



The effect of the quality of diet on the functional response of *Mytilus galloprovincialis* (Lamarck, 1819): Implications for integrated multitrophic aquaculture (IMTA) and marine spatial planning



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ABSTRACT

The integrated multi-trophic aquaculture (i.e., IMTA) is a practice combining organisms with different trophic levels with the final purpose of transforming the continuous waste of food by targeting species into nutrient input for other non-target species. This practice very often involves filter feeders, such as bivalves, by the use of which bioenergetics budgets are strongly influenced by the quality and quantity of different foods. However, to date, scant information is available, to really understand the rebounds of food availability on the growth performances of these harvested biomasses in the natural environment. By choosing the mussel *Mytilus galloprovincialis* as a model, this study aims to (1) characterize the functional response of the species to define all parameters related to food intake strategies and (2) to investigate how responses change as a function of varying food sources. Laboratory procedures have been designed to evaluate the clearance rates (CR) and assimilation efficiencies (AE) of *M. galloprovincialis* with varying food concentrations, while different diets (i.e., seagrass, phytoplankton, and pellets) have been provided to investigate how differently they reach saturation. Results show that in the presence of phytoplankton and seagrass as food sources, the feeding strategies of *M. galloprovincialis* follow a II-type Holling's curve, while it shows a I-type Holling's curve when pellet food is provided. Investigating the behavioural components of functional responses may improve our ability to predict where to place shellfish cultures, as it may be useful in the context of IMTA management and in addressing siting studies.

Statement of relevance: Our paper focuses on a question central to understanding and predicting the likely impacts of one among the most important human economic activity like the aquaculture in coastal habitats. The main question deals with the possibility to combine experimental procedures with the new mechanistic functional trait based bioenergetic models in order to effectively predict life history traits of cultivated species.

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1. Introduction

Coastal habitats are characterized by high concentrations of nutrients and organic matter and as such, they represent elective areas for the development of shellfish farms such as bivalve molluscs. In a context of marine spatial planning, integrated multi-trophic aquaculture (i.e., IMTA; Troell et al., 2003) is an increasing and largely used practice, as it combines organisms from different trophic levels with the ultimate purpose of transforming dejections and surplus food of carnivorous species into nutritive input for other non-target species (Reid et al., 2010; Troell et al., 2009, 2003). Suspension feeders such as bivalves are prominent organism species involved in IMTA (Sarà et al., 2012, 2009) and in some coastal areas, they are subjected to a continuous flux of particulate organic matter of different origin (e.g., seagrass) that dilute the

energetic value of pellets and phytoplankton, which represent the main food items for those species. The recent isotopic research supports the theory that suspension feeders such as bivalves are able to also assimilate, as secondary food energetic sources, fractions of refractory food coming from seagrass detritus (Cabanelas-Reboredo et al., 2009). Some studies have reported the possible effects of different types of food on the bivalve's growth through short-term experiments in the field and mesocosms (e.g., Sarà et al., 1998). Even as some predictive relationships between feeding rate and environmental factors such as, temperature, current speed, and food availability and composition, are available for the major aquaculture species, the information available is still complicated by low reliability in establishing the mechanistic link between the quality of diet and organismal growth processes. Nonetheless, increasing our mechanistic (*sensu* Kearney and Porter, 2009) ability to predict where and when organisms better exploit different types of food at varying densities may be particularly useful in IMTA. For example, such information could be useful to feed recent

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farm-scale models (Ferreira et al., 2011) or may be needed for selection of IMTA locations and optimization of layouts, to predict growth performances and site production-carrying capacity, and for assessing the potential ecological services and impacts of aquaculture operations. Here, we carried out mesocosm experiments to test the effect of three different diets (pure phytoplankton, seagrass detritus, and minced fish pellets) on the feeding behaviour of our model species, the Mediterranean blue mussel *Mytilus galloprovincialis* which represents an important economic resource for the local societies, and outside the native range area it is a highly invasive species (McQuaid and Phillips, 2000). Indeed, on a global scale, the contribution of this species to seafood production rose to about 1 million tons, with China and Spain being the most important producers (FAO, 2014). In the Mediterranean Sea, the annual production attained over 115,00 tons in 2009 mainly produced by Italy and France (as the Spanish production is mostly Atlantic), representing 32% of the marine aquaculture production and ranking *M. galloprovincialis* in the third position after Seabass and Seabream (Gazeau et al., 2014; Goffredo and Dubinsky, 2013).

