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.

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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

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 2017 at 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

microplastics used in each treatment was analogous to that between microplastics and the most common prey of planktivorous fish observed in the field (Moore et al., 2001; Ory et al., 2017). Trawl studies integrate across large areas, sometimes over distances of several kilometres, and thereby underestimate high microplastic abundances across narrow oceanic fronts, where microplastics accumulate, and most biological activity occurs (Acha et al., 2015). We therefore consider that the food-microplastic ratio used herein is representative of conditions encountered by many fish species feeding along frontal systems.

During the same trial, the four plastic colours were given to the same fish in a successive and random order (repeated measurements; Fig. S2). Food pellets and microplastics were first soaked for a few seconds in a 20 ml plastic flask filled with seawater, and then gently released all together at the water surface of the experimental tank. All particles quickly sank ($3-4 \, cm \, s^{-1}$; sinking velocity determined during preliminary observations) to the bottom of the tank. During the experiment, fish mostly picked up the food and microplastics while these were sinking or hovering near the bottom of the tank.

During each trial, two observers, placed side by side, observed the fish from the side of the experimental tank not covered by the green plastic film. Each observer focussed on a single fish, randomly assigned before each trial. The two fish of each pair were chosen to slightly differ in size (i.e. <2 cm total length (TL)) so that they could be visually distinguished from one another. Each observer recorded the number of times a fish captured (i.e. took up in the mouth), and spit out or swallowed a microplastic of a specific colour. Observers also recorded whether each microplastic was captured alone (i.e. with no pellet), or with ≥1 food pellets, in a quick sequence (i.e. gobbled up) or at the same time (i.e. gulped up) as the pellets. The number of food pellets captured and spit out or ingested was also recorded. During the experiment, fish mostly picked up the food and microplastics while they were sinking or hovering near the bottom.

If after 5 min, or when all the 10 pellets were consumed by the fish, at least one microplastic remained, another 10 food pellets were introduced in the tank to give the fish the opportunity to ingest the remaining microplastics for five more minutes. When the two microplastics were ingested by the fish, or after a maximum of 5 min, all remaining pellets and/or microplastics were gently removed from the experimental tank using a transfer pipette. The next of the four colour treatments was then started. A maximum of 80 pellets (20 pellets × 4 treatments) and 8 microplastics (2 microplastics × 4 treatments) were used during each trial.

In total, 33 fish were used to analyse microplastic capture, and 29 fish were used to analyse microplastic swallowing (four of the 33 fish did not swallow microplastics). Two additional trials during which both fish in the same experimental tank swallowed at least one microplastic were discarded; these four fish were nevertheless used in the experiment to examine microplastic gut residence time (see below).

2.4. Monitoring of microplastics gut residence time

All of the fish that ingested at least one microplastic were removed from the experimental tanks with a hand net, and placed in translucent plastic tanks (30 × 30 × 30 cm) filled with 30 L of aerated seawater to determine the time required for fish to egest microplastics. All fish that had not ingested microplastics during the experiment were returned to the aquaculture centre of the Universidad Católica del Norte; they were not further used in this study.

Fish with microplastics were monitored daily and maintained until they egested all the microplastics. Two fish that did not egest

all their microplastics after a maximum of 49 days (10 weeks) were killed by quickly snapping their neck to recover the remaining microplastics; these two fish and two other which died before the end of the experiment (see below), were not used to analyse the gut residence time. The water of each monitoring tank was filtered twice a day (10:00 in the morning and 17:00 in the afternoon) using a flexible PVC tube (diameter = 2 cm) connected to a sieve with a 0.1 mm mesh to check for the presence of egested microplastics. The day a microplastic was recovered was used to estimate the maximum time (in days) the microplastic remained in the fish. Fish were fed with 30 food pellets 5 min after the water had been filtered.

2.5. Food digestion time

The time required for fish (not used in the experiments above) to digest food pellets was determined experimentally to be compared with that of microplastics. Nine fish ($15.0 \pm 1.0 \, cm \, TL$) were collected with hand net from the common tank and placed in individual 30 L translucent plastic tanks (30 × 30 × 30 cm) filled with aerated seawater tanks. Fish were starved for 48h and were then fed with 30 pellets each. Twice a day (morning and afternoon), tanks were checked for the presence of faeces. Fish were not fed during the experiment, which ended when no faeces were found for 48 h.

