Note

Microplastic contents from maricultured and natural mussels

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ABSTRACT

Results of this research focuses on microplastic contents (levels, type, size, colour) in maricultured and natural mussels (*Mytilus galloprovincialis*) from different Italian stocks. No significant differences were found among maricultured and natural stocks. All recovered MPs are filaments ranging within 750–6000 μm of maximum length (average values 1150–2290 μm). Feeding raw mussel could produce median MP intakes of 6.2–7.2 items/g w.w. Concerning human exposure by diet, both raw and cooked values are important. Some preliminary tests performed in this study evidenced that the cooking process determined lower MPs levels (−14%) in cooked tissues compared to raw ones, MPs were recorded in cooking water and were characterized by a lower size than in raw mussels. Results obtained by this study represent an important baseline on MPs level to evaluate environmental and human exposure risks by diet.

There is a global concern over the impact of plastic waste, mainly microplastics (MPs, particles of size below 5 mm, according to NOAA, 2008) found in seas and rivers, on natural habitats and wildlife. MPs could represent significant risks for marine habitats and could affect marine species and top predators throughout marine trophic webs. Furthermore, a potential concern is related to the high concentrations of contaminants such as persistent organic pollutants (POPs), which can be absorbed by MPs in the environment (Cole et al., 2011) or may also be present in plastic material since used in packaging, such as bisphenol A (BPA) or phthalates (Fossi et al., 2016). At the best of our knowledge, there are not studies highlighting as chemicals from microplastics can be transferred to organisms along the trophic web and these aspects should be improved by further researches. Intake of MPs by mussels has been reported by the literature (Browne et al., 2008; Santana et al., 2016). Mussels are interesting species to evaluate risks associated to MPs in marine habitats due to they can: -) accumulate MPs in water column by filtering; −) transfer MPs from water column towards the marine trophic web as they are predated by many marine species (i.e. carnivorous gastropods, sea breams, starfish); −) affect human health by diet based on raw or cooked mussels. In spite of mussels importance in MPs transfer from water column through the trophic web towards humans, no data are reported on MPs levels in natural mussels stocks and commercial products to evaluate both ecosystem and humans health risks associated to mussel consumption. In particular, concerning humans, it is important to estimate the average intake; EFSA estimated the amount of MPs contained in a portion of mussels and, at the same time, stated that research should produce data on the presence of microplastics including effects of food processing (EFSA, 2016).

Given the above needs on data collection and in response to the indications of EFSA, this study was performed in order to attain the following purposes: i) defining levels and principal features (type, size, colour) of MPs in mussels (*Mytilus galloprovincialis*, Lamarck 1819) from maricultured and natural stocks; ii) comparing MPs levels and principal features measured in maricultured from various Italian plants to levels in natural stocks; iii) evaluating differences occurring among MPs levels in raw and cooked mussels.

Three different commercial stocks of *M. galloprovincialis* coming from different Italian mariculture plants (Cesenatico, central Adriatic Coast; La Spezia, Ligurian Sea Coast, and Olbia, North East Sardinia, Tyrrhenian Sea) were collected from local markets. A natural stock of the same species was sampled from natural rocky bottom in Tuscany (Talamone, central Tyrrhenian Sea) to evaluate differences among commercial and natural stocks concerning MP levels and principal features. Collected animals were measured to determine biometrics (maximum length, mm; weight, g) to build for each stock the size curve of the sampled population. A representative number of animals were collected from each tested stock selecting them from the same size class and preferring more represented size classes. Animals were analysed individually rather than in pools to improve data representativeness and statistic significant of collected data. Raw animals from the same size class ($n = 10$) were dissected to open valves, hepatopancreas and gills were excised thanking care not to damage them, weighted and extracted. To evaluate the effect of cooking, another group of animals from the same stock ($n = 10$) were cooked independently in an

