

# 1 Modelling mussel (*Mytilus spp.*) microplastic accumulation.

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13  
14 **Abstract:** Microplastics (MPs) are a contaminant of growing concern due to their widespread  
15 distribution and interactions with marine species, such as filter feeders. To investigate the MPs  
16 accumulation in wild and cultured mussels, a Dynamic Energy Budget (DEB) model was developed  
17 and validated with the available field data of *Mytilus edulis* (*M. edulis*, wild) from the North Sea  
18 and *Mytilus galloprovincialis* (*M. galloprovincialis*, cultured) from the Northern Ionian Sea.  
19 Towards a generic DEB model, the site-specific model parameter, half saturation coefficient ( $X_k$ )  
20 was applied as a power function of food density for the cultured mussel, while for the wild mussel it  
21 was calibrated to a constant value. The DEB-accumulation model simulated the uptake and  
22 excretion rate of MPs, taking into account of environmental characteristics (temperature and  
23 chlorophyll-a). An accumulation of MPs equal to 0.53 particles individual<sup>-1</sup> (fresh tissue mass 1.9 g)  
24 and 0.91 particles individual<sup>-1</sup> (fresh tissue mass 3.3 g) was simulated for the wild and cultured  
25 mussel after 4 years and 1 year respectively, in agreement with the field data. The inverse  
26 experiments investigating the depuration time of the wild and cultured mussel in a clean from MPs  
27 environment showed a 90% removal of MPs load after 2.5 and 12 days, respectively. Furthermore,  
28 sensitivity tests on model parameters and forcing functions highlighted that besides MPs  
29 concentration, the accumulation is highly depended on temperature and chlorophyll-a of the  
30 surrounding environment. For this reason, an empirical equation was found, directly relating the  
31 environmental concentration of MPs, with the seawater's temperature, chlorophyll-a and the  
32 mussel's soft tissue MPs load.

# 1. Introduction

Microplastic particles (MPs) are synthetic organic polymers with size below 5 mm (Arthur et al., 2009) that originate from a variety of sources including: those particles that are manufactured for particular household or industrial activities, such as facial scrubs, toothpastes and resin pellets used in the plastic industry (primary MPs), and those formed from the fragmentation of larger plastic items (secondary MPs) (GESAMP, 2015). Eriksen et al. (2014) estimated that more than 5 trillion microplastic particles, weighing over 250,000 tons, float in the oceans. Due to their composition, density and shape, MPs are highly persistent in the environment and are, therefore, accumulating in different marine compartments at increasing rates: surface and deeper layers in the water column, as well as at the seafloor and within the sediments (Moore et al., 2001, Lattin et al., 2004, Thompson, 2004, Lusher, 2015). Since the majority of MPs entering the marine environment, originate from the land (i.e. land-fills, littering of beaches and coastal areas, rivers, floodwaters, untreated municipal sewerage, industrial emissions), the threat of MPs pollution in the coastal zone puts considerable pressure on the coastal ecosystems (Cole et al., 2011, Andrady, 2011). In recent years, initiatives under various projects (i.e. CLAIM, DeFishGear) target at evaluating the threat and impact of marine litter pollution; the European framework of JERICO-RI focuses on a sustainable research infrastructure in the coastal area to support the monitoring, science and management of coastal marine areas (<http://www.jerico-ri.eu/>). In the framework of JERICO-NEXT, a recent study addressed the environmental threats and gaps with monitoring programmes in European coastal waters, including the marine litter (i.e. MPs) as one of the most commonly identified threat to the marine environment and highlighted the need for improved monitoring of the MPs distribution and their impacts in European coastal environments (Painting et al., 2019).

Numerous studies have revealed that MPs are ingested either directly or through lower trophic prey by animals at all levels of the food web; from zooplankton (Cole et al., 2013), small pelagic fish and mussels (Digka et al., 2018a) to mesopelagic fish (Wieczorek et al., 2018) and large predators like tuna and swordfish (Romeo et al., 2015). Microplastic ingestion by marine animals can potentially affect animal health and raises toxicity concerns, since plastics can facilitate the transfer of chemical additives and/or hydrophobic organic contaminants to biota (Mato et al., 2001, Rios et al., 2007, Teuten et al., 2007, 2009, Hirai et al., 2011). Human, as a top predator, is also contaminated by MPs (Schwabl et al., 2019). Mussel and small fish that are commonly consumed whole, without removing digestive tracts, where MPs are concentrated, are among the most likely pathways for MPs to embed in the human diet (Smith et al., 2018). Especially regarding marine organisms (i.e. mussels), it is notable that the levels of their contamination has been added to the

68 European database ([www.ecsafeseafooddbbase.eu](http://www.ecsafeseafooddbbase.eu)) as an environmental variable of growing concern,  
69 reflecting the health status (Marine Strategy Framework Directive (MSFD) Descriptor 10 – Marine  
70 Litter (Decision 2017/848/EU)) (De Witte et al., 2014, Vandermeersch et al., 2015, Digka et al.,  
71 2018a). Today, a series of studies have denoted the presence of MPs in mussels' tissue intended for  
72 human consumption (Van Cauwenberghe and Janssen, 2014, Mathalon and Hill, 2014, Li et al.,  
73 2016, 2018, Hantoro et al., 2019). For instance, in a recent study, Li et al. (2018) sampled mussels  
74 from coastal waters and supermarkets in the U.K and estimated that a plate of 100g mussels  
75 contains 70 MPs that will be ingested by the consumer. The presence of MPs in mussels has been  
76 also demonstrated during laboratory trials in their faeces, intestinal tract (Von Moos et al., 2012,  
77 Van Cauwenberghe et al., 2015, Wegner et al., 2012, Khan and Prezant, 2018), as well as in their  
78 circulatory system (Browne et al., 2008). Other laboratory studies showed several effects of  
79 microplastic ingestion in laboratory exposed mussels, including histological changes, inflammatory  
80 responses, immunological alterations, lysosomal membrane destabilization, reduced filtering  
81 activity, neurotoxic effects, oxidative stress effects, increase in hemocyte mortality, dysplasia,  
82 genotoxicity and transcriptional responses (reviewed by Li et al., 2019). However, the tested  
83 concentrations of MPs in laboratory experiments are frequently unrealistic, being several orders of  
84 magnitude higher (2 to 7 orders of magnitude) than the observed seawater concentrations (Van  
85 Cauwenberge et al., 2015, Lenz et al. 2016).

86 Mussels, through their extensive filtering activity, feed on planktonic organisms that have  
87 similar size with MPs (Browne et al., 2007) and considering also their inability to select particles  
88 with high energy value (i.e. phytoplankton) during filtration (Vahl, 1972, Saraiva et al., 2011a),  
89 they are directly exposed to MPs' contamination. Recent studies suggest a positive linear  
90 correlation between MPs concentration in mussels and surrounding waters (Capolupo et al., 2018,  
91 Qu et al. 2018, Li et al. 2019). The filtering activity of mussels, which directly affects the resulting  
92 MPs accumulation, is a complicated process that is controlled by other factors (food availability,  
93 temperature, tides etc.).

94 The purpose of the present work is to study the accumulation of MPs in mussels and reveal  
95 relations between the accumulated concentrations in mussels' soft parts and environmental features.  
96 In this context, an accumulation model was developed based on Dynamic Energy Budget theory  
97 (DEB, Kooijman , 2000) and applied in two different regions, in two different modes of life (wild  
98 and cultivated): in the North Sea (*Mytilus edulis* (*M. edulis*), wild) and in the Northern Ionian Sea  
99 (*Mytilus galloprovincialis* (*M. galloprovincialis*), cultivated). DEB theory provides all the necessary  
100 detail to model the feeding processes and aspects of the mussel metabolism, taking into account the  
101 impact of the environmental variability on the simulated individual. Apart from modelling the  
102 growth of bivalves (Rosland et al., 2009, Sara et al., 2012, Thomas et al., 2011, Saraiva et al., 2012,

103 Hatzonikolakis et al., 2017, Monaco & McQuaid, 2018), DEB models have been used to study  
104 other processes as well, such as bioaccumulation of PCBs (Polychlorinated Biphenyls) and POPs  
105 (Persistent Organic Compounds) (Zaldivar, 2008), trace metals (Casas and Bacher, 2006) and the  
106 impact of climate change on individual's physiology (Sara et al., 2014). However, to our knowledge  
107 this is the first time that a DEB-based model is used to assess the uptake and excretion rates of MPs  
108 in mussels.

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## 111 **2. Materials and Methods**

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### 113 **2.1 Study areas and field data**

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115 The North Sea is a marginal sea on the continental shelf of north-west Europe with a total  
116 surface area of 850,000 km<sup>2</sup> and is bounded by the coastlines of 9 countries. The sea is shallow  
117 (mean depth 90 m), getting deeper towards the north (up to 725 meters) and the semi-diurnal tide  
118 (tidal range 0-5 m) is the dominant feature of the region (Otto et al., 1990). Major rivers, such as  
119 Rhine, Elbe, Weser, Ems and Thames discharge into the southern part of the sea (Lacroix et al.,  
120 2004), making this area a productive ecosystem. In this study, the area is limited along the French,  
121 Belgian and Dutch North Sea coast (N 50.98°-51.46°, W 1.75°-3.54°). This is located close to  
122 harbors, where shipping, industrial and agricultural activity is high, putting considerable pressure on  
123 the ecological systems of the region (Van Cauwenberghe et al., 2015).

124 The MPs concentration in mussels' tissue and seawater that were used to validate and force  
125 the model respectively at its North Sea implementation were derived from Van Cauwenberghe et al.  
126 (2015). Van Cauwenberghe et al. (2015) examined the presence of MPs in wild mussels (*M. edulis*),  
127 and thus collected both biota and water at 6 sampling stations along the French, Belgian and Dutch  
128 North Sea coast in late summer of 2011. *M. edulis* (mean shell length: 4 ± 0.5 cm and wet weight  
129 (w.w.): 2 ± 0.7 g) and water samples were randomly collected on the local breakwaters, in order to  
130 assess the MPs concentration in the organisms and their habitat. MPs were present in all analyzed  
131 samples, both organisms and water. Seawater samples (N=12) had MPs (<1mm) on average 0.4 ±  
132 0.3 particles L<sup>-1</sup> (range: 0.0 – 0.8 particles L<sup>-1</sup>) and *M. edulis* contained on average 0.2 ± 0.3  
133 particles g<sup>-1</sup>w.w. (or 0.4 ± 0.3 particles individual<sup>-1</sup>) (Van Cauwenberghe et al., 2015). The size  
134 range of MPs found within the mussels was 20-90 µm.

135 The Northern Ionian Sea is located in the transition zone between the Adriatic and Ionian Sea.  
136 The long and complex coastline, presents a high diversity of hydrodynamic and sedimentary  
137 features. Rivers discharging into the Northern Ionian Sea include Kalamas/Thyamis (Greece) and  
138 Butrinto (Albania) (Skoulikidis et al., 2009), making the area suitable for aquaculture. Small  
139 farming sites and shellfish grounds are operating in Thesprotia (northwestern Ionian Sea)  
140 (Theodorou et al., 2011). The main source of marine litter inputs in the area originates from  
141 anthropogenic activities that mainly include shoreline tourism and recreational activities, poor  
142 wastewater management, agriculture, fisheries, aquacultures and shipping (Vlachogianni et al.,  
143 2017; Digka et al., 2018a). According to Politikos et al. (2020), the area around the Corfu island  
144 (Northern Ionian Sea) is characterized as a retention area of litter particles probably due to the  
145 prevailing weak coastal circulation. Furthermore, a northward current on the east Ionian Sea  
146 facilitates the transfer of litter particles towards the Adriatic Sea, which has been characterized as a  
147 hotspot of marine litter and one of the most affected areas in the Mediterranean Sea (Pasquini et al.,  
148 2016, Vlachogianni et al., 2017, Liubartseva et al., 2018, Politikos et al., 2020).

149 The field data used to validate the model output in the N. Ionian Sea were obtained from  
150 Digka et al. (2018b, 2018a). In the framework of the “DeFishGear” project, mussels (*M.*  
151 *galloprovincialis*) were collected by hand from a long line type mussel culture farm in Thesprotia  
152 (N 39.606567° E 20.149421°), in summer 2015 (end of June) at a sampling depth up to 3 m (Digka  
153 et al., 2018a). The average MPs accumulation was calculated from a total population of 40 mussels  
154 originated from the farm, with 18 of them found contaminated with MPs (46.25%). The average  
155 load of MPs (size <1 mm) per mussel (mean shell length  $5.0 \pm 0.3$  cm) was  $0.9 \pm 0.2$  particles  
156 individual<sup>-1</sup> and the size of MPs found in the mussel’s tissue ranged from 55 to 620  $\mu\text{m}$ . Both clean  
157 and contaminated mussels were included in the calculated mean value in order to represent the  
158 mean state of the contamination level for the individual inhabiting the study area.

159 The seawater concentration of MPs for the N. Ionian Sea implementation was obtained from  
160 Digka et al. (2018b) and the DeFishGear project results ([http://www.defishgear.net/project/main-](http://www.defishgear.net/project/main-lines-of-activities)  
161 [lines-of-activities](http://www.defishgear.net/project/main-lines-of-activities)). In total, 12 manta net tows were conducted in the region, collecting a total  
162 number of  $n_1=2,027$  particles on October 2014 and  $n_2=1,332$  on April 2015, leading to an average  
163 of 280 particles per tow with size <1 mm and >330  $\mu\text{m}$  (Digka et al., 2018b). In order to estimate  
164 the mean MPs concentration in the region, expressed as particles per volume, the dimensions of the  
165 manta net (W 60 cm H 24 cm, rectangular frame opening, mesh size 330  $\mu\text{m}$ ) and the sampling  
166 distance of each tow (~2 km) were used by multiplying the sample surface of the net by the trawled  
167 distance in meters (Maes et al., 2017), which resulted in a mean MPs concentration of 1.17 particles  
168  $\text{m}^{-3}$  (233,333 particles  $\text{km}^{-2}$ ). Moreover, in the wider region of the Adriatic Sea, Zeri et al. (2018)  
169 found a mean density of  $315,009 \pm 568,578$  particles  $\text{km}^{-2}$  ( $1.58 \pm 2.84$  particles  $\text{m}^{-3}$ ), out of which

170 34% sized <1 mm. A relatively high value of standard deviation (one order of magnitude higher  
171 than the mean value) is adopted ( $0.0012 \pm 0.024$  particles  $L^{-1}$ ), considering that the mussel farm is  
172 established in an enclosed gulf and close to the coast, since, according to Zeri et al. (2018), the  
173 abundance of MPs is one order of magnitude higher in inshore (<4 km) compared to offshore  
174 waters (>4 km). Furthermore, it may be assumed that the adopted range (standard deviation is also  
175 multiplied by a factor of 2) includes also the smaller particles sized between 50  $\mu m$  and < 330  $\mu m$ ,  
176 which have been found in mussel's tissue (Digka et al., 2018a), but were overlooked during the  
177 seawater sampling due to the manta net's mesh size (> 330  $\mu m$ ). According to Enders et al. (2015)  
178 the relative abundance of small particles (50- 300  $\mu m$ ) compared to particles larger than 300  $\mu m$  is  
179 approximately 50%.

