Modeling of Microplastics accumulation by mussels (Mytilus spp.) based on a Dynamic Energy Budget (DEB) model

Μοντελοποίηση συσσώρευσης μικροπλαστικών από μύδια (Mytilus spp.) με χρήση του Dynamic Energy Budget (DEB) μοντέλου

Ναταλία Σταματάκη (ΑΜ: 216007)

Πτυχιακή εργασία για το ΔΠΜΣ Ωκεανογραφίας και Διαχείρισης Θαλάσσιου Περιβάλλοντος, ΕΚΠΑ

Επιβλέπων Καθηγητής: Σαράντης Σοφιανός

ΕΘΝΙΚΟ ΚΑΙ
ΚΑΠΟΔΙΣΤΡΙΑΚΟ
ΠΑΝΕΠΙΣΤΗΜΙΟ
ΑΘΗΝΩΝ

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Abstract

Microplastics (MPs) are a contaminant of emerging concern accumulating in marine ecosystems. Due to their ubiquitous nature and small dimensions the ingestion and impact of microplastics on marine life are a cause of concern, notably for filter feeders. One of the tasks of EU Horizon 2020 CLAIM (Cleaning Litter by developing and Applying Innovative Methods in European Seas) project is to assess the degree of threat of plastic pollution to the ecosystem. Inside this framework, a Dynamic Energy Budget (DEB)-accumulation model was developed to investigate the growth and MPs accumulation of wild and cultured bivalve species raised under different environmental conditions (varying chlorophyll-a and microplastics concentration and temperature) and validated against field data for *Mytilus edulis* (wild) from the North Sea and *Mytilus galloprovincialis* (cultured) from the Northern Ionian Sea (eastern Mediterranean Sea). The environmental data (chlorophyll-a and temperature) used for the forcing of the DEB model were extracted from satellite images. Towards a generic DEB model, the site-specific parameter, half saturation (X_k) was applied as a power function of food density availability for the cultured mussel, while for the wild it was calibrated to a constant value. The DEB-accumulation model extended with all feeding processes, dynamically simulates the uptake and excretion of MPs by the mussels showing good agreement with the field data. The MPs accumulation by the wild mussel (fresh tissue mass 2.02g) is 0.52 particles/individual and the cultured mussel (fresh tissue mass 3.96g) is 2.27 particles/individual. Additionally, the inverse experiments performed, investigate the depuration time for both mussels in their clean from MPs environment and result in different times. In particular at North Sea simulation the mussel was cleaned by 90% after 11 hours, while at N. Ionian Sea after 8 hours. A nonlinear regression model was developed, in order to predict the MPs accumulation by both mussels, regarding the prevailing environmental conditions of each area (Chl-a and MPs

concentration of seawater and temperature) and mussel's fresh tissue mass and resulted in good correlation between them, consistent with DEB-accumulation model results and field data. All the experiments performed, highlight the strong dependence between the resulted MPs accumulation by the mussels and the feeding-excretion rates based on the environmental conditions occurring in each area. Finally, the developed DEB-accumulation model was applied at Thermaikos Gulf (North Aegean Sea) to investigate scenarios such as the estimation and uncertainty of microplastics accumulation by cultivated mussels at the region.

Περίληψη

Τα μικροπλαστικά είναι ρύποι που συσσωρεύονται στα θαλάσσια οικοσυστήματα, προκαλώντας αυξανόμενη ανησυχία. Λόγω της ευρέως χρήσης και των μικρών διαστάσεών τους, η κατάποση και ο αντίκτυπος των μικροπλαστικών στη θαλάσσια ζωή αποτελούν αιτία ανησυχίας, ιδίως για τους διηθητές οργανισμούς. Ένα από τα καθήκοντα του προγράμματος EU Horizon 2020 CLAIM (Καθαρισμός απορριμάτων με την ανάπτυξη και εφαρμογή καινοτόμων μεθόδων στην Ευρωπαϊκή θάλασσα) είναι να εκτιμηθεί ο βαθμός απειλής της πλαστικής ρύπανσης στο οικοσύστημα. Μέσα σε αυτό το πλαίσιο, μελετήθηκε η ανάπτυξη και συσσώρευση μικροπλαστικών στα άγρια και καλλιεργούμενα δίθυρα είδη, που αναπτύχθηκαν υπό διαφορετικές περιβαλλοντικές συνθήκες (μεταβαλλόμενη συγκέντρωση χλωροφύλλης, μικροπλαστικών στο περιβαλλον και θερμοκρασία) μέσω ενός Dynamic Energy Budget (DEB)-συσσώρευσης μοντέλου και παραμετροποιήθηκε (tuned) για το άγριο μύδι Mytilus edulis από τη Βόρεια Θάλασσα και το καλλιεργούμενο μύδι Mytilus galloprovincialis από το Βόρειο Ιόνιο Πέλαγος (Ανατολική Μεσόγειος Θάλασσα) σύμφωνα με τα δεδομένα πεδίου. Τα περιβαλλοντικά δεδομένα (χλωροφύλλη και θερμοκρασία) που χρησιμοποιήθηκαν για το forcing του μοντέλου εξήχθησαν απο δορυφορικές εικόνες. Οδεύοντας προς ένα γενικευμένο μοντέλο DEB, η χωροεξαρτούμενη παράμετρος ημικορεσμού (X_κ) εφαρμόστηκε ως συνάρτηση δύναμης της διαθέσιμης πυκνότητας τροφής για το καλλιεργούμενο μύδι, ενώ για το άγριο μύδι παραμετροποιήθηκε σε σταθερή τιμή. Το μοντέλο DEB-συσσώρευσης που επεκτάθηκε ώστε να περιλαμβάνει όλες τις διαδικασίες πρόσληψης τροφής, προσομοιώνει δυναμικά την πρόσληψη και απέκκριση των μικροπλαστικών από τα μύδια, δείχνοντας καλή συμφωνία με τα δεδομένα πεδίου. Η συσσώρευση των μικροπλαστικών από τον άγριο μύδι (μάζα νωπού ιστού 2,02g) είναι 0,52 σωματίδια / άτομο και από το καλλιεργούμενο μύδι (μάζα νωπού ιστού 3,96g) είναι 2,27 σωματίδια / άτομο. Επιπλέον, πραγματοποιήθηκαν τα αντίστροφα πειράματα ώστε να διερευνηθεί ο χρόνος καθαρισμού των μυδιών απο τα

μικροπλαστικά στο καθαρό περιβάλλον τους, και προέκυψαν διαφορετικοί χρόνοι για κάθε μύδι. Συγκεκριμένα στη προσομοίωση της Βόρειας Θάλασσας το μύδι καθάρισε κατά 90% μετά από 11 ώρες, ενώ στο Β. Ιόνιο Πέλαγος μετά από 8 ώρες. Ένα μοντέλο μη γραμμικής συσχέτισης/παλινδρόμησης αναπτύχθηκε για την πρόβλεψη της συσσώρευσης μικροπλαστικών από αμφότερα τα μύδια, αναφορικά με τις επικρατούσες περιβαλλοντικές συνθήκες κάθε περιοχής (συγκέντρωση χλωροφύλλης και μικροπλαστικών στο θαλασσινό νερό, θερμοκρασία) και τη μάζα νωπού ιστού των μυδιών και είχε ως αποτέλεσμα τη καλή συσχέτιση μεταξύ τους και την συμφωνία με τα αποτελέσματα του DEB-συσσώρευσης μοντέλου και των δεδομένων πεδίου. Όλα τα πειράματα που πραγματοποιήθηκαν υπογραμμίζουν την έντονη εξάρτηση μεταξύ της συσσώρευσης μικροπλαστικών από τα μύδια και των ρυθμών πρόσληψης-απέκκρισης τροφής σε σχέση με τις περιβαλλοντικές συνθήκες που επικρατούν σε κάθε περιοχή. Τέλος, το DEB-συσσώρευσης μοντέλο εφαρμόστηκε στη περιοχή του Θερμαϊκού Κόλπου (Βόρειο Αιγαίο Πέλαγος) για τη διερεύνηση σεναρίων όπως η εκτίμηση και αβεβαιότητα της συσσώρευσης μικροπλαστικών από καλλιεργούμενα μύδια στην περιοχή αυτή.

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 mainly: those that are manufactured for particular industrial or household 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). MPs pollution in the world's oceans has been recently estimated at over 5 trillion floating particles, corresponding to 250,000 tons (Eriksen et al., 2014). 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 (Lattin et al., 2004; Moore et al., 2001; Thompson et al., 2004; Lusher, 2015). Numerous studies have revealed that these are ingested by animals from all levels of the food web; from zooplankton (Cole et al., 2013), small pelagic fishes and mussels (Digka et al., 2018) to mesopelagic fishes (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 are known to contain and/or adsorb high concentrations of organic contaminants (Hirai et al., 2011; Mato et al., 2001; Rios et al., 2007; Teuten et al., 2007, 2009).

MPs have similar size with many planktonic organisms and other suspended particles that consist the diet of filter feeders. Bivalves are of particular interest because of their extensive filter activity, which, combined with their inability to select particles with high energy value (i.e phytoplankton) during filtration (Vahl, 1972; Saraiva et al., 2011), exposes them directly to MPs contamination. Recently, many researchers demonstrated that mussels can ingest MPs during

laboratory trials. The presence of microplastics in the mussel's gut, and consequently the ingestion of these particles, was demonstrated using analysis of faeces and dissection of the intestinal tract (Von Moos et al., 2012; Van Cauwenberghe et al., 2015; Wegner et al., 2012; Khan and Prezant, 2018), as well as histological techniques (Browne et al., 2008). It was also shown that very small plastic particles (<10 μm) can translocate to the circulatory system of the bivalve *Mytilus edulis* (Browne et al., 2008). Additionally, it was observed that smaller particles are ingested and retained in greater quantities than larger particles and feeding activity of mussels was reduced after ingestion and/or translocation (Browne et al., 2008). More recent studies showed several effects of microplastic ingestion in laboratory exposed mussels including histological changes, inflammatory responses, immunological alterations, lysosomal membrane destabilization, reduced filtering activity, neurotoxic effects, oxidative stress effects, increase in hemocyte mortality, dysplasia, genotoxicity and transcriptional responses (reviewed by Li et al. 2019). However, the tested concentrations of MPs in laboratory experiments are frequently several orders of magnitude higher (up to 7 times) than what has been documented in the environment (Van Cauwenberge et al., 2015; Lenz et al. 2016). Field studies suggest a positive and quantitative correlation between microplastics in mussels and surrounding waters (Li et al. 2019, Qu et al. 2018). Furthermore, recent field studies confirmed that the plastics abundance may be higher in cultured mussels, as compared to wild populations, because farmed mussels are grown in plastic lines (Mathalon and Hill, 2014), although, other studies showed no difference or the opposite ((De Witte et al., 2014; Vandermeersch et al., 2015; Phuong et al., 2018; Digka et al., 2018; Li et al., 2018). Microplastic contamination in mussels has been proposed as a marine health status parameter (De Witte et al., 2014), and added to the European database on environmental contaminants of emerging concern in seafood (Vandermeersch et al., 2015).

The present study aims to evaluate the MPs contents of two species of the *Mytilidae* family, in different modes of life (cultivated and wild): the blue mussel (*Mytilus edulis*: wild) and the Mediterranean mussel (*Mytilus galloprovincialis*: cultivated). For this purpose, a Dynamic Energy

Budget (DEB)-accumulation model was developed and applied in two different regions: the North Sea (*M. edulis*) and the Northern Ionian Sea (*M. galloprovincialis*). Although DEB models have been used previously as a basis for modeling other processes, apart from the growth of an individual, such as concentrations of contaminants (Zaldivar, 2008) and bioaccumulation of trace metals (Casas and Bacher, 2006), this is the first time that a DEB-based model is used to assess the uptake and excretion rates of MPs in mussels. The MPs accumulation by mussels was described dynamically, based on mussels' physiological rates and validated/tuned against literature data (Digka et al, 2018; Van Cauwenberghe et al., 2015). Finally, a pilot study at Thermaikos Gulf (Aegean Sea) was conducted and led to the estimation of microplastics accumulation and its uncertainty by the mussels of the region.

2. Materials and Methods

2.1 Study areas and validation data

The North Sea is a large semi-enclosed sea on the continental shelf of north-west Europe with a total surface area 850000 km^2 and is bounded by the coastlines of 9 countries. The sea is shallow, getting deeper towards the north (up to 725 meters). Atlantic water enters the North Sea mainly from the north and moves eastward along the Belgian/Dutch coast through an anti-clockwise circulation (Otto et al., 1990). Most sources of nutrients are linked to anthropogenic activities, while major rivers, such as Rhine, Elbe, Weser, Ems and Thames discharge into the southern part of the sea (Lacroix et al.,2004), making this area a productive ecosystem. In this study, the area is limited along the French, Belgian and Dutch North Sea coast (N 50.98^{o}-51.46^{o}, W 1.75^{o}-3.54^{o}) (Figure 1). This is located close to harbors, where shipping and industrial activity is high, putting considerable pressure on the ecological systems of the region (Van Cauwenberghe et al., 2015).