2.6. Statistical analyses

We tested the null hypothesis that the number of times fish captured and (i) swallowed or (ii) spit out food pellets did not vary among the four treatments (microplastic colour) of a trial using two different exact Kendall non-parametric tests for related samples in SPSS version 21.

Two Generalized Linear Mixed Models (GLMM) were developed with the package 'lme4' (Bates, 2010) in R (3.4.3) to test the null hypotheses that (i) the number of times fish captured a microplastic, and (ii) the total number of microplastics swallowed by fish were independent of microplastic colour (i.e. a fixed factor with four levels: black, blue, translucent, and yellow), and whether microplastic was co-captured with or without food (i.e. a categorical covariate with two levels: presence or absence of food pellets). Fish individuals were included in the model as a random factor to account for response variability among fish. The sequence in which the differently coloured microplastics were offered (repetitions) was also included as a random factor to account for the possible influence of repetitions on the response variables. The effects of the fixed factors and their interaction were tested with a Wald chi-square test at the conservative α-level error of 1%. For each dependent variable tested, the model with the lowest Akaike information criterion corrected for small samples (AICc) was chosen to best fit the distribution of the data (Pan, 2001). Randomness and normality of the residuals of the model was assessed graphically to verify the validity of each final model (Chang, 2000; see supplementary materials).

The first GLMM (to analyse microplastic capture) was best-fitted with a negative binomial distribution linked with a log function. The model included the interaction between microplastic colour and food presence, and fish as a random factor. The sequence of the colours was removed from the model, because it did not cover a significant part of the unexplained variation. The second GLMM (to analyse microplastic swallowing) was best fitted with a Poisson distribution linked with a log function; the interaction between microplastic colour and food presence, and the sequence of microplastic colours were not included in the model as they did not cover a significant part of the explained variation. Here, however,

data were unbalanced as the number of microplastics swallowed was only analysed for those that were previously captured; GLMM were specifically preferred over General Estimating Equation because they are more robust to unbalanced data (Omar et al., 1999).

The null hypothesis of no difference in the gut residence time among fish that ingest 1, 2, or 3—5 microplastics (pooled together) was tested with an exact non-parametric Kruskall-Wallis test of rank. All the means and their standard errors ($\alpha = 0.01$) were estimated in SPSS version 21 using bootstrapping method (i.e. random sampling with replacement; see Quinn, 2002) with 1000 resamplings, if not indicated otherwise.

3. Results

3.1. Capture and swallowing of food pellets

Fish ($13.8 \pm SE$ 0.2 cm TL; 31.6 ± 1.5 g, n = 33) ingested a total of $63.3 \pm SE$ 2.8 food pellets per trial, with no difference in the number of food pellets ingested (16.2 ± 0.5) among the different treatment levels (i.e. microplastic colour) of a same trial (W = 0.02, df = 3, p = 0.66). Fish spit out food pellets 8.3 ± 2.5 times per trial, with no difference among the four treatment levels of a same trial (W = 0.02, df = 3, p = 0.62).

3.2. Microplastic capture

All 33 fish captured at least once one or more black microplastics (same colour as pellets), whereas 68%, 55%, and 45% of the fish captured at least one blue, yellow, or translucent microplastic, respectively (Table S1). For the frequency at which microplastics were captured by each fish, the statistical modelling revealed a significant interaction between microplastic colour, and whether the microplastics were captured together with food or not (p < 0.0001; Table 1a and Fig. 1). Furthermore, there was a significant main effect of microplastic colour; each fish captured more often black microplastics (average = $6.8 \pm SE$ 0.9 occurrence per fish) than microplastics of any other colour (Fig. 1, Table S1 and Appendix B in supplementary materials). Microplastics were also captured differently with or without food; black microplastics were captured more often without (4.9 ± 0.9 capture events per trial) than with (1.9 ± 0.3 capture events per trial) food pellets, whereas microplastics of other colours were almost always co-captured with pellets (Fig. 1 and Table S1). Microplastics and food pellets were mostly co-captured in a single gulp (70% of all capture events) than in a quick sequence.
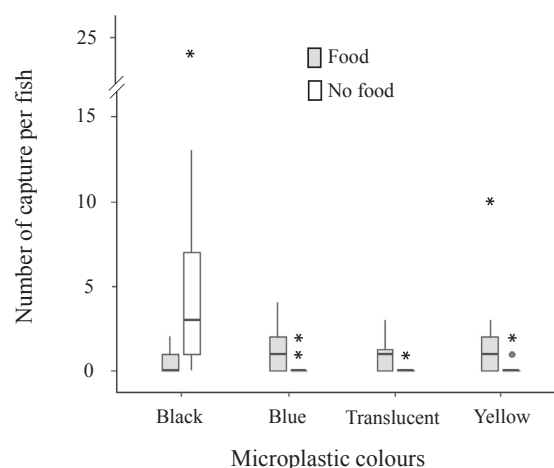