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## 181 **2.2 DEB model description**

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183 In the present study, a DEB (Kooijman, 2000, 2010) model is used as basis to simulate the  
184 accumulation of MPs by mussels. In DEB theory (Kooijman, 2000), the energy assimilated through  
185 food by the simulated individual is stored in a reserve compartment from where a fixed energy  
186 fraction  $\kappa$  is allocated for growth and somatic maintenance, with a priority for maintenance. The  
187 remaining energy ( $I - \kappa$ ) is spent on maturity maintenance and reproduction. The individual's  
188 condition is defined by the dynamics of three state variables: energy reserves E (joules), structural  
189 volume V ( $cm^3$ ) and energy allocated to reproduction R (joules). The energy flow through the  
190 organism is controlled by the fluctuations of the available food density and temperature  
191 characterizing the surrounding environment.

192 The DEB model implemented here is an extended version of the model described in  
193 Hatzonikolakis et al. (2017), where the growth of the Mediterranean mussel is simulated taking into  
194 account only the assimilation rate of the individual. Since the present study focuses on the MPs  
195 accumulation, it is crucial to include a detailed representation of the mussel's feeding mechanism.  
196 In this context, the DEB model was extended by including the clearance ( $C_r$ ), filtration ( $\dot{p}_{XiF}$ ) and  
197 ingestion ( $\dot{p}_{Xil}$ ) rates of the mussel, following Saraiva et al. (2011a), assuming that all parameters  
198 referred to silt (or inedible particles) are applicable also to MPs particles. In this approach, a pre-  
199 ingestive selection occurs between filtration and ingestion, returning the rejected material in the  
200 water through pseudofaeces ( $J_{pfi}$ ). Consequently, energy is assimilated through food while the non-  
201 assimilated particles are excreted through the faeces production ( $J_f$ ). The model's equations,  
202 variables and parameters are shown in Table 1, 2 and 3 respectively. The scaled functional response  
203  $f$  (Eq. 5, Table 1), which regulates the assimilation rate, is modified following Kooijman (2006) to

204 include an inorganic term representing the non-digestible matter i.e. microplastics:  $f =$   
205  $X/(X + K_y)$  and  $K_y = X_K \cdot (1 + Y/Y_K)$  where  $Y$  and  $Y_k$  are the concentration of MPs, converted  
206 from particles  $L^{-1}$  to  $g\ m^{-3}$  (Everaert et al., 2018) and the half saturation coefficient of inorganic  
207 particles here represented by MPs ( $g\ m^{-3}$ ), respectively. Thus, the assimilation rate that is regulated  
208 by  $f$  is decreasing when the concentration of MPs is increased. The same approach is followed by  
209 other authors who considered inedible particles in the mussel's diet (Ren, 2009, Troost et al., 2010).  
210 During the filtration process the same clearance rate for all particles is used ( $\{\dot{C}_R\}$ ), representing the  
211 same searching rate for food that depends on the organism maximum capacity ( $\{\dot{C}_{Rm}\}$ ) and  
212 environmental particle concentrations (Vahl, 1972, Widdows et al., 1979, Cucci et al., 1989).  
213 During the ingestion process the mussel is able to selectively ingest food particles and reject  
214 inedible material, in order to increase the organic content of the ingested material (Kjørboe &  
215 Møhlenberg, 1981, Jørgensen et al., 1990, Prins et al., 1991, Maire et al., 2007, Ren, 2009, Saraiva  
216 et al., 2011a). This selection is reflected by the different binding probabilities adopted for each type  
217 of particle ( $\rho_1$  for algae particles and  $\rho_2$  for inorganic particles i.e. MPs, see Eq. 14 and table 3). The  
218 equations representing the feeding processes handle each type of particle separately, while there is  
219 interference between the simultaneous handling of different particle types (Eq. 12-14, Table 1)  
220 (Saraiva et al., 2011a). Finally, during the assimilation process, suspended matter (i.e. MPs) that the  
221 mussel is not able to assimilate due to its different chemical composition from the reserve  
222 compartment (Saraiva et al., 2011a) or incipient saturation at high algal concentrations (Riisgard et  
223 al., 2011) results in the faeces production (Eq. 16, Table 1).

224

### 225 **2.3 Microplastics accumulation sub-model**

226

227 With the DEB model as a basis, a sub-model describing the MPs accumulation by the mussel  
228 was developed, assuming that the presence of MPs in the ambient water does not cause a significant  
229 adverse effect on the organisms' overall energy budget, in accordance with laboratory experiments,  
230 conducted in mussel species (Van Cauwenberghe et al., 2015: *Mytilus edulis*, Santana et al., 2018:  
231 mussel *Perna perna*). Additionally, it was assumed that the mussel filtrates MPs present in the  
232 water, without the ability of selecting between the high energetic valued particles and the MPs  
233 during the filtration process (Van Cauwenberghe et al., 2015, Von Moos et al., 2012, Browne et al.,  
234 2008, Digka et al., 2018a among others). The uptake of MPs from the environment is taken into  
235 account through the process of clearance/filtration rate, while the excretion of the contaminant is  
236 derived from two processes: (i) pseudofaeces production and (ii) faeces production. The resulting  
237 MPs accumulation is influenced by external environmental factors (MPs concentration, food

238 availability, temperature) and internal biological processes (clearance, filtration, ingestion, growth).  
239 The following differential equation describes the change of the individual MPs accumulation ( $C$ ,  
240 particles individual<sup>-1</sup>), taking into account the processes mentioned above:

241

$$242 \quad \frac{dC}{dt} = C_{env} \cdot \dot{C}_R - \dot{J}_{pf2} - k_f \cdot \frac{J_f}{p_{X1I}} \cdot C \quad (\text{Eq. 18})$$

243 where  $\dot{C}_R$  is the clearance rate for water (L h<sup>-1</sup>), containing a concentration of MPs  $C_{env}$  (particles L<sup>-1</sup>).  
244 The terms of  $\dot{J}_{pf2}$  and  $\frac{J_f}{p_{X1I}}$  represent the elimination rate of MPs through pseudofaeces (particles  
245 h<sup>-1</sup>) and the non-dimensional rate of faeces production with respect to the ingestion rate,  
246 respectively (see Table 1, Eq. 15-16). The parameter  $k_f$  represents the post-ingestive selection  
247 mechanism utilized by the mussel to incorporate indigestible material (i.e. MPs) into faeces and was  
248 calibrated using the available field data of mussel's MPs accumulation from both study areas (Table  
249 3). Mussel is able to discriminate among particles in the gut based on size, density and chemical  
250 properties of the particles (i.e. between microalgae and inorganic material) and thus to eliminate  
251 them through faeces (Ward et al., 2019a and references therein). In this context, the pseudofaeces  
252 production incorporates the rejected MPs prior to the ingestion, while the faeces production  
253 includes MPs that are rejected along with the food particles that are not assimilated by the mussel.  
254 The model's time step has been set to one hour in order to capture the dynamics of the rapidly  
255 changing processes, such as feeding and excretion.

## 256 **2.4 Environmental drivers**

257

258 Besides MPs concentration in the seawater, the DEB model is forced by sea surface  
259 temperature (SST) and food availability, represented by chlorophyll-a concentrations (CHL-a, an  
260 index of phytoplankton biomass). *M. edulis* has been demonstrated to filter suspended particles  
261 greater than 1µm, a size class that includes all of the phytoplankton, zooplankton and much of the  
262 detritus (Vahl, 1972, Mohlenberg and Riisgard, 1978, Saraiva et al., 2011a, Strohmeier et al., 2012),  
263 including even aggregated picoplankton-size particles (i.e. marine snow) (Kach and Ward, 2008,  
264 Ward and Kach, 2009). CHL-a has been considered the most reliable food quantifier for the  
265 calculation of DEB shellfish parameters (Pouvreau et al., 2006, Sara et al., 2012, Hatzonikolakis et  
266 al., 2017 and references therein). Hatzonikolakis et al. (2017) have tested the performance of the  
267 model, considering also particulate organic carbon (POC) in the mussel's diet, which, however, did  
268 not have an important impact on the model's skill against field data in the Mediterranean Sea study  
269 areas. This outcome agrees with Troost et al. (2010) demonstration that POC contributes to the  
270 mussel's diet when CHL-a concentrations are low at the southwest of Netherlands. Thus, in the



271 present study, only CHL-a is considered as the available food source for mussels originated from  
272 the Southern North Sea and the Northern Ionian Sea.. For both study areas SST and CHL-a are  
273 derived from daily satellite data, a method also used by other authors (i.e. Thomas et al., 2011,  
274 Monaco & McQuaid, 2018).

275 In the North Sea, SST data were obtained from daily satellite images provided by Copernicus  
276 Marine Environmental Monitoring Service (CMEMS) at 0.04 degree spatial resolution. CHL-a data  
277 obtained from the Globcolour daily multi-sensor product provided by CMEMS at 1 km spatial  
278 resolution, based on the OC5 algorithm of Gohin et al. (2002) (<http://marine.copernicus.eu/>,  
279 generated using CMEMS Products, production center ACRI-ST). The environmental forcing data  
280 (SST, CHL-a) were averaged over the study area (51.08°-51.44° N, 2.19°-3.45° E), covering the  
281 period 2007-2011 (5 years), in order to realistically simulate the wild mussel's growth harvested in  
282 late summer 2011 (Van Cauwenberghe et al., 2015). It is notable that the study area of the North  
283 Sea belongs to CASE II waters (coastal region), where algorithms tend to overestimate CHL-a  
284 concentrations. In optically-complex Case II waters, CHL-a cannot readily be distinguished from  
285 particulate matter and/or yellow substances (dissolved organic matter) and so global chlorophyll  
286 algorithms are less reliable (IOCCG, 2000). However, the CHL-a dataset that was used was found  
287 in good agreement with available in situ data from [ICES database](https://www.ices.dk/data/Pages/default.aspx)  
288 (<https://www.ices.dk/data/Pages/default.aspx>) for the specific study area and time period,(Fig. 1),  
289 showing a relatively smaller bias and better time-space coverage, as compared with other tested  
290 remote sensing datasets (not shown) (i.e. regional chlorophyll product available for the North West  
291 Shelf-Seas in the CMEMS catalogue, <https://resources.marine.copernicus.eu/>).

292 In the North Ionian Sea, daily satellite SST data were also obtained from the CMEMS  
293 database for the Mediterranean Sea with 0.04 degree spatial resolution, while CHL-a daily data  
294 were derived from the Globcolour multi-sensor (i.e. SeaWiFS, MERIS, MODIS, VIIRS and OLCI-  
295 a) merged product (<http://globcolour.info>) at 1 km spatial resolution based on the OC5 algorithm  
296 suitable for coastal regions (Gohin et al., 2002). The forcing data were averaged over the study area  
297 (N 39.49°-39.65°, E 20.09°-20.23°) covering the period 2014-2015 (2 years), when the cultured  
298 mussel is ready for the market. The chosen CHL-a dataset was found preferable, as compared with  
299 other available remote sensing datasets (i.e. CMEMS chlorophyll product for Mediterranean Sea),  
300 since it presented a better spatial and temporal coverage (Hourany et al., 2019, Garnesson et al.,  
301 2019) and a slightly lower error, as compared with the very few available in situ data in the study  
302 area (not shown). Unfortunately, these were very scarce and therefore an extended comparison  
303 between remote and in situ data could not be conducted. Satellite data have facilitated large scale  
304 ecological studies by providing maps of phytoplankton functional types and sea surface temperature  
305 (Raitsos et al., 2005, 2008, 2012, 2014, Palacz et al., 2013, Di Cicco et al., 2017, Brewin et al.,

306 2017). The daily environmental forcing data are shown in Fig. 1 and Fig. 2 for the North Sea and  
307 the N. Ionian Sea, respectively. The two coastal environments present some important differences  
308 regarding both CHL-a and SST. Specifically, in the N. Ionian Sea, CHL-a is relatively low (annual  
309 mean  $\sim 0.88 \text{ mg m}^{-3}$ ) and peaks during winter (maximum  $\sim 2.64 \text{ mg m}^{-3}$  at December 2014), while  
310 in the North Sea CHL-a is about four times higher (annual mean  $4.25 \text{ mg m}^{-3}$ ), peaking in April  
311 every year (maximum range  $29.44\text{-}33.38 \text{ mg m}^{-3}$ ), as soon as light availability reaches a critical  
312 level (Van Beusekom et al., 2009). The higher productivity during spring season in the North Sea is  
313 related with the nutrient inputs from the English Channel, the North Atlantic and particularly the  
314 river discharge of nutrient-rich waters along the Belgian-French-Dutch coastline which peaks  
315 earlier, during winter period (Van Beusekom et al., 2009). The SST peaks during August in both  
316 areas (Fig. 1 and Fig. 2), but is significantly higher in the N. Ionian Sea (maximum  $28.8^\circ\text{C}$ ), as  
317 compared to the North Sea (maximum  $18\text{-}19.3^\circ\text{C}$ ).

318 The environmental concentration of MPs,  $C_{env}$  (particles  $\text{L}^{-1}$ ) was obtained also at a daily time  
319 step as randomly generated values of the Gaussian distribution that is determined by the mean value  
320 and standard deviation of the observed field data ( $0.4 \pm 0.3$  particles  $\text{L}^{-1}$ , North Sea, Van  
321 Cauwenberghe et al., 2015,  $0.0012 \pm 0.024$  particles  $\text{L}^{-1}$ , N. Ionian Sea, Digka et al., 2018a).  
322 Considering that these values originate from surface waters and that mussels live in the near surface  
323 layer (0-5 m),  $C_{env}$  is estimated as a mean value of the upper layer with the methods described by  
324 Kooi et al. (2016), who studied the vertical distribution of MPs, considering an exponential  
325 decrease with depth. Specifically, in the N. Ionian Sea, mussels were collected from a depth up to 3  
326 m (Digka et al., 2018a), while in the North Sea (Van Cauwenberghe et al., 2015), there is no  
327 information and thus a maximum depth of 5 m is adopted.