Van Cauwenberghe et al. (2015) examined the presence of MPs in 'naturallly exposed' mussels (*M. edulis*), and thus biota and water were collected at 6 sampling stations along the French, Belgian and Dutch North Sea coast, in late summer of 2011. *M. edulis* (size: 4-4.5cm and weight: 2 ± 0.7g) were randomly collected on the local breakwaters and additionally water samples were taken near the breakwaters, in order to assess the MPs concentration in the organisms and their habitat. Seawater samples had Mps (<1mm) on average 0.4 ±0.3 particles L^{-1} (range: 0.0–0.8 particlesL^{-1}) and *M. edulis* contained on average 0.2 ± 0.3 particles g^{-1} tissue (or 0.4 ± 0.3 particles/individual) (with highest concentration 1.1 particles g^{-1} tissue) (Van Cauwenberghe et al., 2015). The size range of MPs found within the mussels was 20-90 μm (size <1mm). These data

were used for validation of the DEB-accumulation model for *M. edulis* in the North Sea. MPs concentrations of the seawater for both study areas are originated by sampling the sea surface, despite the different measurement units. The value of MPs concentration in the Northern Ionian Sea is approximately equal when expressed also as particles/L considering that a layer (surface area $1m^2$) with depth 0.1cm contains 1L of seawater.

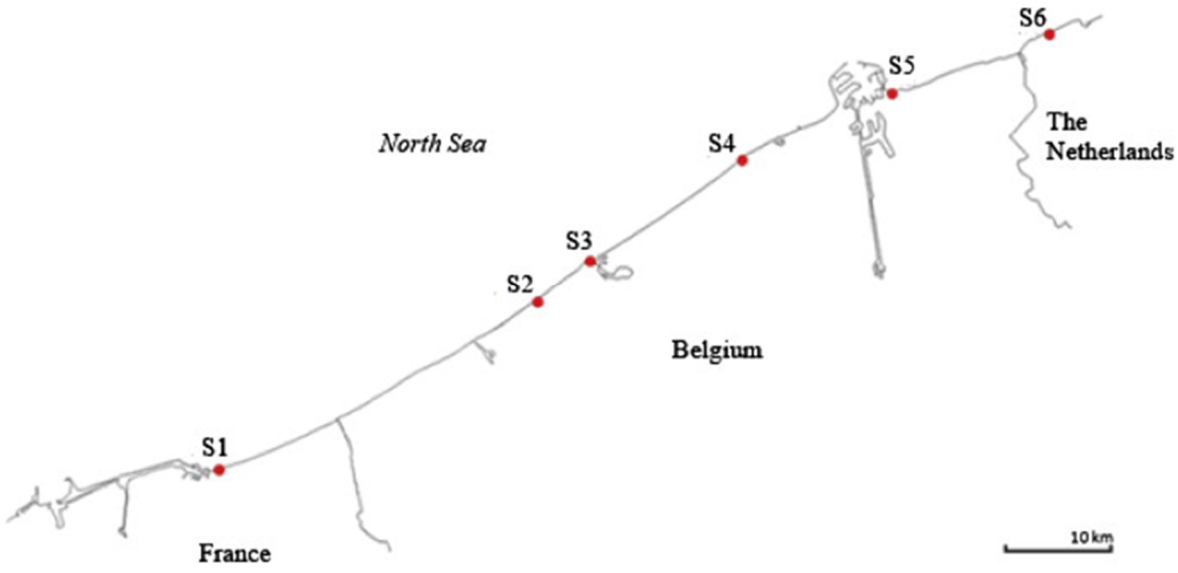

Figure.1. Map of the study area showing the sampling locations along the French–Belgian–Dutch coastline (S1: Dunkerque, S2: Middelkerke, S3: Oostende, S4: Wenduine, S5: Heist, S6: Cadzand) (Van Cauwenberghe et al., 2015).

The Northern Ionian Sea is in the transition zone between the Adriatic and Ionian Seas. The Adriatic-Ionian coastline, being long and complex, creates a high diversity of hydrodynamic and sedimentary environments. River outflows into the Northern Ionian Sea include Kalamas/Thyamis (Greece) and Butrinto (Albania) rivers (Skoulikidis et al., 2009). Shoreline tourism and recreational activities, including poor wastewater management practices, as well as fisheries, aquaculture, and shipping are the main anthropogenic activities related to marine litter inputs in the Northern Ionian Sea (Vlachogianni et al., 2017; Digka et al., 2018). Small farming sites and shellfish grounds are operating in Thesprotia (northwestern Ionian Sea) (Theodorou et al., 2011). The wider area of

Adriatic Sea has been mentioned as a hotspot for marine litter and one of the most affected areas in the Mediterranean Sea (Pasquini et al., 2016; Vlachogianni et al., 2017; Liubartseva et al., 2018), with a potential risk for floating marine litter being transported to the Ionian Sea.

The validation data for the Ionian Sea simulation are provided by Digka et al. (2018). According to Digka et al. (2018), mussels (*Mytilus galloprovincialis*) were sampled in this area (Northern Ionian Sea) in the framework of the "DeFishGear" project. The mussels were collected by hand from a long line type mussel culture farm in Thesprotia, in summer 2015 (end of June) and the sampling depth was up to 3 m (Digka et al., 2018) (Figure 2). The average load of MPs (size < 1 mm) per mussel (mean shell length 5.3±0.3 cm) was 2 ± 0.2 particles/individual. The size of MPs found within the mussels ranged from 55 to 600 μm. The average MPs accumulation was calculated from 18 mussels detected as contaminated with MPs out of a total population of 40 mussels, originated from the farm, with shell length range 4.8-5.6 cm. The abundance of MPs (size <1mm) in sea surface water ranged from 0 to 1.61 particles/m^2 with an average value 0.18 particles/m^2 (Digka et al., 2017b).

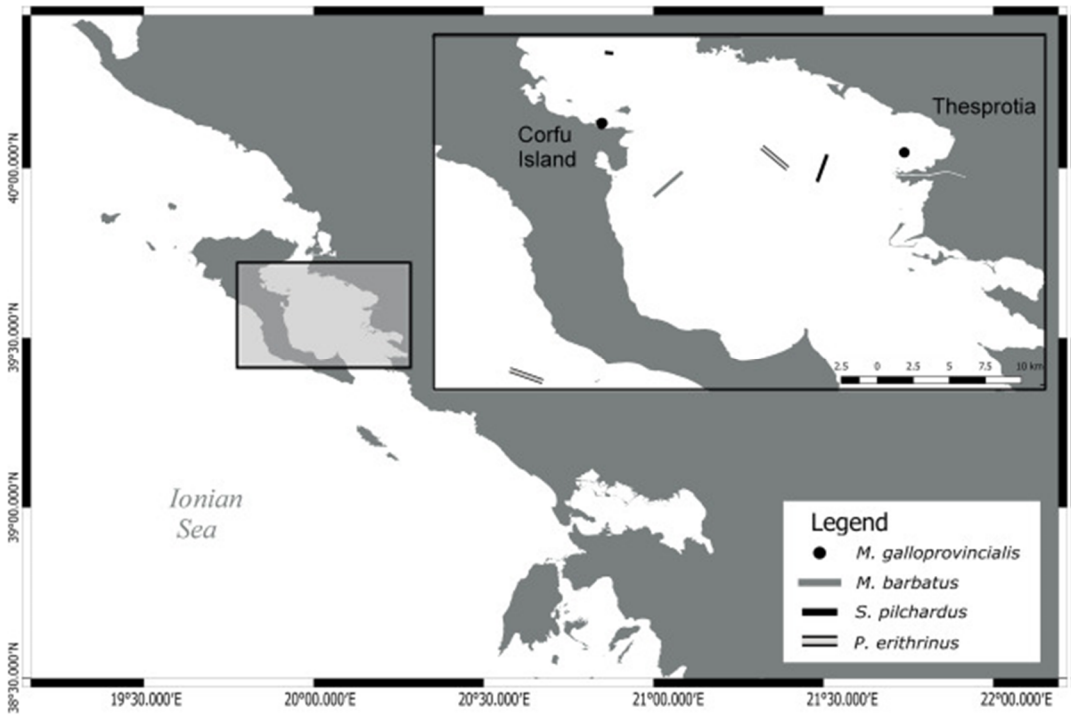

Figure 2. Sampling area in the Northern Ionian Sea and location of sampling sites for Mytilus galloprovincialis and other species. The sampled cultured mussels of Thesprotia site were considered in the present study.

An implementation of the model was conducted at Thermaikos Gulf, which has a total surface of 5,100 km^2, lies to the North-West Aegean and provides an example of a semi-enclosed basin. The depth varies from 10 to 75 meters and the tidal range is 0.25m. The inner Thermaikos Gulf is one of the few areas in Greece that can be characterized as eutrophic (Papakonstantinou et al., 2007; Pagou 2005) and hosts the most extended and productive mussel aquacultures, reaching a 70% of total production in Greece (Catsiki and Florou 2006).

Mussel growth data that have been used for the tuning of the model at Thermaikos Gulf are samples from the coastal area of Chalastra, provided by Kravva, (2000). At Chalastra, the main rivers of Axios and Aliakmon discharge particulate matter and dissolved constituents (Price et al., 2005) making the area ideal for the cultivation of mussels. Since the present study did not focus on the mussel growth simulation at the specific region, the parameterization of the model developed and implemented by Hatzonikolakis et al. (2017) was used and coupled with the accumulation sub-model in order to estimate the MPs accumulation by the cultivated mussels (see Hatzonikolakis et al., 2017 for mussel growth simulation). The area of Thermaikos Gulf has been characterized as heavily polluted by plastic marine debris (400 items/m^3, Valavanidis and Vlachogianni, 2012). Various sources of contaminants contribute to this, such as harbor facilities, industrial activities originating from the adjacent industrial zone, partially treated domestic effluents, intensively agricultural plains and aquaculture facilities. However, since there are not yet available field data regarding the MPs concentration in the seawater and/or MPs accumulation by the mussels at Thermaikos Gulf, an ensemble forecasting was conducted for the estimation of MPs accumulation by cultured mussels of the area.

2.2 DEB model

2.2.1 Description of a DEB model

In the present study, a Dynamic Energy Budget (DEB, Kooijman, 2000, 2010) model was developed to simulate the accumulation of MPs by mussels. This was based on the model described in Hatzonikolakis et al. (2017) that simulates the growth of the Mediterranean mussel. Dynamic energy budget theory has been developed by Bas Kooijman (2000) and since then has been extensively tested for different kinds of organisms, e.g. mollusks, fish, birds etc. (Kooijman, 2000), describing the energy flow through individual organisms from the assimilation of food to the utilization for maintenance, growth, development and reproduction. The basic assumption of a DEB model is that the assimilated food first enters a reserve pool and then allocated between the other compartments: a fixed part κ is spent on somatic maintenance and growth, while the remaining 1-κ, on maturity maintenance and reproduction (Figure 3). This rule is known as the κ-rule. The individual is characterized by three state variables: Structural Volume V (cm^3), Energy reserves E (Joule) and Energy allocated to development and reproduction R (Joule). The environment of the individual is described by food density and temperature and their fluctuations change the energy flow through the organism.

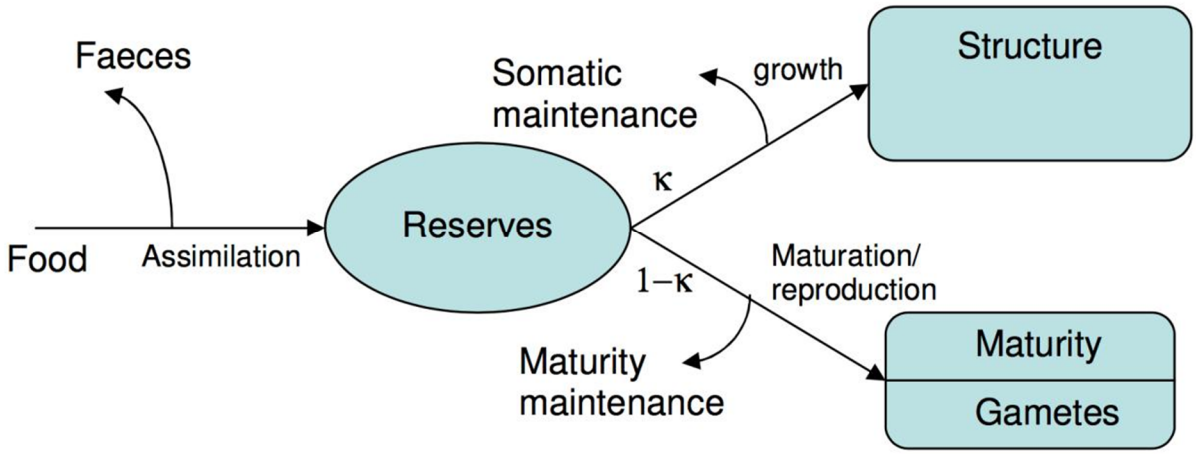

Figure 3: Representation of the energy fluxes following the DEB approach (Zaldivar, 2008; Kooijman, 2000).

