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Baseline

Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean

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ABSTRACT

The gut contents of 292 planktivorous fish, from four families (Atherinopsidae, Clupeidae, Engraulidae and Scombridae) and seven species, captured along the coast of the southeast Pacific, were examined for microplastic contamination. Only a small fraction of all studied fish (2.1%; 6 individuals) contained microplastic particles in their digestive tract. Microplastics found were degraded hard fragments and threads, ranging from 1.1 to 4.9 (3.8 ± SD 2.4) mm in length, and of various colours, which suggests that the planktivorous fish species examined herein did not capture microplastics on the basis of their colour. The low prevalence of microplastic contamination in planktivorous fishes found in this study suggests that the risk of accidental ingestion by these species might be limited in the coastal upwelled waters of the southeast Pacific, perhaps due to small human population and highly dynamic oceanographic processes.

Microplastics (< 5 mm) are ubiquitous in most marine environments, raising increasing concerns as their impacts on the ecosystems are still unknown (Bergmann et al., 2015). Floating microplastics can remain for many years at the surface of the ocean, where they can be ingested by a wide variety of organisms (reviewed by Lusher, 2015). Numerous recent studies have reported microplastic ingestion by marine fish (e.g. Collard et al., 2017; Lusher, 2015; Rummel et al., 2016). The proportion of fish found with small plastic fragments in their gastrointestinal tract ranges from a few percent (e.g. Bråte et al., 2016; Cannon et al., 2016; Foekema et al., 2013; Liboiron et al., 2016) to more than two-third of all fish examined (e.g. McGoran et al., 2017; Naidoo et al., 2016; Ory et al., 2017; Tanaka et al., 2013). The reasons of such contrasting microplastic prevalence among marine fish species are still unclear, and need to be clarified to better understanding the pathways of microplastics within marine food webs.

The abundance of microplastic in fish guts is often high in river and estuarine systems near urban areas (e.g. McGoran et al., 2017; Naidoo et al., 2016), in coastal seas with high anthropogenic activity (e.g. Bellas et al., 2016; Foekema et al., 2013; Rummel et al., 2016), or in the open ocean close to the gyre accumulation zones of microplastics (e.g.

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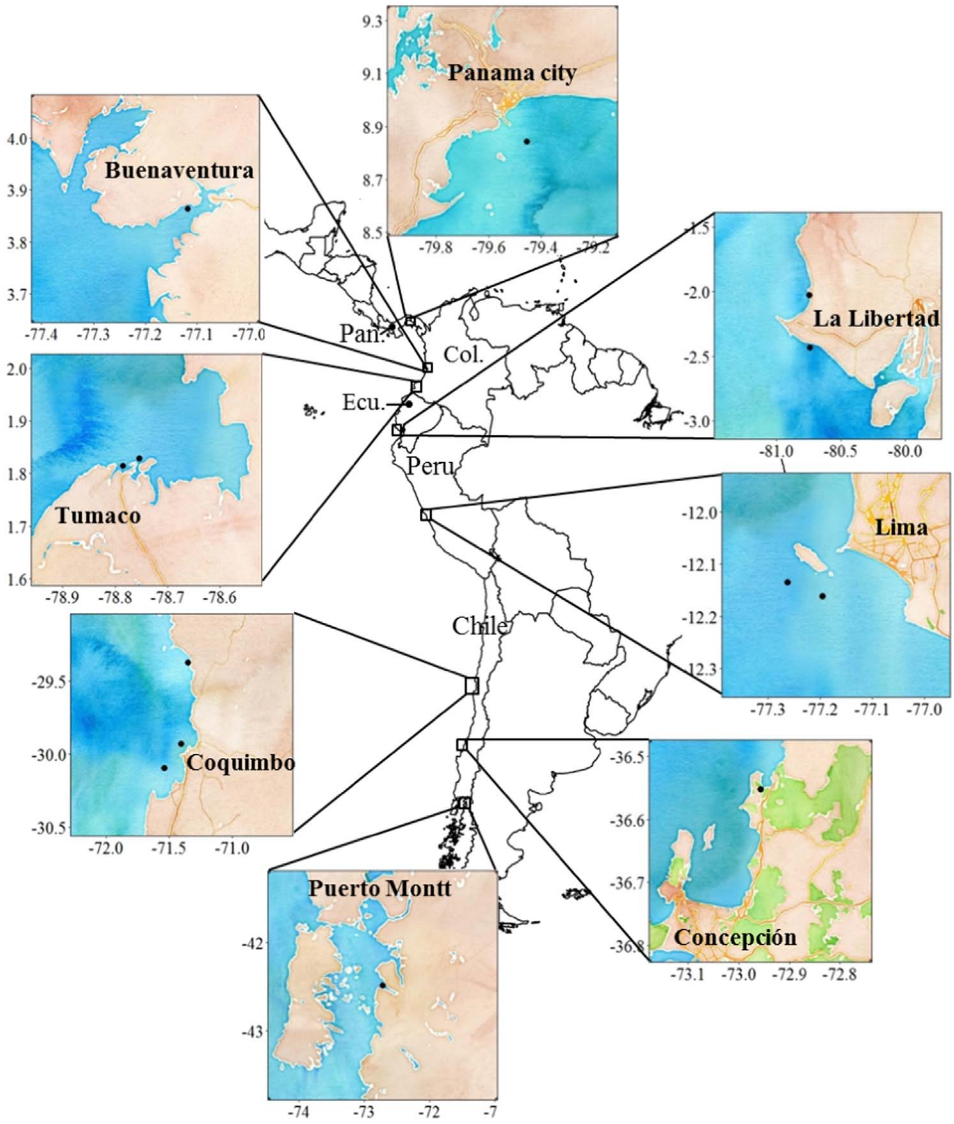

