

Accepted refereed manuscript of:

Graham P, Palazzo L, de Lucia GA, Telfer TC, Baroli M & Carboni S (2019) Microplastics Uptake and Egestion Dynamics in Pacific Oysters, *Magallana gigas* (Thunberg, 1793), Under Controlled Conditions. *Environmental Pollution*, 252 (Part A), pp. 742-748.

DOI: <https://doi.org/10.1016/j.envpol.2019.06.002>

© 2019, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International

<http://creativecommons.org/licenses/by-nc-nd/4.0/>

1 **Microplastics Uptake and Egestion Dynamics in Pacific Oysters, *Magallana gigas***
2 **(Thunberg, 1793), Under Controlled Conditions.**

3 Philip Graham^{ab}, Luca Palazzo^{cd}, Giuseppe Andrea de Lucia^c, Trevor Telfer^a, Maura Baroli^b, Stefano Carboni^{a*}

4 ^a University of Stirling, Institute of Aquaculture, Pathfoot Building FK94LA, Stirling, UK.

5 ^b IMC - International Marine Centre, Loc. Sa Mardini, 09170 Oristano, Italy.

6 ^c IAS-CNR, National Research Council, 09170 Oristano, Italy.

7 ^d Department of Ecology and Biology, University of Tuscia, 01100 Viterbo, Italy.

8
9 **Abstract**

10 Microplastics debris (<5 mm) are increasingly abundant in the marine environment, therefore,
11 potentially becoming a growing threat for different marine organisms. Through aquatic
12 animals, these can enter in the human food chain, and can be perceived as a risk for
13 consumers' health.

14 Different studies report the presence of particles in marketable shellfish including the world
15 wide commercially grown Pacific oyster *Magallana gigas* (Thunberg, 1793). The aim of this
16 study is to examine the potential risk of microplastics entering in the human food chain
17 through this shellfish species, investigating the dynamics of the uptake, egestion (faeces) and
18 rejection (pseudofaeces) of microplastics in Pacific oysters under controlled conditions.

19 *M. gigas* collected from a farm in the San Teodoro lagoon (Italy), were exposed to 60
20 fluorescent orange polystyrene particles L⁻¹ of known sizes (100, 250 and 500 µm). The
21 uptake of each particle size was 19.4 ± 1.1%, 19.4 ± 2 % and 12.9 ± 2 % respectively. After
22 exposure *M. gigas* were left to depurate for 72 hrs, during which 84.6 ± 2 % of the particles
23 taken up were released whilst 15.4 ± 2 % were retained inside the shell cavity. No
24 microplastic particles were found in the animals' soft tissues.

25 The results of this study, suggest that depuration is an effective method to reduce presence of
26 large microplastic particles, in the size range 100 to 500 µm, in *M. gigas*. Importantly, the

27 data suggests that the burden that could theoretically be up taken by consumers from these
28 shellfish is negligible when compared to other routes.

29

30 **Capsule**

31 Microplastic of tested sizes were not retained in the tissues but can be retained in the shell
32 cavity; Depuration is an effective method to reduce microplastics in farmed Pacific oysters

33

34 **Keywords**

35 Microplastic; Pacific Oyster; Uptake dynamics; Depuration; Consumers risks

36

37 **1. Introduction**

38 Plastics are ubiquitously present throughout the world's oceans. In 2016 it was estimated that
39 the production of plastics reached 335 million metric tonnes (Mt) globally (PlasticsEurope,
40 2018). In 2015, 6300 Mt of plastic waste was generated and, if plastic production trends and
41 waste management will remain similar, it is expected that 12,000 Mt of plastic waste will be
42 released to the environment by 2050 (Gündoğdu *et al.*, 2018; Jambeck *et al.*, 2015).

43 Plastics are believed to be one of the main contributors to ocean pollution with some areas of
44 the ocean presenting very high concentrations, as a result in 2013 it was estimated that a
45 minimum of 268,940 tons of plastics were present in the oceans (Eriksen *et al.*, 2014).

46 Microplastics are becoming ever more present in the marine environments due to human
47 population growth. Therefore, an increase in this type of pollution is expected over the
48 coming years and decades. Plastics and micro-plastics (particles <5mm in size) are part of
49 everyday life and can be found in many products used daily such as packaging for food and
50 drinks, shopping bags, toothbrushes and cosmetics (Cole *et al.*, 2011; Browne *et al.*, 2011,
51 Hidalgo-Ruz *et al.*, 2012). Microplastics can be classified into primary microplastics which
52 are intentionally produced at a microscopic scale (Costa *et al.*, 2010; Browne, 2015) and
53 secondary microplastics resulting from the degradation of larger plastics into smaller pieces

54 by environmental processes such as weathering and photo-oxidation (Mathalon and Hill,
55 2014; Gewert *et al.*, 2015).

56 Because primary microplastics are present in cosmetics and medical applications, a major
57 source in the sea and fresh water bodies is waste water from depuration plants (Browne *et al.*,
58 2011, Cole *et al.*, 2011; Duis and Coors, 2016, Carr *et al.*, 2016).

59 Microplastics have been considered to be dangerous for aquatic organisms' health (Alomar,
60 2017). Indeed, their accumulation by ingestion can lead to increased exposure to pollutants
61 and pathogens, and effects on physiological activities linked to nutrient uptake, growth and
62 survival (Browne *et al.*, 2011; Sussarellu *et al.*, 2016; Fendall and Sewell, 2009; Van
63 Cauwenberghe and Janssen, 2014).

64 Nonetheless, when environmental toxicity tests were performed in different marine
65 invertebrates, for example in larvae of *Tripneustes gratilla* (Linnaeus, 1758) exposed to 10 -
66 45 μm microspheres and *Mytilus edulis* (Linnaeus, 1758) exposed to microspheres with
67 diameters between 3 and 90 μm , it became apparent that only very high concentrations of
68 microplastics (10,000 times higher than the maximum concentration of microplastic particles
69 currently found in the sea water) generated significant adverse physiological effects (Duis and
70 Coors, 2016). Still, some considerations would warrant caution since very high concentrations
71 of microplastics have already been observed at some sites; plastics are extremely persistent in
72 the environment and, due to further fragmentation, their presence is expected to further
73 increase (Auta, 2017).