The Energy reserves can be expressed as the difference between the assimilation energy rate ($\dot{p}_a$) and the energy utilization ($\dot{p}_c$):

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

According to Kooijman (2000) a fixed fraction of energy is allocated to somatic maintenance and growth while the rest is used for maturation and reproduction. However, maintenance has priority over growth and when there is not enough food growth stops. Therefore, the change in structural volume, V, is given by:

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

$[\dot{p}_M]$ is the maintenance costs and the $[E_g]$ volume specific costs of growth. Concerning the energy allocated to reproduction, Kooijman (2000) showed that it can be expressed as:

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

where V_p is the mussel's volume at start of reproductive stage.

In this study the symbols and notation follow those that Kooijman (2000) used: Quantities that refer to unit of volume are expressed within brackets [] and those that refer to unit of biosurface area within braces {}, while rates have dots. All physiological rates are characterized by temperature dependence defined as (Kooijman, 2000):

$$k(T) = \frac{exp\left(\frac{T_A}{T_I} - \frac{T_A}{T}\right)}{1 + exp\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right) + exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right)} \quad (4)$$

, thus the assimilation energy rate is given by:

$$\dot{p}_a = \{\dot{p}_{Am}\} \cdot f \cdot k(T) \cdot V^{2/3} \quad (5)$$

where $\{\dot{p}_{Am}\}$ is the maximum surface area-specific assimilation rate and *f* is the functional response of assimilation to food concentration *X*:

$$f = \frac{X}{X + X_k} \quad (6)$$

where X_k is the half saturation coefficient. Further discussion of the half saturation coefficient will be made in section 2.4. Energy utilization rate is given by:

$$\dot{p}_c = \frac{[E]}{[E_g] + k \cdot [E]} \cdot \left(\frac{[E_g] \cdot \{\dot{p}_{Am}\} \cdot k(T) \cdot V^{2/3}}{[E_m]} + [\dot{p}_M] \cdot V \right) \quad (7)$$

where $[E]$ is the energy density: $[E] = \frac{E}{V}$ (8) and [Em] is the maximum energy density in the reserve compartment. Maintenance costs $[\dot{p}_M]$ are given by:

$$[\dot{p}_M] = k(T) \cdot [\dot{p}_M]_m \quad (9)$$

$[\dot{p}_M]_m$ is volume specific maintenance costs. Shell length (cm) is given by: $L = \frac{V^{1/3}}{\delta_m}$ (10)

and δ_m is the shape coefficient. Fresh tissue mass (g) is given by: $W = d \cdot \left(V + \frac{E}{[E_g]} \right) + \frac{R}{\mu_E}$ (11)

where d is the specific density and μ_E the energy content of reserves.

2.2.2 Feeding Processes

The DEB model described simulates the growth of the mussel taking into account only the assimilation rate of the individual. Since the present study focuses on simulating the MPs accumulation by the mussel, it was crucial to include a complete representation of its feeding mechanism. Therefore, the model presented in Hatzonikolakis et al. (2017) was extended to include

also the clearance, filtration and ingestion rate following Saraiva et al. (2011), assuming that MPs are represented by the silt variable. Thus, the mussels feeding mechanism is now described in four steps: (i) water is cleared: $\dot{C}_R = \frac{\{\dot{C}_{Rm}\}}{1+\frac{X\cdot\{\dot{C}_{Rm}\}}{\{\dot{p}_{Fm}\}}+\frac{C_{env}\cdot\{\dot{C}_{Rm}\}}{\{\dot{p}_{pFm}\}}} \cdot V^{2/3}$ (12)

where $\{\dot{C}_{Rm}\}$ is maximum surface area-specific clearance rate, $\{\dot{p}_{Fm}\}$ algal maximum surface area-specific filtration rate, C_{env} the microplastics concentration in the environment and $\{\dot{p}_{pFm}\}$ the silt maximum surface area-specific filtration rate. It was assumed that the cleared water contains algae and microplastics particles. The variable of environmental microplastics concentration C_{env}, was expressed from particles/volume to mass/volume in this equation according to Everaert et al. (2018).

(ii) Its content is then filtered and the particles are retained in the gills: $\dot{p}_F = \dot{C}_R \cdot X \cdot \lambda$ (13)

where λ is a conversion factor for the units. The filtration process handles separately each type of food and this equation refers to algae particles.

(iii) Particles are ingested: $\dot{p}_I = \{\dot{p}_{Im}\} \cdot f \cdot k(T) \cdot V^{2/3}$ (14)

where $\{\dot{p}_{Im}\}$ is the maximum surface area-specific ingestion rate, according to van der Veer et al. (2006). A pre-ingestive selection at the labial palps has as a consequence returning the rejected material into the water column through the pseudofaeces production: $\dot{J}_{pf} = \dot{p}_F - \dot{p}_I$ (15)

(iv) Particles are assimilated (Eq. 5; assimilation rate, $\dot{p}_a$), differential absorption in the gut and incorporation of material into the organism reserves, with the production of faeces:

$\dot{J}_f = \dot{p}_I - \dot{p}_A$ (16). Pseudofaeces and faeces production rates are in this approach not processes themselves but result from the difference between filtration - ingestion and ingestion-assimilation rates, respectively (Figure 4).

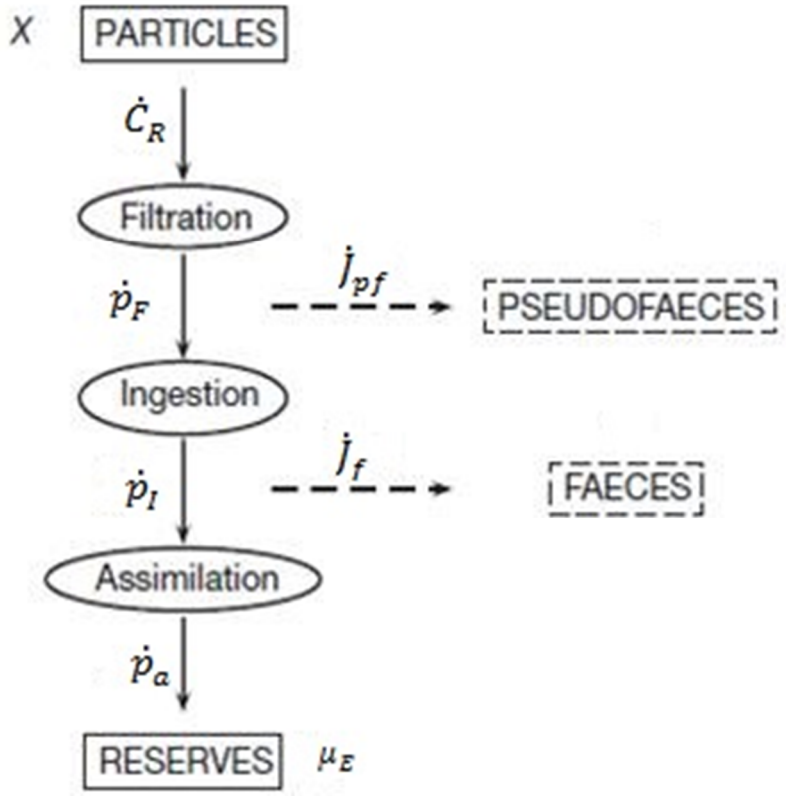

Figure 4. Representation of the feeding mechanism of bivalves following the DEB approach (Saraiva et al., 2012; Kooijman, 2010). See Tables 1 to 3 for equations, variables description and parameter values. Boxes with solid lines represent the organism mass compartments and solid arrows the associated flux; dashed boxes represent a mass compartment outside the organism, and dashed arrows represent the respective outflow from the organism.

It has been reported that *M. edulis* is able to retain in its gill all particles greater than 2–5 µm with 100% efficiency (Vahl, 1972; Strohmeier et al., 2012). In the present study, it is assumed that a mussel will be able to retain all particulate matter suspended in the water column, i.e. be able to filter all particles defined by the clearance rate (Saraiva et al., 2011). Thus, at the filtration level, there is no selection between particles and the same clearance rate for all particles is implied ({$\dot{C}_R$}), representing the same searching rate for food that depends on the organism maximum capacity ({$\dot{C}_{Rm}$}) and environmental particle concentrations (Vahl, 1972; Widdows et al., 1979; Cucci et al., 1989).

All the model equations describing the time evolution of feeding, maintenance, growth development and reproduction are summarized in Table 1. A full description of model variables is

also provided in Table 2. For a more detailed description, the interested reader is referred to the authors cited above (Hatzonikolakis et al., 2017; Saraiva et al., 2011; Kooijman, 2000).

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

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

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

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

$$f = \frac{X}{X + X_k} \quad (5)$$

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

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

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

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

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

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

$$\dot{C}_R = \frac{\{\dot{C}_{Rm}\}}{1 + \frac{X \cdot \{\dot{C}_{Rm}\}}{\{\dot{p}_{Fm}\}} + \frac{C_{env} \cdot \{\dot{C}_{Rm}\}}{\{\dot{p}_{pFm}\}}} \cdot V^{2/3} \quad (12)^*$$

$$\dot{p}_F = \dot{C}_R \cdot X \cdot \lambda \quad (13)$$

$$\dot{p}_I = \{\dot{p}_{Im}\} \cdot f \cdot k(T) \cdot V^{2/3} \quad (14)$$

$$\dot{J}_{pf} = \dot{p}_F - \dot{p}_I \quad (15)$$

$$\dot{J}_f = \dot{p}_I - \dot{p}_A \quad (16)$$

**The variable of environmental microplastics concentration C_{env}, was expressed to mass/volume (g/L) according to Everaert et al. (2018)*

Table 1. Dynamic energy budget model: equations.

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

Table 2. Dynamic energy budget model: variables

2.2.3 Microplastics accumulation sub-model

With the DEB model as a basis, a sub-model describing the microplastics (MPs) accumulation by the mussel was developed. It was assumed that the presence of MPs in the ambient water did not cause a significant adverse effect on the organisms' overall energy budget, in accordance with laboratory experiments, conducted in mussel species (Van Cauwenberghe et al., 2015: *M. edulis*; Santana et al., 2018: mussel *Perna perna*). Therefore, all components of the energy budget remain the same, when the mussel is contaminated by MPs.

The basic assumption of the MPs accumulation sub-model is that the mussel filtrates MPs present in the water, while filtering a great volume of water to ingest available food (Van Cauwenberghe et al., 2015; Von Moos et al., 2012; Browne et al., 2008; Digka et al., 2018 among others), without the ability of selecting between the high energetic valued particles and the MPs during this process. The uptake of MPs from the environment was taken into account through the process of filtration/clearance rate, while the excretion of this contaminant was derived from two processes: (i) pseudofaeces production and (ii) faeces production. The resulting accumulation is influenced by external environmental factors (MPs concentration, food availability, temperature) and internal biological processes (clearance, filtration, ingestion, growth). All these are illustrated on the following differential equation (Hatzonikolakis et al., 2018):

$$\frac{1}{W} \cdot \frac{dC}{dt} = C_{env} \cdot \acute{C}_R - b \cdot \frac{\acute{J}_{pf}}{\acute{p}_f} \cdot C - c \cdot \frac{\acute{J}_f}{\acute{p}_f} \cdot C \qquad \text{(Eq. 17)}$$

where $\acute{C}_R$ is the uptake/filtration rate for water (L/h), containing a concentration of MPs C_{env} (particles/L). The terms of $\frac{\acute{J}_{pf}}{\acute{p}_f}$ and $\frac{\acute{J}_f}{\acute{p}_f}$ represent the non-dimensional elimination rate through pseudofaeces and faeces production respectively, in relation to the filtration rate (see Table 1, Eq. 15-16). The values of b and c were calibrated in order to obtain the best fit with the observed MPs accumulation by mussels in both study areas. The concentration of MPs in the individual is

represented by the state variable C (particles/individual) and is computed at every time step of the model. This was set to one hour, in order to properly resolve the dynamics of rapidly changing processes, such as excretion and feeding. It was assumed that the initial concentration of MPs in the individual is zero.

In order to evaluate the depuration phase (i.e gut clearance), the inverse experiments were conducted, by setting the environmental MPs concentration equal to zero (C_{env}=0), after a period of 1 year in the Ionian Sea, when the mussels have the appropriate size for market, and 4 years in the North Sea, when literature field data are available.

2.3 Environmental data-Forcing of the DEB model

The DEB model is forced by surface temperature and food availability, which is defined as chlorophyll-a concentration. Hatzonikolakis et al. (2017) have tested the performance of the model, taking into account also particulate organic carbon (POC) in the diet of the mussel and concluded that the simulated growth data are on good agreement with field data when considering only chlorophyll-a as available food source. For both study areas temperature and Chl-a were derived from satellite data. Satellite-derived environmental data have also been used as forcing of DEB models in other regions such as Mont Saint-Michel Bay (North Brittany) (Thomas et al., 2011).