Fig. 1. Study areas (insets) and sampling sites (black dots) along the SE Pacific coast.

Boerger et al., 2010; Ory et al., 2017). In studies from remote areas with low human population densities (SE Australia, New Foundland, Norway), only a small proportion of the examined fish had ingested microplastics (Cannon et al., 2016; Liboiron et al., 2016), but incidence of microplastic contamination increased when samples were taken close to populated areas (Bråte et al., 2016).

Pelagic fish feeding on small planktonic organisms are particularly susceptible to accidental ingestion of microplastics suspended in the water column, which can reach abundances exceeding those of plankton (Moore et al., 2001, 2002), and often look similar in colour, size, and shape to many planktonic organisms. For example, microplastics were reported in about 70% of all anchovies (*Engraulis japonicus*; Engraulidae) from Tokyo Bay (Tanaka and Takada, 2016), in 40 – 50% of three clupeiform fish species (*Sardina pilchardus*, *Clupea harengus* and *Engraulis encrasicolus*) from the Atlantic coast of France (Collard et al., 2017), or in 80% of 20 *Decapterus muroadsi* (Carangidae) fish from Easter Island (Ory et al., 2017). Microplastics may enter and be transferred along marine food webs through planktivorous fish species, many of which are of commercial and ecological importance.

Plastic contamination is well documented in many coastal waters of the world's ocean, but still poorly known in the coastal waters of the Eastern Boundary Upwelling Systems (EBUS). Within these current systems, large masses of subsurface waters are upwelled near the coast, and transported offshore once reaching the surface (e.g. Marín et al., 2003); microplastics floating at the sea surface near coastal urban centres may thus be transported offshore by these upwelling currents. A recent study found no difference in microplastic abundances between upwelled and non-upwelled subsurface waters in the eastern Atlantic Ocean, but confirmed relatively low overall microplastic abundance in these coastal waters (Kanhai et al., 2017). Other studies confirmed low microplastic abundance in superficial waters along the eastern Pacific Boundary Currents (Eriksen et al., 2014; Law et al., 2014), suggesting that fish feeding within the water column, such as planktivorous species, may be exposed to low risk of plastic ingestion.

One of the most productive EBUS is the Humboldt Current System (HCS), which extends from southern Chile to Ecuador in the SE Pacific (Thiel et al., 2007). Herein we examined the incidence of microplastic ingestion by planktivorous fish species captured along the coasts of the eastern Pacific, ranging from Panama and Colombia to southern Chile. This study aims to establish the first baseline of microplastic contamination in fish from the South American Pacific coast.