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accurately rinsed glass backer with 50 mL of deionized water. Cooking time was standardized in 2 min of cooking in boiling water, to simulate a real cooking process. Then cooked mussels and cooking water were analysed separately. In particular, cooked tissues were excised to remove hepatopancreas and gills, weighted and extracted to recover litter. Extraction was performed in glass backer with 20 mL of H_2O_2 30% per gram of tissue, backers were put in a Bain-marie at 50 °C for 48 h (raw tissues) but, if needed, the extraction time was extended till the complete digestion of tissues (cooked tissue) as adapted by Nuelle et al. (2014). Extracted sample (raw and cooked tissues) were completely recovered onto paper fibres (0.45 µm filter disks) by the use of a filtrating system, consisting in filtration glass set, vacuum pump, manifold and funnel. Glassware was accurately rinsed to increase recovery efficiency of litter. Filters were stored in glass Petri dish and dried in oven at 40 °C till constant weight. Cooking water was filtered directly taking care to recover by rinsing any plastic items from the backer walls and filters were dried as reported. The plastic items were determined by stereomicroscopy (Nikon SMZ-800 N) and all identified items were divided in shape, colour and dimensional classes reported by the literature (Galgani et al., 2013; Alomar et al., 2016; Blaskovic et al., 2017; Fastelli et al., 2016). The Nikon's software for the imaging analysis was applied to the litter dimensional measurements (Nikon ACT-1). Data on litter items counting and litter dimensions are reported as mean + standard deviation or as median. Population size–class curves were determined by Excel® or Prism® routines. Statistics were performed for data analyses (Prism software, Graph-pad Software) considering a $p < 0.01$ statistically significant. All the filters were checked by the four-eye approach and by an inter-calibration process performed between two operators; in order to minimise accidental contaminations, only glass materials and cotton dresses were used by operators and to treat samples and tests were performed on blanks. Filters ($n = 3$) were left overnight exposed to the laboratory air, putting them on the desk on an opened glass Petri dish and analysed to check air laboratory blanks. Other filters were extracted as reported for raw tissue samples; cooking blanks were, also, performed and checked by the four-eye approach to detect MPs. All blanks analysed resulted free from MPs.

In Fig. 1 the population structure of tested commercial and natural stocks is reported. Tested stocks ranged within 30–90 mm valve length with some differences. Stocks coming from La Spezia and Olbia are similar and range respectively within 40–75 mm (mode 50–55 mm) and 40–85 mm (mode 60–65 mm). Specimens from Cesenatico are larger and it ranges within 60–90 mm (mode 65–70 mm) while these coming from Talamone are smaller and ranges within 30–65 mm (mode 45–50 mm). In Fig. 2 (blue bars), average (standard deviation) items of MPs recovered per animals in each tested stock are reported. Cesenatico stock showed highest items values per animals recorded. Significant differences are recorded between the following couples: Cesenatico-La Spezia ($p = 0.002$), Cesenatico-Olbia ($p < 0.001$), Cesenatico-Talamone ($p < 0.001$). Low significant is reported for the couple Talamone-La Spezia ($p = 0.027$). To standardize collected data, in Fig. 2 (red bars), average (standard deviation) items of MPs recovered per gram of tissue in each tested stock are reported. Cesenatico stock showed the lower variability among specimens on the contrary, the stock from La Spezia showed the highest variability recorded. Any difference was recorded between Olbia and Talamone stocks. Except for the couple Cesenatico-Olbia ($p < 0.05$), statistics evidence no significant differences among average values concerning tested stocks. In the scientific literature, few studies focus on the measurements of the concentrations of MP in molluscs both present in the environment and maricultured. Van Cauwenberghe and Janssen (2014), for example, reported, for *Mytilus edulis*, therefore comparable with the organism investigated in this study (never studied before for what concerns the presence of MPs, for the authors knowledge), average levels of 0.36 MP items/g w.w. Therefore the average value found in this study is, although for a different species from that of comparison, much higher than reported by the above authors. Further researches are needed to clarify the reason of observed data even if some hypotheses are related to MPs load differences among geographical areas of origin of the analysed molluscs, or to differences among different stocks MPs accumulation/excretion, which must be further investigated. MPs transfer through the food web has been demonstrated for mussel (Farrell and Nelson, 2013), with important implications for the health of marine organisms, food chains, and for human health. Commercial and natural stocks are not significantly different concerning MP contents in raw animals with the exception of Cesenatico and Olbia. This difference could be probably due to different MPs pollution levels reported by the literature between the Adriatic Sea and the Tyrrhenian Sea (Blašković et al., 2017; Guerranti et al., 2017; Cannas et al., 2017). Colour dominances are similar to dominances recorded by the above-mentioned literature in sediments. Black and Blue are the most represented colours in all tested stocks. Red and Pink show low frequencies, while Green, Orange, Yellow, Grey and Brown coloured microplastics were only occasionally recorded. All recovered MPs are filaments ranging within 0.75–6.00 mm of length even if average values are included within 1.15–2.29 mm (Table 1). The fact that filaments are the only MPs type recorded in tested stocks could probably be due to the geometry of these plastics that allow them to better reach the considered species. Furthermore, filaments get trapped into gills and hepatopancreas and cannot be easily removed by animals accumulating into them.