In the North Sea, environmental forcing data (temperature, chl-a) were obtained from NASA-Modis (Aqua) satellite measurements at a monthly time step and spatial resolution at 4km (https://podaac.jpl.nasa.gov/). These data cover the period of 2014 and 2015 (2 years). The specific satellite used, offer spatially and temporally extensive data, useful for sea surface temperature and phytoplankton monitoring in coastal waters and has facilitated large-scale ecological studies (Barre et al., 2006; Blondeau-Patissier et al., 2014; Sutton et al., 2017).

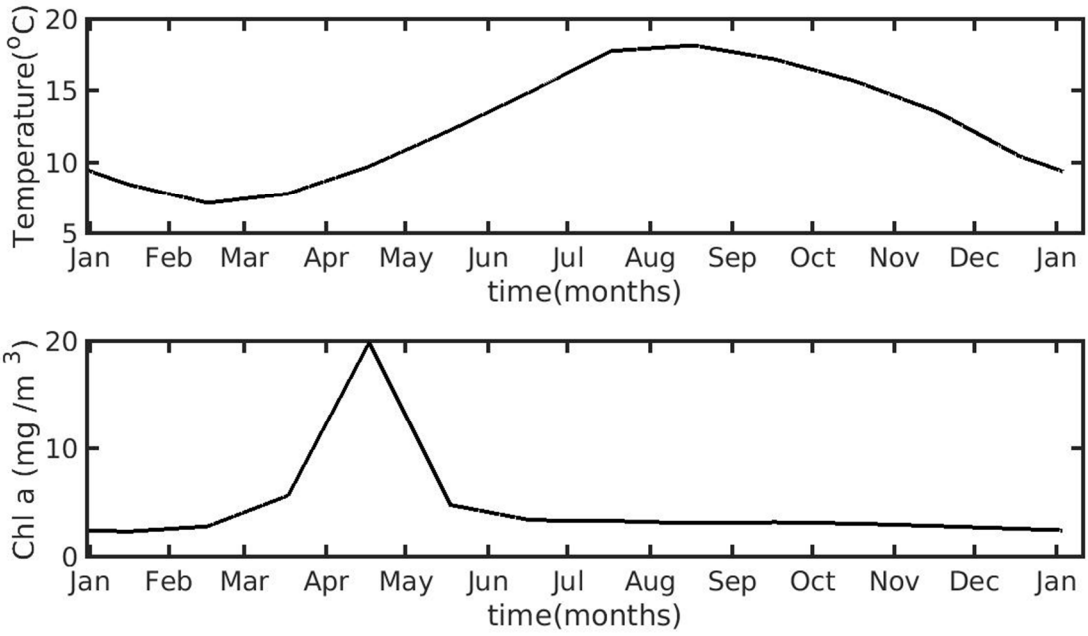

Figure5. Environmental data used for the forcing of the dynamic energy budget model in the North Sea simulation, showing temperature (top) and chlorophyll a concentration (bottom).

Seawater surface temperature (SST) data of the North Ionian Sea were obtained from NASA-Modis (Aqua) satellite measurements. The temporal resolution was monthly and spatial resolution was at 4km for 2 consecutive years, 2010 and 2011. Chl-a concentration data of the region were obtained from the Globcolour database (http://globcolour.info). The temporal resolution was monthly and the spatial resolution was 1km, covering the same time period (2010-2011) with the temperature data. The merged product from many satellites (SeaWiFs, Meris, Modis, Viirs and Olci-a) was used for the Chl-a concentration because it has good spatial resolution specifically in coastal scale, comparing with single satellite product. The sea surface temperature and the chlorophyll-a concentration (Chl-a) were used to force the model, and their values at each model time step were obtained by linear interpolation from the monthly data. The environmental forcing data of monthly averaged values are shown in Figure 5 and Figure 6 for the North Sea and the N. Ionian Sea, respectively.

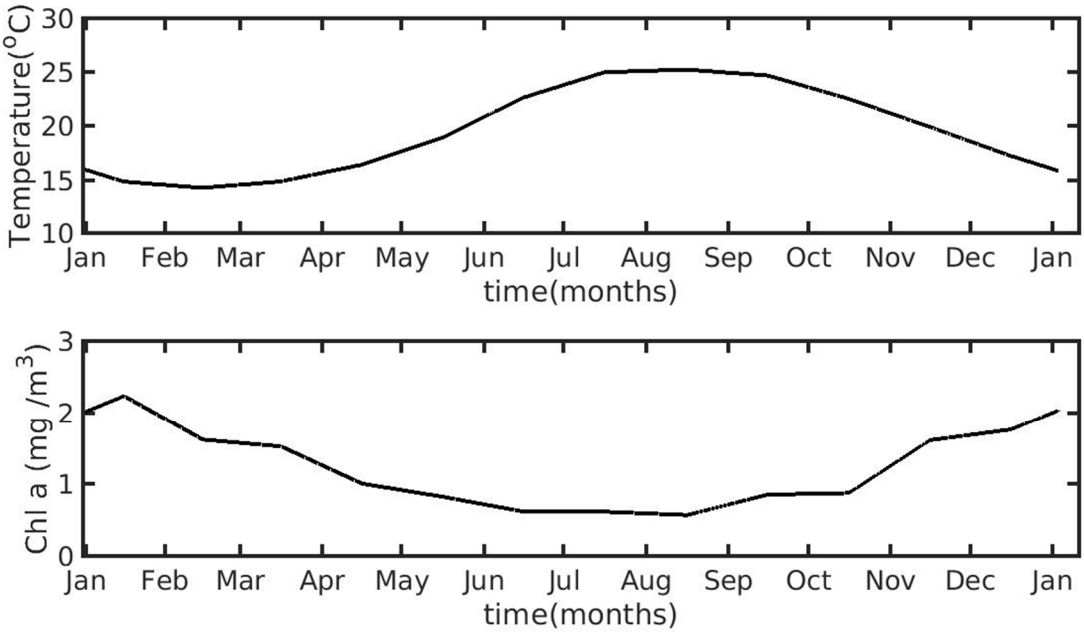

Figure 6. Environmental data used for the forcing of the dynamic energy budget model in the Northern Ionian Sea simulation, showing temperature (top) and chlorophyll a concentration(bottom).

The two coastal environments present some important differences regarding both Chl-a and SST. Specifically, in the Ionian Sea, Chl-a is relatively low (annual mean ~1.29 mg chl-a m^{-3}) and peaks during winter (maximum ~2.24 mg chl-a m^{-3}), while in the North Sea Chl-a is about four times higher (annual mean 4.70 mg chl-a m^{-3}), peaking in April (maximum ~19.87 mg chl-a m^{-3}), as soon as light availability reaches a critical level (Van Beusekom et al., 2009). The higher productivity in the North Sea is related with the nutrient inputs from the English Channel, the North Atlantic and particularly the river discharge of nutrient-rich waters along the Belgian-French-Dutch coastline. River loads are higher in winter and lower in summer (Van Beusekom et al., 2009). Regarding the sea surface temperature, in the Ionian Sea, the maximum value of temperature is 25°C in August, while in the North Sea the maximum value is 18°C at the same month (Fig. 1 and Fig. 2). Several studies have documented that east Mediterranean waters are characterized as

oligotrophic, while the northeast Atlantic waters of the coastal zone are considered eutrophic (Krom et al., 1991; Kress et al., 2003; Brockmann et al., 1993, among others).

The environmental concentration of MPs (C_{env}) at each model time step was set as a function of randomly generated values of the Gaussian distribution that is determined by the mean value and standard deviation of the observed field data (0.18 particles/L, Ionian Sea, Digka et al., 2017b; 0.4±0.3 particles/L, North Sea, Van Cauwenberghe et al., 2015). Considering that these values originate from surface waters and that mussels live in the near surface layer (0-5m), C_{env} was divided by a factor equal to 6.18 to obtain the mean MPs concentration in the upper 5m layer (Kooi et al., 2016).

Input data i.e. temperature, chlorophyll-a used as forcing functions for the DEB model at Thermaikos Gulf simulations are obtained from a three-dimensional coupled hydrodynamic/biogeochemical model (~10 km horizontal resolution), developed by Tsiaras et al.(2012). A long-term simulation over the 1980–2000 period was performed (Tsiaras et al. 2014) providing sufficient environmental data for this study.

The hydrodynamic model is based on the Princeton Ocean Model (Blumberg and Mellor, 1983), a widely spread community model that has been previously implemented in the N. Aegean area (Kourafalou and Barbopoulos, 2003; Kourafalou and Tsiaras, 2007). The biogeochemical model is based on the European Regional Seas Ecosystem Model (ERSEM, Baretta et al., 1995), a generic comprehensive model that has been successfully implemented across a wide range of coastal and open ocean ecosystems, such as the North Sea continental shelf (Pätsch and Radach, 1997), the oligotrophic Mediterranean (Allen et al., 2002; Petihakis et al., 2002) and the Arabian Sea (Blackford and Burkill, 2002), among others. A full description of the 3-D hydrodynamic/biochemical model can be found in Tsiaras et al. (2012) and Tsiaras et al. (2014).

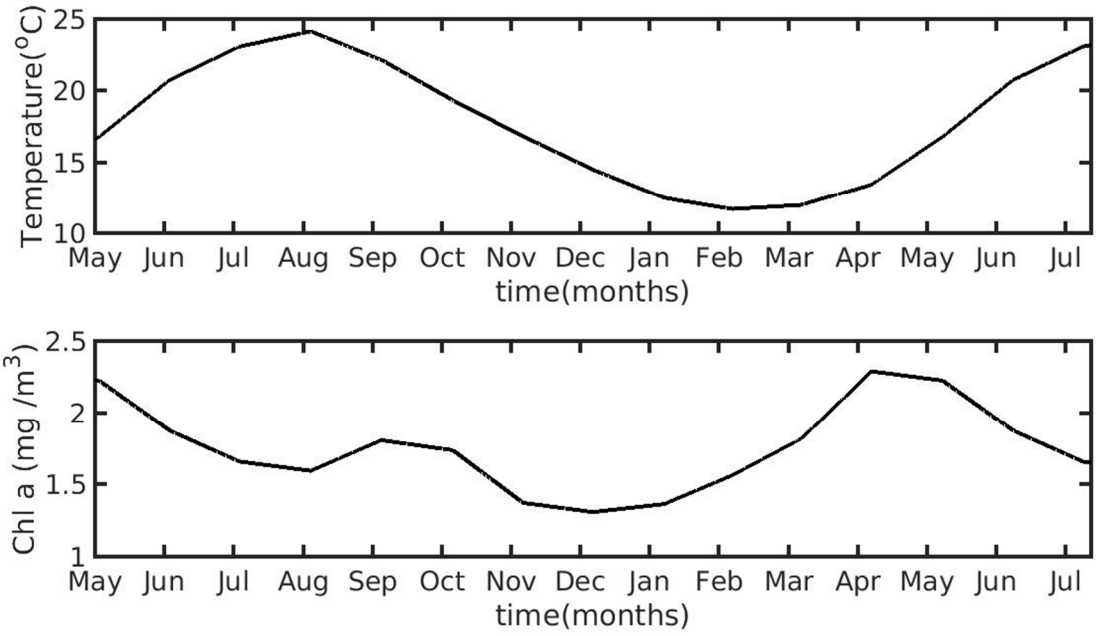

Figure 7. Environmental data used for the forcing of the dynamic energy budget model in Thermaikos Gulf simulation, showing temperature (top) and chlorophyll a concentration(bottom).

2.4 Parameter values

Most of the model parameters are obtained from Van der Veer et al. (2006) and Saraiva et al. (2011) and are referred to the blue mussel *Mytilus edulis* in the northeast Atlantic (Table 3). In previous studies it has been shown that this parameter set for *M. edulis* works sufficiently also for *M. galloprovincialis* (Casas and Bacher, 2006) with the small differences between the two species physiological responses being captured only by the half saturation coefficient X_k (Hatzonikolakis et al., 2017), which should be treated as a site – specific parameter (Pouvreau et al., 2006; Troost et al., 2010). The half saturation coefficient (X_k) represents the density of food at which the food uptake rate reaches half of its maximum value. In order to estimate the value of X_k, a different approach was followed for each study area.