The present study was thus designed to (1) study how the feeding responses of *M. galloprovincialis* changed as a function of diet quality and quantity, and (2) use such information to feed a functional trait-based model as the Dynamic Energy Budget theory (Kooijman, 2010) in order to investigate how the presence of different diets could ideally affect the growth rates of mussels cultivated under different temperature regimes (i.e., simulating six different levels, from 13 to 18 °C).

2. Materials and methods

2.1. Animal collection and maintenance

About 400 individuals of *M. galloprovincialis* (45–75 mm) were collected in February 2014 from the Faro Lake in eastern Sicily (Lat. 38°15'39" North, Long. 15°37'02" East), placed in containers with absorbent material, and cooled with dry ice, in order to keep them moist during the transport to the laboratory. Once in the laboratory, they were cleaned off epibionts and sediments, and then placed in special tanks (60 l) to allow them to acclimatize for approximately one week. They were fed daily with alga *Isochrysis galbana*, equal to 2–3% of the wet biomass (Sarà et al., 2013). Subsequently, the organisms were randomly divided into three equal-sized groups ($n = 120$; mean size (\pm SD) 4.71 ± 0.45 cm), individually labeled and randomly placed into six 300-l tanks (two tanks per each treatment maintained under 12:12 h Light-Dark regime), where they were fed ad libitum three times per day. Once acclimated, the experiments were started and the specimens were constantly fed with three diets prepared to simulate three different trophic scenarios:

1. A phytoplankton diet represented by a culture of microalgae, *I. galbana*, mimicking the natural condition of the pelagic-oceanic environment (Sarà et al., 2011) was used. To this purpose, a pure culture of *I. galbana* was prepared by inoculating a small aliquot of microalgae in beakers containing one l of pre-filtered and sterilized seawater and placed in a thermal room (constant temperature of 18 °C), where the culture was maintained under a suitable period on a light table (day-light UVA heat lamps, Model Repti Zoo, 75W; Italy). Growth of the *I. galbana* strain was ensured by adding aliquots (2 ml l^{-1}) of Walne medium and vitamins (i.e., B1 and B12; 0.2 ml l^{-1}) (FAO, 1996)

2. A solution of minced pellet food and seawater was used, mimicking the incoming eutrophic downstream waters from a farm (Sarà et al., 2012, 2009). In this regard, pellet aliquots of 6.5 g (such as those used within fish farms) were ground and dried in an oven at 60 °C for 24 h. After this period, the food was placed in a beaker glass with filtered seawater and the solution was filtered through filters with a mesh size equal to 40 μm .

3. A solution of refractory organic matter with a high C/N ratio ($>15\text{--}20$; Vizzini et al., 2003) was obtained using fresh leaves of *Posidonia oceanica*, properly dried and minced (mimicking oligotrophic

waters subsidized with highly refractory organic matter for secondary consumers). In doing so, fresh leaves of *P. oceanica* were cleaned off epiphytes and later dried in an oven at 60 °C for 24 h. Dry leaves were ground and reduced to a powder and passed through a 100 μm mesh. The solution was prepared by diluting the mince of *P. oceanica* in 1 l of seawater previously filtered (Whatman GF/F, 0.45 μm).