Fig. 1. Number of microplastics of different colours captured by a fish during a trial together with (food) and without food pellets (no food). The horizontal lines inside the boxes represent the median; boxes and whiskers extend from the 25th to the 75th percentile and from the 10th to the 90th percentiles, respectively; circles represent outlier cases with values between 1.5 and 3 times the interquartile range (IQR); asterisks represent extreme values > 3 times the IQR.

3.3. Microplastic swallowing

Twenty-nine of the 33 fish (88%) tested in the experiment swallowed one to five microplastics out of the eight available (two microplastics of each of the four colour treatments) during a trial (Table S2). Microplastics were swallowed depending on whether they were captured with food or not (p < 0.0001; Table 1b), but not depending on their colour; fish swallowed more often microplastics that were co-captured with food (Fig. 2). Most of the microplastics captured without food pellets (96%) were spit out rather than swallowed by the fish, independently of their colour (Fig. 2 and Table 1b, S2 and S3). About half of the microplastics captured with food were spit out (52%), while in the other half of cases they were swallowed (48%).

3.4. Microplastic gut residence time

Two fish of the 33 fish analysed (29 fish that ingested a microplastic during the experiment + 4 fish from 2 experimental pairs in which both fish ingested microplastics; see methods above) died before the end of the experiment, one of unknown cause, and the other of fungus or bacterial infection of the tail. Twenty-nine of the

Table 1
Effects of microplastic colour and the presence of food on (a) the frequency of capture (fitted with negative binomial distribution linked with log function; AICc (intercept-only model) = 835.1; AICc (best-fitted model, full factorial model with colour as fixed factors and food as categorical covariate, and fish as random factor) = 602.2, n = 33), and (b) the swallowing (fitted with Poisson distribution linked with log function; AICc (intercept-only model) = 215.6; AICc (best-fitted model, categorical covariate: food, random factor: fish) = 167.6, number of fish = 29) of microplastics. Values in bold are significant at the α-level error of 1%. See appendix B for the details of the models.

Parameters	Wald chi-square (Type III)	df	p values
a) Microplastic capture			
Intercept	**12.7**	**1**	**0.0003**
Microplastic colour	**24.6**	**3**	**<0.0001**
Presence of food	**17.3**	**1**	**<0.0001**
Microplastic colour × presence of food	**39.3**	**3**	**<0.0001**
b) Microplastic swallow			
Intercept	0.9	1	0.33
Microplastic colour[a]	1.55	3	0.67
Presence of food	**28.93**	**1**	**<0.0001**

[a] Factor not included in the final model. Displayed for information. Values obtained from an initial model including the two main effects of the fixed factors 'microplastic colour' and 'food', and 'fish' as random factor.
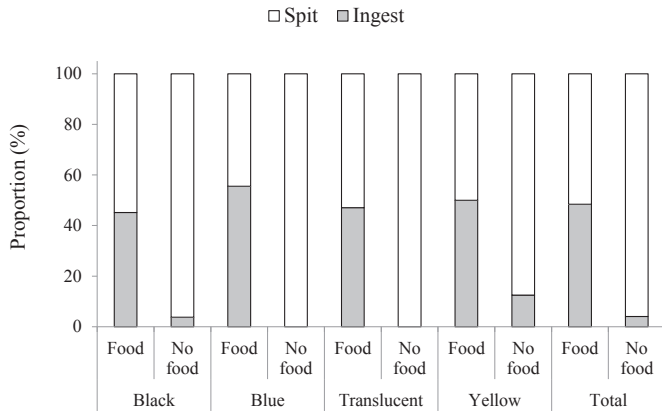
Fig. 2. Number (percentage of the total of each colour and food categories) of microplastics spit out and swallowed by fish after having been captured with or without food.