Parameter	Units	Description	Value	Reference
$\{\dot{p}_{Am}\}$	J cm^{-2} d^{-1}	Maximum surface area-specific assimilation rate	147.6	Van der Veer et al. (2006)
$\{\dot{C}_{Rm}\}$	m^3 cm^{-2}d^{-1}	Maximum surface area-specific clearance rate	0.096	Saraiva et al. (2011)
$\{\dot{p}_{Fm}\}$	mg chla cm^{-2}d^{-1}	Algal maximum surface area-specific filtration rate	0.1152	Rosland et al. (2009)
$\{\dot{p}_{pFm}\}$	g cm^{-2}d^{-1}	Silt maximum surface area-specific filtration rate	3.5	Saraiva et al. (2011)
$\{\dot{p}_{Im}\}$	J cm^{-2} d^{-1}	Maximum surface area-specific ingestion rate	196.8	Van der Veer et al. (2006)
x_K	mg chla m^{-3}	Half saturation coefficient	Calibrated	-
T_A	K	Arrhenius temperature	5800	Van der Veer et al. (2006)
T_I	K	Reference temperature	293	Van der Veer et al. (2006)
T_L	K	Lower boundary of tolerance rate	275	Van der Veer et al. (2006)
T_H	K	Upper boundary of tolerance rate	296	Van der Veer et al. (2006)
T_{AL}	K	Rate of decrease of upper boundary	45430	Van der Veer et al. (2006)
T_{AH}	K	Rate of decrease of lower boundary	31376	Van der Veer et al. (2006)
$[\dot{p}_M]_m$	Jcm^{-3}d^{-1}	Volume specific maintenance costs	24	Van der Veer et al. (2006)
$[E_G]$	Jcm^{-3}	Volume specific growth costs	1900	Van der Veer et al. (2006)
$[E_m]$	Jcm^{-3}	Maximum energy density	2190	Van der Veer et al. (2006)
k	-	Fraction of utilized energy spent on maintenance/growth	0.7	Van der Veer et al. (2006)
V_p	cm^3	Volume at start of reproductive stage	0.06	Van der Veer et al. (2006)
δ_m	-	Shape coefficient	0.25	Casas & Bacher (2006)
d	g cm^{-3}	Specific density	1.0	Kooijman (2000)
μ_E	J g^{-1}	Energy content of reserves	6750	Casas & Bacher (2006)
λ	J mg chla^{-1}	Conversion factor	2387.73	Rosland et al. (2009)

Table 3. Dynamic energy budget model: parameters

At North Sea simulation X_k was tuned so that the simulated individual has the recorded size at the corresponding estimated age (Van Cauwenberghe et al., 2015) growing with the representative growth rates of wild *M. edulis* at the region (Saraiva et al., 2012; Sukhotin et al., 2007). At Ionian Sea simulation a new method was implemented aiming to generalize the DEB model to overcome the problem of site-specific parameterization. The DEB model was tuned against literature field data from environments similar to Ionian Sea, with similar levels of Chl-a concentration (1.0-5.0 mg chl-a m^{-3}), and one X_k value was found for each area. The four areas used and the corresponding value of X_k adopted, are shown on Table 4. These values of X_k were related to the prevailing Chl-a concentration of each area ([Chl-a]) through three different functions: linear: $f(x) = a * x + b$, exponential: $f(x) = a * \exp(b * x)$ and power: $f(x) = a * x^b + c$. The curve fitting app of Matlab (Matlab R2015a) was used for the parameterization of a, b and c of each function taking into account the 95% confidence level. The score of each function regarding the somatic/mussel growth simulation in all four regions was tested through target diagrams (Jolliff et al., 2009) by computing the bias and unbiased root-mean-square-deviation (RMSD) between field and simulated data of all 4 regions and the function with the best score was adopted. A similar approach was followed by Alluno-Bruscia et al. (2011) for the oyster *Crassostrea gigas* in six Atlantic ecosystems who expressed the X_k as a linear function of food density (e.g. phytoplankton). Unfortunately, the approach described for the Ionian Sea simulation could not be applied in the North Sea, as the limited amount of growth data from the literature for wild *M. edulis* in similar environments did not permit a statistically significant fit of a similar function ($X_k = f(chl - a)$).

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

Table 4. Half saturation tuned values (Xk) and mussel growth data (Length) in different areas with similar environmental data (Chl-a, Temperature).

2.5 Simulation of reproduction-Initialization of the model

The reproductive buffer (R) is assumed to be totally emptied at spawning (R=0) (Sprung, 1983; Van Haren et al., 1994). The spawning day was set to occur at a particular time of the year as this is indicated by the literature for each region. According to Theodorou et al. (2011), in the mussel farms in Greece the spawning event occurs during winter for *M. galloprovincialis* (between December and March) and thus the spawning day was chosen to be at the middle of this season for the mussel of the Ionian Sea. The same approach was followed by Hatzonikolakis et al. (2017) for *M. galloprovincialis* in Thermaikos and Maliakos Gulfs (eastern Mediterranean). On the other hand, in the North Sea the spawning event for *M. edulis* was set at the end of May since according with Sprung (1983) the spawning period in this area is extended from the end of April until the end of June.

In both areas, the model was initialized so that the simulated individual is in the juvenile phase (V<Vp; Table 3) and the reproductive buffer can be considered to be empty (R=0) (Thomas et al., 2011). According to Jacobs et al. (2015) amongst others, juvenile mussels (*M. edulis*) range between 1.5-25 mm in size. Specifically, at the North Sea the settlement of mussel larvae (*M.*

edulis) takes place in June and the juveniles grow to a maximum size of 25mm within 4 months (Jacobs et al., 2014). At N. Ionian Sea the operating mussel farms follow the life cycle of *M. galloprovincialis*, starting the operational cycle each year by dropping seed collectors from late November until March and the juvenile mussels grow up to 6-6.5cm after approximately a year according to the information obtained from the local farms in the region and Theodorou et al. (2011). The initial fresh tissue mass was distributed between structural volume (V) and reserves energy (E). Energy allocated to those two compartments was firstly constrained by the initial length (L) and then energy allocated to V was calculated according to Eq.10 (Table 1). The initial value of E was set so that the simulated individual has an initial weight that corresponds to the juvenile phase (V<Vp). The initial accumulation of MPs by mussels was considered zero for both areas. The initial values of the state variables for the 2 regions implementations are shown in Table 5.

North Ionian Sea		North Sea	
Variable	Value	Variable	Value
Start date	20 Nov 2010	Start date	1 Aug 2014
L	0.85cm	L	0.15cm
W	0.1938g	W	0.0055g
V	$0.0096cm^3$	V	$5.3 \cdot 10^{-5} cm^3$
E	350J	E	10J
R	0J	R	0J
C	0 particles/individual	C	0 particles/individual

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

2.6 Regression analysis

Preliminary sensitivity experiments with DEB-accumulation model showed that the MPs accumulation by the mussel is highly depended on the prevailing environmental conditions ([Chl-a], temperature and C_{env}). According to Eq. 17, the MPs accumulation by the mussel depends on the difference between the uptake (first term) and the excretion (second and third term) rate of MPs by the individual. These rates are mainly controlled by the amount of existing food, the temperature (see table 1; Eq. 12, 15, 16) and the MPs concentration in the ambient water (Eq. 17). All physiological rates (i.e clearance rate, filtration rate, pseudofaeces and faeces production rate) also depend on the environmental temperature and the structural volume $V^{2/3}$ and thus of the mussel's fresh tissue mass. Additionally, according to Catarino et al. (2018) the mean number of MPs/individual in *Mytilus spp*. is weight dependent and an allometric relationship was observed between the number of MPs and the mussel's wet weight. Furthermore, Qu et al. (2018) found a strong linear relationship between microplastic levels in the water and in the mussels. An attempt to relate directly MPs accumulation with [Chl-a], temperature, environmental MPs concentration and fresh tissue mass of the mussel was made, through a custom regression model:

$$y = b1 * W + b2 * exp\left(\frac{1}{T}\right) + b3 * \frac{C_{env}}{[Chl-a]} \quad \text{(Eq. 18)}$$

where y (particles/individual) is the response variable and represent the predicted MPs accumulation by the mussel; W (g) the mussel's fresh tissue mass, T (K) the sea surface temperature. $[Chl-a]$ and C_{env} are the concentrations of chlorophyll-a and MPs in the water respectively, which are the predictor variables. The formulation of the regression equation (Eq. 18) was derived taking into account the dependence of the feeding-excretion rates with the environmental data (see Table 1; Eq. 12-16). The values of coefficients $b1$, $b2$ and $b3$ were calculated using the nonlinear regression function (nlinfit, Matlab R2015a) which attempts to find values of the parameters b that minimize the mean squared differences between the responses y and the predictions of the regression model f

(*W, T, [chl a]*, *Cenv*, *b*). The values of *b* coefficients were fatherly tuned in order to best fit with the field data. Finally, at the simulations used for the regression analysis the environmental MPs concentration was kept constant and equal to the values referred from the literature for the two study areas (Digka et al., 2017b; Van Cauwenberghe et al., 2015; see section 2.1 'Study area and validation data' above).

3. Results

3.1 Growth simulations

The growth simulations of *M. edulis* and *M. galloprovincialis* for North Sea and Ionian Sea are shown in Figure 9 and Figure 10 respectively. At North Sea implementation, the X_k was tuned to a constant value: X_k=11.3 mg chl-a m^{-3}. The relatively high value of X_k in the North Sea can be attributed to the Chl-a variability. The fitted value is much higher, as compared to the one (X_k=3.88 μg chla l^{-1}) found by Casas and Bacher (2006) in productive areas of the French Mediterranean shoreline (average chl-a concentration 1.45 μg chl-a l^{-1} maximum peak at 20 μg chl-a l^{-1}). However, a higher value of X_k is expected in even more productive environments, such as the North Sea (average chl-a concentration 4.9 μg chl-a l^{-1}; maximum peak at ~20 μg chl-a l^{-1}). Furthermore, it has been reported that wild mussels grow considerably slower than farmed mussels (~1.7 times) (Sukhotin and Kulakowski, 1992) and thus, a higher value of X_k promotes a lower mussel growth. The resulting mussel shell length after 4 years simulation at August in the North Sea is 4.57 cm and the fresh tissue mass is 2.02 gr in agreement with other studies conducted in wild mussels (Sukhotin et al., 2007; Saraiva et al., 2012) and within the common range of lengths found in field observations (MarLIN, 2016, *www.marlin.ac.uk*) . In particular, Saraiva et al. (2012) found that after 16 years of simulation, the wild mussel of the Wadden Sea (North Sea) is 7 cm long. Also, according to Bayne and Worral (1980) a mussel with shell length 4 cm corresponds to the age of 4 years, in agreement with the current study. The model results indicate a strong seasonal pattern with higher growth during spring/summer season and lower growth during autumn/winter season. This pattern is consistent with the seasonal cycle of temperature and chl-a concentration for a typical year in the region (Fig. 1). The increase of food availability and temperature during the beginning of spring (April) promotes high growth for the mussel during a period of about 4 months. On the other hand, the decline in chl-a during summer until the end of the year and temperature decrease in

autumn, result in a lower mussel growth. Spawning events occurring at the specified time of the year are responsible for the sharp decline in mussel's fresh tissue mass, shown in Fig. 4 (Handa et al., 2011; Zaldivar, 2008).

At Ionian Sea implementation, X_k was applied as a function of Chl-a as described in 'Parameter values' section. The target diagrams showing the performance of each of the tested functions (linear: $f(x) = a * [Chl - a] + b$, where $a = 0.959$ and $b = -1.420$; exponential: $f(x) = a * \exp(b * [Chl - a])$ where $a = 0.2$ and $b = 0.567$; power: $f(x) = a * [Chl - a]^b + c$ where $a = 0.01$, $b = 3.529$ and $c = 0.480$) are shown in Figures 8a, 8b , 8c respectively. The diagrams' marks which are referred to exponential and power function of X_k are closer to the center of the diagram, indicating that one of these functions leads to the most successful simulation of mussel growth in all four areas. Based on the measured distance from the target's center, the power function gave the best results and it was adopted to Ionian Sea simulation.

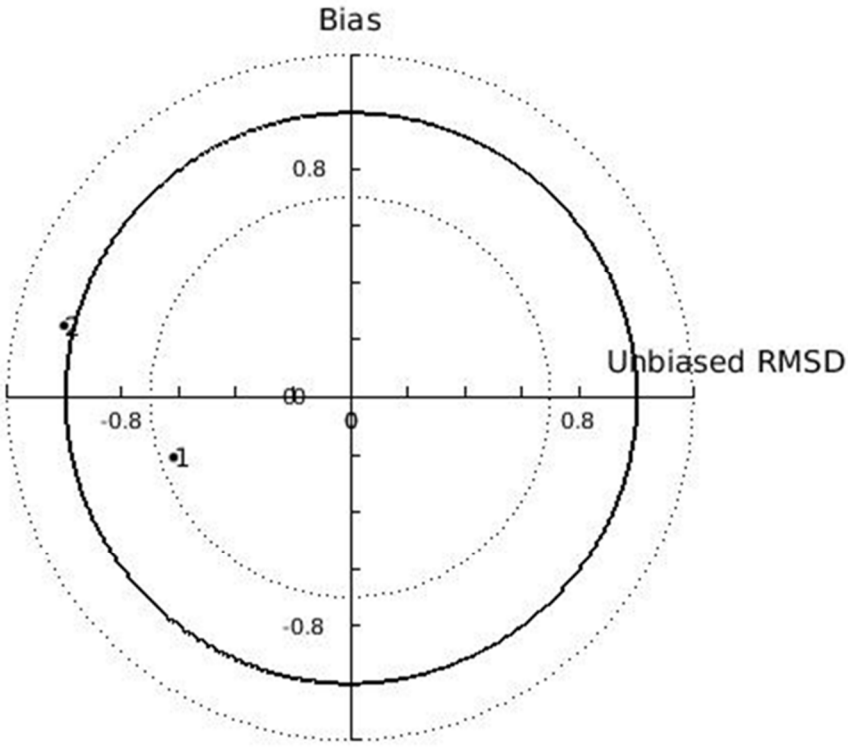

Figure 8a. Target diagram of simulated shell length (L: 1) and fresh mass tissue weight (W: 2) against field data from: Thermaikos and Maliakos Gulf (eastern Mediterranean Sea), Black Sea and Bizerte Lagoon (southwestern Mediterranean Sea), using the linear function of the half saturation coefficient: $X_k = 0.959 * [Chl - a] - 1.420$. The model bias is indicated on the y-axis while the unbiased root-mean-square-deviation (RMSD) is indicated on the x-axis.

