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Microplastics impair the feeding performance of a Mediterranean habitat-forming coral

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ABSTRACT

The impact of plastic debris, and in particular of microplastics (here referred as particles smaller than 5 mm) on aquatic environments has now become a topic of raising concern. Microplastics are particularly abundant in the Mediterranean Sea, potentially exerting substantial pressures on marine organisms at different levels of organization. Ingestion of microplastics has been observed in a large number of marine species. The aim of this work is to test if microplastics produce a feeding impairment in *Astroides calycularis*, a shallow water, habitat-forming coral endemic to the Mediterranean Sea. Our findings suggest a lack of any avoidance mechanism allowing the polyps to discern between food items and microplastics when occurring simultaneously. Moreover, polyps spend a considerable amount of time on handling microplastic particles. As a consequence, microplastics impair the feeding efficiency in *A. calycularis,* since polyps may not be fully able to profit from the drifting plankton aggregations. Therefore, we suggest that microplastics can cause a reduction of fitness in *A. calycularis*, and presumably also in other species characterized by suspension feeding strategy.

1. Introduction

The global production of plastic has increased dramatically in the last decades, reaching 348 million tonnes in 2017 (Plastics Europe, 2015). Its high durability, coupled with inadequate disposal and indiscriminate discards have made plastic one of the most critical and ubiquitous sources of pollution, in particular for marine ecosystems (Moore, 2008; Wright et al., 2013b; Eriksen et al., 2014). Plastic typically ends up in the oceans because of stormwater runoff, direct dumping and loss of fishing and aquaculture gears (Andrady, 2011). Although it is complex to obtain a precise estimate of the plastic litter that reaches the ocean (Andrady, 2011), studies have estimated that there are at least 5.25 trillion plastic particles, weighing more than 268 tonnes, floating in the sea (Wright et al., 2013b). The projected amount of plastic debris at sea by 2025 is about 250×10^6 tonnes (Jovanović, 2017).

Researchers have recently started to investigate the environmental impacts that smaller plastic debris, broadly defined "microplastics", may have on marine organisms (Murray and Cowie, 2011). For the purpose of the present study, microplastics are here referred to as particles equal or smaller than 5 mm (Arthur et al., 2009; Kershaw, 2015) that result either from the fragmentation of larger plastic items or directly from cosmetic products and synthetic fabric residues that enter the oceans through sewage runoff (Fendall and Sewell, 2009; Barnes et al., 2009; Browne et al., 2011). However, there is currently a debate on the dimensional definition of microplastics, which can take a variety of names across the range from millimeters to nanometers (Andrady, 2011; Thompson, 2015).

In recent years, a growing body of literature has acknowledged the adverse effects of microplastics on aquatic organisms (Burns and Boxall, 2018; de Sá et al., 2018). Laist (1997) estimated that 267 marine species were affected by plastic ingestion and entanglement, and corals were

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found to be particularly sensitive to derelict fishing gear, which is a consistent component of the plastic left at sea (Ballesteros et al., 2018). Traditionally, the attention has been focused mainly on how plastics affect large marine animals, such as commercial fish species, mammals, turtles and seabirds (Laist, 1997; Robards et al., 1997; Bugoni et al., 2001; Baulch and Perry, 2014). However, because of their small size and ubiquitous distribution microplastic particles can be potentially ingested by a wide range of marine animals other than the largest ones (Lusher, 2015; Allen et al., 2017).

As microplastic particles fall within the same size range as sediments and several planktonic organisms (Fendall and Sewell, 2009) they can be ingested by filter and suspension feeders, as well as sediment-ingesting organisms, leading to unpredictable cascading effects across trophic levels (Browne et al., 2008; Nadal et al., 2016; Franzellitti et al., 2019). In fact, the impact of microplastics ingestion by invertebrates is a developing topic of research (Lusher, 2015). For example, Cole et al. (2015) reported a significant decrease in energy storage rates in the copepod *Calanus helgolandicus* exposed to microplastic particles because of a decreased feeding capacity. Diminished feeding activity, coupled with a depleted energy reserve, were observed in the green crab *Carcinus maenas* and the annelid *Arenicola marina* following microplastics ingestion (Wright et al., 2013a; Watts et al., 2015). Moreover, microplastics ingestion was observed to cause tissue damage and feeding impairment in the blue mussel *Mytilus edulis* (Von Moos et al., 2012; Wegner et al., 2012) and false satiation in the Norway lobster *Nephrops norvegicus* (Murray and Cowie, 2011). Microplastics may provide substratum for harmful microbial communities and act as a source or vector of contaminants through the food web (Mato et al., 2001; Teuten et al., 2009; Zettler et al., 2013; Avio et al., 2015). Overall, the response of organisms to microplastics ingestion appears to be species-specific and depends on environmental conditions (Hall et al., 2015). Corals are traditionally considered non-selective suspension feeders (Jørgensen, 1966; Rajkurmar and Parulekar, 2001, p. 472) that mostly feed on zooplankton (Palardy et al., 2008; Houlbrèque and Ferrier-Pagès, 2009). Since microplastics fall within the same size range of their normal prey, they may be accidently ingested by corals (Hall et al., 2015).