31 remaining fish (88%) egested within 7 weeks all the microplastics that they had ingested during the experiment (Fig. 3). The two remaining fish fed and egested faecal material like the other fish, but only egested one and two out of the three microplastics that they each had ingested, respectively; the remaining microplastics were found near the entrance of the pyloric sphincter of these two fish (Fig. S3).

Overall, fish egested the first microplastic after 1–19 days, with an average of $4.4 \pm SE\ 0.9$ days (Fig. 3). Fish that had ingested >1 microplastics egested the first microplastic faster (2.4 ± 0.3 days, n = 17) than fish that had ingested a single microplastic (7.2 ± 1.8 days, n = 12; H = 5.3, df = 1, p = 0.02). The last microplastics were egested after 3–49 days (mean = 10.6 ± 2.5 days; n = 23; Fig. 3) by fish with >1 microplastic. According to the sequence at which colours appeared in the monitoring tanks, the order of microplastic egestion did not follow the order of microplastic ingestion. In the experiment to determine the digestive duration of plastic-free food pellets, the last faeces were found after one day for all 8 but one fish, which egested the last faeces after two days.

4. Discussion

4.1. Factors influencing microplastic capture and ingestion

As expected, juvenile *Seriolella violacea* captured preferentially black microplastics over other colours. In clear waters, the visibility of a particle to a fish depends mostly of its contrast with the background (Utne-Palm, 2002). Although colour vision of juveniles *S. violacea* has, to our knowledge, not been documented, the results of our study clearly indicate that fish can discriminate between, at least, black microplastics and other colours. It is unlikely that black microplastics were preferentially captured only because they were the most visible to the fish, as they were less contrasted and bright against the green background of the experimental tanks than yellow or translucent microplastics (see Fig. S1a and Fig. S1b). Fish captured preferentially black microplastics probably because they appear more similar to food pellets to the fish. This result supports the assumption that visually-oriented planktivorous fish accidentally capture microplastics due to their resemblance with their prey, as had also been shown experimentally (Colton et al., 1974; de Sá et al., 2015; Hoss and Settle, 1990) and from observations in the field (Ory et al., 2017) in other fish species.

A large proportion of marine plastic debris is composed of millimetre-sized fragments (Eriksen et al., 2016), many of which have similar colour, size and shape as planktonic prey. Juvenile fish, which are mostly zooplanktivorous, are therefore particularly susceptible to microplastic ingestion, which is worrisome as early ontogenic stages are the bottleneck for successful recruitment (Houde, 2008). Unlike most fish in the wild, the captive-born fish used herein were only fed with one type of food; more experiments comparing the ingestion of different colours of microplastics and natural prey pairs should thus be valuable to provide further evidence that visually oriented planktivorous fish ingest microplastics resembling their natural prey.

Blue, translucent and yellow microplastics were rarely captured alone, but only together with food pellets. Fish probably avoided such microplastics that they did not perceive as prey, but rather accidentally co-captured the microplastics that were close to food pellets, the actual target of the fish. Planktivorous particle-feeding fish adapt their foraging strategy to the abundance of their prey (Lazzaro, 1987). For example, when foraging on abundant prey, fish switch from attacking individual prey at high velocity to slowly gulping up various prey simultaneously (Lammens and Hoogenboezem, 1991), and may accidentally co-ingest microplastics floating close to the prey. The possibility that fish co-capture microplastics with their prey may be enhanced in areas where the proportion of microplastics accounts for a large part of the plankton (Lima et al., 2014; Moore et al., 2001, 2002).