2.2. Routine laboratory measurements

According to the recent literature (e.g., Fields et al., 2012), in order to make negligible the effect of acclimation to each food treatment on *M. galloprovincialis* responses, we conditioned mussels to treatments for 4 weeks prior the physiological measurements. For each aquarium, 40 animals were weighed (0.001 g; Sartorius Inc.) every two weeks, measured for the umbral shell length (nearest 1 mm; Vernier caliper), and the mussels growth performances were monitored for the following two months. Before measuring the wet mass (total wet weight comprising shells) they were externally dried with paper tissue and then placed on paper tissue to air dry for about 10 min in order to minimize the contribution of water content within the mantle cavity. The growth rates for each diet have been calculated taking into account the differences in both shell lengths and wet mass measured on individual organisms at the beginning and at the end of the two months period. Once the exposure period was completed, experiments to estimate the feeding behaviour started and they consisted of exposing independent animals to increasing concentrations of food (from very low to very high; see below for details), to seek that value of ingestion rate corresponding to the saturation threshold for every type of diet. We carried out feeding rate experiments based on the classical estimates of clearance rate (Sarà et al., 2013) to obtain the corresponding ingestion rate ($\text{IR} = \text{CR} \times \text{food}$) for every diet type. Solutions at different concentrations of food were prepared as follows: starting from the same initial solution (mentioned earlier in the text under the diet preparation section), seven different dilutions (from 0.1 ml l^{-1} to 20 ml l^{-1}) were used to estimate the feeding response of *M. galloprovincialis*. In doing so, we obtained concentrations ranging between 6.40 and 10.19 mg l^{-1} for phytoplankton, between 2.68 and 20.50 mg l^{-1} for seagrass-based diet, and between 5.76 and 12.75 mg l^{-1} when pellets were provided (see Supplementary file, Table S1). Ingestion rates (IR) and absorption efficiencies (AE) were measured in individual mussels ($n = 10$). The IR (mg h^{-1}) was calculated through estimates of clearance rates (l h^{-1}) measured by placing single specimens into a 1 l beaker, while a beaker was intentionally left without animals and used as the control (Sarà et al., 2013). Solutions were constantly mixed in each beaker during the experimental phases with a magnetic stirrer, by placing them onto a stirrer plates. For each experiment the organisms were left for about 5 min, to acclimatize, before adding food, and the decrease in food concentrations was monitored over 2 h by means of an electronic particle counter (Beckman Coulter Counter, Z2), fitted with a 100 μm aperture tube and set to count particles ranging between 2 and 6.5 μm . In order to estimate the hourly maximum ingestion rate, aliquots of 20 ml from every beaker were sub-sampled at 30 minute intervals. The individual maximum clearance rate was then calculated following the equation:

$$\text{CR}(\text{l h}^{-1}) = (\text{Vol}) * (\ln C_1 - \ln C_2) / \text{time interval}$$

where Vol is the volume of the beaker used in the experiments and C_1 and C_2 are the initial and subsequent concentrations minus the decline in the cell concentrations in the control tank in each time interval (Ezgeta-Balic et al., 2011; Sarà et al., 2013; Widdows and Staff, 2006). Accordingly, the decreasing cell concentrations were converted in terms of mass concentration and the resulting maximum ingestion rate was then calculated as the maximum density of food (particulate organic matter, mg l^{-1} POM) ingested per hour.

Following the measurement of CR, the mussels were placed into new 1 l beakers containing filtered seawater and left undisturbed overnight (12 h), after which the faeces produced by each individual as separately collected and filtered onto pre-weighed glass-fiber filters (Whatman GF/F). After filtration, filter papers were accurately washed by alternating solutions of 0.5 M ammonium formate and distilled water for three times. The absorption efficiency (AE) for each treatment was then calculated using the Conover ratio (Conover, 1966), which took into account the ratio of the amount of organic matter in the faeces and that in the food. Accordingly the AE was estimated using the following formula:

$$AE = (F - E) / [(1 - E) F]$$

where F is the ratio between the dry weight (DW) and ash-free dry weight of food (AFDW), and E is the ratio between the dry weight (DW) and ash-free dry weight of the faeces (AFDW). When estimating, the filters containing the food and faeces were dried in an oven at 90 °C, re-weighed, and then placed into a furnace at 450 °C, for 4 h, after which the filters were weighed again (Ezgeta-Balic et al., 2011, Sarà et al., 2013).

