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FIRST RECORD OF MICROPLASTICS IN THE ENDANGERED MARINE OTTER (*Lontra felina*)

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ABSTRACT. *Lontra felina* inhabits the coast of Peru and Chile frequently closed to human beach resorts and fishing ports which are common focus of anthropogenic pollution like solid waste and plastics are part of this as persistent material. In consequence the habitat for *L. felina* reduce its quality and indirect potential intake of plastic is a high risk. Here we present the first documented evidence of the occurrence of microplastics in the scats of *L. felina*. This evidence gives a first insight on the trophic transfer of microplastics in the marine web, where top predators like *L. felina* are also included.

RESUMEN. Primer registro de microplásticos en la nutria marina (*Lontra felina*). *Lontra felina* habita la costa de Perú y Chile frecuentemente cerca de balnearios y puertos que representan focos comunes de contaminación antropogénica; los residuos sólidos son parte de la contaminación incluidos los plásticos que son considerados como material persistente. Consecuentemente el hábitat para la especie reduce su calidad y el riesgo de ingesta incidental de plásticos es alto. Presentamos la primera evidencia documentada de la ocurrencia de microplásticos en las heces de *L.felina*. Esta evidencia ofrece un primer vistazo sobre la transferencia trófica de microplásticos en la red trófica marina donde los depredadores tope como *L. felina* están también incluidos.

Key words: microplastics, marine otter, pollution, marine debris, Peru, Punta Corrientes.

Palabras clave: microplásticos, nutria marina, contaminación, desechos marinos, Perú, Punta Corrientes.

Degradation of plastics is a sequence of chemical changes and can vary according the type of polymer; weather is also an important factor in this process because cold seawater might slow the degradation (Avio et al. 2017). In the environment, plastics can be found in different sizes like megaplastics (>100 mm), macroplastics (>20 – 100 mm), mesoplastics (5 – 20 mm) and microplastics (<5 mm) and types (bags, fragments and pellets) (Machovsky-Capuska et al. 2019). Microplastics are solid synthetic organic polymer particles with a size between 100 nm and 5 mm (Duis & Coors 2016). Physical abrasion degrades larger plastics into fragments and microsized plastics named secondary microplastics (Barnes et al. 2009; Avio et al. 2017). The other group of microplastics are primary microplastics or engineered micro-sized plastics (Shim & Thompson 2015), conformed by microscopically particles added to personal care products like exfoliants, cosmetic products, toothpaste, air-blasting technologies for clearing surfaces like boats and machinery from rust and human medicine, serving as vectors for drug delivery (Nerland et al. 2014). Primary microplastics reach the sea through drainage water disposed to rivers or directly to the sea (Nerland et al. 2014; Shim & Thompson 2015; Thiel et al. 2018).

Along its distribution, the habitat of *L. felina* is frequently affected by environment perturbation

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from anthropogenic activities, causing habitat loss, pollution, displacement by invasive species, among others (Sielfeld & Castilla 1999). At the coasts of the Southeast Pacific, the solid waste discharge is one of the most important sources for marine pollution. Plastics comes from two sources, terrestrial (mainly from cities) and marine (fisheries, transportation-related activities), being terrestrial the most representative (CPSS 2007). Debris from terrestrial sources reach the sea after an inadequately manage at land, and non-degradable solid waste reaches the sea through several agents like wind, rain, animals and even by the direct disposition in rivers. CPSS (2007) indicates that plastics are a minor fraction of debris produced by Chile, Perú, Ecuador, Colombia and Panama, but once they reach the sea, they become the most persistent solid waste at beaches (i.e. bottles, bags, bottle caps). The amount of debris discharged to the sea from terrestrial sources in the Southeast Pacific is estimated between 12,304 – 36,909 tons/year, Ecuador and Peru being the countries with more garbage generation at coasts (CPSS 2007). Marine biodiversity is threatened by anthropogenic disturbance produced by overfishing, climate change and others, debris at sea add another factor to increase the risk to marine biodiversity (Derraik 2002).