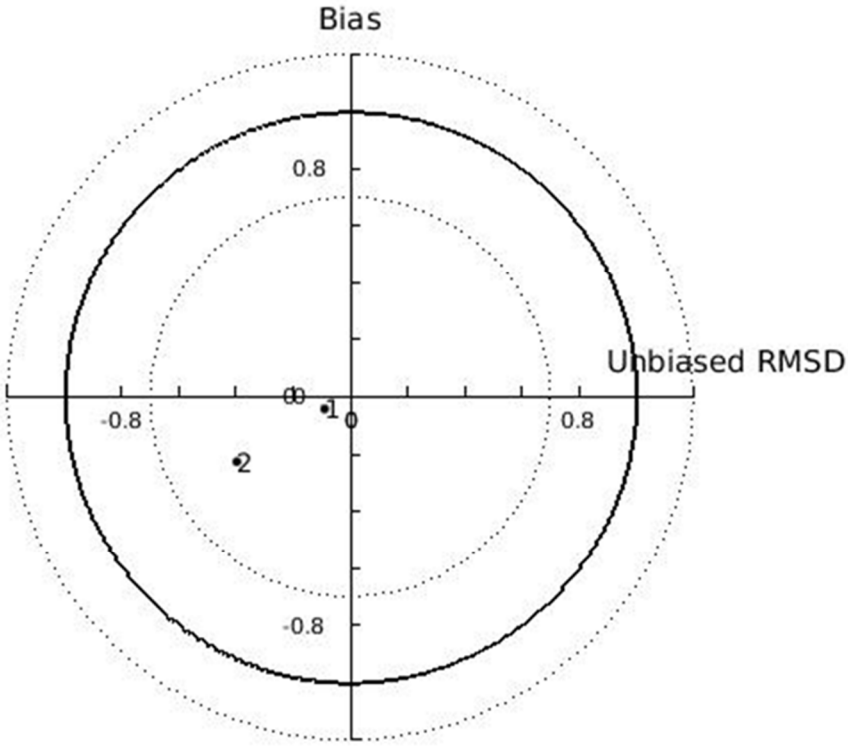

Figure 8b. Target diagram of simulated shell length (L: 1) and fresh mass tissue weight (W: 2) against field data from the Thermaikos and Maliakos Gulf (eastern Mediterranean Sea), Black Sea and Bizerte Lagoon (southwestern Mediterranean Sea), using the exponential function of the half saturation coefficient: $Xk = 0.2 * exp(0.567 * [Chl - a])$. The model bias is indicated on the y-axis while the unbiased root-mean-square-deviation (RMSD) is indicated on the x-axis. The measured distance of point 2 from the target's center is 0.46.

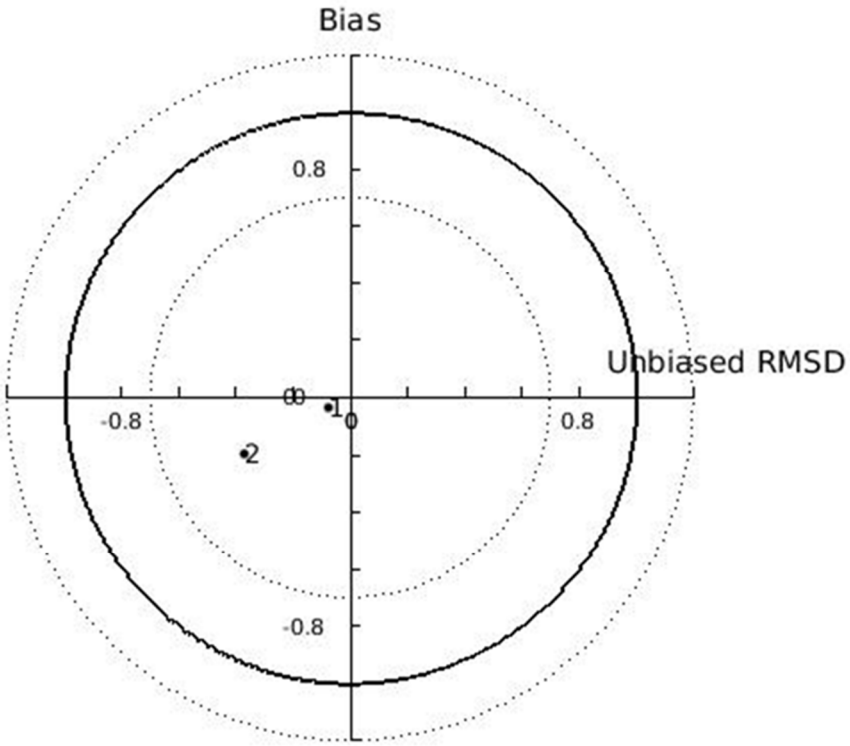

Figure 8c. Target diagram of simulated shell length (L: 1) and fresh mass tissue weight (W: 2) against field data from the Thermaikos and Maliakos Gulf (eastern Mediterranean Sea), Black Sea and Bizerte Lagoon (southwestern Mediterranean Sea), using the power function of the half saturation coefficient: $Xk = 0.01 * [Chl - a]^{3.529} + 0.480$. *The model bias is indicated on the y-axis while the unbiased root-mean-square-deviation (RMSD) is indicated on the x-axis. The measured distance of point 2 from target's center is 0.41.*

According to Theodorou et al. (2011) cultivated mussels in Greek farms, including the farms of N. Ionian Sea, are ready for the market after approximately a year, when they get about 6-6.5 cm long. The power function gave good results at the Ionian Sea simulation, as the simulated mussel reaches shell length 6.21 cm and fresh tissue mass 7.65 gr in a year.

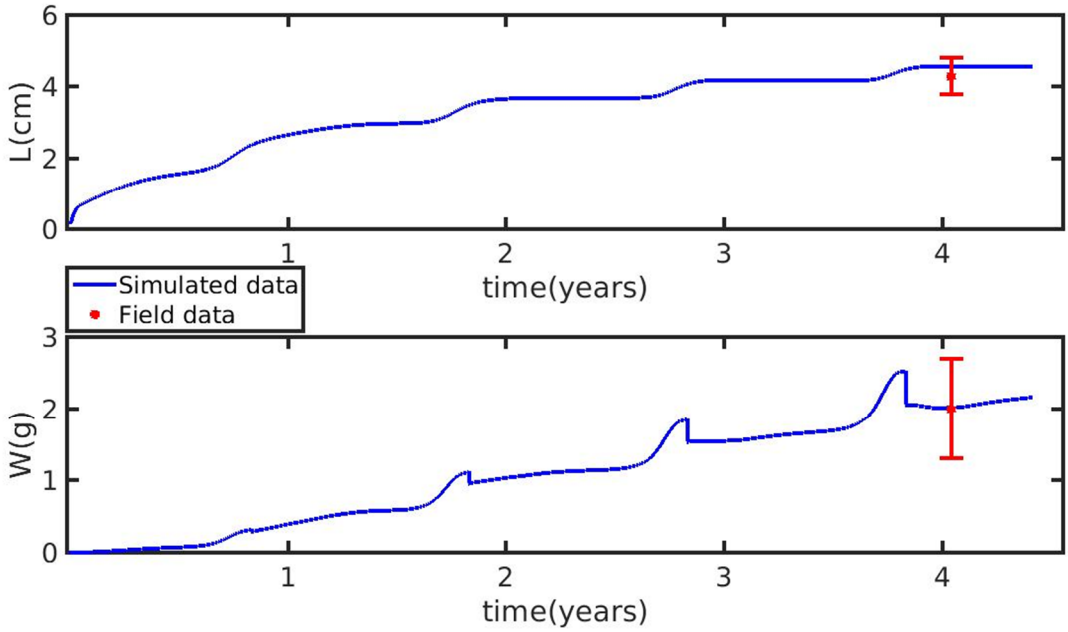

Figure 9. Simulated mussel shell length (L) (top) and fresh tissue mass (W) (bottom) against field data (red star), using chlorophyll a (chl-a) (X= [chl-a]) in the North Sea.

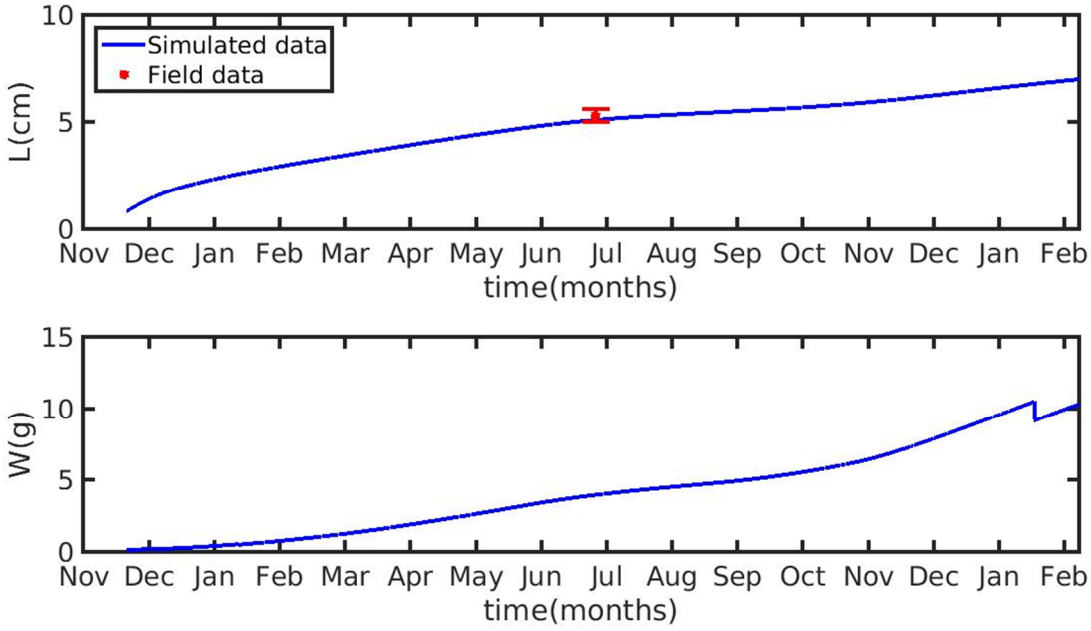

Figure 10. Simulated mussel shell length (L) (top) and fresh tissue mass (W) against field data (red star), using chlorophyll a (chl-a) (X= [chl-a]) in the Northern Ionian Sea.

3.2 Microplastics accumulation by the mussel

The results of the MPs accumulation sub-model applied in North Sea and Ionian Sea are shown in Figure 11 and Figure 12 respectively. In the North Sea, a 4-year-old wild mussel (L=4.57 cm, W=2.02 g) contains 0.52 particles / individual in agreement with Van Cauwenberghe et al. (2015) (0.4±0.3 particles/individual). At N. Ionian Sea simulation, the MPs accumulation by the cultured mussel with L =5.12 cm and W =3.96 g was found 2.27 particles/individual in agreement with field observations obtained from Digka et al. (2018) (2±0.2 particles/individual). It is worth noting that Digka et al. (2018) found up to 4 microplastics per individual, which is also in agreement with the model's results. In both regions, the model was tuned and the same values of parameters b and c in Eq. 17 were used (b=3.4 and c=2.4). The resulting values are based on the assumption that the majority of MPs are rejected through pseudofaeces and fewer through faeces production. Woods et al., 2018 found that most microplastic fibers (71%) were quickly rejected as pseudofaeces and < 1% excreted in faeces, which comes in agreement with model's result.

The small-scale (hourly) fluctuations of MPs in the mussel (wild and cultivated) reflect the random variability of the environmental MPs concentration C_{env} that was adopted, while the large-scale (seasonal) variability follows mainly the variability of the clearance rate. The variability of the food concentration and temperature determines greatly the variability of the clearance rate and hence the variability of MPs in the individual. In particular, when the chl-a concentration in the seawater is decreased (< 1 mg/m^3) and the temperature increased until a threshold value (~ 20^{o}C), the mussel's filtration rate is increased in an attempt of searching for food, resulting in a rapid increase of MPs accumulation on the mussel (see section 2.3 'Environmental data'). Moreover, as the mussel grows, its energy needs are increased and therefore the clearance/filtration rate is increased, resulting in higher MPs accumulation.