2.3. Estimating the functional response and modeling of the effects on an individual's ultimate fitness

Ecological theory offers strong principles which, if used in a reliable modeling framework, are able to increase our understanding of how to predict growth performances of bivalves under varying conditions of food and temperature. Particularly the Scaled Functional Response (SFR; Holling, 1959), has been recently called as a prominent mechanism to describe how the consumption rate of a predator changes with prey density. SFR involves information about the trade-off between two important behavioural components, i.e. searching and handling, together being used as an estimate of the ability to get food from the environment (Lang et al., 2012). In some species, handling time may prevent or limit subsequent food acquisition; however in bivalves, both processes occur simultaneously (Saraiva et al., 2011) and in the particular case of filter feeder modeling, several studies have highlighted the need to investigate the magnitude of the half saturation coefficient -which correspond to the searching:handling ratio- to improve our ability to predict where to place shellfish cultures (e.g., Ren et al., 2012).

Here, we estimated the differences in the functional responses (f) of *M. galloprovincialis* as a proxy of different intake strategies, under natural environmental conditions. Specifically, the f values for each diet were estimated according to Sarà et al. (2014), according to which $f = x / (X_k + x)$ where x was the food density (mg POM l^{-1}), while the parameter X_k corresponded to the concentration where the value of the ingestion rate was equal to half of the maximum. Such information together with the manner in which *M. galloprovincialis* managed the energy from different types of food (i.e., IR and AE), have been used in combination with the bioenergetic parameters of the species (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet), based on the recent Dynamic Energy Budget modeling (DEB, Kooijman, 2010), to perform simulations aimed to investigate the potential variations in growth performance and the individual ultimate fitness. Certainly, being based on individual bioenergetics, the DEB theory provided a general framework that could be utilized to describe physiological mechanisms by which temperature and food availability combine to drive growth and reproductive performances in organisms (Monaco et al., 2014). The present DEB model allowed us to quantify some of the most important response variables, such as, Maximal Habitat Individual Size (MHIS; cm), Maturation Time (MT; day), which refers to the time required to reach the minimal size threshold for sexual maturity and first spawning, the Number of Reproductive Events per life span (RE; #), and the Total Reproductive Output (TRO, #), which is the total number of eggs per life

span (please see Montalto et al., 2015 for further details). To incorporate the effects of different food sources, we performed a sensitivity analysis, to predict the dynamics of the mussel's energetic fluxes by combining the experimentally-derived feeding functional responses with six different levels of body temperatures (from 13 °C to 18 °C, chosen as representatives of the average annual BT experienced by this species throughout its entire biogeographic distributional range; Marshall and Gofas, 2015) constantly maintained throughout the life span. For the sake of simplicity, models were run by putting in a fixed food density (i.e., $10 \text{ mg l}^{-1} \text{ h}^{-1}$) such that the f value was always above a threshold of 0.5 for each diet, and the entire simulated period was cut off when the individual growth reached commercial size (here fixed to 5 cm according to the Food and Agriculture Organization (FAO) statistics).

3. Statistical analysis

A two-way analysis of variance (ANOVA; Underwood, 1997) was carried out to test the differences in the feeding responses and in the assimilation efficiencies of *M. galloprovincialis* fed with diets varying both qualitatively and quantitatively. Both factors (DIET, three levels and CONCENTRATION, seven levels) were chosen as fixed factors and ten replicates were used for each experimental session. The assumption of the homogeneity of variance was tested a priori by mean of the Cochran's test. When significant differences were observed, the appropriate means were compared using the Student-Newman-Keuls (SNK) tests (Underwood, 1997). ANOVA was carried out by using the GMAV software (version 5.0). Also, to establish whether the relationship between prey density and the number of prey eaten is best described by type I, a type II or a type III response, a phenomenological approach focusing on the overall shape of the response curve was used. Specifically, we compared the relationship between food density and the number of cells eaten for each diet by means of an integrated package (frair) for functional response analysis in R (v. 3.0.3), as recently done in Paterson et al. (2015). Lastly, the differences in the shell and mass growth of *M. galloprovincialis* exposed to different food sources at the end of the two-month period, were instead tested by mean of a PERMANOVA due to the ease of use, with unbalanced design and to avoid the usual normality assumptions (Perkol-Finkel et al., 2012). PERMANOVA was carried out by using the PRIMER software (version 6.0).