To date, only a few studies have investigated microplastics ingestion in corals. Hall et al. (2015) demonstrated that the tropical *Dipsastrea pallida* ingests microplastics, which may be retained by the corals for a relevant amount of time. The polyp mesenterial tissue, which is responsible for digestion in corals, can trap the non-edible particles leading to a possible impairment of food digestion (Allen et al., 2017; Hall et al., 2015). Such a mechanism has been observed in corals such as *Pocillopora verrucosa* and *Lophelia pertusa* (Chapron et al., 2018; Reichert et al., 2018). Furthermore, microplastics exposure may cause tissue necrosis in *P. verrucosa* (Reichert et al., 2018).

The orange stony coral *Astroides calycularis* (Pallas, 1766) is an azooxanthellate scleractinian coral endemic to the Mediterranean Sea. It occurs mainly from the Strait of Gibraltar to Sicily and its surrounding islands, and it has been observed sporadically in the Adriatic Sea (Musco et al., 2016; Terrón Sigler et al., 2016; Ingrosso et al., 2018). Typically, *A. calycularis* aggregates in dense colonies, and occurs from the surface down to 50 m, but it is more abundant within the upper 15 m (Ingrosso et al., 2018). It occupies rocky substrates preferentially subject to intense water turbulence in both well-lit and dark habitats (Ingrosso et al., 2018). This shallow-water coral is a reef building species, strictly protected under the Bern Convention (Annex II), and listed as endangered or threatened within the Barcelona (Annex II) and the CITES Conventions (https://www.cites.org/eng/node/19812 accessed 13/01/2020). The species falls within the LC (Least Concern) category of the IUCN Italian Committee (Ocaña et al., 2015), although Prada et al. (2019) observed a drop in the health status of populations of this species (reduction of percent cover/polyp length) exposed to drivers of change of human origin. As other azooxanthellate corals, *A. calycularis* seems to be a non-selective suspension feeder that is thought to prey on mesozooplankton, although the composition of its diet is still unclear and more research is needed to determine it. In fact, it has been recently observed to prey on megaplankton by means of a cooperative behaviour among polyps and colonies (Musco et al., 2018). The Mediterranean basin is considered the sixth greatest accumulation zone of marine litter in the world, holding 7% of all global microplastics in only 1% of the world marine waters, with record levels of concentration of 1.25 million fragments per square km (Cózar et al., 2015).

The aim of this study was to investigate the potential effect of microplastics on the feeding performance of the above mentioned Mediterranean scleractinian coral species. In particular, we predicted that *A. calycularis* exposed to microplastics would be able to recognize, and eventually avoid, microplastic particles. Instead, if *A. calycularis* is a non-selective suspension feeder like it is assumed for most coral species (Palardy et al., 2008; Houlbrèque and Ferrier-Pagès, 2009), then polyps would not discern between microplastics and food items. Therefore, the null hypotheses experimentally tested were that: (1) catches of food particles and microplastics would be made at the same rate when occurring alone (i.e. independently); (2) the amount of catches of food items when occurring alone does not differ from the amount of catches of food items when occurring mixed with microplastics; conversely (3) the amount of catches of microplastics when occurring alone does not differ from the amount of catches of microplastics when occurring mixed with food items.

Should any of the null hypotheses be rejected, the coral would exhibit discrimination between food types. Should the presence of microplastics interfere with food uptake by the coral, it would incur in a reduced feeding performance (if microplastics promote a lower catch rate of food particles) or an enhanced one (if microplastics promote a higher catch rate of food items).