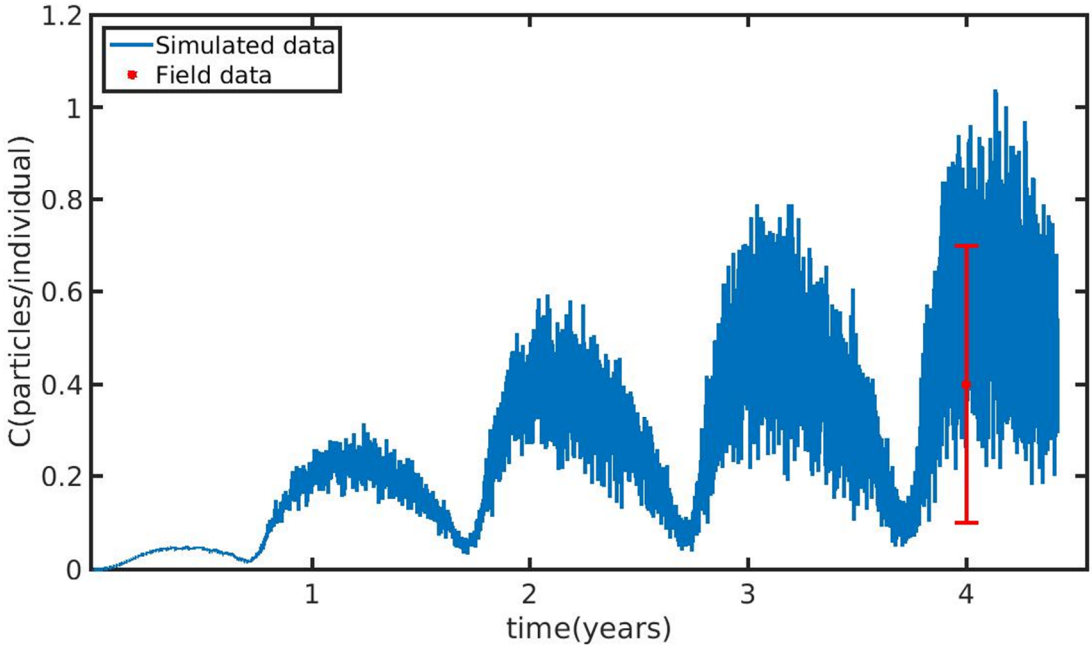

Figure 11. Microplastics accumulation by the mussel (blue line) against field data (red star), using environmental concentration of MPs (C_{env} mean value: 0.4+-0.3 particles/L) in the North Sea.

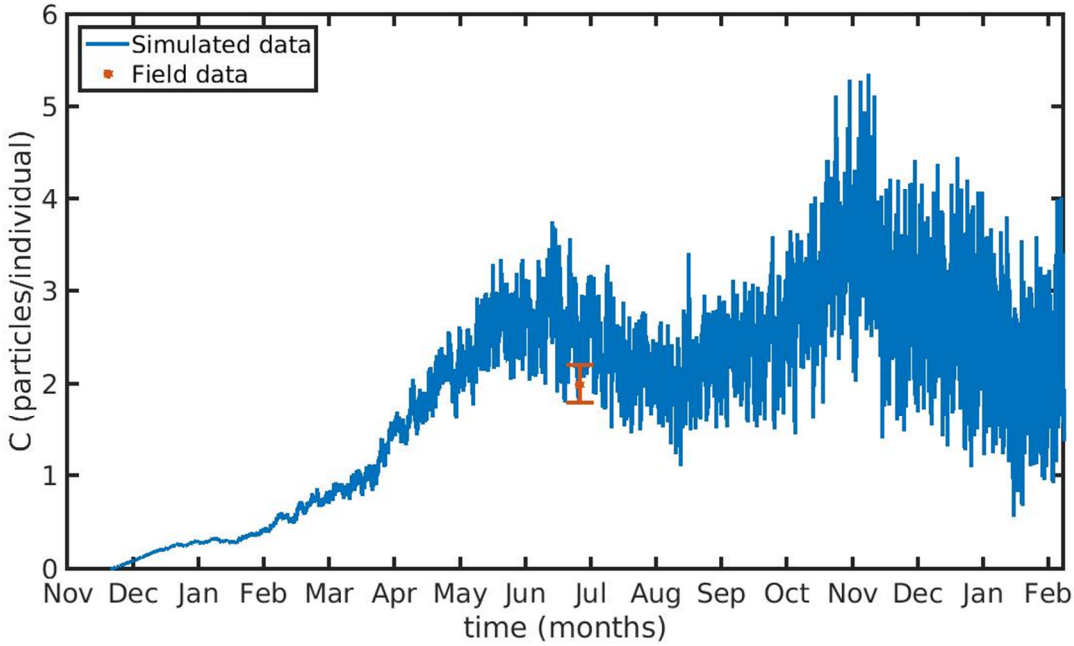

Figure 12. Microplastics accumulation by the mussel (blue line) against field data (red star), using environmental concentration of MPs (Cenv range: 0-1.61 particles/L and mean value: 0.18 particles/L) in the Northern Ionian Sea.

The time needed to clean mussel's gut from the MPs load for both areas is shown on Figure 13. At both areas the cleaning follows an exponential decay in agreement with Woods et al. (2018). However, the cleaning is more rapid in N. Ionian Sea despite the fact that the mussel had initially higher MPs concentration than the North Sea simulation. In particular at N. Ionian Sea simulation the mussel was cleaned by 90% after 8 hours while at North Sea simulation after 11 hours. This is related to the lower Chl-a concentration found in the N. Ionian Sea which leads to increased clearance rates, making all feeding processes more rapid.

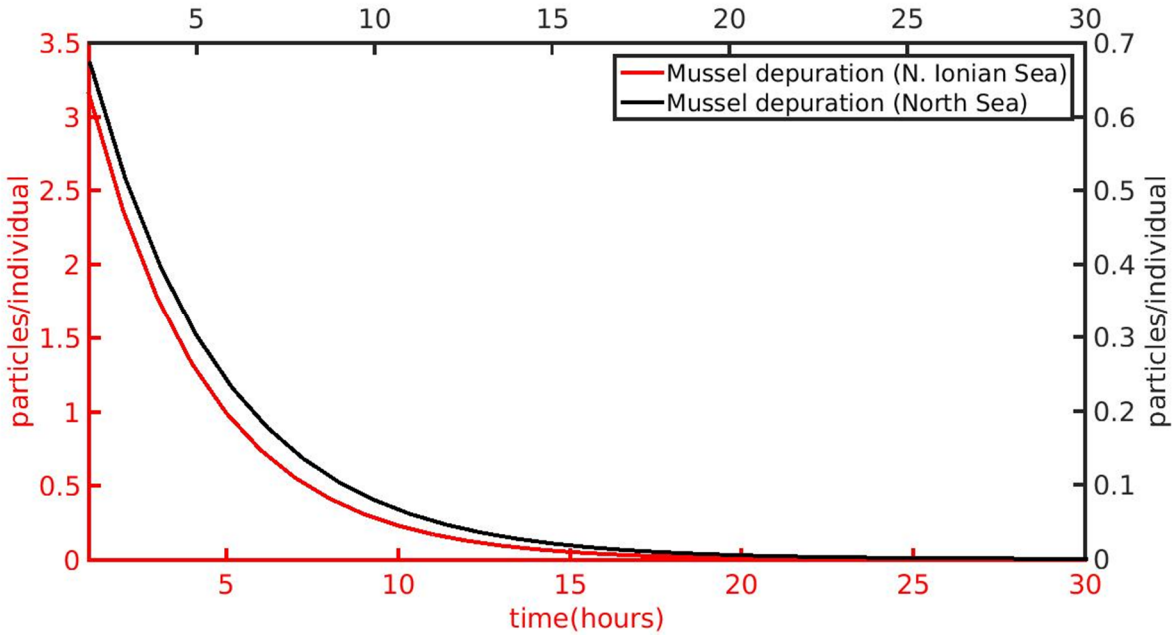

Figure 13. Depuration phase of the cultured (red line) and wild (black line) mussel using zero environmental concentration of MPs (C_{env}=0) after 1 year and 4 years of simulation time at the Northern Ionian Sea and North Sea respectively.

3.3 Regression analysis results

An estimate of the MPs accumulation in mussel as a function of environmental variables [Chl-a], temperature, MPs concentration) and the fresh tissue mass of the mussel was obtained, through least-squares fit to the model simulated MPs accumulation. The predicted MPs accumulation for both N. Ionian Sea and North Sea, as predicted by the nonlinear regression model (Eq. 18), is shown in Figure 14, together with the model's output for MPs accumulation (C) and the observed values of MPs (field data) in the mussel. The obtained function fits quite satisfactorily to the simulated MPs accumulation by the cultivated and wild mussel. The estimated coefficients of the regression model (Eq. 18) are: b1= 0.3229, b2= -0.638 and b3= 30.370. The confidence intervals for the estimated coefficients (*b1, b2, b3*) are small enough which indicates an accurate estimation of them. Based on the results, the regression model (Eq. 18) seems to predict accurately the observed MPs accumulation by both the cultivated mussel in the N. Ionian Sea and the wild in the North Sea.

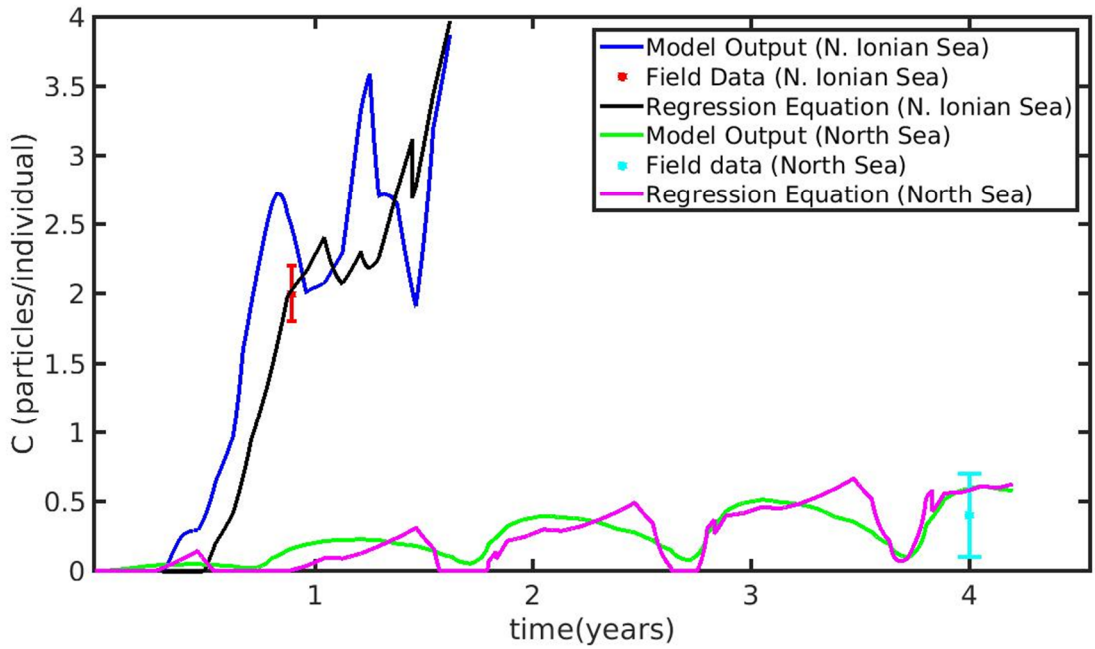

Figure 14. Nonlinear regression model (Eq. 18: $y = b1 * W + b2 * exp\left(\frac{1}{T}\right) + b3 * \frac{C_{env}}{[Chl-a]}$) (black line: N. Ionian Sea; purple line: North Sea), Model's output (blue line: N. Ionian Sea; green line: North Sea) and Field observation ± SD (red star: N. Ionian Sea; cyan star: North Sea) for MPs accumulation by the mussel.

3.4 Estimation and uncertainty of microplastics accumulation by mussels at Thermaikos Gulf

To quantitatively investigate the MPs accumulation and its uncertainty by the cultured mussel of Thermaikos Gulf, a series of simulations was performed with different environmental MPs concentration. The MPs concentration of the sea surface water was set to take constant values from 0.01 to 0.4 particles/L with a time step of 0.02 particles/L, resulting in total 20 model runs. Following the same approach used in the other two areas, the values were corrected by a factor (equal to 6.18) according to Kooi et al. (2016), in order to represent the MPs concentration of the upper sea layer (0-5m), where the mussels usually live. The mean value and standard deviation from all model results were then calculated, as shown in Figure 15.