4. Results

The IR values ranged between $3.94 \pm 0.30 \text{ mg l}^{-1} \text{ h}^{-1}$ with pellet diet and $4.85 \pm 0.34 \text{ mg l}^{-1} \text{ h}^{-1}$ when the mussels were fed with *P. oceanica* detritus, and the ANOVA showed significant differences between the IR when response with the pellet was compared with the IR estimated in the presence of seagrass and algae diets ($p < 0.05$ and $p < 0.01$ for *P. oceanica* and *I. galbana*, respectively; Fig. 1 and Table 1). AE differed significantly ($p < 0.01$) and contrary to IR, the lower value was estimated for the *P. oceanica* diet ($AE = 0.31 \pm 0.02$) compared to the other two diets (mean $AE = 0.51 \pm 0.01$) (Fig. 2 and Table 1). Overall the IR not changed significantly when different phytoplankton concentrations were provided, while significant differences resulted among IR of organisms fed with seagrass and pellet; indeed as showed by the pairwise comparison, *Mytilus*' responses differed respectively at lowest and intermediate levels of the seagrass diet and from the intermediate to the highest concentrations of pelleted food provided in this study (Table S2). No evident patterns resulted by comparing AE at different concentrations of pellet and phytoplankton while they differed significantly when elevated concentrations of seagrass-base diets were available (Table S2). The phenomenological analysis revealed significant evidences of a Holling type II curve in the presence of algae ($p < 0.05$) and seagrass ($p < 0.001$), while mussel's SFR met the Holling type I curve with the pellet ($p < 0.001$). The saturation coefficient was about 6 mg l^{-1} with algae and seagrass and 8.04 mg l^{-1} with pellet

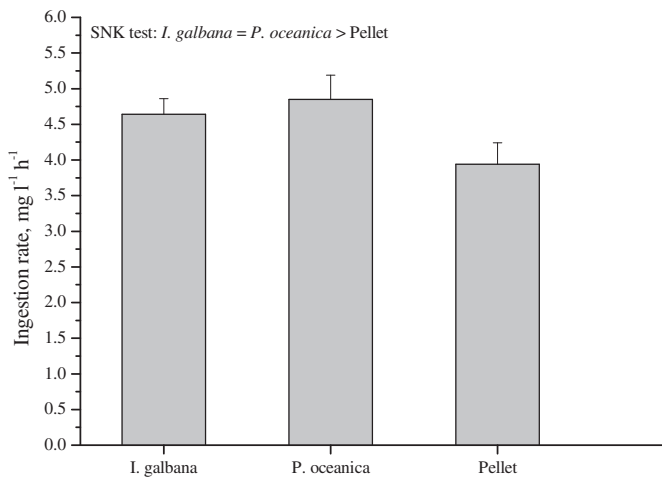


Fig. 1. Ingestion rates (IR, mg l⁻¹ h⁻¹) of *M. galloprovincialis* experimentally derived under three different trophic conditions.