2. Material and methods

2.1. Coral collection and rearing

Thirty-two colonies of *A. calycularis* were collected from April to June 2015 along 10 km of the Zingaro coast (NW Sicily, central Mediterranean), where the coral usually is a dominant species from 0 to 5 m depth. The marine environment is not subject to protection in the area of collection: artisanal fishing, boat anchoring and recreational activities (including diving and fishing) are permitted. During periodical surveys conducted every three days, detached colonies found on the bottom were collected. Colonies dislodged due to mechanical disturbance (such as snorkeling, diving or storms) are normally found lying on the bottom where the species is present and abundant (Musco et al., 2017). Only the apparently healthy colonies (i.e. those with active polyps and no evidently damaged tissues) were transported to the Marine Ecology Lab (Consiglio Nazionale delle Ricerche) in Castellammare del Golfo (NW Sicily) and reared in seawater tanks. Once in the laboratory the colonies were glued to marble tiles using epoxy putty and kept in three 80 l tanks filled with filtered seawater. Before the experiment, colonies were left to acclimatize for 72 h under starvation.

2.2. Experimental design and set-up

In order to test the experimental hypotheses, a factor named Food type was considered, fixed with four levels: (S) 20 dehydrated euphausiid shrimps, (M) 20 microplastic particles, (S(M)) 20 shrimps in presence of 20 microplastics, and (M(S)) 20 microplastics in presence of 20 shrimps. The difference between the last two groups is that in S(M) only the ingestion of shrimps was quantified and in M(S) only that of microplastics, in order to get independent measurements. The total density of food items was not kept constant in order to mimic natural conditions, assuming that zooplankton density does not vary in the short term as a consequence of microplastics presence in the environment. Therefore, particle density was double in groups S(M) and M(S) than it was in groups S and M. Eight coral colonies were randomly chosen from those collected and allocated into one of the four experimental groups. Dehydrated euphausiid shrimps measured approximately 1.3 mm–6.3 mm in length. Microplastics were obtained by sorting shreds of polyethylene (PE) plastic bags, since PE is the major polymer type in the oceans (Cózar et al., 2015). Shreds with a size between 2 and 3 mm were selected, according to the classification of microplastics and to allow visual identification during the video analysis of trials. Microplastics were conditioned for three days in filtered, aerated seawater tanks before the experiment, in order to make them similar to those occurring along the coastline. Three response variables were recorded: (1) "Contact" – the number of times each item entered in contact with a polyp of the colony during 30-min tests; (2) "Ingestion" – the number of times each item was ingested by a polyp of the colony during 30-min trials; (3) "Handling time" - the time occurring between the catch, ingestion and expulsion of microplastics by the polyp during 90-min trials. Handling time was recorded in nine polyps randomly chosen among those that ingested a microplastic particle from the M(S) group, assumed to mime natural conditions at sea. All three response variables were quantified *a posteriori* through video analysis.

The experiment was run in four tanks (32 × 22 × 22 cm each) filled with 15 l of filtered seawater and equipped with one air bubbler to create turbulent water movement resembling the native shallow habitat conditions, and allow an even distribution of microplastics and shrimps. Each colony attached to its own marble tile (25 cm^2) was placed perpendicularly to the tank bottom simulating the most common position in the native environment. One replicate consisted of a trial made with a colony exposed to one of the four Food types. Food was left on the water surface directly onto the bubbles, thereby obtaining rapid diffusion of the particles within the water mass. Four trials were run simultaneously, each one with a colony from a different treatment, for a total of 32 trials (eight replicates per treatment). The order of the colonies in the four arenas was randomly assigned each time, and every colony ran a single trial. At the end of each trial, the tanks were carefully cleaned and rinsed with freshwater. Two video cameras linked to a CCTV recording apparatus allowed to record the trials in the four tanks simultaneously. The video files were analysed using the BORIS (Behavioural Observation Research Interactive Software) event logging software. Colony surface areas were also measured, in mm^2, from frames of the videos and analysed.