328 In the North Sea simulation, the effect of tides is taken into account by considering that the  
329 mussel originated from the intertidal zone, is submerged 12 hours during the day (Van  
330 Cauwenberghe et al., 2015). In the N. Ionian Sea simulation, tides are not considered, given the  
331 very small tide amplitude (few centimeters) in the Mediterranean (i.e. Sara et al., 2011;  
332 Hatzonikolakis et al., 2017) and thus the cultured mussel is assumed permanently submerged. *In*  
333 *situ* hourly tide data (2007-2011) from the coastal zone of the region (Dunkerque station N  
334  $51.04820^\circ$ , E  $2.36650^\circ$ ) obtained from Coriolis and Copernicus data provider  
335 (<http://marine.copernicus.eu>, <http://www.coriolis.eu.org>), showed that mussels experience  
336 alternating periods of aerial exposure and submergence at approximately every 6 hours (2 high and  
337 2 low tides). During aerial exposure the model suspends the feeding processes (Sara et al., 2011)  
338 and simulates metabolic depression (Monaco & McQuaid, 2018) where, the Arrhenius thermal  
339 sensitivity equation (Eq. 9) is corrected by a metabolic depression constant ( $M_d = 0.15$ ), a value  
340 representative for *M. galloprovincialis* and here applied also for *M. edulis*. In the present study, the

341 mussel's body temperature change during low tide is ignored, inducing a model error. The mussel's  
342 body temperature (i.e. surrounding water temperature for submerged mussels) during air exposure  
343 depends on many factors, such as solar radiation, air's temperature, wind speed and wave height,  
344 according to studies investigating the temperature effect on intertidal mussels (Kearney et al., 2010,  
345 Sara et al., 2011). However, the present study aims to primarily examine the MPs accumulation and  
346 thus the intertidal mussel's body temperature was not thoroughly examined. Nonetheless, the time  
347 that the mussel is able to filter, ingest and excrete the suspended matter (i.e. food and MPs particles)  
348 and the effect on the mussel's growth through the modified relation of  $k(T)$  are included, since the  
349 assimilation process occurs whether the mussel is submerged or not (Kearney et al., 2010).

## 350 2.5 Parameter values

351

352 Most of the DEB model parameters were obtained from Van der Veer et al. (2006) and are  
353 referred to the blue mussel *M. edulis* in the northeast Atlantic (see Table 3 for the exceptions). This  
354 assumption has also been adopted in previous studies which showed that this parameter set for *M.*  
355 *edulis* applies also for *M. galloprovincialis* (i.e. Casas and Bacher, 2006, Hatzonikolakis et al.,  
356 2017). The half saturation coefficient  $X_k$  represents the density of food at which the food uptake rate  
357 reaches half of its maximum value and should be treated as a site – specific parameter (Troost et al.,  
358 2010, Pouvreau et al., 2006). In order to estimate the value of  $X_k$ , a different approach was followed  
359 for each study area.

360 For the North Sea simulation,  $X_k$  was tuned so that the simulated individual has the recorded  
361 size at the corresponding estimated age (Van Cauwenberghe et al., 2015) growing with the  
362 representative growth rates of wild *M. edulis* at the region (Saraiva et al., 2012, Sukhotin et al.,  
363 2007). For the N. Ionian Sea simulation, an alternative method was adopted, aiming to generalize  
364 the DEB model to overcome the problem of site-specific parameterization. The DEB model was  
365 tuned against literature field data for cultured mussels originated from different areas in the  
366 Mediterranean and Black Seas, where the average CHL-a concentration ranged between 1.0 and 5.0  
367  $\text{mg m}^{-3}$ , and one  $X_k$  value was found for each area. The four areas used, their characteristics and the  
368 corresponding value of  $X_k$  adopted, are shown in Table 4. These values of  $X_k$  are related to the  
369 prevailing CHL-a concentration of each area ([CHL-a]) through three different functions: linear:  
370  $f(x) = a * [CHL - a] + b$  exponential:  $f(x) = a * \exp(b * [CHL - a])$  and power:  $f(x) = a * [CHL - a]^b + c$ . The curve fitting app of Matlab (Matlab R2015a) was used for the determination  
371 of  $a, b$  and  $c$  of each function taking into account the 95% confidence level. The score of each  
372 function regarding the somatic/mussel growth simulation in all four regions is tested through target  
373 diagrams (Jolliff et al., 2009) by computing the bias and unbiased root-mean-square-deviation  
374

375 (RMSD) between field and simulated data of all 4 regions and the function with the best score is  
376 adopted. A similar approach was followed by Alunno-Bruscia et al. (2011) for the oyster  
377 *Crassostrea gigas* in six Atlantic ecosystems who expressed the  $X_k$  as a linear function of food  
378 density (e.g. phytoplankton). Unfortunately, the approach described for the N. Ionian Sea  
379 simulation could not be applied in the North Sea, as the limited amount of growth data from the  
380 literature for wild *M. edulis* in similar environments did not permit a statistically significant fit of a  
381 similar function ( $X_k = f(chl - a)$ ).

382

## 383 **2.6 Simulation of reproduction-Initialization of the model**

384

385 The reproductive buffer (R) is assumed to be completely emptied at spawning ( $R = 0$ )  
386 (Sprung, 1983, Van Haren et al., 1994). In order to simulate mussel's spawning, the gonado-somatic  
387 index (GSI) defined as gonad dry mass over total dry flesh mass was computed at every model's  
388 time step (Eq. 17 Table 1; the water content of the fresh tissue mass was assumed 80% according to  
389 Thomas et al. (2011)). Spawning was induced by a critical value of GSI ( $GSI_{th}$ , Table 3) and a  
390 minimum temperature threshold ( $T_{th}$ ) at each study area, obtained from the literature. In the North  
391 Sea implementation,  $T_{th}$  was set at 9.6 °C (Saraiva et al., 2012), while in the N. Ionian Sea, at 15 °C  
392 (Honkoop and Van der Meer, 1998). This kind of formulation for the spawning event in bivalves  
393 has been used in previous studies (i.e. Pouvreau et al., 2006, Troost et al., 2010, Thomas et al.,  
394 2011, Monaco & McQuaid, 2018). The simulated abrupt losses of the mussel's tissue mass  
395 correspond to spawning events and the model's prediction was compared with the available  
396 literature data regarding the spawning period in each study area. Theodorou et al. (2011)  
397 demonstrated that the spawning events occur during winter for *M. galloprovincialis* in the mussel  
398 farms of Greece, while in the North Sea the spawning period for *M. edulis* is extended from the end  
399 of April until the end of June (Sprung, 1983, Cardoso et al., 2007).

400 In both areas, the model was initialized so that the simulated individual is in the juvenile phase  
401 ( $V < V_p$ ; Table 3) and the reproductive buffer can be considered to be empty ( $R = 0$ ) (Thomas et al.,  
402 2011). As stated by Jacobs et al. (2015) amongst others, juvenile mussels (*M. edulis*) range between  
403 1.5-25 mm in size. Specifically, in the North Sea the settlement of mussel larvae (*M. edulis*) takes  
404 place in June and the juveniles grow to a maximum size of 25 mm within 4 months (Jacobs et al.,  
405 2014). In the N. Ionian Sea, the operating mussel farms follow the life cycle of *M.*  
406 *galloprovincialis*, starting the operational cycle each year by dropping seed collectors from late  
407 November until March and the juvenile mussels grow up to 6-6.5cm after approximately one year  
408 according to the information obtained from the local farms in the region and Theodorou et al.

409 (2011). The initial fresh tissue mass was distributed between the structural volume (V) and reserves  
410 energy (E). Energy allocated to those two compartments was firstly constrained by the initial length  
411 (L) and then energy allocated to V was in Eq.10 (Table 1). The initial value of E was set so that the  
412 simulated individual has an initial weight that corresponds to the juvenile phase ( $V < V_p$ ) (Table 5).  
413 Finally, for both model implementations, the initial accumulation of MPs in the mussel's tissue (C)  
414 was set to zero.

415

## 416 **2.7 Simulation Runs**

417 The DEB-accumulation model simulates at an hourly basis the growth and MPs accumulation  
418 of the wild mussel from the North Sea and the cultured mussel from the N. Ionian Sea. Initially, a  
419 model run is performed at each study area during the periods July 2007 to August 2011 (4 years) for  
420 the North Sea simulation and late November 2014 to January 2016 (~ 1 year) for the N. Ionian Sea  
421 simulation. Additionally, the inverse simulations were performed in order to evaluate the depuration  
422 phase of both cultured and wild mussel, by setting the environmental MPs concentration equal to  
423 zero ( $C_{env}=0$ ), after a period of 1 year simulation at the N. Ionian Sea, when the cultured mussel has  
424 the appropriate size for market, and after 4 years at the North Sea, when literature field data are  
425 available (Van Cauwenberghe et al., 2015). In this simulation, the mussel's gut clearance is  
426 achieved by the excretion of MPs through faeces (3<sup>rd</sup> term of Eq. 18), and thus it is necessary to  
427 maintain the existence of food in the mussel's environment in order to ensure that the feeding-  
428 excretion processes will occur.

429 Furthermore, to examine the model's uncertainty related to the environmental MPs  
430 concentration, a series of 15 and 13 simulations were performed in the North Sea and N. Ionian Sea  
431 respectively, adopting different constant values of  $C_{env}$  within the observed range of each area.  
432 Finally, the effect of the environmental forcing data and some model's parameters on the resulting  
433 MPs accumulation by both mussels was explored through sensitivity experiments. These were used  
434 to derive a new function that predicts the level of MPs pollution in the environment.

435

## 436 **2.8 Sensitivity tests and Regression analysis**

437 The effect of the environmental data (CHL-a, temperature,  $C_{env}$ ) and two parameters  
438 representative of mussel's growth ( $X_k, Y_k$ ) on the MPs accumulation by the mussel for each study  
439 area was examined through sensitivity experiments with the DEB-accumulation model. Each  
440 variable (CHL-a, T,  $C_{env}$ ) and parameter ( $X_k, Y_k$ ) was perturbed by  $\pm 10\%$  to examine their effect on

441 the simulated MPs accumulation, and the results of each run were analyzed using a sensitivity index  
 442 (SI). SI calculates the percentage change of the mussel's MPs accumulation  $SI = \frac{1}{n} \sum_{t=1}^n \frac{|C_t^1 - C_t^0|}{C_t^0}$ .  
 443 100 (%), where  $n$  is the simulated time steps,  $C_t^0$  is the MPs accumulation predicted with the  
 444 standard simulation at time  $t$  and  $C_t^1$  is the MPs accumulation with a perturbed variable/parameter at  
 445 time  $t$ ; for details see Bacher and Gangnery, (2006). The same method has been also applied to  
 446 other studies, which examined the model's sensitivity on specific variables/parameters regarding the  
 447 mussel growth (Casas and Bacher, 2006, Rosland et al., 2009, Béjaoui-Omri et al., 2014,  
 448 Hatzonikolakis et al., 2017). In order to also examine the effect of tides, in the North Sea  
 449 implementation, the sensitivity experiments were conducted twice: the first time assuming that the  
 450 mussel is permanently submerged and the second time assuming that the mussel is periodically  
 451 exposed to the air.

452 Preliminary sensitivity experiments showed that the MPs accumulation is highly depended on  
 453 the prevailing conditions regarding the CHL-a, temperature and  $C_{env}$  and the mussel's growth that is  
 454 regulated by the half saturation coefficient ( $X_k$ ). Therefore an attempt was made using the model's  
 455 output to describe the MPs accumulation as a function of these variables through a custom  
 456 regression model:

$$457 \quad y = b_1 * W + b_2 * \exp\left(\frac{1}{T}\right) + b_3 * \frac{1}{[CHL-a]} + b_4 * C_{env} \quad (\text{Eq. 19})$$

458 where  $y$  (particles/individual) is the response variable and represent the predicted MPs  
 459 accumulation by the mussel;  $W$  (g) the mussel's fresh tissue mass,  $T$  (K) the sea surface  
 460 temperature, CHL-a and  $C_{env}$  are the concentrations of chlorophyll-a and MPs in the water  
 461 respectively, which are the predictor variables. The values of coefficients  $b_1$ ,  $b_2$ ,  $b_3$ , and  $b_4$  are  
 462 calculated using the nonlinear regression function (nlinfit, Matlab R2015a) which attempts to find  
 463 values of the parameters  $b$  that minimize the least squared differences between the model's MPs  
 464 accumulation output  $C$  and the predictions of the regression model  $y = f(W, T, [chl a], C_{env}, b)$ .

465 The ultimate aim of this analysis, once coefficients are determined, is to use Eq. 19 to obtain  
 466 the environmental MPs concentration:

$$467 \quad C_{env} = \frac{1}{b_4} * \left( C - b_1 * W - b_2 * \exp\left(\frac{1}{T}\right) - b_3 * \frac{1}{[CHL-a]} \right) \quad (\text{Eq. 20})$$

468 which could be a very useful tool to predict the MPs concentration in the environment, when all  
 469 involved variables are known ( mussel's accumulated MPs ( $C$ ), wet weight ( $W$ ) temperature ( $T$ ) and  
 470 CHL-a), using the mussel as a potential bioindicator (Li et al., 2016, Li et al., 2019). The score of

471 this custom model was tested by applying Eq. 20 in our study areas and 6 more areas around the  
472 U.K., where information of mussel's wet weight and both mussels' and environment's MPs load is  
473 available (Li et al., 2018). CHL-a and temperature, which were not included in Li et al. (2018),  
474 were obtained from daily satellite images (same source as in the North Sea, see 2.4 section),  
475 covering the period that the mussels were harvested (Li et al., 2018).

476

## 477 **3. Results**

478

### 479 **3.1 Growth simulations**

480

481 The growth simulations of *M. edulis* and *M. galloprovincialis* for the North Sea and the N.  
482 Ionian Sea are shown in Fig. 3 and Fig. 4 respectively. In the North Sea implementation,  $X_k$  was  
483 tuned to a constant value:  $X_k=8 \text{ mg m}^{-3} (\pm 1.5 \text{ mg m}^{-3})$ . The fitted value was higher, as compared to  
484 the one ( $X_k=3.88 \text{ mg m}^{-3}$ ) used by Casas and Bacher (2006) in productive areas of the French  
485 Mediterranean shoreline (average CHL-a concentration  $1.45 \text{ mg m}^{-3}$  maximum peak at  $20 \text{ mg m}^{-3}$ ),  
486 as a consequence of the higher productivity in the North Sea (average CHL-a concentration  $4.25 \text{ mg}$   
487  $\text{m}^{-3}$ ; maximum peak at  $\sim 33.40 \text{ mg m}^{-3}$ ). The high value of  $X_k$  could also be explained by the  
488 presence of inedible particles (i.e. MPs) which led to lower quality food in the mussel's diet  
489 compared with an assumed clean of inedible particles environment (Kooijman, 2006, Ren, 2009). In  
490 the present study the inedible particles (i.e. MPs) have been incorporated in the mussel's diet  
491 through the modified relation of the functional response  $f$  (Eq. 5, Table 1), which regulates the  
492 assimilation rate and thus the mussel's growth. However, the DEB model applied at the French site,  
493 did not account inedible particles in the mussel's food. Furthermore, it has been reported that wild  
494 mussels grow considerably slower than farmed mussels ( $\sim 1.7$  times) (Sukhotin and Kulakowski,  
495 1992) and thus, a higher value of  $X_k$  promotes a lower mussel growth, which is the case of the North  
496 Sea mussel. The simulated mussel shell length after 4 years, in August, is 4.35 cm and the fresh  
497 tissue mass is 1.87 gr, in agreement with Van Cauwenberghe et al. (2015) and other studies  
498 conducted on wild mussels (Sukhotin et al., 2007, Saraiva et al., 2012, MarLIN, 2016). In  
499 particular, Saraiva et al. (2012) found that after 16 years of simulation, the wild mussel of the  
500 Wadden Sea (North Sea) is 7 cm long, while according to Bayne and Worrall (1980) a mussel with  
501 shell length 4 cm corresponds to the age of 4 years, in agreement with the current study. The  
502 simulated growth presents a strong seasonal pattern, being higher during spring and summer season,  
503 as compared to autumn and winter, which is consistent with the seasonal cycle of temperature and

504 CHL-a concentration, for a typical year in the region (Fig. 1). The increase of food availability and  
505 temperature during spring (April) results in high mussel growth for a 4-month period, while the  
506 decrease of CHL-a from summer until the end of the year, in conjunction with the temperature  
507 decrease in autumn, result in a lower mussel growth. Spawning events that occurred each year in  
508 late April-early May (30 April-2 May) each year, are responsible for the sharp decline in mussel's  
509 fresh tissue mass, shown in Fig. 4 (Handa et al., 2011; Zaldivar, 2008) and in agreement with the  
510 literature (Sprung, 1983, Cardoso et al., 2007, Saraiva et al., 2012). The predicted weight loss due to  
511 spawning was around 7% at the first year of simulation, while the second, third and fourth year the  
512 percentage of weight loss increased gradually to 8.3%, 12.6% and 14.4% respectively. Bayne and  
513 Worrall (1980) demonstrated that the weight losses on spawning for individuals of 1 g weight vary  
514 between 2.1% and 39.8%, presenting a weight-specific increase with size.