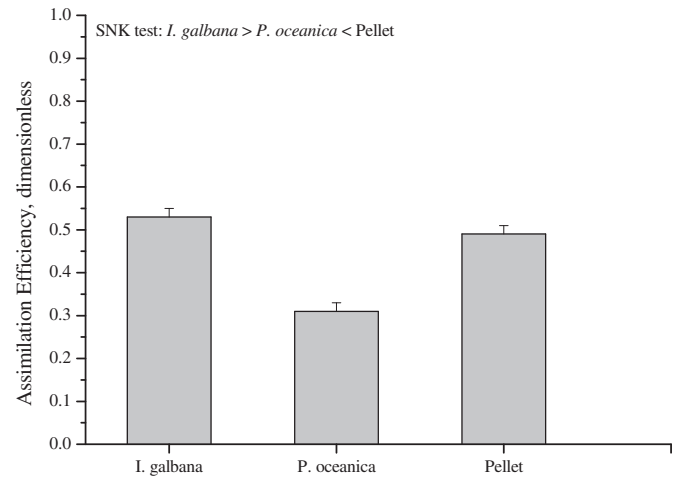


Fig. 2. Assimilation efficiencies (AE) of *M. galloprovincialis* experimentally derived under three different trophic conditions.

(Table 2). Our analysis did not show significant differences (PERMANOVA, $p > 0.05$; see Supplementary file Table S3) in the growth rate of individuals fed with the three different diets. Indeed, within the experimental period *Mytilus* increased on average (\pm S. E.) its size of 1.02 ± 0.11 mm, 1.06 ± 0.09 mm, and 1.41 ± 0.28 mm when fed with *I. galbana*, *P. oceanica*, and pellet, respectively. On the other hand, the estimated increase in weights was similar for organisms fed with *I. galbana* and pellet, being equal to 1.21 ± 0.17 g, and 0.91 ± 0.17 g while it resulted significantly lower for organisms fed with seagrass (i.e., 0.47 ± 0.07 g). Finally, DEB allowed us to get information of rebounds of the three different diets and the SFR values. Indeed, as shown in Figs. 3–5, for the entire range of simulated body temperatures, the amount of time needed to reach the commercial sizes varied between about four years in the presence of both phytoplankton and pellet and up to eight years in case of seagrass. Also, changes in the reproductive features were detected within the simulated period; specifically under all the trophic conditions (food quality) tested, the results showed that when the BT increased, *M. galloprovincialis* was able to invest more energy (about 25%) to reach its maturation size and to reproduce (Table 2). Such a pattern was observed when the temperature range fell between 13 °C and 17 °C, while a failure was evident under the maximum simulated body temperature.

5. Discussion and conclusions

According to previous studies, our results showed that *M. galloprovincialis* might be able to ingest food—in the form of particulate matter—of different origins (Manganaro et al., 2009; Sarà, 2006), although, as shown here, the response could be different depending on the food source provided. Indeed, based on the significantly higher

ingestion rates, our results suggested that the mono-algal and the seagrass diets were the best energy providers for the mussel's intake compared to pelleted food. The increase in ingestion rates on increasing food densities could be partly explained by the differences in dimension of the microalgae species (4–7 μ m), which perfectly match the size range at which they realise maximum filtration efficiency of microalgal component (Dame, 1993) and confirmed a non-selective filter-feeding of this species when fed with algae (e.g., Vahl, 1972). Also, when analysed in terms of optimal foraging (sensu Lehman, 1976) mussels would benefit from the constancy of higher IR to cope with the low quality diet. After all, the existence of an upper limit to ingestion in bivalve molluscs has been generally acknowledged (Bayne and Newell, 1983; Navarro et al., 1992) and demonstrated, either in bivalves fed with algae (Sarà et al., 2013; Saraiva et al., 2011) or in those fed with vascular plants (Arambalza et al., 2010; Charles, 1993).

Table 2

Reports the experimental results for each diet and is used as the input for the modeling exercise and lists out the outputs obtained through the DEB simulations carried out at varying organismal body temperatures (BT). IR, ingestion rate; AE, assimilation efficiency; Xk, saturation constant; f , simulated functional responses at constant food availability (density). For each simulated body temperature (BT) we modeled (i) the days to reach commercial size (Comm. Time), (ii) size of first reproduction (MT), and (iii) the reproductive ability in terms of numbers of eggs (TRO) produced and of number of spawning events (RE).