74 Von Moos *et al.*, (2012) studied the effect of exposure and ingestion of microplastics
75 ($\leq 80\mu\text{m}$) in Blue mussel (*Mytilus edulis*, Linnaeus, 1758). These authors reported that the
76 smallest particle sizes were accumulated in gills and digestive gland with a consequent strong
77 inflammatory response and a lysosomal membrane destabilization. Unfortunately, no
78 information on excretion was provided by these authors and conclusions on the fate of the
79 larger particles cannot be made. Cole *et al.*, (2011) investigated the presence of microplastics

80 (between 1 and 10 μm) and their effect on food intake and growth of Pacific oyster larvae.
81 They found that microplastics were ingested with only limited impact on feed intake and no
82 consequences on growth rates being observed. Van Cauwenberghe and Janessen, (2014),
83 investigated the presence of different microplastics particles (size class 5-10, 11-15, 16-20,
84 21-25, >25 μm) in farmed blue mussel and Pacific oyster, showing that these were present in
85 both species at concentration of 0.36 ± 0.07 particles g^{-1} and 0.47 ± 0.16 particles g^{-1} soft
86 tissue, respectively. The same authors also depurated animals from the same batches for 72
87 hrs observing a significant reduction in the abundance of microplastics, concluding that
88 although depuration was an effective procedure, the consumption of farmed bivalves could
89 potentially represent a risk to consumers' health. Nonetheless, Wright and Kelly (2017), in
90 their review, report that there is still no clear evidence that the absorption of microplastics has
91 a direct impact on human health, but that their accumulation could exert dose-dependent
92 toxicity, due to the leaching of other pollutants or the presence of pathogens on their surface,
93 therefore suggesting that the assessment of exposure levels is of fundamental importance.
94 Still, the concomitant evidence of microplastics being accumulated in bivalve soft tissue and
95 the presence of wastewater effluent (one of the major sources of microplastics in the
96 environment) in the same water catchment areas as shellfish farming activities deserves
97 further studies (Rochman *et al.*, 2015). Indeed, Sussarellu *et al.*, (2016) studied possible
98 influence of microplastics (2 and 6 μm) on the physiology of Pacific oysters, finding that
99 individuals exposed to microplastics showed lower fecundity, possibly linked to the
100 substances leached by the microplastics during digestion process if not directly caused by
101 their accumulation. This study also indicated that although microplastics were observed in the
102 digestive system, no tissue accumulation was observed, therefore suggesting an efficient
103 egestion process.
104 The presence of microplastics in commercially relevant bivalves, including Pacific oysters,
105 has been reported by different studies (Van Cauwenberghe and Janessen, 2014, Li *et al.*,

106 2015, Cole and Galloway, 2015, Phuong *et al.*, 2018, Sussarellu *et al.*, 2016, Fernández *et al.*,
107 2018, Von Moos *et al.*, 2012, Pont *et al.*, 2016, Silva *et al.*, 2016). Two main objectives have
108 been pursued by previous investigations: 1) determination of the presence and the abundance
109 of microplastics in individuals collected from the wild, farms and retailers to establish
110 potential risks for consumers; 2) the determination of the potential adverse effects to animals'
111 physiology caused by the exposure to plastics under controlled conditions.

112 However to date, there is still limited knowledge on the relationship between plastics uptake
113 and egestion (Van Cauwenberghe and Janessen, 2014). Therefore, the first aim of this present
114 study was to investigate the adult oysters' egestion dynamics after exposure to known
115 concentration of microplastics under controlled conditions. Moreover, previous studies have
116 so far used microplastics of sizes comparable to phytoplankton cells. However, in the marine
117 environment, microplastics are present in sizes often larger than microalgae cells and there
118 are evidence suggesting that bivalves could potentially up-take particles as large as 500 μm
119 (O'Donohue and McDeromtt, 2014). Still, no information on the ability of oysters to uptake,
120 retain and egest larger particles is currently available. Consequently, the second aim of this
121 study was to determine whether larger particles had the potential to remain in the marketable
122 product post depuration by employing sizes larger than those commonly used in previous
123 microplastics absorption studies. The size classes of 100 ± 7.42 , 250 ± 23.2 and $500 \pm 52,34$
124 μm were chosen because Van Cauwenberghe and Janssen (2014), found that *Crassostrea*
125 *gigas* reared in the Atlantic Ocean (average shell length of 9.0 ± 5.0 cm), showed a
126 prevalence of microplastics size $> 25 \mu\text{m}$, and because studies on mussels and Pacific oysters
127 so far were focused only on microplastics of a size comparable to phytoplankton or in general
128 at size between 0.5 and 90 μm (Sussarellu *et al.*, 2016, Cole and Galloway, 2015, Van
129 Cauwenberghe *et al.*, 2015, Farrell and Nelson, 2013, Browne *et al.*, 2008, Von Moos *et al.*,
130 2012), without taking in to account that in the marine environment microplastics are present

131 in different sizes and adults' Pacific oysters can uptake larger size microplastics from the
132 environment.

133

134 **2. Materials and Methods**

135 2.1. Pacific Oyster source and experimental set-up

136 Pacific oysters (20 oysters $85 \pm 2.3\text{g/ind.}$) were collected from a farm in the San Teodoro
137 Lagoon (Italy) ($40^{\circ}48'39.18''\text{N}$, $9^{\circ}40'24.42''\text{E}$), and kept in a cold box until arrival to the
138 laboratory. Oysters were then transferred to an aerated rectangular tank and left to acclimatize
139 for 48hrs at 22°C temperature and 36 ppm salinity (Choi *et al.*, 2008). For the purpose of this
140 study, oysters were individually deployed in individually deployed in 20 glass spherical
141 aquariums of 1.5 L, filled with filtered sea water.

142 With the aim to keep the water in movement each aquarium was supplied with an air-stone
143 connected to a valve and an air pump. Water temperature, salinity and dissolved oxygen were
144 monitored and maintained (by daily water exchange) respectively at 22°C , 36 ppm and 8.5
145 mg/L.