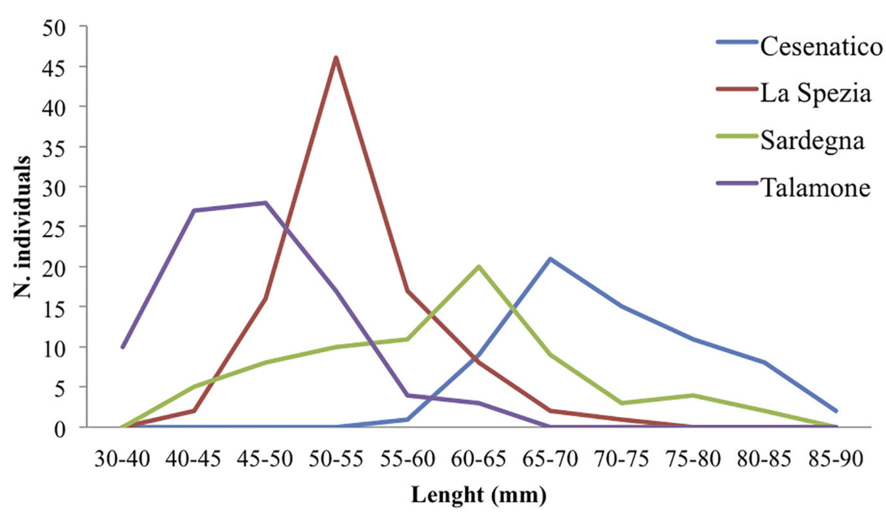

Fig. 1. Population structure of tested stocks. The number of individuals per each dimension class (5 mm length) are represented in tested stocks.

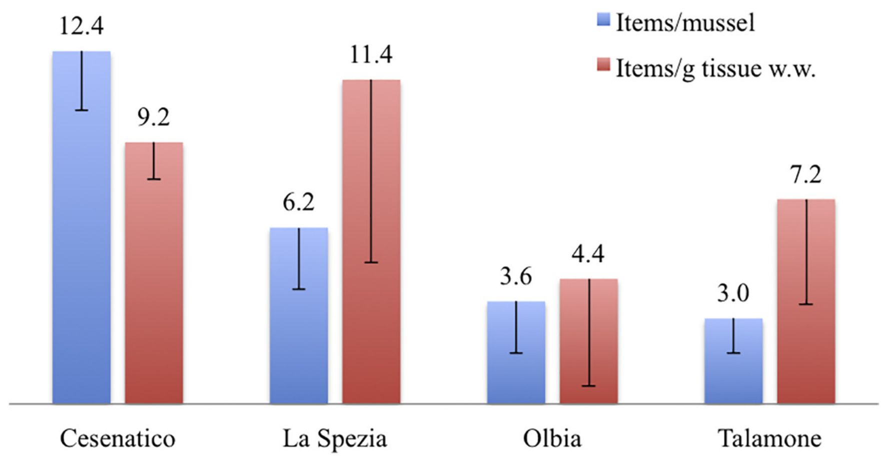

Fig. 2. Average values (SD) of microplastics recovered in raw animals from different commercial and natural stocks. Data are reported both as average items per animal and as average items per gram of tissue.

Table 1
Microplastic items mean length and standard deviation (SD) in tested mussels stocks.

	Stock	Mean length (mm)	SD
Raw	Cesenatico	1.70	0.37
	La Spezia	1.90	0.75
	Olbia	1.71	0.52
	Talamone	1.89	0.57
Cooked	Cesenatico	1.40	0.56
	La Spezia	2.04	0.80
	Olbia	1.65	0.91
	Talamone	2.27	1.14
Cooking water	Cesenatico	1.00	0.11
	La Spezia	1.15	0.34
	Olbia	2.29	0.71
	Talamone	1.83	0.17

Concerning human exposure to MPs ingestion by diet and associated health risks, the European Food Safety Authority (EFSA) has recently examined the existing literature on the subject (EFSA, 2016, noting the inadequacy of available data on the presence, toxicity and fate, what happens for instance during and after the digestion of such materials, aiming a complete risk assessment. In Fig. 3a, average (+SD) value of recovered number of items per gram of tissue is reported for cooked tissues and cooking water as percentage compared to levels in raw tissues. Median levels are included within 6.2–7.2 items/g of tissue. Cooked tissues show on average lower MPs levels than raw ones. This data is associated to the presence of litter in cooking water. It should be noted (Table 1) that the length of MPs measured in raw tissues (1.80 mm) is similar to values recorded in cooked tissues (1.84 mm) even if standard deviations differ significantly (0.11 mm vs. 0.39 mm respectively). On the contrary, average value recorded in cooking water is lower and associated to a higher variability (1.76 mm; SD = 0.57 mm). In the cooking water, we find relevant level of MPs expressed as number of items/g tissue, which added to the values, found in cooked organisms, they give a total levels higher than founded in raw mussels. The observed results could be probably due to two factors able to determine observations performed: i) natural variability of items inside individuals from the same stock; ii) thermal degradation of microplastics during cooking which could produce a higher number of items/g in cooking water and cooked tissues associated to a lower size as reported in Fig. 3b (average, mm; +SD). Temperature closed to water boiling point, in fact, could determine micro fractures or fusion of the polymeric structure of some plastic materials (i.e. fusion temperature for PVC 100–260 °C; PE 115–140 °C) and can probably induce fragmentation. Nevertheless, further studies could be performed to