A total of 292 planktivorous fish from 7 species and 4 families were captured between the 3rd of July and the 7th of December 2016 off the coasts of Panama, Colombia, Ecuador, Peru, and Chile (Fig. 1, Table 1 and Supplementary Table 1). Fish were captured with throw net or gillnet, between 1 and 220 m water depth (Table 1 and Supplementary Table 1). All fish were acquired directly from fishermen or in local markets < 8 h after their capture, stored in a clean cooler box, and

Table 1
Family, species, diet and feeding behaviour (exclusively filter-feeders or mixed particulate and filter-feeder), habitat, country and date of capture, individual number, and size (total length) ± standard deviation (SD) of the 292 fish analysed herein. Percentage of fish sampled with microplastics, maximum and mean (± SE) number of microplastics per fish.*Multiple fragments were found in the fish, but probably came from at least one larger item; a single fragment was thus accounted in the analysis to be conservative. Bootstrap (random resampling of the original observations with replacement) analysis based on [b]646, [c]951, [e]629, [f]622, [h]635, [i]663 resamplings. References of the studies used to determine the diet of each fish species can be found in Table 3 of Supplementary data.

Fish							Microplastics		
Family	Species	Diet (feeding behaviour)	Habitat	Sampling country	n	size (SD)	% (number) of total fish	Max. number	Mean number per fish (SE)
Atherinopsidae	*Odontesthes regia*	Planktivorous (mixed[1])	Pelagic-neritic	Chile	9	22.0 (1.4)	11.1 (1*)	1	0.1 (0.1)[b]
Clupeidae	*Strangomera bentincki*	Planktivorous (filter[2])	Estuarine	Chile	10	13.7 (0.2)	0	–	0
	Sardinops sagax	Planktivorous (mixed[3])	Pelagic-neritic	Chile	7	18.1 (1.8)	0	–	0
	Opisthonema libertate	Planktivorous (filter[4,5])	Pelagic-neritic	Colombia	26	20.2 (1.1)	0	–	0
				Colombia	14	21.2 (1.0)	0	–	0
				Ecuador	20	25.6 (0.9)	5.0 (1)	1	0.05 (0.04)[e]
				Ecuador	20	24.9 (1.7)	5.0 (1)	1	0.05 (0.04)[c]
Engraulidae	*Cetengraulis mysticetus*	Planktivorous (filter[5])	Pelagic-neritic	Colombia	30	15.9 (0.5)	3.3 (1)	1	0.03 (0.03)[f]
				Panama	10	17.5 (0.6)	0	–	0
	Engraulis ringens	Planktivorous (mixed[3,5])	Pelagic-neritic	Chile	23	12.8 (1.1)	0	–	0
				Chile	13	15.0 (0.2)	7.7 (1)	1	0.1 (0.1)[h]
				Chile	40	14.5 (0.6)	0	–	0
				Peru	40	13.5 (0.6)	0	–	0
Scombridae	*Scomber japonicus*	Planktivorous (mixed[5])	Pelagic-neritic	Peru	30	21.4 (1.6)	3.3 (1)	1	0.03 (0.03)[i]

brought to the laboratory within 2 h; some samples were also frozen immediately after capture. There, fish were measured to the nearest 0.5 cm (total length, TL), weighed (± 1 g), and their gut (oesophagus, stomach and intestine, and pyloric caeca when present) removed, and fixed in 70% ethanol.

Fish guts were weighed to the nearest 0.1 g, placed on a petri dish and carefully cut longitudinally with a micro-dissecting scissor; the gut content was then emptied onto the petri dish filled with seawater, which was previously filtered through a 100 μm mesh sieve. Organic matter remaining on the internal walls of the guts was removed with a wash bottle of filtered seawater, and transferred to the same petri dish. Gut contents were thoroughly examined under the dissecting microscope at 6.5 × to 50 × magnification to differentiate microplastics from other non-anthropogenic food particles. Before use, all tools used for sample processing and sorting were thoroughly cleaned with ethanol and checked under the dissecting microscope to verify the absence of plastic contamination.

