Here, a single T. albacares fish (2%) had ingested mesoplastics 15–26 mm long; a low prevalence of mesoplastics (>5 mm) had also been reported in T. albacares (0% of total individuals) and Thunnus obesus (3%) from the NPCG (Choy and Drazen, 2013), and in Thunnus alalunga in the Mediterranean Sea (0%; Romeo et al., 2015). A higher incidence of mesoplastics was found in Thunnus thynnus in the Mediterranean Sea (Romeo et al., 2015), indicating that mesoplastic ingestion varies between conspecifics inhabiting different geographical areas, and among similar species from the same environment. One reason for such differences might be that the proportion of plastics >5 mm is substantially higher in the Mediterranean Sea than in the open ocean (Cózar et al., 2015), thereby being more available to fish in those waters. On the other hand, T. albacares feeds mostly in the epipelagic layer (Duffy et al., 2017), where most floating plastic are found, meaning that this species should be more susceptible to ingest mesoplastics than other tuna species that forage across a broad vertical range, such as T. thynnus and T. alalunga (Battaglia et al., 2012; Duffy et al., 2017). The factors influencing the ingestion of large plastic fragments by top-predators still need to be clarified.

The mesoplastics we found in a T. albacares were most likely not transferred from its prey because they are substantially larger than what planktivorous fish can ingest. These mesoplastics had therefore probably been accidentally captured by T. albacares. Three of these plastics were hard fragments of similar sizes and shapes, which suggests that the fish misidentified these items as potential prey on several independent occasions. The ingestion of the green threads, which probably come from synthetic ropes used by local fishermen, is more surprising as they do not resemble the typical fish and squid prey of T. albacares; these threads may have been captured with another prey, or may have been transferred after having been ingested by one of the tuna’s prey.

Out of the five mesoplastics found in the T. albacares individual, three exceeded the size of the pylorus, which is likely to prevent them from being egested: mesoplastics may thus have accumulated in the stomach of the fish. Other large predatory species such as Lampris spp. and Alepisaurus ferox were also found to have several mesoplastics in their gut (average 2.8 ± 2.2 items/individuals, Choy and Drazen, 2013), which may have been accumulating in the fish digestive tract for some time. The retention of larger plastic debris can potentially block the digestive tract and induce false satiation, and the irregular edges of some mesoplastics found herein might harm the stomach wall (Wright et al., 2013). Nonetheless, despite potential accumulation of large plastic particles in the digestive tract of tuna, the results of this study indicate a low incidence of plastics in such large predators in the clear waters of the South Pacific Subtropical Gyre.

5. Conclusion

We confirmed ingestion of microplastics by planktivorous fish around Rapa Nui, which are susceptible to be transferred to their predators, such as T. albacares. However, no microplastics were found in the empty stomachs of T. albacares, even though the transfer of plastic may occur between the predator and its prey; microplastics seem to be rapidly egested and do not accumulate in the tunas. In contrast, five mesoplastics were found in one T. albacares, suggesting that plastic fragments that exceed the size of the fish’s pylorus may accumulate in the stomach and harm the fish. Nevertheless, this and other studies found low prevalence of mesoplastics in tunas. More studies are thus needed to determine the actual threat of plastic contamination on top predator fish.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.08.042.

References