515 In the N. Ionian Sea implementation,  $X_k$  is applied as a function of CHL-a concentration  
516 through the method described in section 2.5. The target diagram showing the performance of each  
517 tested function (linear:  $f(x) = a * [CHL - a] + b$ , where  $a = 0.959$  and  $b = -1.420$ ; exponential:  
518  $f(x) = a * \exp(b * [CHL - a])$  where  $a = 0.2$  and  $b = 0.567$ ; power:  $f(x) = a * [CHL - a]^b +$   
519  $c$  where  $a = 0.01$ ,  $b = 3.529$  and  $c = 0.480$ ) is shown in Fig. 5. The linear and power function of  
520  $X_k$  present a good skill, with the power function leading to the most successful simulation of the  
521 cultured mussel's growth in all four areas (diagram marks for mussel length and fresh tissue mass  
522 are closer to the target's center). The power function applied in the N. Ionian Sea, resulted in  
523 mussel's shell length 5.8 cm and fresh tissue mass 5.92 gr after one year simulation, in agreement  
524 with Theodorou et al. (2011). The spawning event occurred at the beginning of December  
525 (Theodorou et al., 2011) and was illustrated by a 12.6% tissue mass decline.

526

### 527 **3.2 Microplastics accumulation and depuration phase**

528

529 The hourly simulated MPs accumulation by the mussel in the North Sea and N. Ionian Sea are  
530 shown in Fig. 6 and Fig. 7 respectively. Calibration of the parameter  $k_f$  ( $1.2 \text{ d}^{-1}$ ) led to a model  
531 which was well fitted to the observed MPs accumulation in the mussel of both study areas. In the  
532 North Sea, a 4-year-old wild mussel ( $L=4.35 \text{ cm}$ ,  $W=1.87 \text{ g}$ ) contains  $0.53 \text{ particles individual}^{-1}$  in  
533 August within the range value found by Van Cauwenberghe et al. (2015) ( $0.4 \pm 0.3 \text{ particles}$   
534  $\text{individual}^{-1}$ ), although the model overestimated the data range reproducing a seasonal increase that  
535 was not observed. This is most likely due to the fact that Van Cauwenberghe et al. (2015) allowed a  
536 24 h clearance period, before analyzing the mussels' tissue for MPs, resulting in slightly lower MPs  
537 accumulation than the model's prediction. The MPs egested through faeces by the 4 year old mussel



538 after 24 h were  $0.2 \pm 0.2$  particles individual<sup>-1</sup> (Van Cauwenberghe et al., 2015), which agree also  
539 with model's output ( $0.3$  particles individual<sup>-1</sup>, Fig. 8) regarding the depuration phase and could  
540 compensate for the observed difference in mussel's MPs load between the simulated and field data.  
541 In the N. Ionian Sea, the simulated MPs accumulation by the cultured mussel with  $L = 4.85$  cm and  
542  $W = 3.33$  g was  $0.91$  particles individual<sup>-1</sup> in the end of June, in agreement with field observations  
543 obtained from Digka et al. (2018a) ( $0.9 \pm 0.2$  particles individual<sup>-1</sup>). Overall, the developed model  
544 simulated the MPs accumulation by both mussels in the two different areas, using the same  
545 parameter set (see Table 3 for the exceptions), under the assumption that parameters referred to silt  
546 particles (i.e. inedible particles) may be used to describe also the MPs accumulation. Both  
547 simulations were in good agreement with the available field data, with a small deviation for the  
548 North Sea. This may lead to the assumption that mussels present a common behavior against all  
549 inedible particles. In model's results, based on the uptake and excretion rates of MPs by the mussels  
550 in both study areas, the majority of MPs are rejected through pseudofaeces and fewer through  
551 faeces production (not shown). This is in agreement with Woods et al. (2018) who found that most  
552 microplastic fibers (71%) were quickly rejected as pseudofaeces and  $< 1\%$  excreted in faeces.

553 The small-scale (daily) fluctuations of MPs in the mussel (wild and cultivated) reflect the  
554 adopted random variability of the environmental MPs concentration  $C_{env}$  and the daily fluctuations  
555 of the environmental forcing (CHL-a, temperature). The large-scale (seasonal) variability follows  
556 mainly the variability of the clearance rate. The seasonal variability of the CHL-a concentration and  
557 temperature greatly determines the variability of the clearance rate and hence the variability of MPs  
558 in the individual. Moreover, the model predicts that mussel's energy needs are increased as it grows  
559 and therefore the clearance rate is increased, resulting in higher MPs accumulation.

560 The simulated time needed to clean the mussel's gut from the MPs load for both areas is  
561 shown in Fig. 8. In both areas, the cleaning follows an exponential decay, in agreement with  
562 laboratory experiments by Woods et al. (2018). In particular, the model predicts a 90% mussel's  
563 cleaning after 284 hours (~12 days) and 56 hours (~2.5 days) for the N. Ionian Sea and North Sea  
564 respectively. The cleaning process is more rapid in the North Sea simulation, which can be  
565 attributed to the higher CHL-a concentration found in this area, leading to increased production of  
566 faeces by the mussel and hence faster excretion of the accumulated MPs. In the N. Ionian Sea, on  
567 the other hand, the rate of the mussel's cleaning is slower, due to the limited food availability.

568

### 569 **3.3 Model's uncertainty regarding the environmental microplastics concentration**

570

571 The MPs concentration in the environment presents a strong variability in both temporal and  
572 spatial scales. To examine the model's uncertainty related to the environmental MPs concentration  
573 ( $C_{env}$ ), a series of 15 and 13 simulations were performed in the North Sea and N. Ionian Sea  
574 respectively, adopting different values of  $C_{env}$  within the observed range of each area. In the North  
575 Sea, the adopted  $C_{env}$  ranged between 0.1 and 0.8 particles  $L^{-1}$  with a step of 0.05 (15 runs), while in  
576 the N. Ionian Sea  $C_{env}$  ranged between 0.0012 and 0.0252 particles  $L^{-1}$  with a step of 0.002 (13  
577 runs). The mean seasonal values and standard deviation of the 15 simulations in the North Sea and  
578 the mean monthly values and standard deviation of the 13 simulations in the N. Ionian Sea were  
579 computed and plotted in Fig. 9 and Fig. 10, respectively. Each error bar represents the uncertainty  
580 of the simulated accumulation at the specific time, related to the environmental MPs concentration.

581 In both case studies, the uncertainty of the model appears to increase as the MPs accumulation  
582 is increased. As the mussel grows in the North Sea, the mean value and standard deviation of MPs  
583 accumulation is increased during the same season every year, illustrating the effect of the mussel's  
584 weight. Moreover, the seasonal variability of the MPs accumulation appears to be related with the  
585 seasonality of CHL-a concentration. This is apparent during each year's spring: when CHL-a  
586 concentration peaks at its maximum value ( $\sim 30 \text{ mg m}^{-3}$ ; see Fig. 1), the filtration rate is decreased  
587 (Riisgard et al., 2003, 2011), leading to lower MPs accumulation by the mussel and thus lower  
588 model's uncertainty. In the N. Ionian Sea, the effect of the mussel's weight is more apparent in the  
589 early months ( $\sim 6$  months), resulting on higher MPs accumulation and model uncertainty as the  
590 mussel grows. Afterwards, the seasonality of both CHL-a concentration and temperature plays the  
591 major role. During summer, when the CHL-a concentration is progressively decreased, reaching  
592 minimum values ( $\sim 0.7 \text{ mg m}^{-3}$ ) and temperature is increased ( $>20^\circ \text{ C}$ ), the filtration rate is  
593 significantly decreased or stopped, resulting in lower MPs accumulation and lower model's  
594 uncertainty. This is in line with studies reporting that the mussel suspends the filtering activity and  
595 thus closes its valves until better conditions occur (Pascoe et al., 2009, Riisgard et al., 2011).  
596 Overall, the available field data lie within the model's uncertainty, apart from the North Sea case,  
597 where the range of field data variability and model uncertainty do not overlap significantly at the  
598 time of the observations.

599 Moreover, to evaluate the scenario adopted with the set-up of the previous experiments  
600 (random  $C_{env}$  at a daily time step) 3 additional model runs are performed in each study area,  
601 adopting each time different stochastic sequences of daily random  $C_{env}$  values within the observed  
602 range, which is considered to reflect the high spatial and temporal variability of the environmental  
603 MPs concentration. The mean value and standard deviation of these "stochastic" runs lie most of the  
604 time within the standard deviation of the overall model's uncertainty in both case study areas (Fig. 9  
605 and Fig. 10).

### 607 3.4 Sensitivity and Regression analysis results

608

609 The results of the sensitivity experiments regarding the MPs accumulation by the mussels are  
610 shown in Fig. 11 and 12 for the North Sea and N. Ionian Sea respectively. The comparison between  
611 the intertidal and subtidal mussel of the North Sea revealed that both +10% and -10% perturbation  
612 of CHL-a and  $X_k$  have a slightly lower effect on the MPs accumulation by the intertidal mussel  
613 which is probably attributed to the intermittent feeding periods experienced by the individual due to  
614 the tide effect. As far as the temperature effect, both +10% and -10% perturbed value led to higher  
615 sensitivity on the MPs accumulation by the intertidal mussel, due to the adopted modified  
616 temperature relation during low tide. Especially, if the mussel's body temperature change during air  
617 exposure would be considered, the perturbed temperature will probably affect even more the MPs  
618 accumulation on the intertidal than the subtidal mussel. The sensitivity of the  $C_{env}$  on the MPs  
619 accumulation when perturbed either +10% or -10% is almost the same for the intertidal and  
620 subtidal mussel, indicating that the environmental MPs concentration affects similarly both mussels,  
621 regardless the continuous or intermittent feeding-excretion process.

622 The comparison between the mussel sensitivity indexes in the N. Ionian and the North Sea  
623 (in conditions of submergence) study areas reveals some important differences. Generally, most of  
624 the perturbed (either +10% or -10%) variables and parameters (i.e. CHL-a, temperature,  $X_k$ ) present  
625 higher sensitivity on the MPs accumulation by the mussel from the N. Ionian Sea. This is attributed  
626 to the prevailing environmental conditions and specifically the lower food availability (CHL-a) and  
627 the higher temperature range in the N. Ionian Sea compared to the North Sea, which greatly  
628 determine the feeding processes, the mussel's growth and hence the MPs accumulation. The  
629 perturbed  $C_{env}$  in both study areas appears to affect similarly the MPs accumulation on both mussels  
630 (~10%), with the small difference (<2%) probably attributed to the higher abundance of seawater's  
631 MPs present in the North Sea compared to the N. Ionian Sea. Finally, the half saturation coefficient  
632 for the inorganic particles ( $Y_k$ ) has no effect on the MPs accumulation of both North Sea and N.  
633 Ionian Sea mussels, indicating that the amount of inedible particles (i.e. MPs) is relatively low in  
634 both areas and thus the  $Y_k$  does not affect the way that the organic particles are being ingested  
635 (Kooijman, 2006). According to Ren (2009), when the inorganic matter is low, the  $K(y)$  (Eq. 5;  
636 Table 1) is approximately equal to  $X_k$  and then  $Y_k$  is the least sensitive parameter for the ingestion  
637 rate and thus growth.

638 The DEB-accumulation model output was used to determine the coefficients in Eq. 19 by the  
639 nonlinear regression analysis:  $b_1 = 0.1909 (\pm 0.0006)$ ,  $b_2 = 0.0412 (\pm 0.0019)$ ,  $b_3 = 0.1315 (\pm$

640 0.0021) and  $b_4=1.1060 (\pm 0.0253)$ . The accurate estimation of the coefficients ( $b_1, b_2, b_3, b_4$ ) is  
641 indicated by the low confidence intervals, while the mean squared error of the regression model  
642 appears also sufficiently small (MSE=0.0523). Subsequently, as shown in Figure 13, Eq. 20 may be  
643 used to predict the MPs concentration of the environment where mussels live. In most cases, the  
644 predicted MPs concentration is found within the standard deviation of the field data. Two  
645 exceptions are shown in Hastings-A and Plymouth areas. The reasons behind these discrepancies  
646 may be related to the environmental conditions prevailing in each area at the sampling time. For  
647 example, Eq. 20 does not take into account the impact of tides that may affected the mussel's MPs  
648 load ( $C$ ) and the lack of information on the exact sampling date led to using a mean SST and CHL-a  
649 value representative of the given sampling time period (Li et al., 2018). Although, Eq. 20 does not  
650 account for the tide effect, however, the sensitivity analysis (Fig. 11) showed that the effect of  $C_{env}$   
651 on the mussel's MPs accumulation was the same for both intertidal and subtidal mussel in the North  
652 Sea. This result may also apply at the two exceptions areas, leading to the assumption that the  
653 discrepancies are due to the lack of the ambient temperature and CHL-a information during the  
654 sampling date. In any case, this is a first rough demonstration of the method and should be  
655 implemented in more environments in order to be further validated.

656  
657

## 658 4. Discussion

659

660 A DEB-accumulation model was developed and validated with data available from the  
661 North Sea and the N. Ionian Sea, to study the MPs accumulation by wild *M. edulis* and cultured *M.*  
662 *galloprovincialis*, grown in different, representative environments. Although the study is limited by  
663 scarce validation data, it should be noted the MPs accumulation model parameter set, except one  
664 tuning parameter ( $k_f$ ), was extracted from the literature (Table 3), assuming that mussels adopt a  
665 common defensive mechanism against inedible particles (i.e. silt, MPs). Thus, the theoretical  
666 background constructed by Saraiva et al., (2011a) (based on Kooijman, 2010) regarding the feeding  
667 and excretion processes of the mussel remains unspoiled. Through the strong theoretical  
668 background of DEB theory, this study highlights that the accumulation of MPs by the mussel is  
669 highly depended on the prevailing environmental conditions which control the amount of MPs that  
670 the mussel filtrates and excretes.