2.3. Statistical analysis

Potential differences among Food type levels were tested. Being the number of contacts and the ingestion rate (IR) possibly dependent on the colony size, the latter was treated as a covariate in a 1-way ANCOVA. The analysed variables were number of contacts and IR, expressed as the ratio between number of ingestions and number of contacts recorded in the same trial. Subsequently to the main analysis, a set of planned comparisons were computed in order to test for the experimental hypotheses (Ruxton and Beauchamp, 2008). Contrast were coded for comparing IR in (1) S *versus* M; (2) S *versus* S(M); and (3) M *versus* M(S). All statistical analyses were performed using the software Statistica v.7.1.

3. Results

Colony surface (810.12 [mean] ± 385.52 [S.D.] mm) had no effect among the levels of the factor Food type.

The average number of contacts were 25 ± 7.52 for treatment M (mean ± 1 S.E.), 18.25 ± 5.68 for M(S), 19.25 ± 9.07 for S(M), and 15.13 ± 5.68 for S. No significant differences were found in the number of contacts among any of the four experimental groups.

Polyps ingested shrimps as well as microplastics (Fig. 1; Supplementary Video 1, Supplementary Video 2). The highest average IR was recorded for treatment S with 75.72 ± 4.04% (mean ± 1 S.E.), the lowest for treatment M with 31.92 ± 6.55% (Fig. 2). IR of food items was found to be significantly different between S and S(M) (Table 1, Fig. 2).

In particular, IR in S(M) decreased by 21.24% in average, from 75.72 ± 4.04% to 54.48 ± 4.65% (Fig. 2). Inversely, IR significantly increased for microplastics in presence of shrimps (M(S)) with respect to the values scored in the group with only microplastics (M). Such increase was 26.44% on average, from 31.92 ± 6.55% in M to 58.36 ± 5.19% in M(S) (Table 1, Fig. 2).

The handling time of microplastics lasted on average 86.12 ± 4.73 min. The observation time ended with the *a priori* fixed conclusion of the trial and not with the actual microplastics expulsion, which in some cases lasted more than 90 min. This implied an underestimation of the time wasted by the polyps for catching, swallowing, and regurgitating microplastics (Fig. 1; Supplementary Video 1, Supplementary Video 2).

4. Discussion

Astroides calycularis displays a certain degree of feeding selectivity when microplastics and food items are not mixed, as proven by the higher ingestion rate of food items compared to that of microplastics, thus rejecting the null-hypothesis 1 (see Fig. 2, Table 1). The coral consumed food particles when they were offered together with microplastics, with a reduced rate in comparison to the catches of food items alone (see Fig. 2, Table 1). This result leads to the rejection of the null hypothesis 2, and demonstrates that the presence of microplastics causes a lower food intake in *A. calycularis* polyps. However, *A. calycularis* did

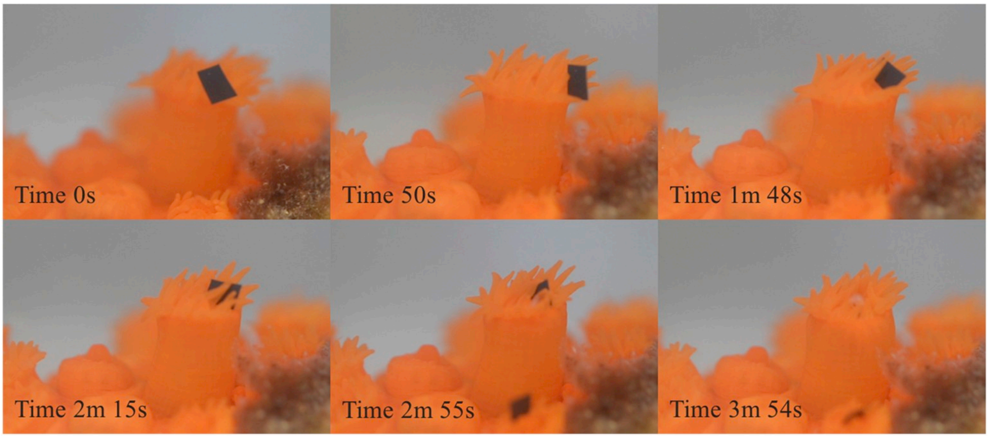

Fig. 1. Video frame sequence of capture and ingestion of a microplastic particle by a polyp of *Astroides calycularis*. Full length videos of the complete capture, ingestion and regurgitation sequence are available as supplementary material (photo credits: LM and AZ).