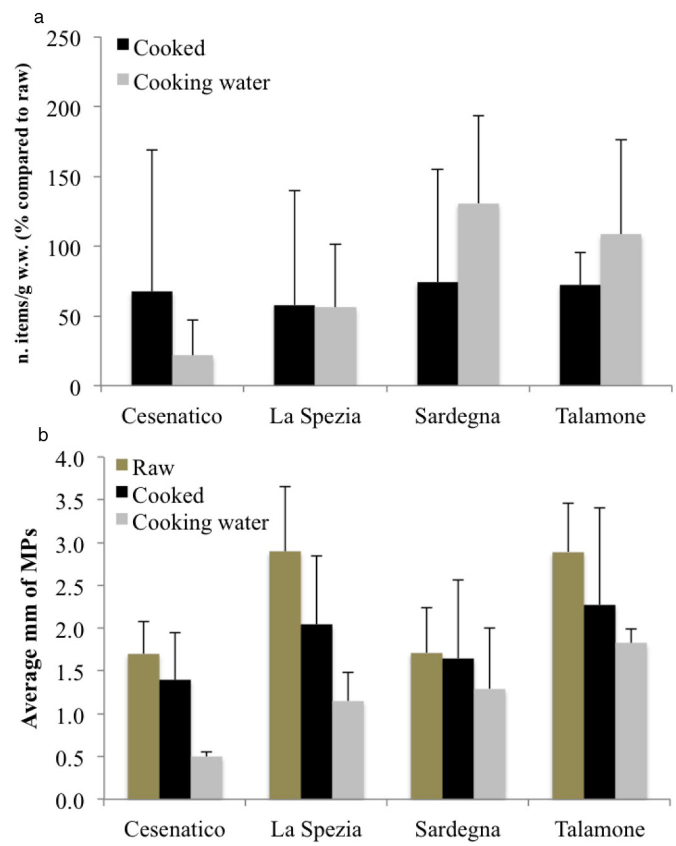

Fig. 3. a) Average (SD) values of microplastics recovered in cooked tissues and cooking water. Data are reported as percentage of number of items per gram of tissue w.w. compared to raw samples. b) Average values of maximum length (mm + SD) of microplastics collected in raw, cooked tissues and cooking water.

better clarify this aspect. High plastic debris concentrations have been found in fish, but since the MPs are present mostly in the stomach and intestine, which are usually removed before the human consumption, resulting in an absence of exposure for consumers. However, in the case of crustaceans and bivalve mollusks, such as oysters and mussels, the digestive tract is eaten, leading to some exposure (EFSA, 2016). In bivalves in general, the average number of items of MPs is 0.2–4.0 items/g (EFSA, 2016), even if extraction methods are different, values reported by the literature are lower if compared to the average value measured in this study. EFSA (2016), also, estimated that the consumption of 225 g of mussels is associated to a MPs assumption of 900 items (using the highest value found in mussels). On the basis of our results, the consumption of a portion of 225 g of mussels analysed in this study, the MPs intakes are 1395 (cooked) – 1620 (raw) items. Intakes can be reduced of about 14% per meal in case of cooked tissues if the cooking water is discharged and not consumed. Beyond the quantification of ingested MP, which have a negative effect demonstrated with regard to their interference as foreign matter (Lam et al., 1993; Hussain et al., 2001; Nemmar et al., 2003), important implications for human health derive from the fact that MPs contain contaminants, both added during the manufacturing process and absorbed from water (e.g.: Teuten et al., 2009; Fossi et al., 2016). Chemical substances, which are harmful to health, have been found to be present in foods, which often represent the main route of exposure for humans, which in many cases accumulates them (Liem, 1999). Microplastics as food contaminants, in turn, carriers of these compounds, represent an additional and emerging route of contamination for humans, to POPs and other toxic substances. Furthermore, some studies indicate that MPs, following assumption with contaminated foods, can move into the tissues. As reported by EFSA (EFSA, 2016), there is no literature available on the fate of MPs during the cooking process, so this study provides some important preliminary indication helping to fill an important information gaps and to define objectives of new further researches.

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