All microplastics were counted, and their type (hard or soft fragments, thread, film), dominant colour (representing > 50% of the particle surface), and degradation (new, weathered, degraded) were described following Table S2 in Ory et al. (2017). When > 1 similar fragments were found in a fish gut, only one microplastic was accounted for in the analysis as a conservative measure, because they probably came from the fragmentation of a larger item.

Although fibres are often the dominant type of microplastics reported from fish guts (e.g. Bellas et al., 2016; McGoran et al., 2017; Neves et al., 2015; Rochman et al., 2015), they were not counted herein because of the risk of airborne contamination during sample processing and analysis (see Foekema et al., 2013; Torre et al., 2016), or confusion of plastic fibres with non-synthetic (e.g. cotton) or organic particles (Song et al., 2015). A photograph of each microplastic found was taken to measure its maximum length to the nearest 0.1 mm with the program Image J (imagej.nih.gov/ij/).

Particles were analysed by infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR). The spectra 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. All spectra were obtained with a resolution 4 cm^{-1} and 32 scans. The identification of the samples was based on best expert judgment by the presence of specific absorption bands. Data were compared with a reference spectra library and accepted with a match > 72%.

Microplastics were found in six (2.1%) of 292 fish analysed, belonging to five (71%) of the seven species examined (Table 1 and Fig. 2). One out of nine (11.1%) Atherinopsidae, two out of 97 (2.1%) Clupeidae, two out of 156 (1.3%) Engraulidae, and one out of 30 (3.3%) Scombridae fish ingested microplastic fragments. *Odontesthes regia* was the species with the highest proportion of individuals that had ingested microplastics (1 individual out of 9; 11.1%), followed by *Scomber japonicus* (1 out of 30; 3.3%), *Opisthonema libertate* (2 out of 80; 2.5%), *Cetengraulis mysticetus* (1 out of 40; 2.5%), and *Engraulis ringens* (1 out of 116; 0.8%). No microplastic was found in *Strangomera bentincki* ($n = 10$) nor in *Sardinops sagax* ($n = 7$).

Two microplastics found in the fish were green threads (Fig. 2a,b), and three were hard fragments (1 blue, 1 black and 1 red-orange; Fig. 2c–e). The average size of these five microplastics was 3.8 ± SD 2.4 mm, with a total length ranging from 1.1 to 4.9 mm (Fig. 2 and Table 2). All these microplastics had their surface degraded (Fig. 2) and were positively buoyant in seawater.

A large number (> 100) of green, yellow, blue, and red fragments, some of which < 0.3 mm, were found in the stomach of an *Odontesthes regia* (Atherinopsidae) individual (Fig. 2f). These fragments were very brittle and broke into powder when manipulated; they may have come from the fragmentation of at least one larger item, and were counted as a single particle of which size was therefore not measurable. These fragments were not analysed, but because they were very similar to