146 Preliminary trials were performed to determine both the level of aeration required and the
147 most suitable type of microplastics polymer. For this purpose, three polymers of the following
148 densities were tested: polystyrene $1.04\text{-}1.1\text{ g/cm}^3$; polyamide $1.12\text{-}1.15\text{ g/cm}^3$; polycarbonate
149 $1.20\text{-}1.22\text{ g/cm}^3$ (Avio *et al.*, 2016, Enders *et al.*, 2015). With the aim to keep the
150 microplastics beads suspended in the water column to maximise their chances to be filtered
151 by the oysters, batches of 30 microplastics per polymer were deployed to an experimental
152 tank and aeration was adjusted by a valve. Once the appropriate aeration was identified by
153 observing the microplastics distribution on the water column, the ability of the chosen
154 polymer to withstand the tissue digestion procedure (Li *et al.*, 2015) was tested. This was
155 conducted using a sterile container containing soft tissues of 3 Pacific oysters (80 ± 3.5
156 g/ind.) plus 9 plastic beads per size class (100 ± 7.42 , 250 ± 23.2 and $500 \pm 52.34\text{ }\mu\text{m}$) of the

157 microplastics chosen for the study (3 replicates). The soft tissue was covered with hydrogen
158 peroxide 15%, this was added until the oyster was completely digested (Avio *et al.*, 2015).

159 Once the oysters were digested the remaining solution was filtered using 47 mm Whatman
160 GF/F filters (0.6 – 0.8 μm) and then analysed under the dissecting microscope (Leica Mz8).

161

162 2.2. Microplastics

163 The selected microplastics were fluorescent polystyrene microspheres purchased from
164 Degradex Hopkinton (MA 01748). These particular beads were selected because of their
165 colour (fluorescent orange with Excitation/Emission 530/582 nm) and because their density
166 was similar to seawater (UNESCO,1981, Capolupo *et al.*, 2018).

167 Three microplastics sizes were used: 100 ± 7.42 , 250 ± 23.2 and $500 \pm 52,34$ μm (Fig. 1A)
168 and 600 microplastics of each size, were individually counted under a stereo microscope,
169 using an UV lamp (Surenhap 100 LED) to enhance fluorescence (Fig. 1B), and micro-
170 dissecting tweezers (World Precision Instruments, FL 34240-9258 USA).

171 Beads were then allocated (thirty beads per size) to twenty 1.5 ml Eppendorf tubes, (Fig. 1C).

172

173 2.3. Exposure and Microplastics uptake

174 The experiment was carried out in 2 parts: 24hrs exposure (Cole and Galloway, 2015) and
175 72hrs depuration (Van Cauwenberghe and Janessen, 2014). During the first 24hrs
176 experimental individuals (n=20) were individually exposed to 30 Microplastic particles of
177 each size (100, 250 and 500 μm) with a density of 60 particles per litre. This particles density
178 despite being higher than the ones commonly reported in sea water (De Lucia *et al.*, 2014)
179 was chosen for analytical and practical reasons.

180 At the end of the exposure period the aeration was stopped and each oyster was collected
181 using long tweezers, oysters and tools were carefully observed using a UV lamp to increase
182 beads fluorescence and washed taking care that no microplastics adhered to the oysters' shell

183 and to the tools used. The water used for the exposure was, at this point, filtered through a 47
184 mm GF/F filter using a filtration unit Millipore and a vacuum pump. Again, all filtration
185 equipment was checked for the presence of adhered beads. Post filtration each filter was
186 individually stored inside labelled 50 mm petri dishes. Uptake was measured subtracting the
187 final number of beads recovered onto the filters from the initial number used for exposure.

188

189 2.4. Depuration and egestion

190 The oysters collected after exposure were transferred to a new tank, again filled with 1.5 L of
191 filtered sea water. Aeration was not supplied in order to avoid faeces and pseudo-faeces
192 mixing.

193 At 24hrs intervals over a total of 72hrs, each oyster was removed from each tank using the
194 same procedure described earlier, and transferred to a new tank under the same environmental
195 conditions.

196 The water left in the original tank during the 24, 48 and 72 hrs after exposition, was filtered
197 and beads counted using the same procedure described before.

198 Finally, at the end of the trial (72 hrs after exposure) oysters were collected from the
199 experimental tanks and externally washed and dissected taking care that the water contained
200 in the shell cavity was stored in a plastic tray.

201 The Digestive gland, gills and mantle of each oyster were dissected, washed and placed in
202 labelled sterile containers. The water contained in the shell and the water used to wash the
203 tissues was collected and filtered as described previously.

204 All dissected tissues of each individual were digested using hydrogen peroxide 15%, at room
205 temperature of 22°C for 7 days, and the resulting digestate was filtered as described
206 previously.

207

208 2.5. Statistical Analysis

209 Prior to analyses, percentage data were arc-sine transformed, and all data were checked for
210 normality and homogeneity of variance. Uptake and residual microplastics post depuration
211 data were analysed by one-way ANOVA followed by post-hoc Tukey's Multiple Comparison
212 tests where significant differences occurred. Egestion over time for particles of all sizes was
213 analysed by general linear model followed by a Tukey post-hoc test where significant
214 differences occurred.

215 Statistical analyses were performed using Minitab v.18 with a significance level of 5 % ($p <$
216 0.05). All results are presented as mean \pm SE.

217

218 **3. Results**

219 3.1. Microplastics uptake

220 At the end of the 24 hrs exposure, the uptake (% of missing beads) of the different sizes (100,
221 250 and 500 μm), was $19.4 \pm 1.1\%$, $19.4 \pm 2\%$ and $12.9 \pm 2\%$ respectively. No significant
222 difference in uptake between the microplastics of 100 and 250 μm was observed, however
223 beads of 500 μm in size had a significant lower uptake when compared with the others sizes
224 ($P = 0.009$) (Figure 2).

225

226 3.2. Depuration and egestion

227 Table 1 illustrates the percentage of microplastics recovered from the depuration water, and
228 tissues at the different time points over the depuration period. A significant effect of time ($p <$
229 0.001) and a significant interaction between time and treatment ($p < 0.02$) was observed. The
230 excretion of microplastics beads of all sizes was significantly higher during the first 24 hrs in
231 comparison with the later time points. Furthermore, no significant difference was recorded in
232 the excretion of microplastic particles of 100 μm and 500 μm between 48 and 72 hrs of
233 depuration, whilst significantly more beads of 250 were released after 48hrs in comparison to
234 72hrs of exposure. (Fig. 3).