671 Towards a generic DEB model, the applied function of the half saturation coefficient  
672 ( $f(x) = a * [CHL - a]^b + c$ ) successfully captures the physiological responses and thus the growth  
673 rate of the cultured mussel at the N. Ionian Sea implementation. In the current study, this method

674 led to a robust and generic DEB growth model able to simulate the mussel growth in representative  
675 mussel habitats of the Mediterranean Sea, covering a range of productivity and sea surface  
676 temperature. This approach supports and takes a step further of Bourles et al. (2008) suggestion  
677 about a seasonally varied half saturation coefficient, demonstrating an improvement of the food  
678 quantifier. The applied function of  $X_k$  considers the daily CHL-a fluctuations, and thus the seasonal  
679 variation of the seawater composition. As more field data becomes available from various  
680 environments, the applied approach could result to more generic formulations for the site-specific  
681 parameter  $X_k$ , so that the model could be applied in several areas of interest, where field growth data  
682 are absent and/or to simulate the potential mussel growth in the 2D space.

683 The simulation of MPs accumulation by the mussels, using the DEB-accumulation model, is  
684 in good agreement with the available field data (Fig. 3 and Fig. 4). The simulated values lie within  
685 the observed field data range (mean  $\pm$ SD), although the seasonal increase reproduced by the model  
686 at the North Sea implementation did not exactly overlap with the field data at the time of  
687 observations. This could be attributed to the clearance period (24 h) that allowed mussels to excrete  
688 MPs through faeces ( $0.2 \pm 0.2$  particles individual<sup>-1</sup>) before the mussel's tissue analysis (Van  
689 Cauwenberghe et al., 2015). The measured loss of mussel's MPs is in agreement with the model's  
690 result on the depuration experiment after 24 h. The MPs accumulation by the cultivated mussel  
691 (fresh tissue mass 3.33 g) originated from the N. Ionian Sea with mean  $C_{env} = 0.0012 \pm 0.024$   
692 particles L<sup>-1</sup>, is 0.91 particles individual<sup>-1</sup> and by the wild mussel (fresh tissue mass 1.87 g) from the  
693 North Sea with mean  $C_{env} = 0.4 \pm 0.3$  particles L<sup>-1</sup> is 0.53 particles individual<sup>-1</sup>. If these  
694 concentrations are expressed per gram of wet tissue of mussels, the cultivated mussel contamination  
695 ( $0.27$  particles g<sup>-1</sup>w.w.) is comparable with the wild mussel ( $0.28$  particles g<sup>-1</sup>w.w.), despite the  
696 much lower environmental MPs concentration ( $C_{env}$ ) in the N. Ionian Sea than the North Sea. This  
697 comparison aims to highlight the significant impact of the prevailing environmental conditions  
698 (CHL-a and temperature) on the MPs accumulation by the mussels, although they originate from  
699 different areas and lived different time period. The generally high abundance of CHL-a in the North  
700 Sea simulation, contributes to a reduction of the filtering activity and hence of the MPs  
701 accumulation. The threshold algal concentration for reduction of the mussel's filtration rate  
702 (incipient saturation) has been found to lie between 6.3 and 10.0 mg m<sup>-3</sup> (Riisgard et al., 2011),  
703 which is a range comparable to the CHL-a concentrations in the North Sea. Van Cauwenberghe and  
704 Janssen (2014) found that cultivated *M. edulis* from the North Sea contained on average  $0.36 \pm 0.07$   
705 particles g<sup>-1</sup>w.w., a slightly higher value with that found in the present study for the wild mussel of  
706 the North Sea ( $0.28$  particles g<sup>-1</sup>w.w.). This could be attributed to mussel farms acting as a potential  
707 source of MPs contamination for the mussels due to plastic materials (i.e. plastic sock nets and  
708 polypropylene long lines) used during cultivation (Mathalon and Hill, 2014, Santana et al., 2018).

709 Moreover, the intertidal wild mussel (present study) is assumed to filter and excrete MPs half of the  
710 time in comparison with the submerged cultured mussel in the North Sea, resulting though in  
711 similar accumulation level. The model also predicts the time needed for the 90% gut clearance of  
712 both cultured (N. Ionian Sea) and wild (North Sea) mussel to be almost 284 hours and 56 hours  
713 (equivalent to 12 and 2.5 days) respectively, when MPs contamination is removed from their  
714 habitat. This is in line with a series of studies which demonstrated that the depuration time varies  
715 between 6-72 hours and can last up to 40 days depending on several factors such as species,  
716 environmental conditions (Bayne et al., 1987), size and type of MPs (Browne et al., 2008, Ward and  
717 Kach, 2009, Woods et al., 2018, Birnstiel et al., 2019).

718 The strong dependence of food (CHL-a), temperature and seawater's MPs concentration on  
719 the MPs accumulation by the mussel, regarding its wet weight, is demonstrated through sensitivity  
720 experiments that were used to derive a rather simple nonlinear regression model (Eq. 19). The  
721 comparison of the regression model's with the DEB model's output resulted in a quite accurate  
722 estimation of the coefficients, which in turn sparked the idea of a 'new' relationship (Eq. 20) that  
723 could potentially predict the MPs concentration in the environment ( $C_{env}$ ) when certain conditions  
724 are known (CHL-a, T, C, W). The latter equation was applied in 8 areas in total (2 from the present  
725 study areas and 6 from Li et al. (2018)), with relatively good results since there is general  
726 overlapping of regressed and observed MPs concentration in the environment ( $C_{env}$ ), except for  
727 Hastings-A and Plymouth areas, probably due to missing information on the environmental  
728 conditions (CHL-a, SST) during the sampling, suggesting that the mussels can be used as potential  
729 bioindicators. Mussels have been previously proposed as bioindicators for marine microplastic  
730 pollution (<1 mm), although the efficient gut clearance and selective feeding behavior limit their  
731 quantitative ability (Lusher et al., 2017, Brate et al., 2018, Beyer et al., 2017, Fossi et al., 2018, Li  
732 et al., 2019). The recent study by Ward et al. (2019b) demonstrated that bivalves are poor  
733 bioindicators of MPs pollution due to the particle selection during feeding and excretion processes  
734 that is based on the physical characteristics of the MPs. Considering that the MPs accumulation is  
735 site-dependent and that sampling of mussels is usually easier than seawater (Karlsson et al., 2017,  
736 Brate et al., 2018), models like the one described in Eq. 20, besides the MPs accumulation, take into  
737 account also characteristics of the environment that are crucial for the way that mussels accumulate  
738 MPs. This method could be possibly used at global level and allow comparisons between various  
739 environments. However, the method described should be validated in more environments with more  
740 frequent field data to be able to provide secure results.

741 In addition to the scarce validation data regarding the MPs accumulation in mussels, this  
742 study has some more limitations. First of all, the data regarding the concentration of MPs in the  
743 mussels' environment are also scarce; since MPs is a relatively recent subject of study, the existing

744 knowledge of the spatial and temporal distribution is still quite limited (Law and Thompson, 2014,  
745 Browne, 2015, Anderson et al., 2016, de Sa et al., 2018, Smith et al., 2018, Troost et al., 2018). To  
746 overcome the lack of environmental MPs time series, a function of randomly generated values  
747 within the observed range of each area was applied and its uncertainty was examined through an  
748 ensemble forecasting. Specifically, the model's uncertainty due to the environmental MPs  
749 concentration ( $C_{env}$ ) was tested by performing a series of model runs forced by an envelope of  
750 representative values of  $C_{env}$ . The results (section 3.3) showed that the adopted stochastic scenario  
751 simulated quite satisfactorily the MPs accumulation by the mussels, lying within the observed field  
752 range, although a slight overestimation was found in the North Sea. The approach used is assumed  
753 to represent the natural variability since it has been reported that tides, wind, wave action, ocean  
754 currents, river inputs and hydrodynamic features lead to high spatial and temporal variability of  
755 MPs distribution even in very small scales (Messinetti et al., 2018, Goldstein et al., 2013). In  
756 addition, the nature of the variable  $C_{env}$  makes it difficult to estimate, presenting large observational  
757 errors, not only due to the intense physical variation but also due to different sampling and analysis  
758 techniques that were used. In a future work the DEB-accumulation model could be coupled with a  
759 high-resolution MPs distribution model (Kalaroni et al., 2019), being extensively validated against  
760 field data that will have been collected and processed according to a common scientifically defined  
761 protocol, to overcome this limitation. Moreover, the approach followed in calculating the value of  
762 MPs concentration in the near surface layer (0-5m depth) (Kooi et al., 2016), resulted in a  
763 representative value of the upper ocean layer. In depth knowledge of the MPs distribution, both  
764 horizontally and vertically, is essential to understand and mitigate their impact not only on the  
765 various marine compartments but also on the organisms inhabiting those compartments (Van  
766 Sebille et al., 2015, Kooi et al., 2016). For that reason, it is important to enhance the monitoring  
767 activity especially in the vulnerable coastal environments, adopting integrated cross-disciplinary  
768 approaches and monitoring of biological, physical and chemical parameters which provide  
769 information on the ecosystem function, in order to improve the assessment of emerging pollutants  
770 (i.e. MPs) and their impacts on biota (objective of JERICO-RI framework).

771 Our assumption that the mussel has the same filtration rate for all particles independently of  
772 their chemical composition, size and shape, is a simplification and an open theme of discussion (see  
773 Saraiva et al., 2011a for details). However, in our model application, a pre-ingestive particle  
774 selection by the mussel is implied based on the organic-inorganic content of the suspended matter  
775 illustrating the different binding probabilities applied for algal and MPs particles during the  
776 ingestion process. Through an investigation of wild mussel's faeces and pseudofaeces production in  
777 laboratory conditions, Zhao et al. (2018) found that the length of MPs was significantly longer in  
778 pseudofaeces than in the digestive gland and faeces. Furthermore, Van Cauwenberghe et al. (2015)

779 demonstrated that mussel's faeces contained larger MPs (15–500  $\mu\text{m}$ ) compared to the mussel's  
780 tissue (20–90  $\mu\text{m}$ ). Apparently, smaller sized MPs seem to be dominant within the mussels in  
781 comparison with the size of the MPs in the ambient environment (Li et al., 2018, Qu et al., 2018,  
782 Digka et al., 2018b), implying that the mussel is more prone to ingest and retain smaller sized MPs.  
783 As an example, Digka et al. (2018b) confirmed that the smaller MPs (<1 mm) occupy the 62.3%,  
784 96.9% and 100% of the total MPs in seawater, sediments and mussels from the N. Ionian Sea  
785 respectively. In a future work this selection pattern regarding size, could be simulated by suitable  
786 preference weights among different MPs sizes. This will improve the knowledge of the feeding and  
787 excretion mechanisms used by the mussels against MPs pollution and the assessment of the  
788 ecological footprint (Rist et al., 2019).

789 Our assumption that the contamination by MPs does not affect the energy budget in terms  
790 of growth might also be a simplification as this is a subject currently under investigation. Van  
791 Cauwenberghe et al. (2015) found that although mussels *M. edulis* exposed to MPs increased their  
792 energy consumption, the energy reserves was not affected compared to the control organisms,  
793 implying that mussels are able to adopt a defensive mechanism against the suspended inorganic  
794 particles (i.e. MPs) (Ward and Shumway, 2004). Furthermore, MPs exposure showed no significant  
795 effect on mussel's (*Perna perna*) energy budget, despite its long duration and relatively realistic  
796 intensity, leading to the hypothesis that mussels can acclimate to the MPs exposure to maintain their  
797 health (Santana et al., 2018). On the contrary, other authors suggested a significant energy shift  
798 from reproduction to structural growth and elevated maintenance costs, probably attributed to the  
799 reduced energy intake, when the organisms (i.e. oyster *Crassostrea gigas*) were contaminated with  
800 high and unrealistic concentration of MPs (Sussarellu et al., 2016). Moreover, Gardon et al. (2018)  
801 showed that the overall energy balance of oyster *Pinctada margaritifera* was significantly impacted  
802 by the reduced assimilation efficiency in correlation with the exposed dose of MPs and for that  
803 reason energy had to be withdrawn from reproduction to compensate for the energy loss. In future  
804 dedicated experiments exploring the effects on all components of a DEB model should be carried  
805 out considering long-term realistic MPs exposure.

806 Our use of the tide data led to some model bias, since the model does not take into account of  
807 the mussel's body temperature change when this is exposed to air. Assessing the mussel's body  
808 temperature requires extended experiments in field conditions (Tagliarolo and McQuaid, 2015,  
809 Monaco and McQuaid, 2018). The study by Seuront et al. (2019) along the French coast of the  
810 eastern English Channel found no significant correlation between air and mussel's body  
811 temperature, but demonstrated a significant positive correlation between the body temperature and  
812 the hard substrate (i.e. rocks) temperature. However, in the present study the tide effect on  
813 processes that are affected by the thermal equation ( $k(T)$ ) is considered indirectly through the



814 metabolic depression (details in section 2.4). Sara et al. (2011) coupled a DEB model with a  
815 biophysical model (Kearney et al., 2010), incorporating the change of mussel's body temperature  
816 during emersion by using information of various climatological variables (i.e. solar radiation, air  
817 temperature, wind speed, wave height), but ignored the temperature sensitivity on the physiological  
818 processes. In a future study, a combined approach by coupling the present DEB-accumulation  
819 model with a biophysical model, which includes both the tide effect on the physiological processes  
820 and the mussel's body temperature respectively, could be followed and lead to a more detailed  
821 simulation of the intertidal mussel.

## 822 **5. Conclusions**

823  
824 In a future study the model should be corroborated further by using a larger dataset of MPs  
825 accumulation, with sampling of mussels of various sizes and life stages. Currently, the model is  
826 mainly limited by the insufficient validation, as a larger dataset could be also used for a better model  
827 calibration. However, this study provides a new approach in studying the accumulation of MPs by  
828 filter feeders and reveals the relations between characteristics of the mussel's surrounding  
829 environment and the MPs accumulation, which is presented with high seasonal fluctuations.  
830 Additionally, in a future study the DEB-accumulation model will be coupled to a hydrodynamic-  
831 biochemical model (e.g., Petihakis et al., 2002, 2012, Triantafyllou et al., 2003, Tsiaras et al., 2014,  
832 Ciavatta et al., 2019, Kalaroni et al., 2020) and a MPs distribution model (Kalaroni et al., 2019) that  
833 will provide fields of temperature, food availability and MPs concentration respectively at the  
834 Mediterranean scale, and eventually lead to an integrated representation of the MPs accumulation  
835 by mussels (Daewel et al., 2008). This fully coupled model will be downscaled to the Cretan Sea  
836 SuperSite, while the parameterization of important biological processes will be redesigned based on  
837 the new data which will be acquired in the framework of the JERICO S3 project  
838 (<http://www.jerico-ri.eu>). The present study highlights the urgent need for adopting a multi-  
839 disciplinary monitoring activity by measuring physical, biological and chemical parameters that are  
840 crucial for mapping the MPs distribution, assessing the contamination level of the marine organisms  
841 and investigating the impact on the health status. Overall, despite the limitations mentioned, taken  
842 into account that plastics are one of the global hot issues, this particular study could help design  
843 next efforts, since it provides indications on the future priority related issues.