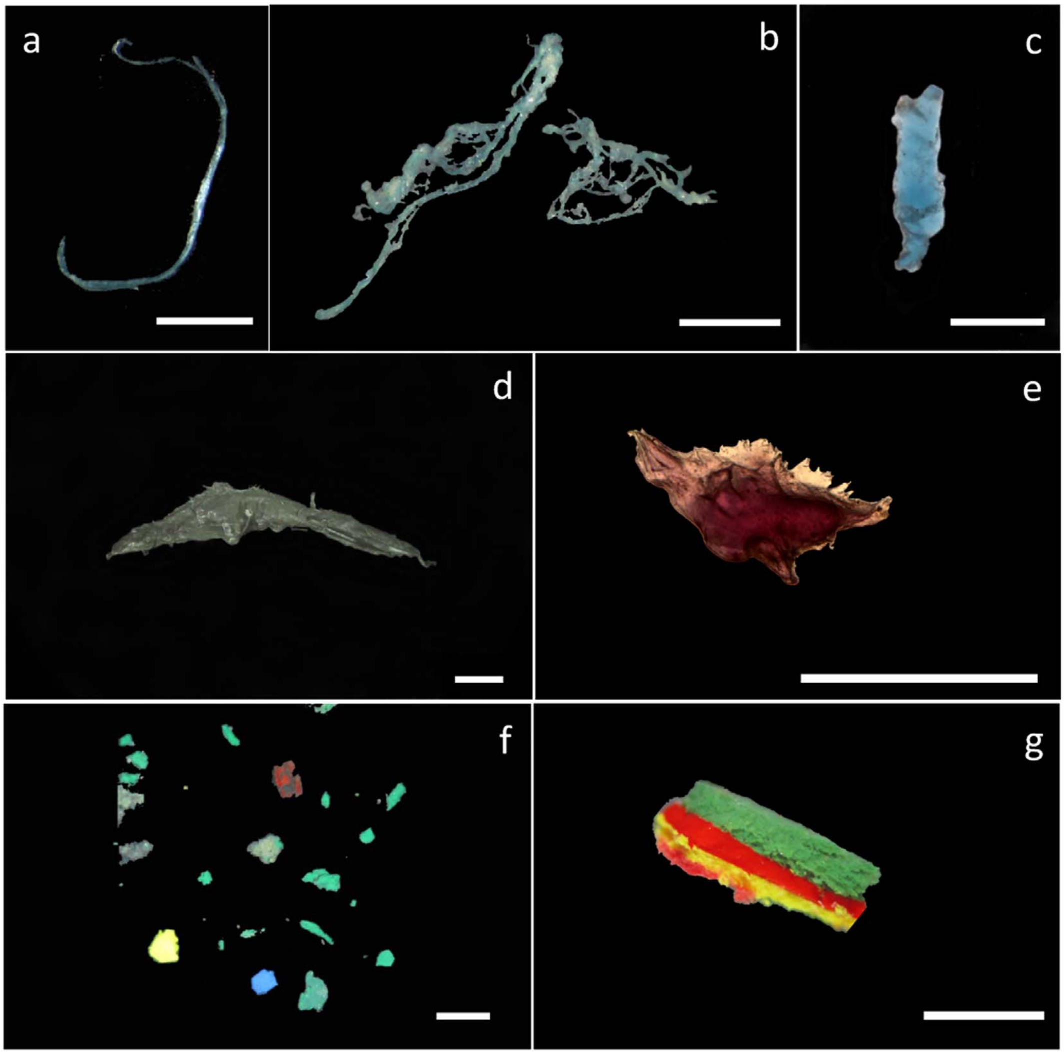

Fig. 2. (a–f) Microplastics found in fish guts in this study. (g) Example of alkyd resin fragment made of three layers of different paint coating collected in surface waters of the South Pacific Gyre. Scale bars represent 1 mm. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

Table 2
Physical features of the 6 microplastics found in fish.

Fish species	Microplastic							
	Nbr.	Type	Colour	Maximum size (mm)	Edges	Surface erosion	Hardness	Photo ID
O. libertate	1	Thread	Green	4.9	Torn	Degraded	Fragile	Fig. 2a
	1	Thread	Green	3.7	Torn	Degraded	Hard	Fig. 2b
S. japonicus	1	Hard fragment	Blue	2.1	Angular	Degraded	Hard	Fig. 2c
E. ringens	1	Hard fragment	Black	7.3	Angular	Degraded	Hard	Fig. 2d
C. mysticetus	1	Hard fragment	Red-orange	1.1	Angular	Degraded	Hard	Fig. 2e
O. regia	1	Soft fragment	Green, blue, yellow, red	Multiple fragments	Angular	Degraded	Brittle	Fig. 2f

other particles found in water samples analysed by the authors (e.g. Ory et al., 2017; NCO, PS and MT personal observations; Fig. 2g), we suggest that these particles may correspond to an alkyd resin.

Microplastics identified were polyethylene PE (3 particles) and polypropylene PP (2 threads), which are widely used plastic polymers. Infra-red assignment and selected spectra of the 5 samples identified as plastic polymers are shown in Supplementary Table 2 and Supplementary Fig. 1.