235 Although the vast majority of ingested microplastic particles were released during the 72hrs
236 of depuration, 17.7 ± 3.8 , 16.7 ± 2.4 and 5.4 ± 2.7 % of microplastic particles of 100, 250 and
237 500 μm respectively were still present in the water contained inside the shell cavity. At this
238 location a significant difference in the abundance of each particle size class was observed,
239 with the largest size class being significantly less abundant than the other two ($p = 0.007$)
240 (Fig. 4). Importantly, no microplastic particles were found in the digestive gland and in the
241 other tissues post digestion.

242 Taking into account each time step there was a decreasing egestion of microplastic particles
243 during the depuration time: $63.9 \pm 3\%$, $17 \pm 2.2\%$ and 3.7 ± 0.9 % in 24, 48 and 72 hrs,
244 respectively. Only $15.4 \pm 2\%$ of the microplastic particles were retained within the oysters
245 after 72 hrs of depuration (Tab. 1).

246

247 **4. Discussion**

248 The aim of this study was to investigate the uptake and egestion dynamics of known sizes
249 (100, 250 and 500 μm diameter) of microplastic particles in Pacific oysters, during a 24hrs
250 exposure and a subsequent 72hrs depuration period. Depuration is a common practice in
251 bivalve aquaculture whereby bacteria are egested to comply with European food safety
252 legislation (regulation 853/2004, 852/2004 and 2073/2005) (Who, 2019, Martínez *et al.*,
253 2009, Doré and Lees, 1995). In this study, Pacific oysters showed an efficient egestion rate,
254 egesting 84.6 ± 2 % of the microplastic particles taken up, while only the 15.4 ± 2 % of beads
255 taken up were retained within the shell cavity, post depuration.

256 To date, studies on microplastic uptake have been conducted mainly to investigate their
257 potential negative physiological effects on marine life, including bivalves, or to establish
258 whether animals entering the human food chain could be a carrier of these particles and
259 therefore represent a risk for consumers (Sussarellu *et al.*, 2016, Fernández *et al.*, 2018, Von
260 Moos *et al.*, 2012, Pont *et al.*, 2016, Silva *et al.*, 2016, Van Cauwenberghe and Janessen,

261 2014). The main difference between these approaches has been the controlled nature of the
262 studies. The former employed controlled conditions (known density, type and size of the
263 microplastics employed), whilst the latter focused on the abundance of plastics in marketable
264 products without considering levels of exposure, uptake or the nature of the polymers.

265 In contrast, our study investigated both the uptake and egestion dynamics under controlled
266 conditions to more robustly describe the fate of microplastic particles of 100 to 500 μm
267 diameters during exposure and depuration therefore contributing to the collective knowledge
268 on these dynamics in shellfish produced for human consumption.

269 Amongst the studies focused on the risks for consumers, the one conducted by Van
270 Cauwenberghe and Janessen (2014) provides the only comparable platform for the
271 interpretation of the results presented here. Comparison of the studies shows a slight
272 difference in egestion rate post-depuration (74.5 % vs 84.6 ± 2 %), this can be attributed to
273 the difference in materials and diameters of the particle used and by the food sorting
274 mechanisms of the Pacific oysters which discriminates not only based on size but also based
275 on chemical cues present on the surface of the particles (Kiørboe, *et al.*, 2012, Ward *et al.*,
276 1997).

277 In this study no microplastic particles were observed within the oysters' tissues, while in the
278 Sussarellu *et al.*, (2016) study, microplastic particles were found in the stomach and the
279 intestine of Pacific oysters. This can be attributed to the difference in the particle size used
280 (100, 250 and 500 vs 2-6 μm), and it is possible that the *C. gigas* food sorting mechanisms
281 recognise only the smaller size as a food source due to similarity in size with phytoplankton
282 (Ward and Shumway, 2004). However, different studies point out that bivalve can ingest
283 larger particle size. For instance, blue mussels can ingest early larval stages of sea lice,
284 *Lepeoptheirus salmonis* (Krøyer 1837), with an average size of roughly 500 μm . Furthermore,
285 during a microplastics survey conducted in the Dutch North Sea, the presence of large plastics
286 (up to 5mm in size) was also observed in Pacific oysters (Molloy *et al.*, 2011, O'Donohe and

287 McDeromtt, 2014, Leslie *et al.*, 2013). Our results suggest that these larger particles could
288 probably be filtered by the oysters but, instead of being ingested, they are retained within the
289 shell cavity by adhesion. Therefore, with the assumption that in the marine environment
290 microplastics of different size have the potential to be accumulated in marketable bivalves
291 (Andardy 2011, Koelmans *et al.*, 2015), the present study further clarifies the uptake and
292 egestion dynamics of larger particles and the associated potential risks for consumers. Indeed,
293 microplastic may not necessarily have to be ingested to represent a potential exposure risk to
294 consumers as adhesion to external tissue may still be considered as a vehicle for trophic
295 transfer.

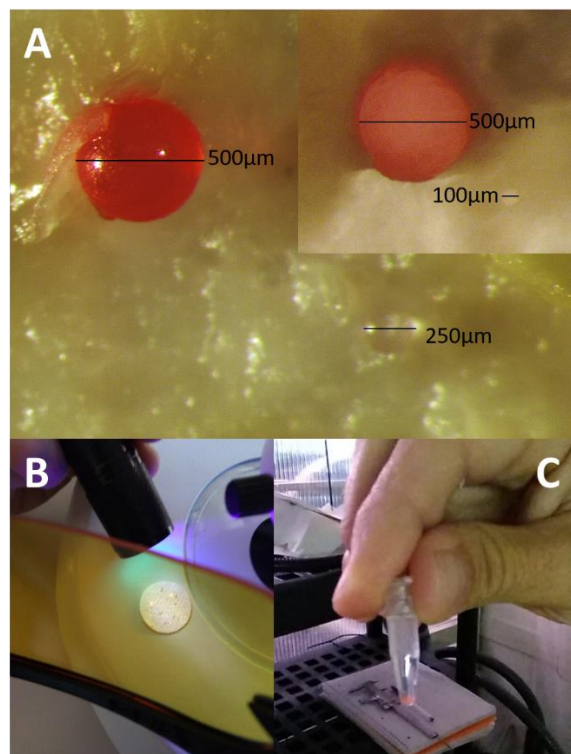
296 Importantly, during the depuration period, microplastic particles were observed in faeces and
297 pseudo-faeces, but it is not possible to conclude here that the beads have been ingested,
298 because these were not observed within the digestive system. Further work focused on the
299 ingestion and excretion of microplastic particles of different sizes class, including particles
300 larger than microalgae cells, should be conducted to estimate gut transit time of these
301 particles.