In our study, *S. violacea* readily rejected almost all of the microplastics captured without food pellets. The fish were thus able to distinguish inedible particles from food items, as had also been observed in juveniles striped killifish (*Fundulus majalis*; Funduli-dae) and tomcods (*Microgadus tomcod*; Gadidae), which spit out most of the millimeter-sized microspheres that they had captured (Colton et al., 1974). *Seriolella violacea* also spit out food pellets, which were nevertheless readily recaptured, once or several times, and ingested before the end of the experiment. Fish usually preferentially ingest soft over harder particles (Houlihan et al., 2001). In our experiment, fish probably initially rejected the food pellets that were too hard, but eventually ingested them when they had softened after contact with water. Hard plastic fragments should thus rarely been swallowed by fish, meaning that other mechanisms underlie the ingestion; future studies are needed to rigorously
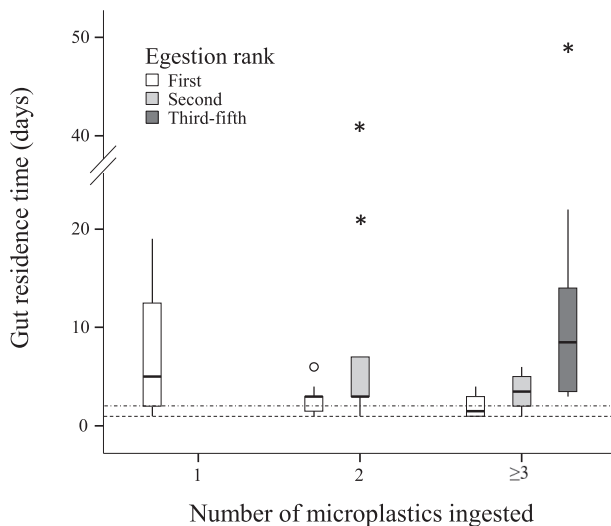

Fig. 3. Gut residence time (days) of the first, second, and third to fifth (pooled together) microplastics in fish that had ingested 1, 2 or ≥3 microplastics during the experiment. Horizontal lines inside the boxes represent the median; the upper and lower limits of the box represent the 3rd and 1st quartiles, respectively; whiskers extend to 1.5 times the interquartile range (IQR); circles represent outlier cases with values between 1.5 and 3 times the IQR; asterisks represent extreme values > 3 times the IQR. Dash-dotted line represents the maximum and dashed line the average time until the last food item was egested by the fish.

examine the role of texture for ingestion of food and microplastics.

Seriolella violacea swallowed microplastics only when co-captured with food pellets, in a single gulp or in quick sequence, meaning that microplastics and food pellets were mixed together, at least for a short moment, in the fish's mouth. Food might thus have impeded the ability of the fish to detect and discriminate against inedible particles, thereby causing planktivorous particle feeders like *S. violacea* to ingest microplastics despite the highly developed gustatory system of fish (Houlihan et al., 2001; Kasumyan and Döving, 2003). Our findings also suggest that planktivorous fish feeding on aggregated prey in areas where microplastics are abundant and account for an important part of the seston (e.g. Moore et al., 2001, 2002) are particularly susceptible to co-ingest microplastics with their prey. Recent studies revealed that chemical cues released by biofilm overgrowing sea-weathered microplastics may trigger foraging activity by some fish (Savoca et al., 2017). Neither food pellets nor the differently coloured microplastics in our study were overgrown by a biofilm, and the assumption that the presence of biofilm on sea-weathered microplastics facilitates microplastic ingestion by fish needs to be tested in future studies.

4.2. Residence time of microplastics in fish

In our experiment, almost all fish egested all the microplastics that they had ingested, as also observed in other studies (Colton et al., 1974; Grigorakis et al., 2017; Hoss and Settle, 1990). For example, many of the microplastics found in juvenile Amberstripe scad *Decapterus muroadsi* (Carangidae) in coastal waters around Easter Island (South Pacific ocean) were in the intestine of the fish, suggesting that those microplastics that passed through the narrow pyloric sphincter would probably have been egested by the fish (Ory et al., 2017). In our experiment, the only microplastics that were not egested by the fish were found near the entrance of the pyloric sphincter (Fig. S3). Overall, unlike some seabird species that accumulate small plastic items in their stomach throughout part of

their lifetime (Furness, 1985; van Franeker and Law, 2015), the current results indicate that microplastics < 2 mm are egested by juvenile *S. violacea*. However, microplastics remained for long time periods in the digestive tract of the fish, leading to the risk of accumulation if microplastics are continuously ingested; this is particularly worrisome for juvenile fish with similar feeding habits and digestive systems as *S. violacea* living in areas where microplastics are abundant (e.g. the subtropical gyre accumulation zones).