The marine otter *Lontra felina*, the smallest marine mammal in the world, is an endemic species of the Southeastern Pacific Coast. Its distribution ranges from northern Peru (9°S) to Cape Horn in Chile (Larivière 1998; Alfaro-Shigueto et al. 2011; Apaza & Romero 2012; Valqui 2011). Habitat preferences of *L. felina* are rocky shore patches that provide dens within caves that animals use for reproduction and rest (Cabello 1983; Ostfeld et al. 1989; Valqui 2011). Marine debris concentrates in rocky shores moved by wind and currents (Thiel et al. 2013). This habitat preferences and the plastic concentration in rocky shores highly increases the potential ingestion of plastics in the diet of coastal species and apex predators like *L. felina* (Thiel et al. 2018). Occurrence of plastics in marine biota (invertebrates, seabirds, sea turtles and marine mammals) has already been documented (Provencher et al. 2014; Davidson & Dudas 2016; Avio et al. 2017; Jiménez et al. 2017; Nelms et al. 2018; Thiel et al. 2018; Herrera et al. 2019; Machovsky-Capuska et al. 2019). Microplastics are often introduced to trophic webs through ingestion by producers (plankton), and low-level consumers (invertebrates like crustaceans and bivalves), and there is a trophic transfer to predators (like fish and cephalopods) and apex predators (birds, reptiles and marine mammals) (Machovsky-Capuska et al. 2019). However, the presence of plastic < 5mm in stomach contents and/or scats is not extensively known, especially for apex predators at the Southeast Pacific coast. Effects of microplastics ingestion in animal health are related to the blockage of digestive tracts, loss of food intake, inducing starvation, and loss of energy (Sharma & Chatterjee 2017). For marine otters there is a lack of evidence of microplastics ingestion on spite of their habitats are highly affected by plastic pollution.

In this work we present qualitative evidence (presence/absence) of the occurrence of microplastics in scats of a top predator *L. felina* in coastal sea waters off Peru.

In December 2018, a survey to assess the impact of coastal perturbation was carried out, including the collection of biological material of *L. felina*, specifically scats. The study site was Punta Corrientes (12° 57.2830' S; 76° 30.914' W) located in the central coast of Peru, this site is easily accessible from land and there are five individual frequently inhabiting in this site Apaza & Romero (2012) and Valqui (2011). It is also affected by anthropogenic perturbation because is surrounded by private beaches and is frequented by artisanal fishermen. One cave was inspected since it was the most accessible and a sighting of at least one otter swimming close by gave evidence of current use. Fecal material was found at the entry and at the middle of the cave, no feces were found at the deeper parts of the cave. Most of the fecal material was collected, totaling approximately 377 grams of material collected with the objective to analyze if human pollution related to microplastics might affect the species in relation to the observed pollution in coastal areas. Scats were store in aluminum cans and frozen for a short time before laboratory analyze.

Extraction and identification of microplastics followed suggestions of Lusher & (2018). Samples were taken to the laboratory and divided in subsamples of 3 g and 6 g. Potassium hydroxide (KOH) was used as solvent of organic material following Dehaut et al. (116:). KOH is reported to cause the least damage to plastic particles (Dehaut et al. 116:; Wagner et al. 2017). We considered three different treatments changing KOH quantity and concentration and amount of samples in standard test tubes: i. Treatment I: 6 g of sample in 10% KOH concentration, ii. Treatment II: 6 g of sample and 20% KOH, and iii. Treatment III: 3 g of sample and 10%KOH. Each treatment was repeated once. The solution in each tube was shaken to ensure homogenization of KOH and the sample. Afterwards, all tubes were incubated overnight at 60°C.