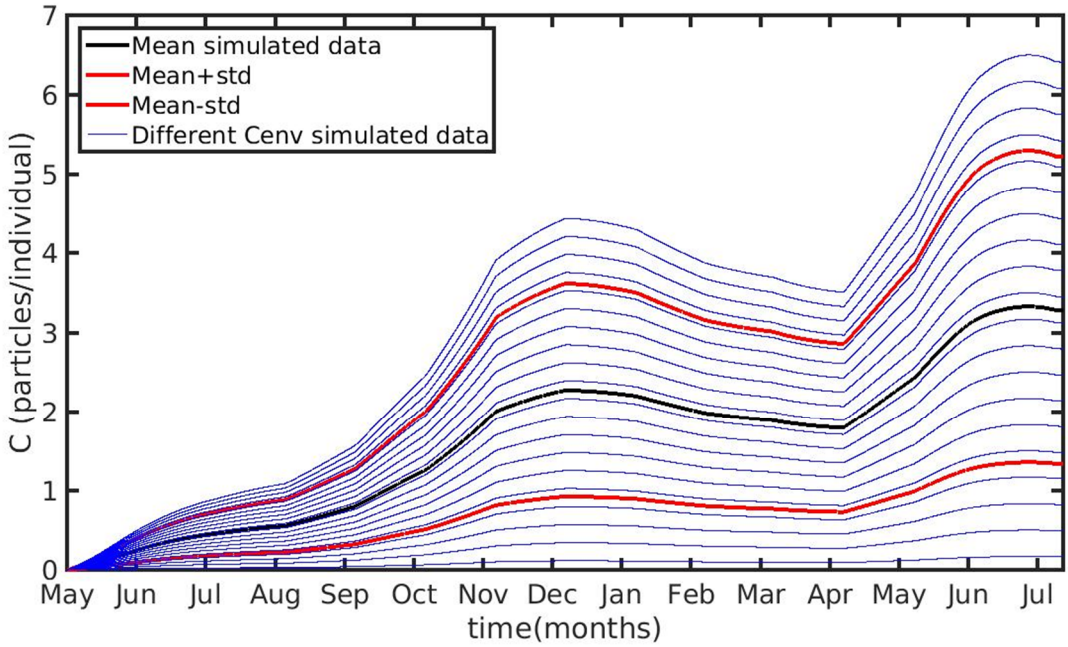

Figure. 15. Estimation of microplastics accumulation by the mussel of Thermaikos Gulf. The blue lines are the 20 model runs with each different constant value of environmental microplastic concentration (0.01-0.4 particles/L); the black line indicates their computed mean value while red lines indicate their mean value ± standard deviation.

The mean MPs accumulation by the cultured mussel of Thermaikos Gulf (mean shell length ~ 7cm and fresh tissue mass ~12g) after approximately 1 year simulation time, was estimated at 3±2

particles/individual when the environmental MPs concentration increased from 0.01 to 0.4 particles/L. The variability of the simulated MPs accumulation observed in each model run is attributed to the variability of the feeding rate (i.e filtration rate) which is highly dependent on the available food density (i.e chl-a concentration). Specifically, as shown also in section 3.3 ('Regression analysis'), the MPs accumulation on the mussel is inversely proportional with the chl-a concentration. Thus, when the chl-a concentration in the ambient seawater is decreased, the MPs load in the mussel tend to increase, because the mussel's filtration rate is increased (see Figure 7 environmental data of Thermaikos Gulf). Furthermore, the simulations at Thermaikos Gulf showed that the relationship of MPs concentration between mussels and their ambient seawater is proportional, and hence when the concentration of seawater increased led to increasing MPs accumulation by the mussel.

4. Discussion

A DEB-accumulation model was developed and tuned against data from the Northern Ionian Sea and the North Sea, to study the MPs accumulation by the cultured *M. galloprovincialis* and the wild *M. edulis*, as they grow in different environments. The 2 environments showed differences in Chl-a fluctuations and maximum values, having an impact on the mussel's growth. In the N. Ionian Sea simulation, the function of the half saturation ($X_k = a \cdot [Chl - a]^b + c$) successfully captures the physiological responses and thus the growth rate of the mussel. In the current study, a demonstration of this method was conducted leading to a DEB growth model robust enough with a sufficiently generic nature for the accurate simulation of the mussel growth in environments similar to the Ionian Sea. Bourles et al. (2008) suggested for oyster growth (*Crassostrea gigas*) that a seasonally varied half saturation coefficient could improve the accuracy of the food quantifier because seawater composition is closely related to season. A future study including more data could result to more generic formulations for the site-specific parameter X_k, so that could be applied in several areas of interest where field growth data are absent and/or simulation of mussel growth in 2D space.

The MPs accumulation sub-model predicts accurately the estimated MPs contents in the mussels of both studying areas. The MPs accumulation by the cultivated mussel (fresh tissue mass 3.96g) originated from the N. Ionian Sea with mean C_{env}=0.18±0.1 particles/m^2 (or particles/L, see 'Study areas and Validation data'), is 2.27 particles/individual and the wild mussel (fresh tissue mass 2.02g) from the North Sea with mean C_{env}=0.4±0.3 particles/L is 0.52 particles/individual. If these concentrations are expressed per gram of wet tissue of mussels, it is obvious that the cultivated mussel has about twice MPs load (0.57 particles/g w.w) comparing with the wild mussel (0.26 particles/g w.w), despite the much lower (~half) environmental MPs concentration (C_{env}) in the N. Ionian Sea, as compared to the North Sea. This could be attributed to the abundance of Chl-a

in the North Sea, which is the major source of food diet for mussel and thus high concentrations of Chl-a contributes to a reduction of filtering activity. Riisgard et al. (2011) found that the threshold of algal concentration for reduction of the filtration rate due to incipient saturation lies between 5000 and 8000 cells/mL, equivalent to 6.3 and 10.0 μg chla/L, which is the case at North Sea. Moreover, at Ionian Sea simulation the production of pseudofaeces is zero for almost 7 months (from April to October) when [Chl-a] has minimum values (<1 μg chl-a/L). Van Cauwenberghe and Janssen (2014) found that cultivated *M. edulis* from North Sea contained on average 0.36±0.07 particles/g w.w at time of human consumption, a higher value than the value found in this study for the wild mussel of the North Sea (0.26 particles/g w.w.). This suggests that farm systems can be a constant source of MPs to mussels, highlighting the importance of the biological risks associated with microplastic long-term exposures to seafood industry and its management (Santana et al., 2018). The model also successfully simulates the time needed for the gut clearance of both cultured (North Ionian Sea) and wild (North Sea) mussel as the mussel has been cleaned at 90% after almost 8 and 11 hours respectively when MPs contamination is removed from their habitat. This is in line with a series of studies which demonstrated that the depuration time varies between 6 and 72 hours depended on several factors including, species, environmental conditions (Bayne et al., 1987), and size and type of MPs (Ward and Kach, 2009; Woods et al., 2018).

Despite the good performance of the model against the available validation data, the model suffers by specific limitations. The assumption that the mussel has the same filtration rate for all particles independently their chemical composition, size and shape, is a simplification. However, through the model, a particle selection by the mussel is implied based on particle type illustrated by a faster rate for egestion in pseudofaeces (b=3.4) and faeces (c=2.4) production. Through an investigation of faeces and pseudofaeces produced by wild mussels in the laboratory while acclimated, Zhao et al. (2018) found that the length of MPs was significantly longer in pseudofaeces than in the digestive gland and faeces. Van Cauwenberghe et al. (2015) found that larger sized (15–500 μm) MPs were detected in mussel's faeces compared to mussel's tissue (20–

90 μm). In addition, smaller size MPs seem to take up a larger proportion in mussels compared to the surrounding environmental media (Li et al., 2018; Qu et al., 2018; Digka et al., 2017b). As an example, the smaller MPs (<1 mm) account for 62.3%, 96.9%, 100% in seawater, sediments and mussels from the N. Ionian Sea respectively (Digka et al., 2017b). In a future work this selection pattern regarding size, could be simulated by suitable preference weights among different MPs sizes. This will increase the ecological relevance and improve the mechanistic understanding of MPs egestion (Rist et al., 2018).

Moreover, the assumption that contamination by MPs does not affect the energy budget might also be a simplification as this is a subject currently under investigation. Van Cauwenberghe et al. (2015) found that the MPs exposed mussels showed increased energy consumption compared to control organisms but this was not reflected in the energy reserves of the exposed organisms, implying that mussels could adjust a defensive mechanism against high quantities of suspended particulate matter (Ward and Shumway, 2004). In contrast, other authors who contaminated organisms (e.g. oysters) with high and unrealistic concentration of MPs in an attempt of predicting future effects suggested a significant shift of energy allocation from reproduction to structural growth, and elevated maintenance costs, which is thought to be caused by interference with energy uptake (Sussarellu et al., 2016). Moreover, Gardon et al. (2018) showed that MPs significantly impact the assimilation efficiency and more broadly the energy balance of oyster *P. margaritifera*, with negative repercussions on reproduction.

The present study is also limited by scarce data regarding the validation of MPs accumulation by the mussel and MPs concentration in surrounding waters, since MPs and their environmental impact are a relatively recent subject of study and the existing knowledge of the spatial and temporal distribution and abundance or effects on biota are still quite limited (Law and Thompson, 2014). In a future study the model should be validated against more frequent field data regarding the MPs accumulation, with sampling of mussels among various sizes and life stages. In order to overcome the lack of environmental MPs time series, a function of randomly generated

values of environmental MPs concentration within the observed range of each area was applied. This approach is close to reality since it has been reported that MPs quantification in the water is a complicated procedure due to the influence of tide, wind, wave action and ocean currents resulting to a high variability of spatial and temporal distribution of microplastic particles even in very small scales (Messinetti et al., 2018). In a future work the DEB-accumulation model could be coupled with a MPs distribution model (Kalaroni et al., 2019) in order to overcome this limitation. Moreover, the approach followed in calculating the value of MPs concentration in the first 5 meters of the sea according to Kooi et al. (2016), resulted in a representative value of the upper layer. He found that the surface MPs concentration decays rapidly with water depth in agreement with other studies (Reisser et al., 2015; Kukulka et al., 2012). Unfortunately, there are still not field data regarding the depth profile of MPs concentration at the study areas. Knowledge of the MPs distribution, both horizontally and vertically, is essential to understand and mitigate their impact on the marine environment (Van Sebille et al., 2015).

The effect of food, temperature and MPs concentration of the water in MPs accumulation by the mussel, regarding its weight, was demonstrated by applying a nonlinear regression model (Eq. 18). This equation predicts quite accurately the simulated MPs accumulation derived from the model and the field observations in both study areas, implying a strong dependence between MPs accumulation in the organism and its weight and environmental conditions (Chl-a, T, C_{env}). The linear relationship between MPs accumulation by the mussel and the concentration of the environment raises future concern, since it is predicted that MPs abundance in the water will only rise in the future. Preliminary simulations of the DEB-accumulation model at N. Ionian Sea showed that increasing the environmental MPs concentration (0.1-0.5 particles/L) leads to increasing MPs accumulation by the mussel up to a maximum of 5.5 particles/individual.

The pilot study at Thermaikos Gulf illustrated the proportional relationship between the MPs accumulation by the mussel and the MPs concentration in the environment. According to the investigating scenarios, the cultivated mussels are severely contaminated and thus more

investigation is urgently needed regarding the marine microplastic pollution, considering that in this area the majority of the cultivated mussels production of Greece is operating.

Given that sampling of mussels is usually easier than sampling water for MPs from an area (Karlsson et al., 2017; Brate et al., 2018), this regression model presented here can be used for the estimation of contamination in the water when sea temperature, chlorophyll-a and the mussel weight and concentration of MPs in mussels' tissue are known, using the mussels as bioindicators. Mussels have been proposed as bioindicators for marine microplastic pollution (<1mm) in many occasions, although efficient gut clearance and selective feeding behavior of mussels limit their quantitative ability (Lusher et al., 2017; Brate et al., 2018; Beyer et al., 2017; Fossi et al., 2018, Li et al., 2019). Considering that the accumulation of MPs by the mussel is site-depended, models like the one described in Eq. 18 that besides the MPs concentration in the water also take into account characteristics of the environment that are crucial for the way that mussels accumulate MPs, are moving towards the direction of global methods that will allow comparisons between different environments. However, the method described should be tested in more environments with more frequent field data to be able to provide secure results.

5. Conclusions

The DEB-accumulation model presented here, describes dynamically the uptake and excretion rate of MPs by cultivated and wild mussel during their life cycle, taking into account the physiological responses to the variability of specific aspects of the mussel's environment (Temperature, Food Density, MPs concentration). Along with field studies and monitoring activities, model tools are necessary to understand the fate transport of contaminants such as MPs and to assess their impacts on communities and ecosystems. The quantitative knowledge of uptake, excretion and depuration processes of MPs in the organism is needed to predict the fate, accumulation and biomagnification of MPs along the food web. Since mussels are an important food source for many marine species and humans, the trophic transfer of MPs could affect other species and raise human health concerns.

The depuration time needed for mussel to clean from MPs contamination could be useful information for the mussel farms in order to implement techniques of transporting mussels in a free of MPs environment before placing them in the market. Finally, the developed regression model can be applied instead of the DEB-accumulation model as a simplified model without requiring knowledge of all the DEB variables and parameters and highlights the strong dependence of MPs uptake, accumulation and excretion rates by the mussels to the seasonal variability of the environmental data. In a future work the DEB-accumulation model has the potential to be coupled with MPs distribution models that are currently being developed and validated in the framework of CLAIM project (EU Horizon 2020) and eventually lead to an integrated dynamic presentation of MPs uptake by the mussels.

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