Microplastics remained in *S. violacea* guts for a week on average, up to 7 weeks, which is substantially longer than the time required by fish to digest and egest food pellets (2 days maximum), meaning that microplastics are less easily egested by the fish than food. Gut clearance rate may have been slower in fish that were starved during our experiment compared to fish under a (natural) continuous feeding regime. Nevertheless, such an effect would make our results more conservative by reducing the difference between food and microplastic gut residence time. These findings contrast with that of another study in which juvenile goldfish *Carassius auratus* egested food and microbeads both within 33 h (Grigorakis et al., 2017). The relatively long (1.2 mm) and tubular microplastics used herein may pass less easily through the pyloric sphincter than smaller and spherical microbeads. This finding underscores the importance to examine microplastic egestion by fish using microplastics similar to those commonly found in the marine environment (e.g. Phuong et al., 2016) and in fish guts (e.g. Battaglia et al., 2016; Ory et al., 2017) rather than microbeads <100 μm, which are most often ignored in marine fish diet studies.

Although microplastics were egested by most *S. violacea*, they remained in the fish longer than what had been reported for juveniles of other fish species. For example, microplastic clearance rates were estimated up to a maximum of four days in juvenile yellowtail, *Seriola lalandi* (Gassel et al., 2013), and 10 days in striped mullet, *Mugil cephalus* (Hoss and Settle, 1990), substantially less long than observed in our experiment. A prolonged exposure of microplastics to gut digestive acids may enhance desorption of
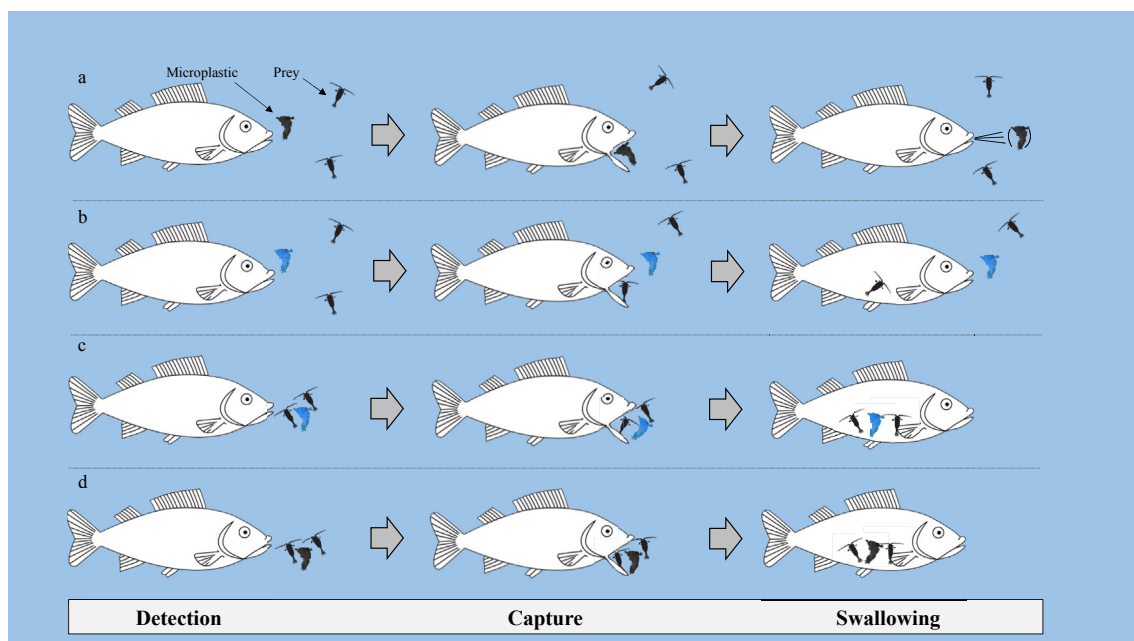