844

845 **Author Contribution:**

846 **Natalia Stamataki, Yannis Hatzonikolakis, Kostas Tsiaras, Catherine Tsangaris, George**  
847 **Petihakis, Sarantis Sofianos, George Triantafyllou**

848

849 G.T. conceived the basic idea of the present study and was responsible for the management and  
850 coordination of the research planning and execution. N.S. and Y.H. developed the model code with  
851 the contribution of K.T.. N.S. collected the existing information on the subject and performed the  
852 simulations of the present study with the help of Y.H. when needed. G.T., G.P., K.T., Y.H. and N.S.  
853 contributed to the interpretation of the results. C.T. provided the field data of the mussel's  
854 microplastic accumulation in the North Ionian Sea. N.S. prepared the manuscript, with critical  
855 review, commentary and revision contributed from all co-authors.

856

#### 857 **Competing interests:**

858 The authors declare that they have no conflict of interest.

859

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861

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1288 Tables & Figures

1289  $\frac{dE}{dt} = \dot{p}_a - \dot{p}_c$  (1)

1290  $\frac{dV}{dt} = \frac{k \cdot \dot{p}_c - [\dot{p}_M] \cdot V}{[E_g]}$  (2)

1291  $\frac{dR}{dt} = (1 - k) \cdot \dot{p}_c - \left[ \frac{1-k}{k} \right] \cdot \min(V, V_p) \cdot [\dot{p}_M]$  (3)

1292  $\dot{p}_a = \{\dot{p}_{Am}\} \cdot f \cdot k(T) \cdot V^{\frac{2}{3}}$  (4)

1293  $f = \frac{X}{X + K_y}$ , where  $K_y = X_K \cdot \left(1 + \frac{Y}{Y_K}\right)$  (5)

1294  $\dot{p}_c = \frac{[E]}{[E_g] + k \cdot [E]} \cdot \left( \frac{[E_g] \cdot \{\dot{p}_{Am}\} \cdot k(T) \cdot V^{\frac{2}{3}}}{[E_m]} + [\dot{p}_M] \cdot V \right)$  (6)

1295  $[E] = \frac{E}{V}$  (7)

1296  $[\dot{p}_M] = k(T) \cdot [\dot{p}_M]_m$  (8)

1297  $k(T) = \frac{\exp\left(\frac{T_A - T_A}{T_I - T}\right)}{1 + \exp\left(\frac{T_{AL} - T_{AL}}{T - T_L}\right) + \exp\left(\frac{T_{AH} - T_{AH}}{T_H - T}\right)}$  (9)

1298  $L = \frac{V^{\frac{1}{3}}}{\delta_m}$  (10)

1299  $W = d \cdot \left( V + \frac{E}{[E_g]} \right) + \frac{R}{\mu_E}$  (11)

1300  $\dot{C}_R = \frac{\{\dot{C}_{Rm}\}}{1 + \sum_i^n \frac{X_i \{\dot{C}_{Rm}\}}{\{\dot{p}_{XiFm}\}}} \cdot k(T) \cdot V^{\frac{2}{3}}$ ,  $i = \begin{cases} 1 \text{ for CHL} - a \\ 2 \text{ for MPs} \end{cases}$  (12)<sup>a</sup>

1301  $\dot{p}_{XiF} = \dot{C}_R \cdot X_i$  (13)<sup>a</sup>

1302  $\dot{p}_{XiU} = \frac{\rho_{Xi} \cdot \dot{p}_{XiF}}{1 + \sum_i^n \frac{\rho_{Xi} \cdot \dot{p}_{XiF}}{\{\dot{p}_{XiUm}\}}}$  (14)<sup>a</sup>

1303  $\dot{J}_{pfi} = \dot{p}_{XiF} - \dot{p}_{XiU}$  (15)<sup>a</sup>

1304  $\dot{J}_f = \dot{p}_{X1U} - \dot{p}_A$  (16)

1305  $GSI = \frac{\frac{R}{\mu_E}}{d \cdot \left( V + \frac{E}{[E_g]} \right) + \frac{R}{\mu_E}}$  (17)

1306 Table 1. Dynamic energy budget model: equations. See Table 2 for model variables, Table 3 for parameters and Table  
1307 4 for initial values

1308 <sup>a</sup> notation refers to feeding equations handling each type of suspended matter separately ( $i=1$  for algae and  $i=2$  for  
1309 microplastics) where units transformation is applied when it is necessary (see Table 3).

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Variable	Description	Units
$V$	Structural volume	$\text{cm}^3$
$E$	Energy reserves	J
$R$	Energy allocated to development and reproduction	J
$C$	Microplastics accumulation	particles individual <sup>-1</sup>
$\dot{p}_a$	Assimilation energy rate	$\text{J d}^{-1}$
$\dot{p}_c$	Utilization energy rate	$\text{J d}^{-1}$
$\dot{C}_R$	Clearance rate	$\text{m}^3 \text{d}^{-1}$
$C_{env}$	Microplastics concentration	particles $\text{L}^{-1}$
$\dot{p}_{XiF}$	Filtration rate	$\text{J d}^{-1}$ or $\text{g d}^{-1}$
$\dot{p}_{XiI}$	Ingestion rate	$\text{J d}^{-1}$ or $\text{g d}^{-1}$
$\dot{J}_{pfi}$	Pseudofaeces production rate	$\text{J d}^{-1}$ or $\text{g d}^{-1}$
$\dot{J}_f$	Faeces production rate	$\text{J d}^{-1}$
$f$	Functional response function	-
$X_i$	Food or MPs density	$\text{mg m}^{-3}$ or $\text{g m}^{-3}$
$[\dot{p}_M]$	Maintenance costs	$\text{J cm}^{-3} \text{d}^{-1}$
$T$	Temperature	K
$k(T)$	Temperature dependence	-
$L$	Shell length	cm
$W$	Fresh tissue mass	g
GSI	Gonado-somatic index	-

1333 *Table 2. Dynamic energy budget model: variables*

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Parameter	Units	Description	Value	Reference
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1338	$\{\dot{p}_{Am}\}$	$\text{J cm}^{-2} \text{d}^{-1}$	Maximum surface area-specific assimilation rate	147.6	Van der Veer et al. (2006)
1339	$\{\dot{C}_{Rm}\}$	$\text{m}^3 \text{cm}^{-2} \text{d}^{-1}$	Maximum surface area-specific clearance rate	0.096	Saraiva et al. (2011a)
1340	$\{\dot{p}_{X_1Fm}\}$	$\text{mg cm}^{-2} \text{d}^{-1}$	Algal maximum surface area-specific filtration rate*	0.1152	Rosland et al. (2009)
1341	$\{\dot{p}_{X_2Fm}\}$	$\text{g cm}^{-2} \text{d}^{-1}$	Silt maximum surface area-specific filtration rate	3.5	Saraiva et al. (2011a)
1342	$\{\dot{p}_{X_1Im}\}$	$\text{mg d}^{-1}$	Algae maximum ingestion rate*	$3.12 \cdot 10^6$	Saraiva et al. (2011b)
1343	$\{\dot{p}_{X_2Im}\}$	$\text{g d}^{-1}$	Silt maximum ingestion rate	0.11	Saraiva et al. (2011b)
1344	$\rho_1$	-	Algae binding probability	0.99	Saraiva et al. (2011a)
1345	$\rho_2$	-	Inorganic material binding probability	0.45	Saraiva et al. (2011a)
1346	$k_f$	$\text{d}^{-1}$	Post-ingestive losses through faeces	Calibrated	-
1347	$X_K$	$\text{mg m}^{-3}$	Half saturation coefficient	Calibrated	-
1348	$T_A$	K	Arrhenius temperature	5800	Van der Veer et al. (2006)
1349	$T_I$	K	Reference temperature	293	Van der Veer et al. (2006)
1350	$T_L$	K	Lower boundary of tolerance rate	275	Van der Veer et al. (2006)
1351	$T_H$	K	Upper boundary of tolerance rate	296	Van der Veer et al. (2006)
1352	$T_{AL}$	K	Rate of decrease of upper boundary	45430	Van der Veer et al. (2006)
1353	$T_{AH}$	K	Rate of decrease of lower boundary	31376	Van der Veer et al. (2006)
1354	$[\dot{p}_M]_m$	$\text{J cm}^{-3} \text{d}^{-1}$	Volume specific maintenance costs	24	Van der Veer et al. (2006)
1355	$[E_G]$	$\text{J cm}^{-3}$	Volume specific growth costs	1900	Van der Veer et al. (2006)
1356	$[E_m]$	$\text{J cm}^{-3}$	Maximum energy density	2190	Van der Veer et al. (2006)
1357	$k$	-	Fraction of utilized energy spent on maintenance/growth	0.7	Van der Veer et al. (2006)
1358	$V_p$	$\text{cm}^3$	Volume at start of reproductive stage	0.06	Van der Veer et al. (2006)
1359	$\text{GSI}_{th}$	-	Gonado-somatic index triggering spawning	0.28	Van der Veer et al. (2006)
1360	$\delta_m$	-	Shape coefficient	0.25	Casas & Bacher (2006)
1361	$d$	$\text{g cm}^{-3}$	Specific density	1.0	Kooijman (2000)
1362	$\mu_E$	$\text{J g}^{-1}$	Energy content of reserves	6750	Casas & Bacher (2006)
1363	$\lambda$	$\text{J mg}^{-1}$	Conversion factor	2387.73	Rosland et al. (2009)

1364 *Table 3. Dynamic energy budget model: parameters*

1365 \*units mol C converted to mg CHL-a by multiplying with the factor  $\frac{12 \cdot 10^3}{50}$  assuming Carbon:CHL-a ratio of 50  
1366 (Hatzonikolakis et al., 2017).

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Area	$X_k$ value ( $\text{mg m}^{-3}$ )	CHL-a range ( $\text{mg m}^{-3}$ )	CHL-a mean ( $\text{mg m}^{-3}$ )	Temperature range( $^{\circ}\text{C}$ )	Length after one year $\pm$ SD (cm)	Reference
<b>Maliakos Gulf</b>	0.72	0.87-5.59	1.80	12.0-26.0	$7.06 \pm 0.46$	Hatzonikolakis et al., 2017
<b>Thermaikos Gulf</b>	0.56	1.04-2.76	1.89	11.5-24.5	$7.0 \pm 0.47$	Hatzonikolakis et al., 2017
<b>Black Sea</b>	Calibrated: 0.96	0.53-16.30	3.07	6.5-25.0	$7.5 \pm 0.1$	Karayucel et al., 2010
<b>Bizerte lagoon</b>	3.829	4.00-7.70	5.20	12.0-28.0	$7.26 \pm 0.46$	Béjaoui-Omri et al., 2014

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Table 4. Half saturation tuned values ( $X_k$ ) and mussel growth data (Length) in different areas of the Mediterranean and Black Seas.

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Northern Ionian Sea		North Sea	
Variable	Value	Variable	Value
Start date	20 Nov 2010	Start date	1 Jul 2007
L	0.85 cm	L	0.15 cm
W	0.1938 g	W	0.0055 g
V	$0.0096 \text{ cm}^3$	V	$5.3 \cdot 10^{-5} \text{ cm}^3$
E	350 J	E	10 J
R	0 J	R	0 J
C	0 particles individual <sup>-1</sup>	C	0 particles individual <sup>-1</sup>

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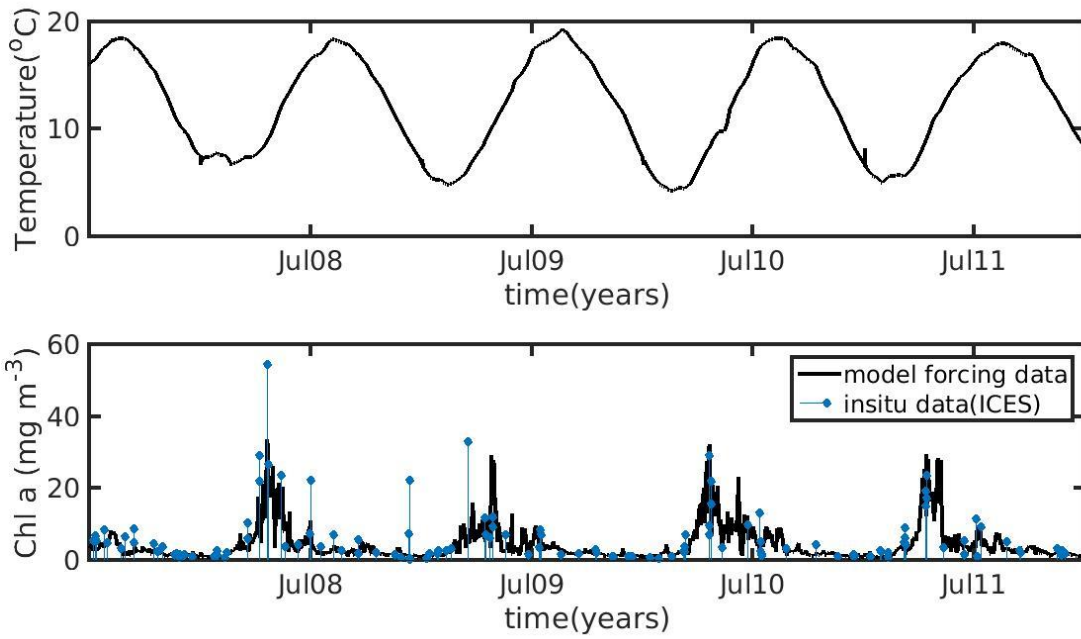
Table 5. Dynamic energy budget-accumulation model: initial values. L: shell length; W: fresh tissue mass; V: structural volume; E: energy reserves; R: energy allocated to reproduction; C: Microplastics accumulation

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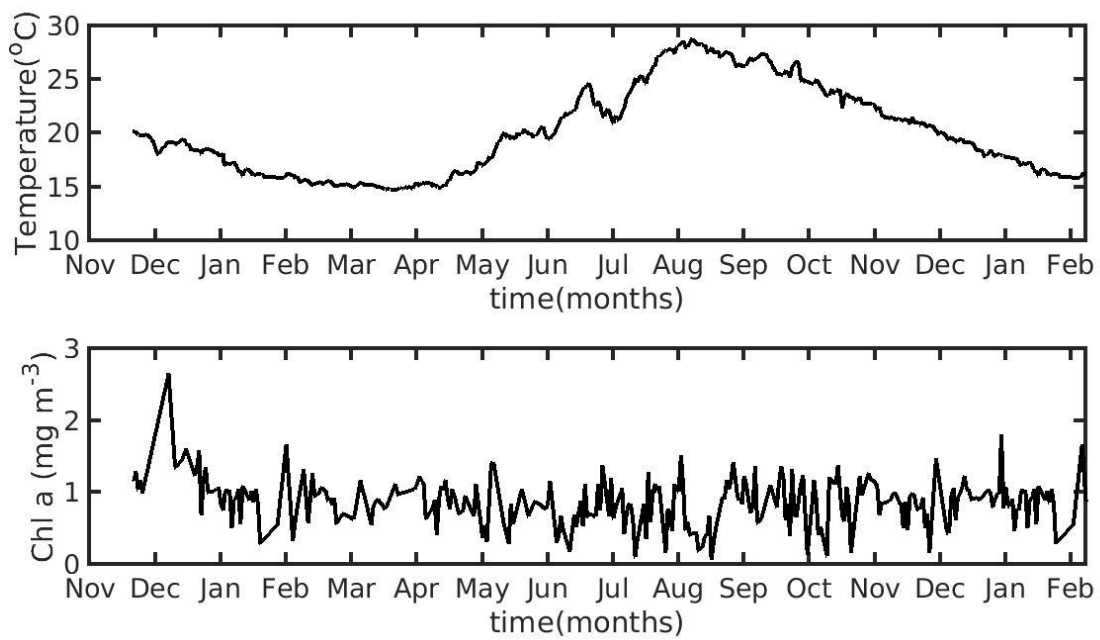
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1392 *Fig. 1. Environmental data used for the forcing of the Dynamic Energy Budget model(DEB) in the North Sea*  
 1393 *simulation, showing temperature (top) and chlorophyll a concentration against in situ data from the ICES database*  
 1394 *(bottom).*