After incubation, the supernatant was extracted from the tubes and passed through a filter paper (Herrera et al. 2019). Filter was a 20 μm pore glass fiber filter paper (Whatman PLC 122 United Kingdom) set in a kitasato flask and connected to a laboratory vacuum pump. We obtained a filter paper with un-dissolved material. To detect microplastics captured in the filter papers we used optical microscopy, using 10 x – 40x objectives.

Treatments I and II presented reduced supernatant inside the test tube and slow filtration when passed through the filter system, remaining fecal material was high and the precipitated was abundant. Treatment III yielded more supernatant, the amount of precipitate was reduced and the filtration process was fast. In relation to efficiency in digestion expressed in amount of supernatant, the most effective was treatment III. A concentration of 10%KOH seemed to be proper to dissolve the organic material in a reduced amount of sample (3 g).

Diverse types of microplastics were found in all samples. **Fig**. 1 (images A to H) shows the types of microplastics found: fibers, fragments (color green and blue) and beads. Occurrence of microplastic was detected in all sub-samples. Our aim was to detect the occurrence of microplastics but not the identification of polymers, consequently, we only use optical microscopy like other authors (Davidson & Dudas 2016; Lusher & 2018; Hernandez-Milian et al. 2019).

Trophic transfer of microplastics is a problem to concern (Sharma & Chatterjee 2017). Nelms et al. (2018) reported the trophic transfer from prey to predators with the analysis of scats from captured grey seals fed with Atlantic mackerel *Scomber scombrus*. In the wild, studies in microplastics on marine mammals is scarce, specifically, evidence of microplastics for *L. felina* has never been reported in the scientific literature. However some authors suggested that due its habitats requirements, *L. felina* has an evident risk of entanglement or ingestion of marine debris (Thiel et al. 2018).

Plastics are the dominant debris reported in shores world-wide (Thiel et al. 2013). Tides play an important role in the accumulation of debris; usually the supralittoral zone is where plastic fragments are more concentrated (Purca & Henostroza 2017). Accumulation of debris in rocky shores is common due to winds and currents (Thiel et al. 2013; 2018), afterwards they are fragmented by waves (Eriksson & Burton 2003). Invertebrates (like decapods, shrimps, crabs and mollusks) and coastal fish could ingest those small particles and *L. felina* prey upon these organisms (Valqui 2011).

Mangel et al. (2010), analyzed scats of *L. felina* and found that fish represents an important percentage of prey in northern than southern populations in Peru. According to this, the occurrence of microplastic that we are reporting here for *L. felina*, might be induced by fish consumption, we support this finding based on results of De-La-Torre et al. (2019) who found microplastics in species commonly preyed by marine otters like Peruvian silverside (*Odontesthes regia*), Peruvian morwong (*Cheilodactylus variegatus*) and Peruvian grunt (*Anisotremus scapularis*).

In our results fiber was the most common detected microplastic (**Fig**. 1), similar to Nelms et al. (2018) for Atlantic mackerel. However, the same authors also found that type, color and size of microplastics differ between the prey and the predator. According to Eriksson & Burton (2003) and Nelms et al. (2018), the majority of particles detected in scats of grey seals were fragments. *L. felina* scats presented several types of microplastics like Nelms et al. (2018) detected for grey seals, but additionally we found potential occurrence of microbeads (Figure 1) a primary microplastic.

Effects of microplastics in marine organisms are not clear, especially in the case of marine mammals. Bioaccumulation and biomagnification of chemical contaminants can have an unhealthy effect on marine organisms. Murphy et al. (2015) found a reproductive failure in harbor porpoises *Phocoena phocoena* due to pollutant exposure. The occurrence of organochlorines was identified as endocrine disruptor (Murphy et al. 2015). This kind of chemicals has long live and wide distribution in the ocean and the ingestion of microplastics may represent another pathway to enter into marine mammals bodies. Our results confirm that plastic in the form of microplastics are already part of the food web in the marine ecosystem. In this particular case, a top predator of the coastal marine habitat has already assimilated primary and secondary microplastics in its metabolic process; consumption would be indirect through the ingestion of prey carrying microplastics inside their bodies. *L. felina* is a coastal top predator living closer to humans. Pollution from terrestrial sources impact directly and indirectly to this predator, directly through the concentration of debris and plastics reducing their habitat quality, and indirectly through the ingestion of prey carrying debris inside and the contamination with bacteria. Valqui (2011) reported five individuals in Punta Corrientes. The