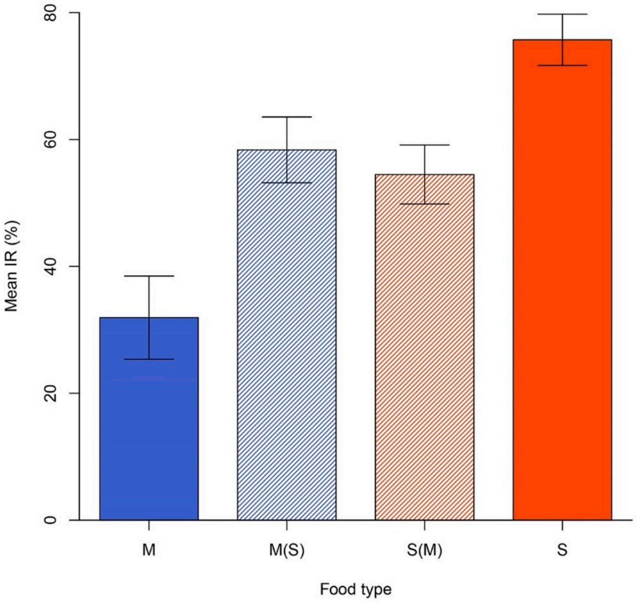

Fig. 2. Mean ingestion rate (IR, in %) of *Astroides calycularis* in the four different experimental groups. Microplastics (M), shrimps in presence of microplastics (M(S)), microplastics in presence of shrimps (S(M)) and shrimps (S). Vertical bars represent 1 S.E.

Table 1
Results of 1-way ANCOVA testing for differences in IR among levels of the factor Food type (top) and subsequent planned comparisons (bottom). M = microplastics, S(M) = shrimps in presence of microplastics, M(S) = microplastics in presence of shrimps, S = shrimps.

Source of variation	df	MS	F	P
Intercept	1	0.94	43.04	<0.0001
Colony size	1	0.01	0.37	0.5496
Food type	3	0.31	14.19	<0.0001
Residual	26			
Total	31			

Experimental group	P-value
S *vs.* M	<0.0001
S *vs.* S(M)	0.0116
M *vs.* M(S)	<0.0001

not disregard microplastics, since it actively captured and ingested microplastics although at a lower rate than food particles (see Fig. 2, Table 1). In absence of food particles, microplastics ingestion was lower than when occurring together with edible particles. Indeed, the presence of shrimps enhanced the ingestion of microplastics by the polyps, hence hypothesis 3 was rejected. In particular, the IR value recorded in the group where microplastics were provided together with shrimps was almost double than that in the group exposed exclusively to microplastics (see Fig. 2, Table 1). Since IR is the ratio between the number of ingested particles and the number of contacts, this finding is not attributable to variation in the density of particles. Our findings are thus in line with what observed in the sea anemone *Aiptasia pallida* (de Orte et al., 2019), since also for this last species the presence of prey stimulated feeding activity of polyps and increased the intake of microplastics. This reveals that polyps of both *A. pallida* and *A. calycularis* display some degree of preference for food particles over microplastics, but do not avoid the latter, particularly when present simultaneously with food items.

The mechanism underlying the ingestion of microplastics by *A. calycularis* is not known. The presented evidence indicates that polyps of *A. calycularis* are somehow able to perceive differences between plastics and shrimps. However, this ability does not allow polyps of *A. calycularis* to avoid non-edible particles. Feeding behaviour is usually mediated by chemical cues in anthozoans (Kamio and Derby, 2017), hence a plausible explanation is that either plastics, the bacterial biofilm settled on plastics, or both, contain and release phagostimulants that trigger their consumption by anthozoans that encounter plastic items (Allen et al., 2017). Indeed, *A. calycularis* polyps in the absence of shrimps captured and ingested microplastic particles, although at a lower rate. Additional chemical cues released by food particles in the surrounding water could be responsible for the enhanced consumption of microplastics by the coral in presence of food. Importantly, ingestion of microplastics impairs food intake in *A. calycularis* polyps, especially whereas food is an ephemeral and rather unpredictable resource, as it is normally the case with sessile suspension feeders predating upon drifting zooplankton aggregations. In such instance, the ingestion and subsequent egestion of inert particles implies polyp inflation, mucus production, ciliary and tentacular action, with significant time and energy costs (Erftemeijer et al., 2012). Eventually this would imply the loss of opportunities to fully exploit a drifting patch of particulate food. This study suggests that the presence of microplastic particles in the water column should be expected to cause a reduction in the fitness of *A. calycularis*, analogous to that observed in other coral species. For example, in the scleractinian coral *Dipsastrea pallida* microplastics ingestion leads to the impairment of natural food digestion (Hall et al., 2015). Two more scleractinian corals, *Montastraea cavernosa* and *Orbicella faveolata* were observed to ingest microplastics, especially those ranging from 425 μm to 2.8 mm (Hankins et al., 2018). Reichert et al. (2018) demonstrated how microplastics not only negatively affected the feeding capacity in five out of six stony coral species analysed, but they also led to bleaching and tissue necrosis. Differently, microplastics exposure did not seem to affect the prey capture efficiency in the deep-water coral *Lophelia pertusa*, while it led to a reduction in the coral skeleton mineralization rate, with a negative impact on the coral fitness (Chapron et al., 2018). It was suggested that in this last species, polyps increased their feeding activity as a compensatory behaviour triggered by the nutrient deficit caused by microplastics ingestion.