Fig. 4. Conceptual model predicting the probabilities that visually-oriented planktivorous fish ingest microplastics depending on the resemblance (colour) of microplastics to fish's prey, and whether microplastics are co-captured with the prey. The model is based on the results of this study and that from Ory et al. (2017): fish have the highest probability to capture microplastics similar to their prey (a), and avoid microplastics different than their prey (b), which are probably captured accidentally when floating beside the prey (c). Microplastics captured alone are almost always spit out (a), and are most likely to be swallowed when they are mixed with food in the fish's mouth (c,d). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contaminants from microplastics to the fish (Bakir et al., 2014), but the adverse effects of the transfer are still under debate (Koelmans et al., 2016). Only two of the 33 fish that had ingested microplastics died before the end of our experiment, one of bacterial or fungus infection of the tail, and the other one from unknown cause. Other studies also observed low mortality of fish that had ingested microplastics (Colton et al., 1974; Grigorakis et al., 2017; Hoss and Settle, 1990). Microplastics did not induce notable acute detrimental effects on fish, but potential behavioural and/or physiological sublethal effects of microplastics on fish have been observed in other experiments (Cedervall et al., 2012; de Sá et al., 2015; Mattsson et al., 2015; Oliveira et al., 2013; Smith et al., 2007), and still need to be tested.

4.3. Conceptual model of microplastic ingestion by planktivorous fish

Based on the results of this study, we propose a theoretical model that predicts the ingestion of microplastics by a visually-oriented particulate-feeding fish, based on the probability for a microplastic to be captured, and subsequently ingested by the fish (Fig. 4). Fish are more susceptible to capture microplastics similar to their prey (Fig. 4a), as also observed in other planktivorous fish (de Sá et al., 2015; Ory et al., 2017). Fish avoid microplastics that appear different from their prey (Fig. 4b), but may co-capture such microplastics when they float in close vicinity to the natural prey (Fig. 4c). Microplastics captured alone (i.e. with no food) are almost always spit out by the fish (Fig. 4a), and are most likely to be swallowed when co-captured with prey (Fig. 4c&d), although fish also often reject inedible particles mixed with food. Overall, our findings suggest that fish are more susceptible to capture microplastics resembling their food items. Microplastics not directly targeted by the fish may, however, be swallowed when captured accidentally together with food.

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Ethical concerns

All experiments involving animals conducted in this study were approved by the committee of bioethics and biosecurity of the Universidad Católica del Norte in Coquimbo, Chile.

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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.2018.04.093.

References

Acha, E.M., Piola, A., Iribarne, O., Mianzan, H., 2015. Ecological Processes at Marine Fronts: Oases in the Ocean. Springer.

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23.

Bates, D.M., 2010. lme4: Mixed-effects Modeling with R. Springer New York.

Battaglia, P., Pedà, C., Musolino, S., Esposito, V., Andaloro, F., Romeo, T., 2016. Diet and first documented data on plastic ingestion of *Trachinotus ovatus* L. 1758 (pisces: carangidae) from the strait of messina (central mediterranean sea). Ital. J. Zool. 83, 121–129.

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the north pacific central gyre. Mar. Pollut. Bull. 60, 2275–2278.

Cedervall, T., Hansson, L.-A., Lard, M., Frohm, B., Linse, S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One 7 e32254.

Chang, Y.C., 2000. Residuals analysis of the generalized linear models for longitudinal data. Stat. Med. 19, 1277–1293.

Colton, J.B., Knapp, F.D., Burns, B.R., 1974. Plastic particles in surface waters of the northwestern Atlantic. Science 185, 491–497.

Davison, P., Asch, R.G., 2011. Plastic ingestion by mesopelagic fishes in the north pacific subtropical gyre. Mar. Ecol. Prog. Ser. 432, 173–180.

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362.

Eriksen, M., Thiel, M., Lebreton, L., 2016. Nature of plastic marine pollution in the subtropical gyres. In: Takada, H., Karapanagioti, H.K. (Eds.), Hazardous Chemicals Associated with Plastics in the Marine Environment. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1–28.

Furness, R.W., 1985. Plastic particle pollution: accumulation by Procellariiform seabirds at Scottish colonies. Mar. Pollut. Bull. 16, 103–106.

Gassel, M., Harwani, S., Park, J.-S., Jahn, A., 2013. Detection of nonylphenol and persistent organic pollutants in fish from the North Pacific Central Gyre. Mar. Pollut. 73, 231–242.

Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). Chemosphere 169, 233–238.

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 286–294.

Hoss, D.E., Settle, L.R., 1990. Ingestion of plastics by teleost fishes. In: Shomura, R.S., Codfrey, H.L. (Eds.), Proceedings of the Second International Conference on Marine Debris. U.S. Dep. Commer., NOAA Tech. Memo. NMFS, NOAA-TM-NMFS-SWFSC-154, Miami, FL, pp. 693–709.

Houde, E.D., 2008. Emerging from Hjort's shadow. J. Northwest Atl. Fish. Sci. 41, 53–70.

Houlihan, D., Boujard, T., Jobling, M., 2001. Food Intake in Fish. Blackwell Science, London.

Kasumyan, A.O., Döving, K.B., 2003. Taste preferences in fishes. Fish Fish. 4, 289–347.

Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environ Sci Technol 50, 3315–3326.

Lamb, C.F., 2001. Gustation and feeding behaviour. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell Science, London, pp. 108–130.

Lammens, E., Hoogenboezem, W., 1991. Diets and feeding behaviour. In: Winfield, I.J., Nelson, J. (Eds.), Cyprinid Fishes: Systematics, Biology and Exploitation. hapman & Hall, London, pp. 23–46.

Law, K.L., 2017. Plastics in the marine environment. Annu. Rev. Mar. Sci 9, 205–229.

Lazzaro, X., 1987. A review of planktivorous fishes: their evolution, feeding behaviours, selectivities, and impacts. Hydrobiologia 146, 97–167.

Lima, A.R.A., Costa, M.F., Barletta, M., 2014. Distribution patterns of microplastics within the plankton of a tropical estuary. Environ. Res. 132, 146–155.

Lusher, A., 2015. Microplastics in the marine environment: distribution, interactions and effects. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine anthropogenic Litter. Springer, pp. 245–307.

Lusher, A.L., O'donnell, C., Officer, R., O'connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES J. Mar. Sci.: J. Conseil 73, 1214–1225.

Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology and metabolism in fish exposed to polystyrene nanoparticles. Environ. Sci. Technol. 49, 553–561.

Medina, M., Vega, C., Araya, M., 2004. Guía de peces marinos de la zona norte de chile. Universidad Arturo Prat, departamento de ciencias del mar, Iquique, Chile.

Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. Mar. Pollut. Bull. 42, 1297–1300.

Moore, C.J., Moore, S.L., Weisberg, S.B., Lattin, G.L., Zellers, A.F., 2002. A comparison of neustonic plastic and zooplankton abundance in southern California's coastal waters. Mar. Pollut. Bull. 44, 1035–1038.

Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indicat. 34, 641–647.

Omar, R.Z., Wright, E.M., Turner, R.M., Thompson, S.G., 1999. Analysing repeated measurements data: a practical comparison of methods. Stat. Med. 18, 1587–1603.

Ory, N.C., Sobral, P., Ferreirad, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437.

Pan, W., 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57, 120–125.

Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: preliminary results. Environ. Pollut. 212, 251–256.

Phuong, N.N., Zalouk-Vergnoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C., Lagarde, F., 2016. Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ. Pollut. 211, 111–123.

Quinn, G.P., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge.

Savoca, M.S., Tyson, C.W., McGill, M., Slager, C.J., 2017. Odours from marine plastic debris induce food search behaviours in a forage fish. Proc. Biol. Sci. 284.

Shaw, D.G., Day, R.H., 1994. Colour-and form-dependent loss of plastic micro-debris from the North Pacific Ocean. Mar. Pollut. Bull. 28, 39–43.

Smith, C.J., Shaw, B.J., Handy, R.D., 2007. Toxicity of single walled carbon nanotubes to rainbow trout, (Oncorhynchus mykiss): respiratory toxicity, organ pathologies, and other physiological effects. Aquat. Toxicol. 82, 94–109.

Utne-Palm, A., 2002. Visual feeding of fish in a turbid environment: physical and behavioural aspects. Mar. Freshw. Behav. Physiol. 35, 111–128.

van Franeker, J.A., Law, K.L., 2015. Seabirds, gyres and global trends in plastic pollution. Environ. Pollut. 203, 89–96.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.