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# Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish☆

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ABSTRACT

Microplastics (<5 mm) have been found in many fish species, from most marine environments. However, the mechanisms underlying microplastic ingestion by fish are still unclear, although they are important to determine the pathway of microplastics along marine food webs. Here we conducted experiments in the laboratory to examine microplastic ingestion (capture and swallowing) and egestion by juveniles of the planktivorous palm ruff, *Seriolella violacea* (Centrolophidae). As expected, fish captured preferentially black microplastics, similar to food pellets, whereas microplastics of other colours (blue, translucent, and yellow) were mostly co-captured when floating close to food pellets. Microplastics captured without food were almost always spit out, and were only swallowed when they were mixed with food in the fish's mouth. Food probably produced a 'gustatory trap' that impeded the fish to discriminate and reject the microplastics. Most fish (93% of total) egested all the microplastics after 7 days, on average, and 49 days at most, substantially longer than food pellets (<2 days). No acute detrimental effects of microplastics on fish were observable, but potential sublethal effects of microplastics on the fish physiological and behavioural responses still need to be tested. This study highlights that visually-oriented planktivorous fish, many species of which are of commercial value and ecological importance within marine food webs, are susceptible to ingest microplastics resembling or floating close to their planktonic prey.


## 1. Introduction

Millimetre-sized plastic fragments are ubiquitous in the world's ocean, where they often represent a major fraction of anthropogenic litter (Law, 2017). Microplastics (<5 mm) are ingested by a wide range of marine organisms (reviewed by Lusher, 2015) to which they can cause deleterious physiological and behavioural effects (e.g. Lusher, 2015; Wright et al., 2013), thereby threatening the integrity of marine ecosystems. Although microplastics have been reported in many fish species from various marine habitats (reviewed by Lusher, 2015), the mechanisms underlying microplastic ingestion still need to be clarified to determine microplastic pathways through marine food webs.

Planktivorous fish feeding on individual prey (particle feeders) use visual cues to detect and identify their prey (Lazzaro, 1987), which they usually capture in a fast and directed attack. Particle feeders are thus susceptible to accidentally target inedible items, such as microplastics, many of which are of similar size, colour and shape as natural planktonic prey (Shaw and Day, 1994; Wright et al., 2013). For example, the planktivorous Amberstripe scad, *Decapterus muroadsi* (Carangidae), selectively ingest blue microplastics resembling their copepod prey in the clear waters around Easter Island in the subtropical South Pacific Ocean (Ory et al., 2017). A laboratory experiment also suggested that the common goby *Pomatoschistus microps* (Gobiidae) ingests microplastics of similar colour as *Artemia* nauplii (de Sá et al., 2015).

Fish feeding on planktonic organisms adjust their attack strategy when foraging on abundant prey (Lazzaro, 1987). Instead of rushing toward a single prey, they approach aggregated prey more slowly, and draw in large volume of water to engulf several prey items at once; microplastics floating among the prey may thereby be accidentally gulped up by the fish. Planktivorous fish inhabiting

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areas where microplastics account for a large part of the plankton, such as coastal waters near urban centres (Lima et al., 2014; Moore et al., 2002) or oceanic waters in the subtropical gyres (Moore et al., 2001), may thus be susceptible to accidentally ingest microplastics when foraging on aggregated prey.

Fish have a highly developed gustatory system that allows them to segregate food from inedible items upon oral uptake (Houlihan et al., 2001; Kasumyan and Döving, 2003; Lamb, 2001). Despite such an advanced sense of taste, microplastics are ingested by many fish species (reviewed in Lusher, 2015), suggesting that some mechanisms impede fish to distinguish inedible items from food particles. The co-occurrence of food together with microplastics in the oral cavity of the fish may result in lower detectability of inedible particles, which may then be swallowed accidentally by the fish.

Once ingested, microplastic fragments may induce deleterious effects to the fish, such as damaging or blocking the digestive tract, or suppressing energy uptake, the severity of which depends on the time the microplastics remain in the digestive tract of the organism (Wright et al., 2013). For example, experiments showed an increase of alterations of the intestinal epithelium in the European sea bass *Dicentrarchus labrax* (Moronidae) in relation to the duration of microplastic exposure (Pedà et al., 2016). The residence time of microplastic fragments in fish is still poorly known (Lusher et al., 2016); some experiments showed that juvenile fish egested microplastics after several hours to a couple of days (Grigorakis et al., 2017; Hoss and Settle, 1990). However, microplastics used in those experiments were spherical, of small (<0.1 mm) and homogeneous size, and probably pass through the digestive tract of the fish more easily than broken plastic fragments commonly found in the environment (Phuong et al., 2016) and in fish guts (Battaglia et al., 2016).

