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Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles

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ABSTRACT

Anthropogenic particles (APs), including microplastics, are ingested by a wide variety of marine organisms. Exposure of *Clupeiformes* (e.g. herrings, anchovies, sardines) is poorly studied despite their economic and ecological importance. This study aims to describe the morphology of the filtration apparatus of three wild-caught *Clupeiformes* (*Sardina pilchardus*, *Clupea harengus* and *Engraulis encrasicolus*) and to relate the results to ingested APs. Consequently, the species with the more efficient filtration apparatus will be more likely to ingest APs. We hypothesized that sardines were the most exposed species. The filtration area and particle retention threshold were determined in the three species, with sardines displaying the highest filtration area and the closest gill rakers. Sardines ingested more fibers and smaller fragments, confirming that it is the most efficient filtering species. These two results lead to the conclusion that, among the three studied, the sardine is the species most exposed to APs.

1. Introduction

It is estimated that in 2010, up to 13 million tons of plastic ended up in oceans (Jambeck et al., 2015). This may cause negative impacts on wildlife (Laist, 1997; Wright et al., 2013) because the plastic can be ingested by many marine organisms (Cole et al., 2011), causing mechanical (Bugoni and Krause, 2001; Boren et al., 2006; Gregory, 2009) and/or toxicological harm (Browne et al., 2013; Rochman et al., 2013). Macroplastics (>5 mm) and microplastics (MPs; 0.1 μm to 5 mm; Klaine et al., 2012; Koelmans et al., 2015) are ingested by a wide range of organisms including marine birds (Brandão et al., 2011; Fife et al., 2015; Jiménez et al., 2015), marine mammals (Walker and Coe, 1989; Secchi and Zarzur, 1999; Jacobsen et al., 2010), marine turtles (Bjorndal et al., 1994; da Silva Mendes et al., 2015), fish (Collard et al., 2015; Romeo et al., 2015), zooplankton (Cole et al., 2013), and mollusks (Van Cauwenberghe and Janssen, 2014). In laboratory experiments, these plastics have been shown to be transferred from one trophic level to another (Farrell and Nelson, 2013; Setälä et al., 2014). Plastic material is considered an endocrine disruptor (Rochman et al., 2014). In addition, once ingested, anthropogenic particles (APs, which include MPs and other particles with certified anthropogenic origins such as artificially dyed fibers), can introduce several types of pollutants, including PCBs, triclosan, PAHs, and PBDEs within the organism (Besseling et al., 2013; Browne et al., 2013; Rochman et al., 2013), also causing, for example, endocrine disruption (Rochman et al., 2014) and hepatic stress (Rochman et al., 2013).

Teleosts have been reported to ingest APs (Foekema et al., 2013; Lusher et al., 2013; Collard et al., 2015; Neves et al., 2015), including MPs. However, they have different feeding mechanisms allowing the seizure of different kinds of prey, which means that the route of exposure might be different. In bony and cartilaginous fishes, gill rakers (GRs) are found at the level of the branchial basket (Gibson, 1988; Gerking, 1994). The primary function of these GRs is to protect the gill epithelium by retaining particles from the water flow during breathing (Lagler et al., 1962; Elsheikh, 2013). In some species, such as *Clupeiformes*, however, GRs have acquired a second function related to feeding (Elsheikh, 2013; Magnuson and Heitz, 1971). Filter-feeder fishes possess numerous and elongated rakers that are used as a net to extract food from the water flow and direct it toward the esophagus (Gibson, 1988). These rakers can be rod-like or fitted with small denticles, also called microspines (Iwata, 1976), microbranchiospines (Smith and Sanderson, 2007) or teeth (Gibson, 1988). These denticles have been reported in distantly teleost families such as *Clupeidae*

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(Gibson, 1988), *Cyprinidae* (Iwata, 1976), *Comephoridae* (Jakubowski, 1996), *Carangidae* (Sanderson et al., 1996), and also in some elasmobranchs (Misty Paig-Tran and Summers, 2014). Differences in mesh reflects the ability to catch different kinds of prey. In parallel, this should also support the fact that filtration efficiency would change species' capacity to consume APs.