302 In conclusion our data, taken together with results from other studies, strongly indicate that
303 *M. gigas* could be a carrier of different microplastic sizes in the human food chain, not only
304 through the absorption and inclusion in tissues (Bricker *et al.*, 2014, Van Cauwenberghe and
305 Janessen, 2014, Li *et al.*, 2015, Rochman, *et al.*, 2015, Wright and Kelly, 2017, Bouwmeester
306 *et al.*, 2015), but also through the adhesion of these particles in different parts of the internal
307 cavity of the oysters shell. Nonetheless, the exposure density of 60 microplastics L⁻¹ used in
308 this study, is higher than the density of microplastic particles (<5 mm) commonly reported in
309 coastal Mediterranean Sea areas 5 *10⁻⁴ microplastic particles L⁻¹ (De Lucia *et al.*, 2014).
310 Assuming that the uptake for all sizes observed in this study (16.2 ± 1.2 %) is applicable to
311 the wider farming context, the number of particles filtered by each individual would be 1.2
312 *10⁻⁴, which would become 4.3 *10⁻⁵ per individual after 24 hrs depuration. This final

313 microplastic burden can be considered lower if compared with the number of microplastic
314 particles found by Schymanski *et al.*, (2018) contained in drinking water (from 11 ± 8 to 118
315 ± 8 particles L^{-1} depending on the type of package). Therefore, the risks for consumers can be
316 considered negligible for the particle size tested if compared to the amount of microplastic
317 particles that can be uptaken in everyday life.

318 Pacific oysters are farmed world-wide for human consumption, and microplastic particles are
319 widely distributed in the environment and therefore available to filter feeders. However, after
320 depuration the number of microplastic particles decreased significantly suggesting that this
321 standard procedure is an effective method to reduce the presence of larger microplastic
322 particles in marketable Pacific oysters even when no depuration would be compulsory due to
323 sanitary reasons such in the case of class A waters.

324



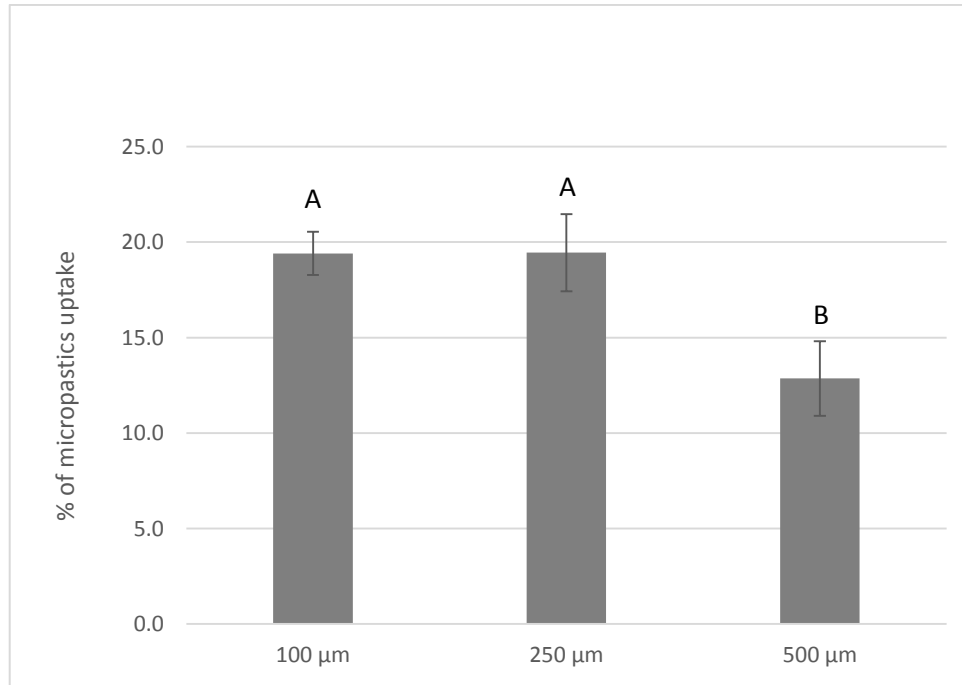
325

326 *Figure 1* A. Different Microplastic particle sizes used during this study. Picture was taken on a 47mm GF/F filter B. 500 µm

327 Microplastics on a 25mm GF/F filter with fluorescence enhanced by a UV light. B. Microplastics with fluorescence enhanced

328 using an UV lamp C. Microplastics mix composed by 30 Microplastics per size class (100, 250 and 500 μm) ready to be
329 deployed for the exposure trial.