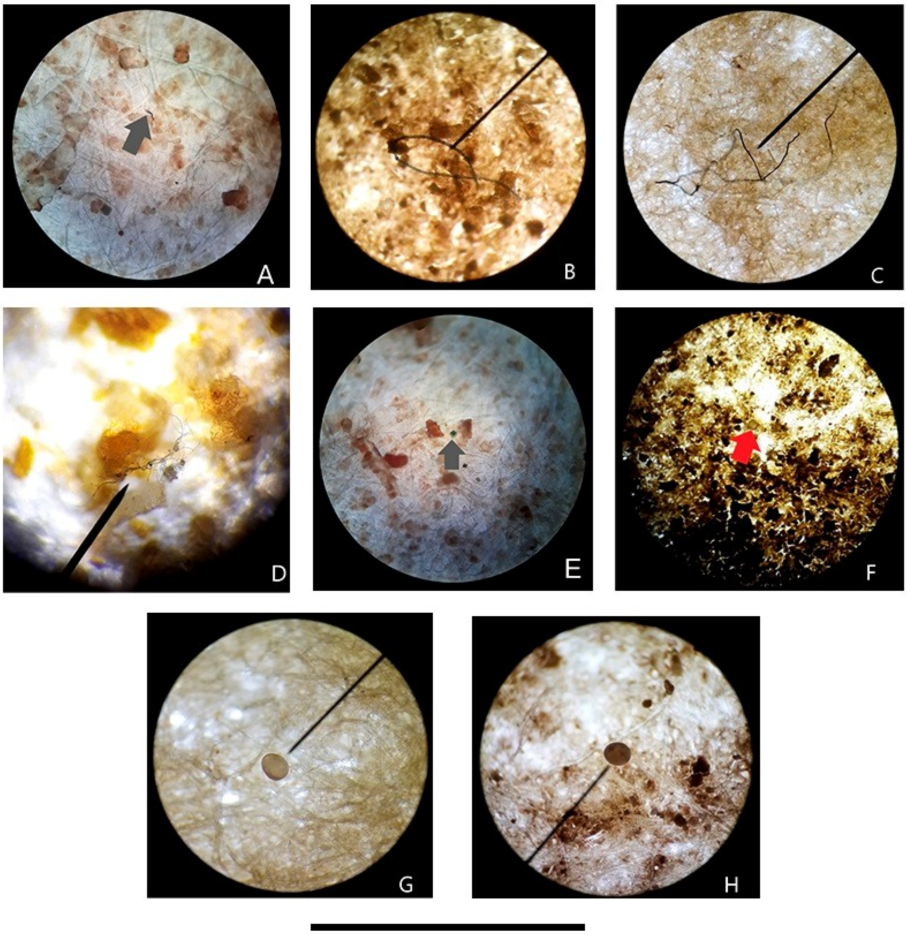

Fig. 1. Microplastics found in the scat of *Lontra felina*, objective 10X, bar scale is 1.8 mm. A) Brown fiber. B) Grey fiber. C) Brown fibers. D) Fibers. E) Green fragment. F) Semi-transparent fragment. G) Possible microbead. H) Possible microbead.

number of individuals using the cave at present is difficult to determine, and ecological information about the species says that home-range overlap and no avoidance are common for marine otters (Medina-Vogel et al. 2007). The amount of sample collected cannot therefore be considered as representative for one individual exclusively and probably would represent the whole population reported for Punta Corrientes. Next steps in this research are establishing the extension of this pollution in terms of populations and seasonality, and the effect in the natural dynamics of the species.

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