The aim of this study was to examine the ingestion (i.e. capture and swallowing) and egestion of microplastics by juveniles of the palm ruff, *Seriolella violacea* (Centrolophidae). More specifically, we tested the hypothesis that fish would ingest preferentially microplastics (black) that appear to the fish similar as food pellets. We also assessed whether microplastics co-captured with food pellets or not were swallowed or spit out. Furthermore, we determined the gut residence time of the microplastics ingested by the fish, and compared it with that of food.

## 2. Materials and methods

### 2.1. Model species

The palm ruff, *Seriolella violacea* (Centrolophidae), is a gregarious fish commonly found along the Pacific coasts from Costa Rica to Chile, feeding principally on planktonic organisms (Medina et al., 2004). A total of 200 four-months old *S. violacea* juveniles were obtained from the laboratory of fish aquaculture of the Universidad Católica del Norte in Coquimbo, Chile, where the fish were born and reared. Only fish without morphological malformations that could affect their feeding behaviour (e.g. jaw or tail bent) were used in the experiments. All fish were kept in a common 500 L circular green fibreglass tank (diameter = 200 cm) with aerated running water pumped from La Herradura bay nearby. Fish were fed ad libitum twice a day (morning and afternoon) since they were two months old with dark colour Protec™ pellets (length × diameter = 1.2 × 0.8 mm; unit weight ~ $2.10^{-3}$ g; Fig. S1).

### 2.2. Microplastic ingestion by *S. violacea*

Laboratory experiments were conducted from 18 March to 31 April 2016 to examine whether the capture (i.e. the take up into the mouth) and the swallowing (i.e. the passage from the mouth into the digestive tract) of microplastics by juveniles *S. violacea* was related to microplastic colour (treatment with four levels: black, blue, translucent, and yellow). We also recorded whether microplastics were differently captured and swallowed together with food pellets or alone.

Microplastics were obtained from black, blue, translucent and yellow new nylon cable ties (density = 1.2 g $cm^{-3}$) cut into small pieces with surgical scissors. Pieces of similar shape (tubular), length (1.2 ± 0.2 mm), and diameter (1.0 ± 0.1 mm) as the food pellets were chosen under a dissecting microscope to be used in the experiment (Fig. S1). Black microplastics were used to mimic the colour of the food pellets, blue and translucent microplastics were used because these colours are often reported in fish stomachs from the field (e.g. Battaglia et al., 2016; Boerger et al., 2010; Davison and Asch, 2011; Güven et al., 2017; Ory et al., 2017), and yellow microplastics were used to contrast with the microplastics of other colours.

### 2.3. Experimental design and setup

All fish were starved for 12 h before an experimental trial started. Two hours before the beginning of a trial, 10 fish were randomly captured from the common tank with a hand net. For acclimation, two fish together were placed in five separate glass tanks (44 × 30 × 30 cm) filled with 30 L of seawater. Fish were used in pairs because preliminary experiments revealed that solitary fish or fish separated by a mesh in an experimental tank were stressed (i.e. showing dark colouration of the skin, rapid breathing and stationary hovering in the water column with rapid movements of the fins) and did not feed, perhaps due to the gregarious behaviour of this species (Medina et al., 2004). Preliminary experiments also revealed that, generally, one of the two fish actively fed during a trial, whereas the other fish remained mainly inactive. Therefore, only the behaviours of the most active fish (i.e. which ingested >75% of all the food pellets) were used in the analysis to compare the ingestion of microplastics among fish with similar behaviours. Also, trials in which both fish in the same experimental tank swallow at least one microplastic were discarded because the probability that the active fish swallow two microplastics of the same colour was null, unlike in trials when only the active fish swallowed microplastics.

The bottom, the two small sides, and one of the large sides of the experimental glass tank were covered with a green plastic film to reduce visual disturbances from outside of the tank. The green colour of the background was chosen because it was similar to that of the circular green fibreglass tank where the fish were kept before the experiment, and because it contrasted best against the four colours of the microplastics tested in the experiment. Experimental tanks were illuminated with artificial neon tube lights. The average illumination was of 43.6 ± 3.8 μmol $m^{-2}$ $s^{-1}$ (measurement taken for reference with a LI-COR® LI-250A light meter on 12 December 2017at 5 different places within the experimental area).

After 2 h, four food pellets were given to the fish to confirm that fish ate normally and were ready to be used in the experiment, which started when the four food pellets were eaten by one or both fish. Pellets that remained after a minute were carefully removed from the tank with a transfer pipette, and the fish were left alone for one more hour before testing again whether they fed or not on four new pellets. If after three attempts (i.e. 4 h), none of the fish had eaten pellets, they were put back in the common tank to be used another day.

At the beginning of a trial, 10 food pellets and 2 microplastics of one of the four colours were introduced in the experimental tank for the fish to eat. The 5:1 ratio between food pellets and