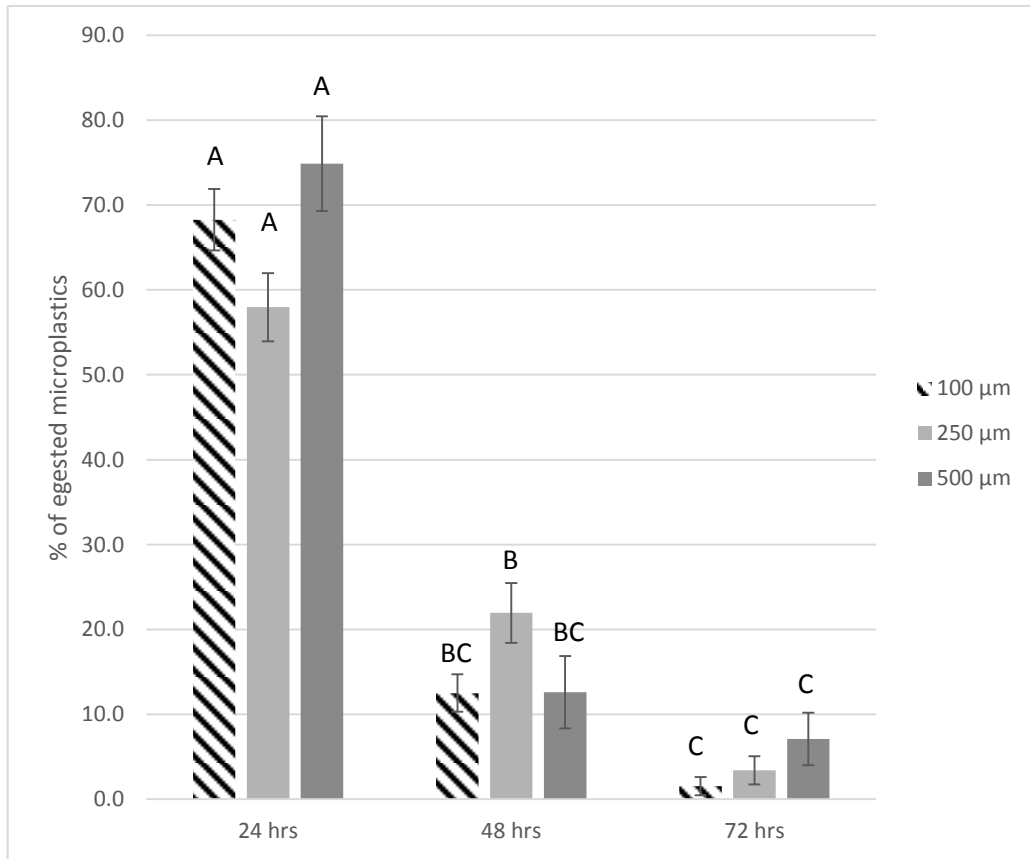
330



331

332 *Figure 2* Uptake of the different Microplastic particle size classes from ambient water. Significant differences (P value >
333 0.05) are showed by different letters, results are presented as mean \pm SE; n=20.

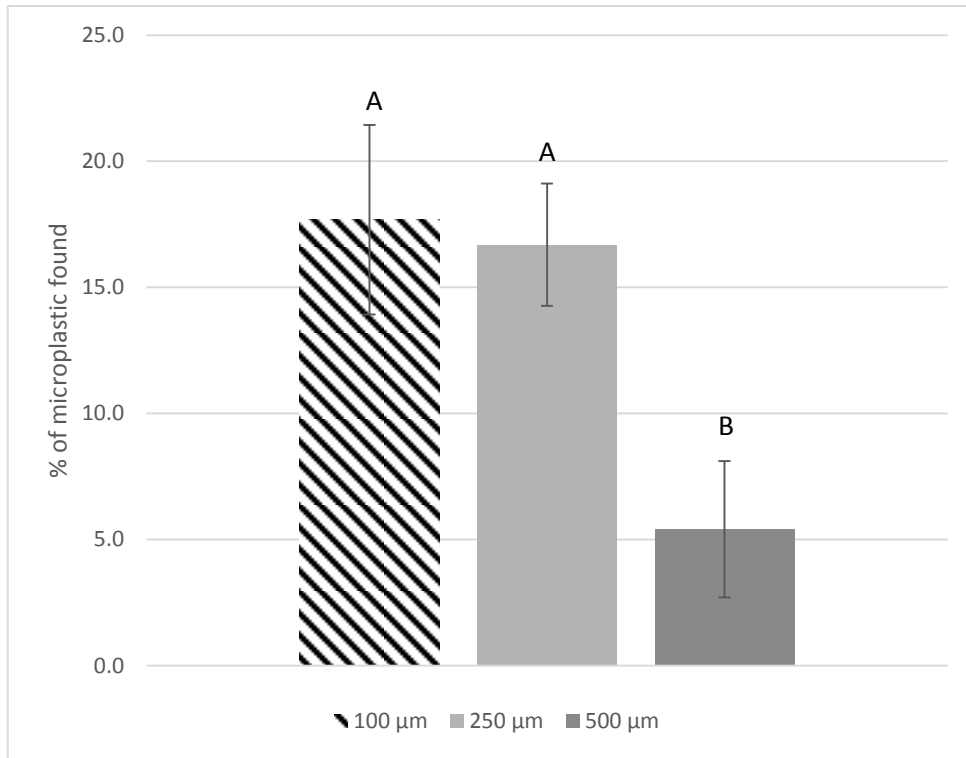
334



335

336 *Figure 3* Egestion dynamics of the different microplastic particle sizes. Significant differences (P value > 0.05) are showed
 337 by different letters, results are presented as mean ± SE; n=20.

338



339

340 *Figure 4* Residual microplastic particles of the different sizes post depuration. Significant differences (P value > 0.05) are

341 showed by different letters, results are presented as mean ± SE; n=20.

342

343 *Table 1* Summary of the percentages of egested during 72 hrs depuration, and non-egested post depuration,
 344 Microplastics, both divided by sizes and as a mix of beads (100, 250 and 500 μm).

<i>Microplastics beads</i>	100μm	250μm	500μm	Mix	Mix
<i>egested and</i>	%	%	%	%	%
<i>non-egested in:</i>					
24 hrs	68.3 \pm 3.6	58 \pm 4.0	74.9 \pm 5.6	63.9 \pm 3.0	84.6 \pm 2
48 hrs	12.5 \pm 2.2	21.9 \pm 3.5	12.6 \pm 4.3	17 \pm 2.2	
72 hrs	1.5 \pm 1.1	3.4 \pm 1.7	7.1 \pm 3.1	3.7 \pm 0.9	
Internal cavity	17.7 \pm 3.8	16.7 \pm 2.4	5.4 \pm 2.7	15.4 \pm 2	15.4 \pm 2
Digestive gland	0	0	0	0	
Other soft tissues	0	0	0	0	

345

346

347 **Acknowledgements**

348 We thank Alessandro Gorla and Francesca Gargiulli of the Compagnia Ostricola Mediteranea
 349 for support and providing us the Pacific oysters. Thanks to Stefano Guerzoni, Gianni Brundu,
 350 Barbara Loi, Gemma Giménez Papiol, Dimitri Bernabè and all the IMC and IAS-CNR staff
 351 for help and providing laboratories space to set up the experiments of this study. We also
 352 would like to thank the anonymous reviewers who helped improving the manuscript.