In the present study, once microplastics were captured by the polyps, an average handling time of 86 min was observed. This result is a likely underestimation of the average handling time, as the variable was recorded for a maximum of 90 min. Other studies reported that microplastics remained in the coral digestive system for at least 24 h (Hall et al., 2015; Allen et al., 2017) or even more before expulsion (Hankins et al., 2018). Even if the average handling time could be longer than measured, our findings show that *A. calycularis* wastes a considerable amount of time (and presumably of energy) handling microplastic particles.

Microplastics ingestion by corals raises additional issues. It has been hypothesized that a protracted microplastics residence time can cause a false satiation sense and the blockage of the digestive tract in coral species (Hall et al., 2015; Allen et al., 2017), as well as in other benthic organisms (Murray and Cowie, 2011; Watts et al., 2015). The ingestion and retention of microplastics may cause the transfer of toxic hydrophobic substances, like persistent organic pollutants (POPs) (Rochman et al., 2013a, 2013b, 2016). Plastic is known to adsorb high concentrations of POPs (Mato et al., 2001; Rios et al., 2007; Teuten et al., 2009; Rochman et al., 2013b), with possible transfer of toxic contaminants to coral tissues after ingestion (Peachey and Crosby, 1996; Negri and Heyward, 2000; Guzmán-Martínez et al., 2007). Our study was not designed for analysing these issues, but we cannot exclude similar detrimental effects on *A. calycularis*. Further studies are needed to assess more precisely the effects of plastic ingestion on the health of *A. calycularis*, such as the possible impairment of its digestive system, its skeletal growth as well as the possible accumulation of toxic pollutants. Nonetheless, the presence of microplastics in the marine environment is detrimental for the feeding performance of the orange stony coral and they represent an additional threat to this species. IUCN has generically classified pollution, human coast exploitation and underwater tourism to be the major threats for *A. calycularis* (Ocaña et al., 2015). Prada et al. (2019) have demonstrated that *A. calycularis* health is negatively related to anthropogenic pressure, identifying sea-based impacts (such as shipping, invasive species, artisanal and commercial fishing) as the main driver for decrease of population abundance and impairment in biometry, which are important indicators of health status in bioconstructor coral species (Jameson et al., 1998; Uychiaoco et al., 2001). Considering that 20%–40% of the plastic marine litter is produced by fishing activity (Sheavly, 2005; Veiga et al., 2016) we hypothesize that the anthropogenic impacts on *A. calycularis* observed by Prada et al. (2019) may at least in part be caused by this kind of plastic debris.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on motivated request.

Declaration of competing interest

The authors have no conflict or competing interests that might be perceived to influence the results or discussion reported in this paper.

CRediT authorship contribution statement

Beatrice Savinelli: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Tomás Vega Fernández:** Conceptualization, Data curation, Formal analysis, Writing - review & editing. **Nicola Maria Galasso:** Methodology, Writing - review & editing. **Giovanni D'Anna:** Methodology, Writing - review & editing. **Carlo Pipitone:** Methodology, Writing - review & editing. **Fiorella Prada:** Methodology, Writing - review & editing. **Arturo Zenone:** Methodology, Writing - review & editing. **Fabio Badalamenti:** Conceptualization, Methodology, Writing - review & editing, Resources. **Luigi Musco:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, Resources, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.104887.

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