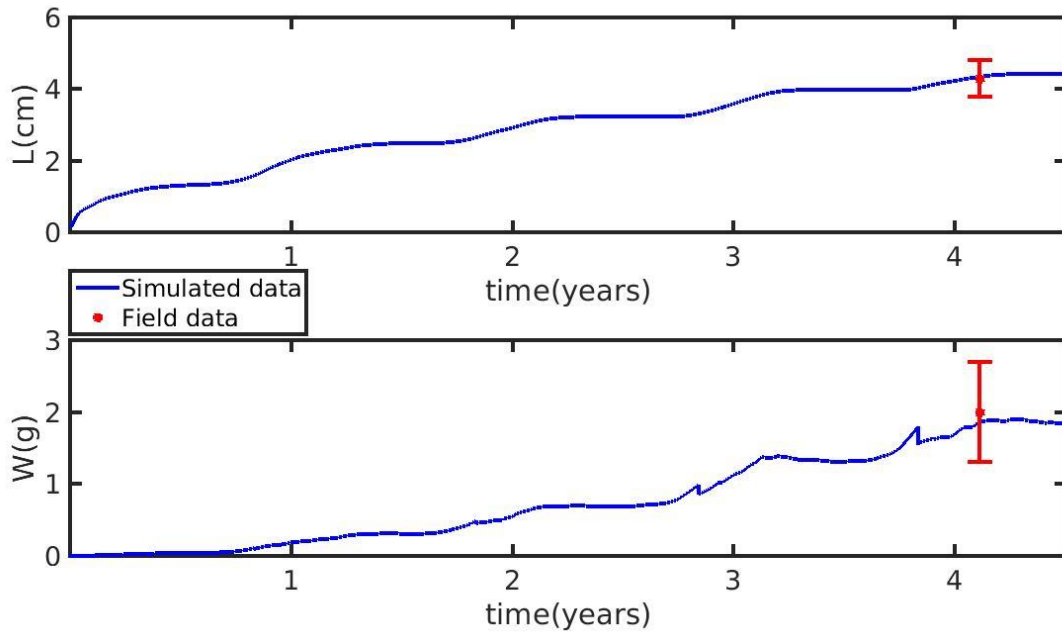
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1397 *Fig. 2. Environmental data used for the forcing of the dynamic energy budget model in the Northern Ionian Sea*  
 1398 *simulation, showing temperature (top) and chlorophyll a concentration (bottom).*

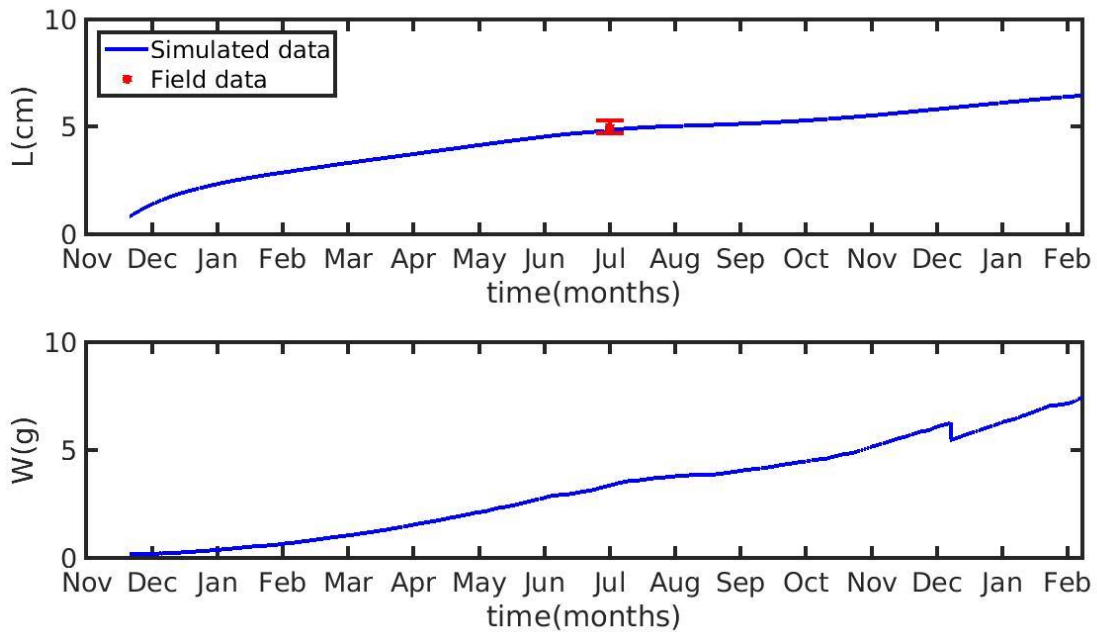
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1400

1401 *Fig. 3. Simulated mussel shell length (L) (top) and fresh tissue mass (W) (bottom) against North Sea data (red star:*  
 1402 *mean ± SD), using chlorophyll a (X =[CHL-a]) in the mussel diet.*

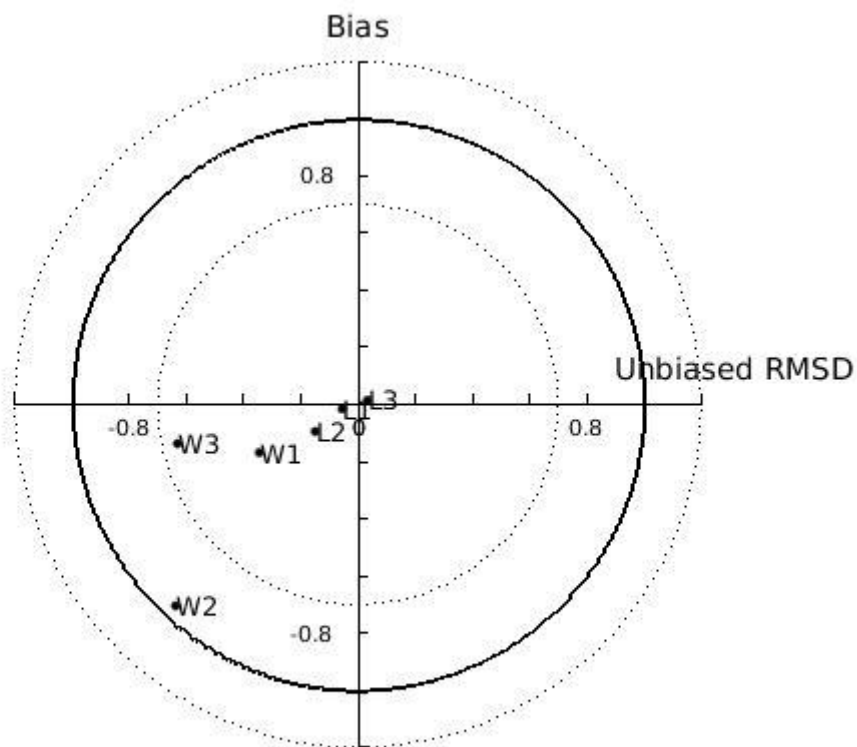
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1404

1405 *Fig. 4. Simulated mussel shell length (L) (top) and fresh tissue mass (W) (bottom) against North Sea data (red star:*  
 1406 *mean ± SD), using chlorophyll a (X =[CHL-a]) in the mussel diet.*

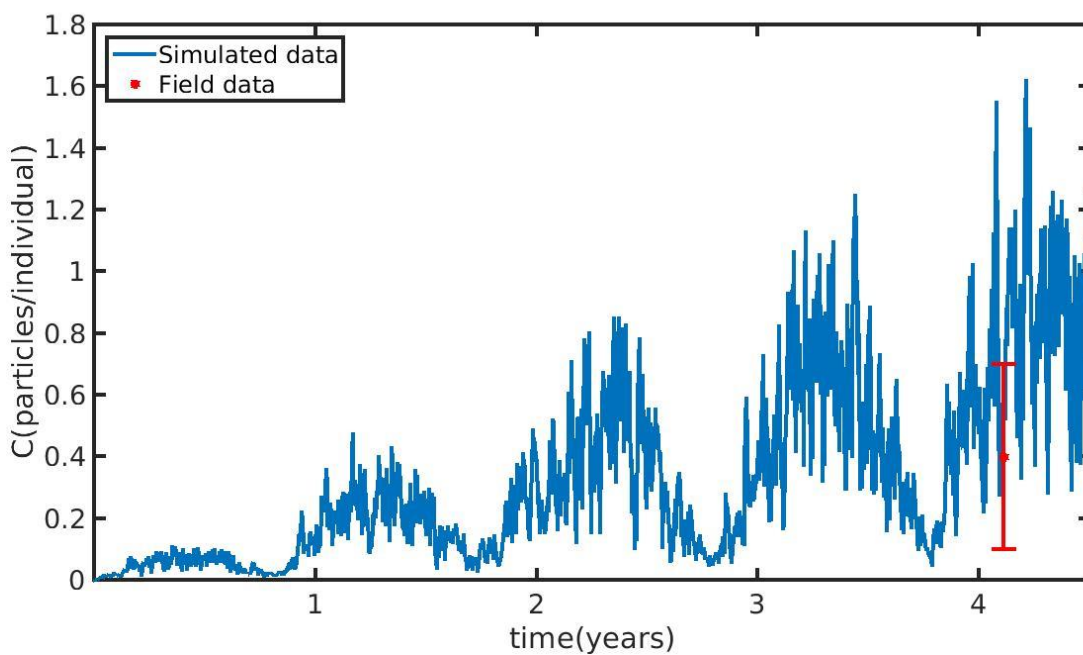




1407

1408 *Fig. 5. Target diagram of simulated shell length (L) and fresh mass tissue weight (W) against field data from*  
 1409 *Thermaikos and Maliakos Gulf (eastern Mediterranean Sea), Black Sea and Bizerte Lagoon (southwestern*  
 1410 *Mediterranean Sea), using the **power** ( $L_1$ ,  $W_1$ ), **exponential** ( $L_2$ ,  $W_2$ ) and **linear** ( $L_3$ ,  $W_3$ ) function of the half saturation*  
 1411 *coefficient. The model bias is indicated on the y-axis while the unbiased root-mean-square-deviation (RMSD) is*  
 1412 *indicated on the x-axis.*

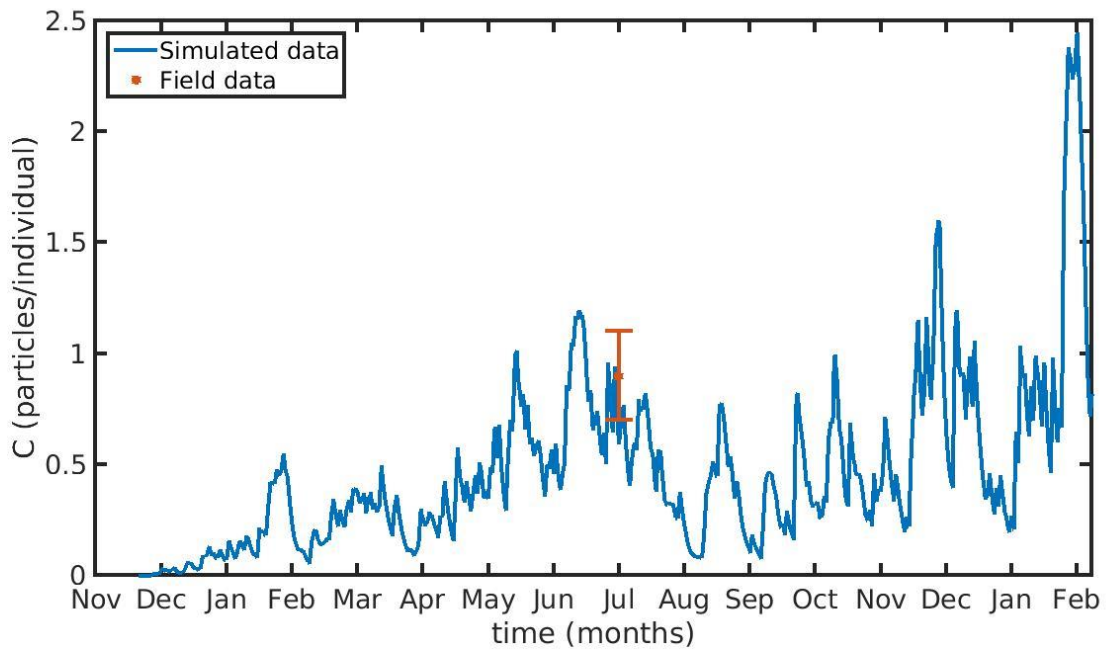
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1415 *Fig.6. Microplastics (MPs) accumulation by the mussel (blue line) against field data (red star: mean  $\pm$  SD), using daily*  
 1416 *environmental concentration of MPs ( $C_{env}$  mean value  $\pm$  SD:  $0.4 \pm 0.3$  particles  $L^{-1}$ ) in the North Sea.*