353

354 **References**

355 Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S. (2017)
356 Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for
357 causing oxidative stress. *Environmental Research* **159**, 135-142.

358 Andrady, A.L. (2011). Microplastics in the marine environment. *Marine Pollution*
359 *Bulletin* **62**, 1596-1605.

360 Auta, H.S., Emenike, C.U., Fauziah, S.H. (2017) Distribution and importance of
361 microplastics in the marine environment: A review of the sources, fate, effects, and
362 potential solutions. *Environment International* **102**, 165-176.

363 Avio, C.G., Gorbi, S., Regoli, F. (2015) Experimental development of a new protocol for
364 extraction and characterization of microplastics in fish tissues: First observations in
365 commercial species from Adriatic Sea. *Marine Environmental Research* **111**, 18-26.

366 Avio, C.G., Gorbi, S., Regoli, F. (2016). Plastics and Microplastics in the oceans: From
367 emerging pollutants to emerged threat. *Marine Environmental Research* **128**, 2-11.

368 Bouwmeester, H., Hollman, P.C.H., Peters, R.J.B. (2015). Potential Health Impact of
369 Environmentally Released Micro- and Nanoplastics in the Human Food Production
370 Chain: Experiences from Nanotoxicology. *Environmental Science and Technology* **49**,
371 8932-8947.

372 Bricker, S., Lauenstein, G. Maruya, K. (2014). NOAA's mussel watch program:
373 incorporating contaminants of emerging concern (CECs) into a long-term monitoring
374 program. *Marine Pollution Bulletin* **81**, 289-290.

375 Browne, M.A. (2015). Sources and Pathways of Microplastics to Habitats. *Marine*
376 *Anthropogenic Litter*, 229-244.

377 Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson,
378 R. (2011) Accumulation of Microplastics on Shorelines Worldwide: Sources and Sinks.
379 *Environmental Science Technology* **45 (21)**, 9175-9179.

380 Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C. (2008).
381 Ingested Microscopic Plastic Translocates to the Circulatory System of the Mussel,
382 *Mytilus edulis* (L.). *Environmental science and technology* **42 (13)**, 5026-5031.

383 Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C.S., Fabbri, E. (2018). Uptake and
384 transcriptional effects of polystyrene Microplastics in larval stages of the Mediterranean
385 mussel *Mytilus galloprovincialis*. *Environmental Pollution* **241**, 1038-1047.

386 Carr, S.A., Liu, J., Tesoro, A.G. (2016). Transport and fate of Microplastics particles in
387 wastewater treatments plants. *Water Research* **91**, 174-182.

388 Choi, Y.K., Jo, P.G., Choi, C.Y. (2008). Cadmium affects the expression of heat shock
389 protein 90 and metallothionein mRNA in the Pacific oyster, *Crassostrea gigas*.
390 *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **147 (3)**,
391 286-292.

392 Cole, M. and Galloway, T. S., (2015) Ingestion of Nanoplastics and Microplastics by
393 Pacific Oyster Larvae. *Environmental science Technology* **49 (24)**, 14625-14632.

394 Cole, M., Lindeque, P., Halsband, C., Galloway, T.S. (2011) Microplastics as
395 contaminants in the marine environment: A review. *Marine Pollution Bulletin* **62 (12)**,
396 2588-2597.

397 Costa, M. F., Ivar do Sul, J.A., Silva-Cavalcanti, J.S., Araújo, M.C.B., Spengler, Â.,
398 Tourinho, P.S. (2010). On the importance of size of plastic fragments and pellets on the
399 strandline: a snapshot of a Brazilian beach. *Environmental Monitoring and Assessment*
400 **168 (1-4)**, 299-304.

401 De Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., Campani, T.,
402 Giannetti, M., Coppola, D., Cicero, A.M., Panti, C., Bainsi, M., Guerranti, C., Marsili, L.,

403 Massaro, G., Fossi, M.C., Matiddi, M. (2014) Amount and distribution of neustonic
404 micro-plastic off the western Sardinian coast (Central-Western Mediterranean Sea).
405 *Marine Environmental Research* **100**, 10-16.

406 Doré, W.J., Lees, D.N. (1995). Behavior of *Escherichia coli* and Male-Specific
407 Bacteriophage in Environmentally Contaminated Bivalve Molluscs before and after
408 Depuration. *Applied and Environmental Microbiology* **61(8)**, 2830-2834.

409 Duis, K., Coors, A. (2016) Microplastics in the aquatic and terrestrial environment: source
410 (with a specific focus on personal care products), fate and effects. *Environmental Science*
411 *Europe* **28 (2)**, 1-25.

412 Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G. (2015). Abundance, size and polymer
413 composition of marine Microplastics $\geq 10 \mu\text{m}$ in the Atlantic Ocean and their modelled
414 vertical distribution. *Marine Pollution Bulletin* **100 (1)**, 70-81.

415 Eriksen, M., Leberon, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C.,
416 Galgani, F., Ryan, P.G., Reisser, J. (2014) Plastic Pollution in the World's Oceans: More
417 than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PloS ONE* **9**
418 **(12)**, 1-15.

419 Farrel, P., Nelson, K. (2013) Trophic level transfer of Microplastics: *Mytilus edulis* (L.) to
420 *Carcinus maenas* (L.). *Environmental Pollution* **177**, 1-3.

421 Fendall, L.S., Sewell, M.A. (2009) Contributing to marine pollution by washing your
422 face: Microplastics in Facial cleaners. *Marine Pollution Bulletin* **58 (8)**, 1225-1228.

423 Fernández, C. G., Tallec, K., Le Goic, N., Lambert, C., Soudant, P., Huvet, A., Suquet,
424 M., Berchel, M., Pont, I. P. (2018). Cellular responses of Pacific oyster (*Crassostrea*
425 *gigas*) gametes exposed *in vitro* to polystyrene nanoparticles. *Chemosphere* **208**, 764-772.

426 Gewert, B., Plassmann, M.M., MacLeod, M. (2015). Pathways for degradation of plastic
427 polymers floating in the marine environment. *Environmental Science Processes Impacts*
428 **17**, 1513-1521.