The proportion of fish with microplastics (2.1%) found herein was low compared to other parts of the world (Fig. 3), but was similar to that reported from other planktivorous fish species captured in coastal regions with low human population densities (Cannon et al., 2016; Liboiron et al., 2016). The highest incidences of microplastics in fish are often found in waters near populated areas where microplastics are abundant. For example, Battaglia et al. (2016) suggested that the high proportion of *Boops boops* (Sparidae) that had ingested microplastics in southern Italy might be due to the accumulation of floating plastics in convergence zones where this species often feeds. Bellas et al. (2016) also reported higher proportions of fish with microplastics from areas where large amounts of microplastics accumulate at the surface and bottom of the sea. In a study from another Eastern Boundary Current (California Current), Rochman et al. (2015) recorded that 30% of the planktivorous Pacific anchovy (*Engraulis mordax*) contained anthropogenic debris in their stomachs, mainly fibres, but the chemical

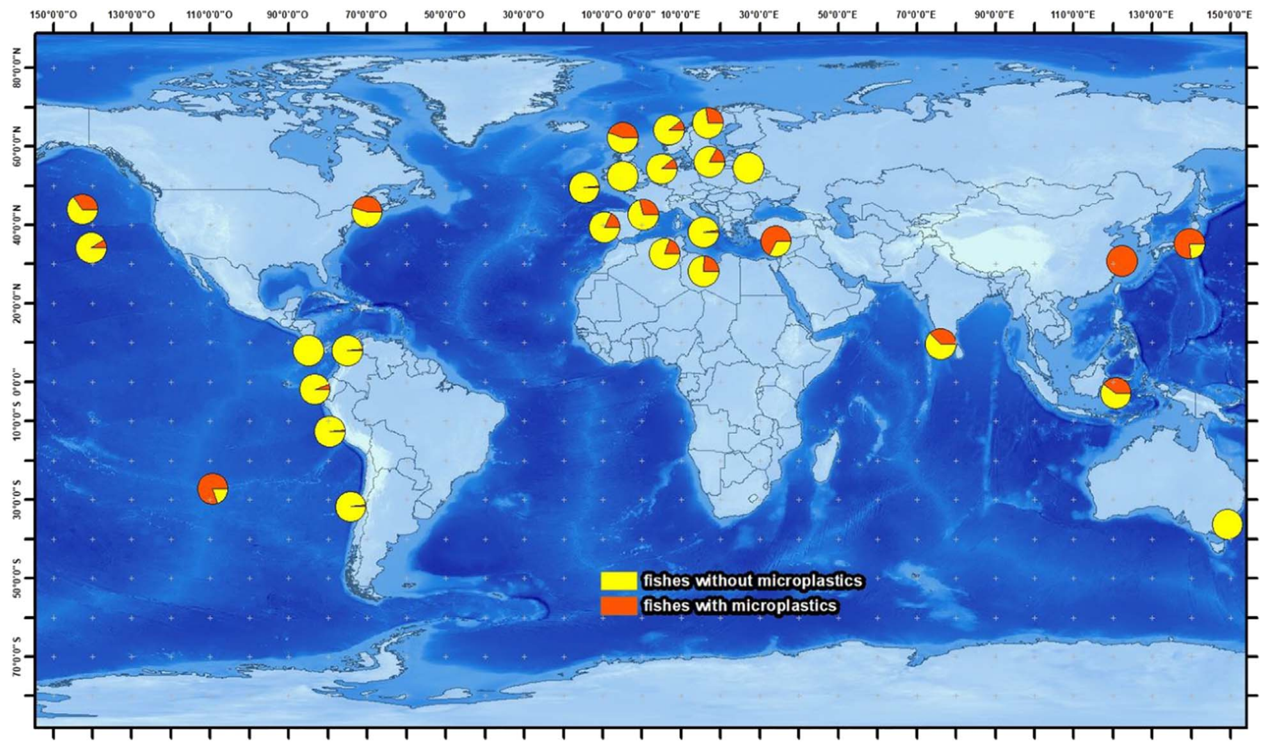

Fig. 3. Proportion of marine planktivorous fish without and with microplastics (pie charts) around the world (see Supplementary Table 4 for the data used to generate the map).

structure of the debris found was not verified. The low proportion of fish with microplastics found herein compared to other studies may thus also be due to the fact that synthetic fibres were not included in the analysis due to the risk of airborne contamination or misidentification (e.g. Torre et al., 2016).