In this study, we compare three planktivorous *Clupeiformes* that are all highly consumed fish products by humans: the Atlantic herring (*Clupea harengus*, Linnaeus 1758), the European pilchard (or sardine; *Sardina pilchardus*, Walbaum 1792) and the European anchovy (*Engraulis encrasicolus*, Linnaeus 1758). For each of these three species, we aim to determine the degree of exposure to AP pollution. To this end, two complementary approaches are used. Based on GRs and denticles morphometry, we define a new method that accurately evaluates the filtration areas and the minimum diameter of particles ingested. The degree of exposure is compared with APs found and characterized in sixty stomach contents from wild fish, providing a first picture of the impact on taxa.

2. Materials and methods

2.1. Sampling

Three planktivorous species (*C. harengus*, *S. pilchardus* and *E. encrasicolus*) were sampled. Fish were caught in three different zones (Fig. 1): the English Channel, the Northwestern Mediterranean Sea and the Northeastern Atlantic (Bay of Biscay), and at three different periods (Table 1). All sampling surveys were organized by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER). Fig. 1 was made with Google Earth (Google, Mountain View, CA, U.S.A.).

Twenty individuals of each species were used for stomach contents analysis and five individuals of each species were used for morphological analysis. Individuals were dissected on-board. Gill baskets and stomachs were directly stored in a 5% formaldehyde solution. Total lengths (TLs) of fish were recorded. The first left gill arch was used for pictures and measurements (Alexandrino et al., 2006; Costalago and Palomera, 2014; Costalago et al., 2015).

2.2. Morphological study

2.2.1. Light microscopy

Gill arches were observed with a stereomicroscope (Zeiss Stemi 2000-C, Edmunds optics, Germany) and photographed with a 5 megapixels camera (Tucsen ISH500 v1.48, Xintu Photonics Co., China). Different measurements (Fig. 2) were carried out using ImageJ v1.48 software (National Institutes of Health, U.S.A.) on lengths of epibranchial, ceratobranchial, hypobranchial and GRs. Length of gill arches was calculated by summing up the epi-, cerato- and hypobranchial lengths.

2.2.2. Scanning electron microscopy

Other structures of the gill arches were observed in scanning electron microscopy (SEM), including the gap between GRs and denticles, the thickness of GRs and denticles, and the length of denticles (Fig. 2). Gill arches were dehydrated through a graded ethanol series then mounted on a glass slide and sputter-coated with a 20 nm Pt in a BALZERS SCD 030 unit. Two individuals from each species were used to measure denticles parameters. Pictures were taken with the Orion software (v 6.60.6) in a SEM Jeol JSM-840A (Japan) working at 20 kV of accelerating voltage.

2.2.3. Filtration area calculation and particle retention

To calculate filtration areas and particle retention, three different calculations were used. The first one, based on the method developed by Magnuson and Heitz (1971), consists of adding the area covered by GRs on the epibranchial (upper area) to the area covered by GRs on both cerato- and hypobranchial (lower area) of one gill arch (Fig. 3). The second calculation, which was developed by Gibson (1988), takes into account the space occupied by the GRs and adds the areas of open spaces between GRs where water flows. Finally, the third calculation, called “alpha” is a formula that we have developed with the aim of taking denticles into account.

The alpha formula uses Gibson's formula with additional parameters in order to include the space occupied by denticles in the calculation:

$$F = (\Sigma L - L_{max}) * (\overline{G} - 2x) \quad \text{where} \quad x = L_d{}^* \sin\alpha$$

Fig. 1. Map presenting all sampling points. Black symbols: sampling points for the contamination study; grey symbols: sampling points for both morphological and contamination studies; white symbols: sampling points for the morphological study.

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