429 Gündoğdu, S., Çevik, C., Ayat, B., Aydoğan, B., Karaca, S. (2018) How Microplastics
430 quantities increase with floods events? An example from Mersin Bay NE Levantine coast
431 of Turkey. *Environmental Pollution* **239**, 342-350.

432 Hidalgo-Ruz, V., Gutow, L. Thompson, R.C., Thiel, M. (2012). Microplastics in the
433 Marine Environment: A Review of the Methods Used for Identification and Quantification.
434 *Environmental Science and Technology* **46**, 3060-3075.

435 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan,
436 R., Law, L.K. (2015). Plastic waste inputs from land into the ocean. *Science* **347** (6223),
437 768-771.

438 Kiørboe, T., Møhlenberg, F., Nøhr, O. (2012). Feeding, particle selection and carbon
439 absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom
440 material. *Ophelia* **19** (2), 193-205.

441 Koelmans, A.A., Besseling, E., Shim, W.J. (2015). Nanoplastics in the Aquatic
442 Environment. Critical Review. In: Bergmann M., Gutow L., Klages M. (eds) Marine
443 Anthropogenic Litter. Springer, Cham. pp 325-340.

444 Leslie, H.A., Van Velzen, M.J.M., Vethaak, A.D. (2013). Microplastics survey of the
445 Dutch environment. IVM Institute for Environmental studies report

446 Li, J., Yang, D., Li, L., Jabeen, K., Shi, H. (2015) Microplastics in commercial bivalves
447 from China. *Environmental Pollution* **207**, 190-195.

448 Martínez, O., Rodríguez-Calleja, J.M., Santos, J.A., Otero, A., García-López, M.L.
449 (2009). Foodborne and Indicator Bacteria in Farmed Molluscan Shellfish before and after
450 Depuration. *Journal of Food Protection* **72** (7), 1443-1449.

451 Mathalon, A., Hill, P. (2014) Microplastic fibers in the intertidal ecosystem surrounding
452 Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin* **81** (1), 69-79.

453 Molloy, S.D., Pietrak, M.R., Bouchard, D.A., Bricknell, I. (2011). Ingestion of
454 *Lepeophtheirus salmonis* by the blue mussel *Mytilus edulis*. *Aquaculture* **311**, 61-64.

455 O'Donohe, P., McDermott, T. (2014). Reducing sea lice re-infestation risk from harvest
456 water at a salmon farm site in Ireland using a bespoke sieving and filtration system.
457 *Aquacultural Engineering* **60**, 73-76.

458 Phuong, N.N., Poirier, L., Pham, Q.T., Lagarde, F., Vergnoux, A.Z. (2018) Factors
459 influencing the Microplastics contamination of bivalves from the French Atlantic coast:
460 Location, season and/or mode of life? *Marine Pollution Bulletin* **129 (2)**, 664-674.

461 PlasticsEurope (2018) Association of Plastics Manufacture[Online] Available from:
462 <https://www.plasticseurope.org/en/resources/publications/274-plastics-facts-2017>
463 [Accessed: 17th September 2018].

464 Pont, I. P., Lacroix, C., Fernández, C. G., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L.,
465 Cassone, A. L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A.,
466 Soudant, P. (2016). Exposure of marine mussels *Mytilus* spp. To polystyrene
467 Microplastics: Toxicity and influence on fluoranthene bioaccumulation. *Environmental*
468 *Pollution* **216**, 724-737.

469 Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C.,
470 Werorilangi, S., The, S.J. (2015). Anthropogenic debris in seafood: Plastic debris and
471 fibers from textiles in fish and bivalves sold for human consumption. *Scientific reports* **5**
472 **(14340)**.

473 Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C.,
474 Werorilangi, S., The, S.J. (2015) Anthropogenic debris in seafood: Plastic debris and
475 fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports* **5**,
476 14340

477 Schymanski, D., Goldbeck, C., Humpf, H-U., Fürst, P. (2018). Analysis of Microplastics
478 in water by micro-Raman spectroscopy: Release of plastic particles from different
479 packaging into mineral water. *Water research* **129**, 154-162.

480 Silva, P.P.G., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F. (2016). Leachate from
481 Microplastics impairs larval development in brown mussels. *Water Research* **106**, 364-
482 370.

483 Sussarellu, R., Suquet, M., Thomas, T., Lambert, C., Fabioux, C., Pernet, M. E. J., Le
484 Goic, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J.,
485 Robbens, J., Pont, I. P., Soudant, P., Huvet, A. (2016) Oyster reproduction is affected by
486 exposure to polystyrene Microplastics. *PNAS* **113** (9), 2430-2435.

487 UNESCO, (1981). Tenth Report of the Joint Panel on Oceanographic Tables and
488 Standards. UNESCO Technical Papers in Marine Science. Paris 25 p.

489 Van Cauwenberghe, L. and Janssen, C., R. (2014) Microplastics in bivalves cultured for
490 human consumption. *Environmental Pollution* **193**, 65-70.

491 Van Cauwenberghe, L. Claessens, M., Vandegehuchte, M.B., Janssen, C. R. (2015).
492 Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*)
493 living in natural habitats. *Environmental Pollution* **199**, 10-17.

494 Von Moos, N., Burkhardt, H.P., Kohler, A. (2012) Uptake and effects of Microplastics on
495 cells and tissue of the blue mussel *Mytilus edulis* L. after experimental exposure.
496 *Environmental Science Technology* **46**, 11327-11335.

497 Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T. (1997). Site of particle selection in a
498 bivalve mollusc. *Nature* **390**, 131-132.

499 Ward, J.E., Shumway, S.E. (2004). Separating the grain from the chaff: particle selection
500 in suspension-and deposit-feeding bivalves. *Journal of Experimental Marine Biology and*
501 *Ecology* **300** (1-2), 83-130.

502 Who (2019) World Health Organization [Online] Available from:
503 https://www.who.int/water_sanitation-health/emerging/depuration.pdf [Accessed: 22th
504 January 2019].

505 Wright, S.L., Kelly, F.J. (2017). Plastic and Human Health: A Micro Issue?
506 *Environmental Science and Technology* **51 (12)**, 6634-6647.