Microplastics found in our study were of several different colours, suggesting that fish do not target microplastics based on their colour. Boerger et al. (2010) also found that microplastic ingestion by pelagic fish in the North Pacific Gyre was not influenced by colour cues as the colour composition of microplastics in fish was similar to that of surface waters. On the other hand, Ory et al. (2017) showed that the planktivorous fish *Decapterus muroadsi* (Carangidae) ingested preferentially blue microplastics resembling their copepod prey. In our study, one individual of *Engraulis ringens* had ingested a microplastic that looks similar in shape and size to an euphausiid (Fig. 2d), common prey items of Peruvian anchoveta *E. ringens* along the Humboldt Current System (Espinoza and Bertrand, 2008). Clupeiformes can switch from filter-feeding to particle feeding (Collard et al., 2017), which may explain that *E. ringens* is susceptible to mistakenly ingest microplastic that resemble their natural prey. However, the number of microplastics found herein is insufficient to test the hypothesis that planktivorous fish preferentially ingest prey-like microplastics; this assumption still need to be tested to better comprehending microplastic pathways along marine food webs.

Herein, fish guts were analysed using visual techniques by trained observers, under dissecting microscopes similar to the one used by Ory et al. (2017) who correctly identified > 90% of the microparticles as plastic polymers. Visual identification of microplastics during sample sorting is a common procedure (e.g. Boerger et al., 2010; Cannon et al., 2016; Liboiron et al., 2016; Rummel et al., 2016), which is appropriate to assess particles > 100 μm (Lenz et al., 2015), as it is the case in our study. We are therefore confident that the low prevalence of microplastics found in fishes in this study is not an artefact of the method of analysis used.

Neuston samples from eastern boundary upwelling systems (EBUS) of the world's oceans revealed generally low abundance of microplastics (e.g. Eriksen et al., 2013, 2014; Law et al., 2014). While microplastic abundance in EBUS can be high in close vicinity to urban areas, especially after rainfall events (Moore et al., 2002), surface waters at only a few kilometres from the coast contained low abundances of microplastic particles (Lattin et al., 2004). This suggests that many floating microplastics, which enter the coastal waters in EBUS, are rapidly moved away from the coast due to offshore transport of surface waters that are replaced by upwelled deeper waters free of microplastic contamination. This suggestion is also supported by the observation that microplastic contamination decreased with distance from the coast in the NE Pacific (Desforges et al., 2014).

Lower microplastic abundance in waters within EBUS should result in fish (especially planktivorous) having a lower probability to ingest microplastics than in other parts of the world's ocean where microplastic densities are higher (e.g. subtropical gyres or urban estuaries). In general, fish caught close to urban areas had ingested more microplastics than those caught in areas far from urban centres (Bråte et al., 2016; Neves et al., 2015). In this context, Foekema et al. (2013) also suggested that in coastal waters near populated areas the risk of microplastic ingestion by fish is higher than in offshore waters. It is interesting that despite the high range of population densities in cities next to the sampled sites in the SE Pacific, from 43 inhabitants km^{-2} in Tumaco, Colombia, to 3840 inhabitants km^{-2} in La Libertad, Ecuador (IOC-UNESCO/CPPS, 2014), prevalence of microplastics was fairly similar within the entire region. Possibly, upwelling is rapidly moving microplastics offshore and consequently fishes feeding in the productive upwelled waters close to the coast might be exposed to lower microplastic contamination than in coastal waters with no upwelling or in enclosed sea areas. Future studies in the region should include samples from areas beyond the direct influence of the coastal upwelling.

Although the low prevalence of microplastics in fish found in this study suggests that the SE Pacific region is not as severely affected as other regions of the world, we recommend to continue monitoring microplastic prevalence in coastal fishes from EBUS to identify changes in time. There are several areas within this region where domestic wastes are not properly disposed, and as a consequence of this it is estimated that between 10 and 30% of the solid waste generated in coastal areas of the SE Pacific countries becomes marine litter (CPPS, 2007) and then microplastics. Targeted sampling close to those areas is recommended.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.12.016.

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