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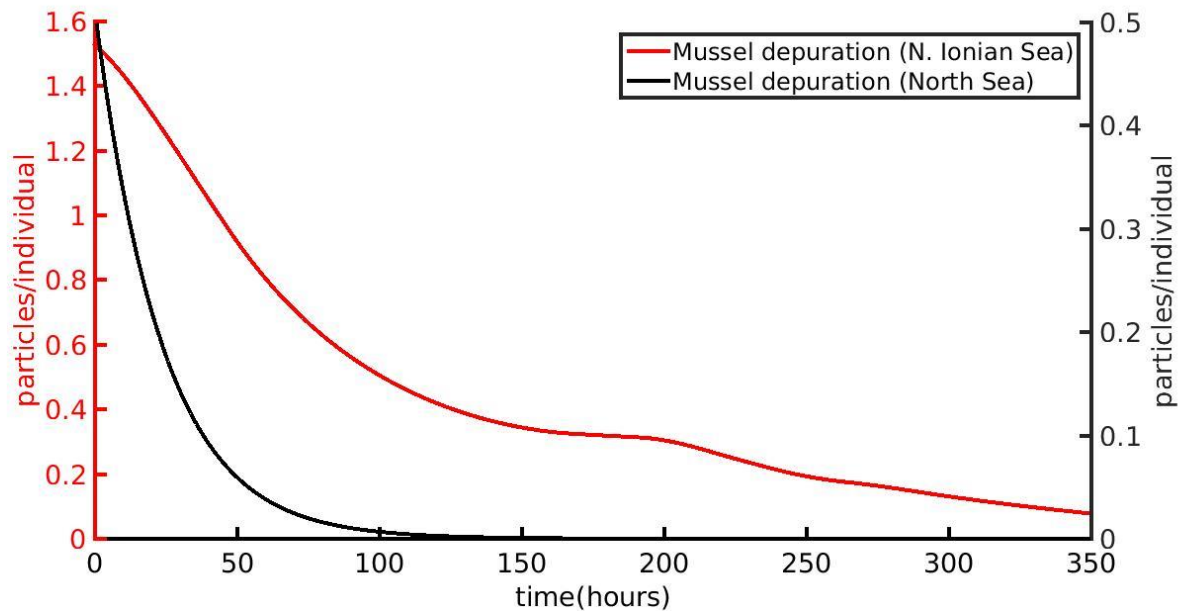


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1419 *Fig. 7. Microplastics (MPs) accumulation by the mussel (blue line) against field data (red star: mean value ± SD),*  
1420 *using daily environmental concentration of MPs ( $C_{env}$  mean value ± SD:  $0.0012 \pm 0.024$  particles  $L^{-1}$ ) in the Northern*  
1421 *Ionian Sea.*

1422

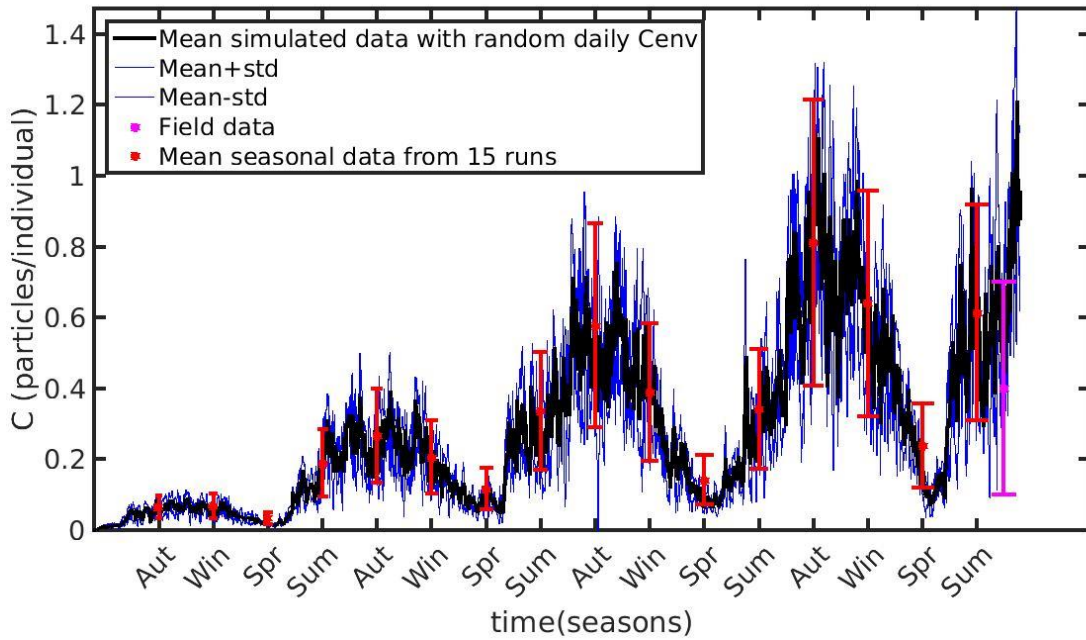
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1424

1425 *Fig. 8. Depuration phase of the cultured *Mytilus galloprovincialis* (red line) and wild *Mytilus edulis* (black line)*  
1426 *using zero environmental concentration of microplastics ( $C_{env}=0$ ) after 1 year and 4 years of simulation time at the Northern*  
1427 *Ionian Sea and North Sea respectively.*

1428

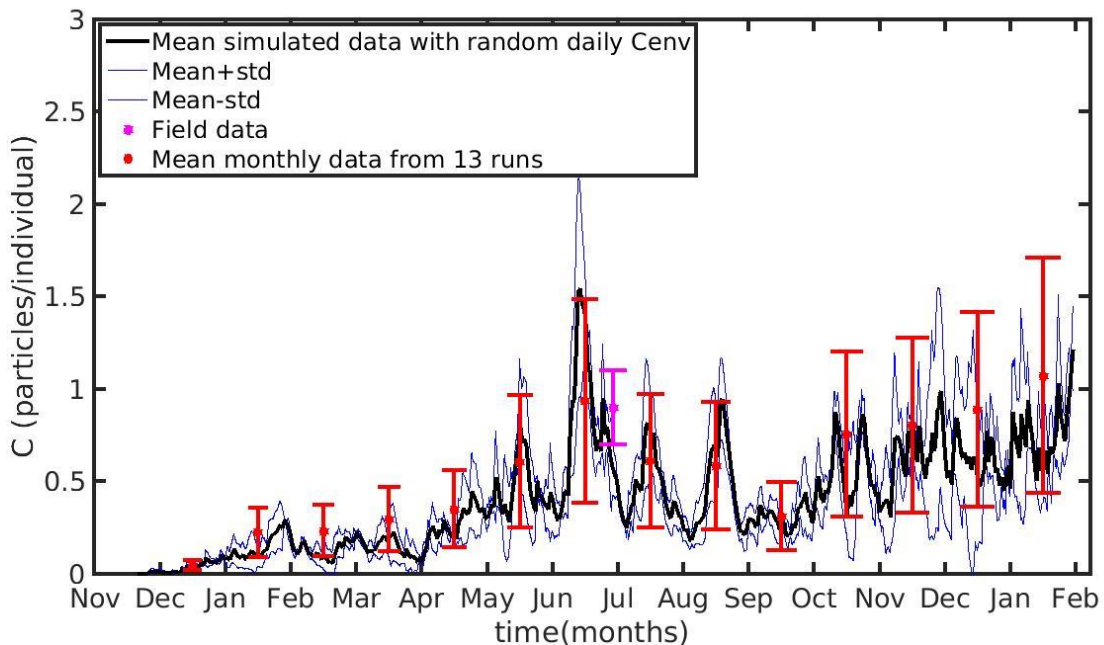


1429

1430 *Fig.9. Mean seasonally values and standard deviation of microplastics (MPs) accumulation (red error bars: mean*  
 1431 *value ± SD) by the mussel in North Sea derived from 15 model runs with different constant values of environmental*  
 1432 *MPs concentration ( $C_{env}$  range: 0.1-0.8 particles  $L^{-1}$ ); Mean hourly simulated data (black line) and standard deviation*  
 1433 *(blue lines) of microplastics accumulation derived from 3 model runs with stochastic sequences of daily random  $C_{env}$*   
 1434 *values.*

1435

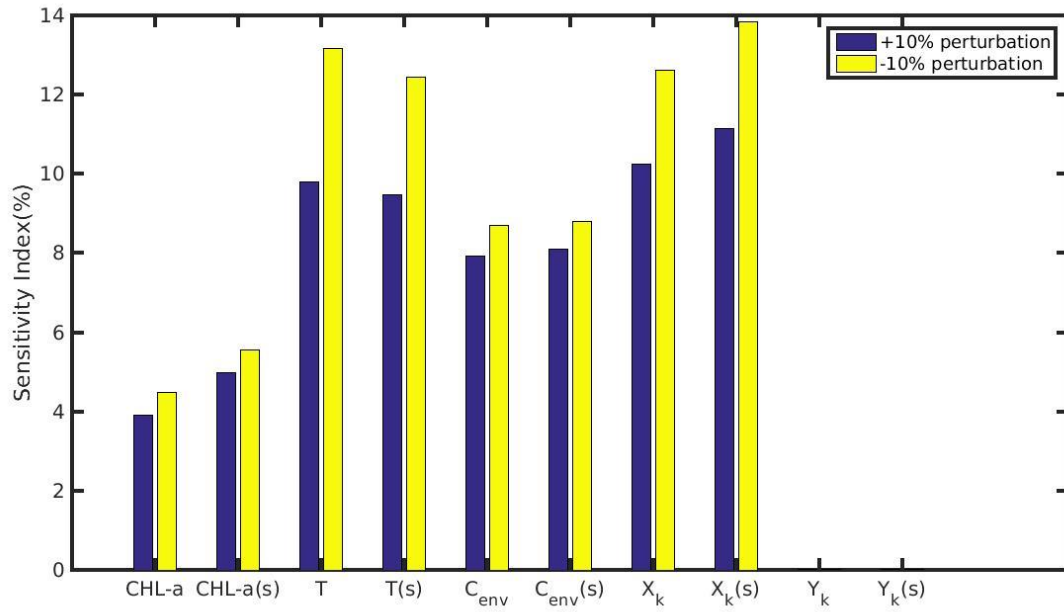
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1438 *Fig. 10. Mean monthly values and standard deviation of microplastics accumulation (red error bars: mean value ± SD)*  
 1439 *by the mussel in Northern Ionian Sea derived from 13 model runs with different constant values of environmental MPs*  
 1440 *concentration ( $C_{env}$  range: 0.0012-0.024 particles  $L^{-1}$ ); Mean hourly simulated data (back line) and standard deviation*  
 1441 *(blue lines) of microplastics accumulation derived from 3 model runs with stochastic sequences of daily random  $C_{env}$*   
 1442 *values.*

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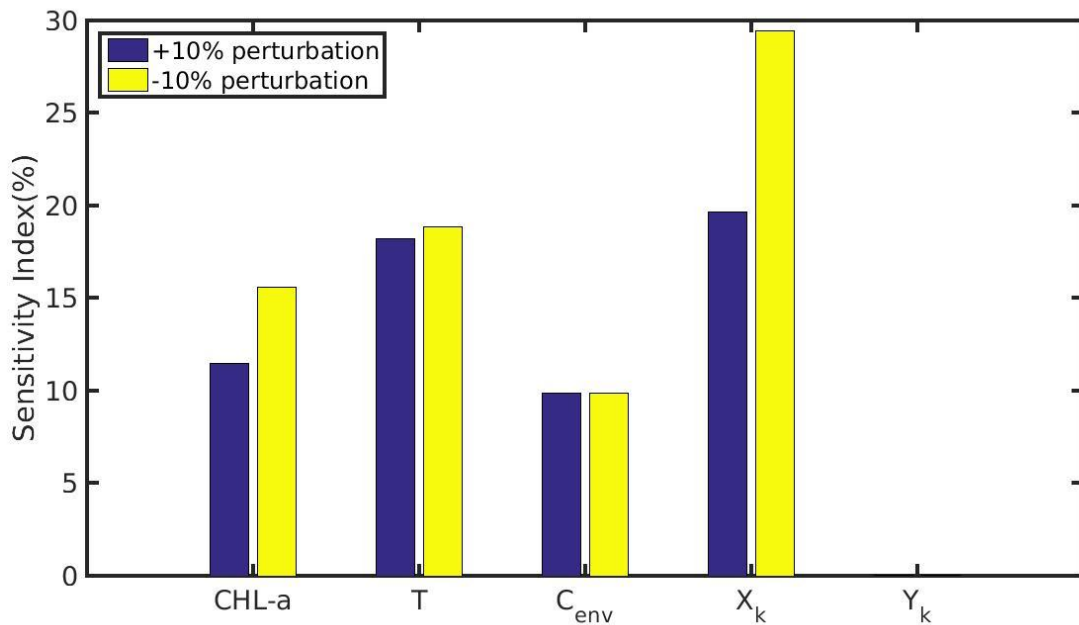


1444

1445 *Fig. 11. Sensitivity index of MPs accumulation on the wild mussel of the North Sea when variables (CHL-a,*  
1446 *temperature, C<sub>env</sub>) and parameters (X<sub>k</sub>, Y<sub>k</sub>) are perturbed ± 10%. The notation (s) refers to the permanently submerged*  
1447 *mussel.*

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1449



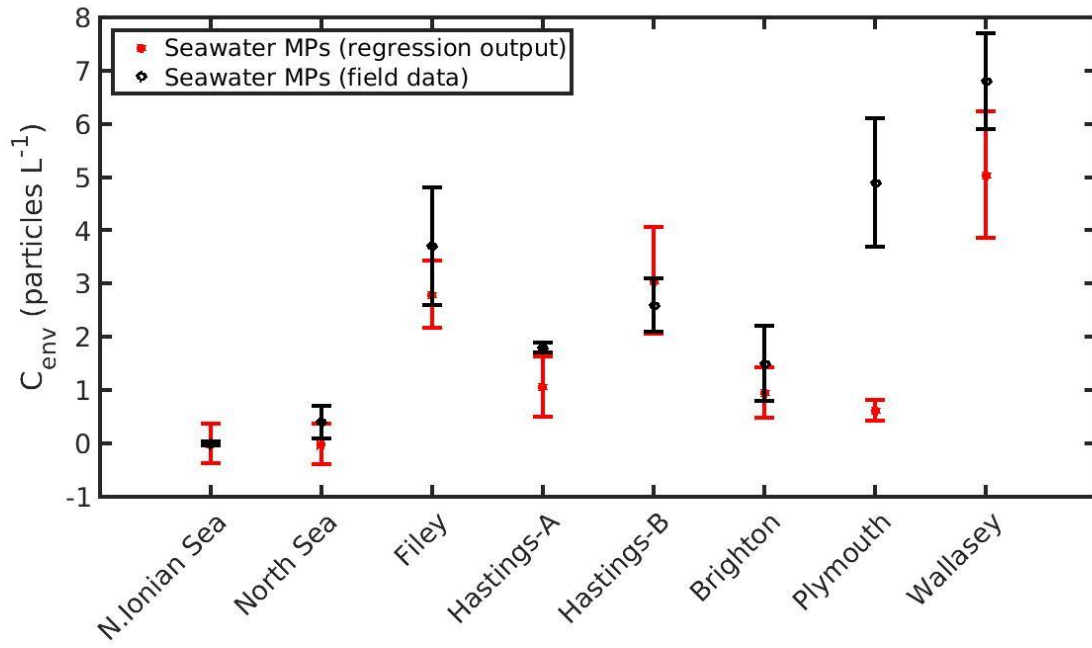
1450

1451 *Fig. 12. Sensitivity index of MPs accumulation on the cultured mussel of the Northern Ionian Sea when variables*  
1452 *(CHL-a, temperature, C<sub>env</sub>) and parameters (X<sub>k</sub>, Y<sub>k</sub>) are perturbed ± 10%.*

1453

1454

1455



1456

1457 *Fig. 13. Prediction of seawater microplastics concentration by applying Eq. 20 in the Northern Ionian Sea, North Sea*  
1458 *(present study) and 6 areas around U.K. (Filey, Hastings-A&B, Brighton, Plymouth, Wallasey; Li et al. (2018)).*

1459