Diet	IR	AE	f	Density	Xk
<i>I. galbana</i>	8.07	0.53	0.63	10	5.75
<i>P. oceanica</i>	7.88	0.31	0.64	10	5.57
Pellet	8.48	0.49	0.55	10	8.04

BT	Diet	Comm. time	MT	TRO	RE
13	<i>I. galbana</i>	1496	947	311,623	4
	<i>P. oceanica</i>	3382	1892	723,691	11
	Pellet	1811	1121	358,101	5
14	<i>I. galbana</i>	1427	903	319,423	5
	<i>P. oceanica</i>	3225	1804	728,320	14
	Pellet	1727	1069	444,886	7
15	<i>I. galbana</i>	1362	862	331,624	6
	<i>P. oceanica</i>	3079	1722	748,040	17
	Pellet	1648	1020	416,389	8
16	<i>I. galbana</i>	1301	824	346,037	7
	<i>P. oceanica</i>	2942	1646	709,547	19
	Pellet	1575	975	403,435	8
17	<i>I. galbana</i>	1245	788	361,832	8
	<i>P. oceanica</i>	2814	1574	689,576	21
	Pellet	1507	933	398,806	10
18	<i>I. galbana</i>	1195	755	0	0
	<i>P. oceanica</i>	2695	1508	0	0
	Pellet	1443	893	0	0

Table 1

ANOVA carried out on IR; and AE under different diet regimes and at varying food concentrations; Sqrt(X + 1) = data transformed; ns = not significant.

Source	CR			AE	
	DF	MS	P	MS	P
DIET	2	0.766	**	0.9914	***
CONC	6	2.6371	***	0.212	***
DIET \times CONC	12	0.7327	***	0.0483	***
RES	189	0.1535		0.0152	ns
TOT	209				
Cochran's test			Sqrt(X + 1)		ns

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

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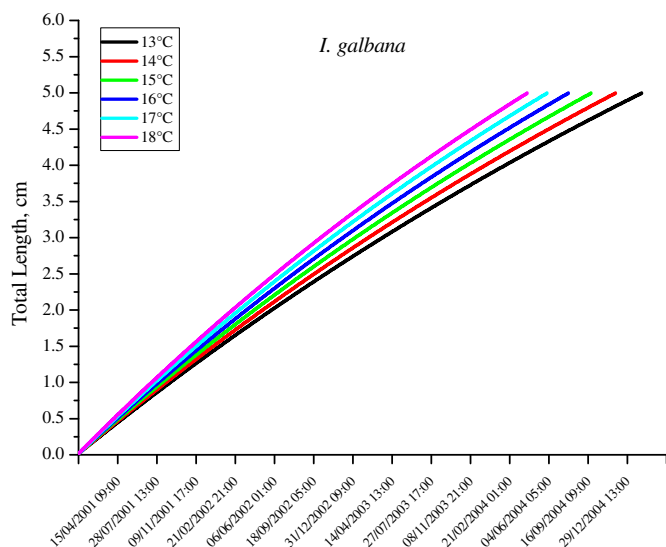


Fig. 3. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with *I. galbana*.

However, similar to what was observed by several authors (Martínez-Fernández et al., 2004; Saraiva et al., 2012), such as having higher ingestion rates, it does not imply that all energy ingested would be also digested. Indeed as showed in Fig. 2, organisms fed with *P. oceanica* increased their ingestion rates and this resulted in significant lower absorption efficiency when compared with the phytoplankton diet. Such a fact could be a probable consequence of constraining the capacity of the digestive system and the residence time of food (Navarro et al., 1994). As showed for other bivalves, by assuming the same probability to process different types of food, differences in the overall retention relate not to gut passage times but to the processing and release strategies of the food material (Saraiva, 2014). Indeed, it is likely that the low digestibility (low nitrogen content) of the phanerogamic organic matter and the high content of phenolic compounds could be the two primary factors that affected the organismal functioning and reduced the efficiency of the digestive enzymes (Charles et al., 1996). Also, our results showed that when

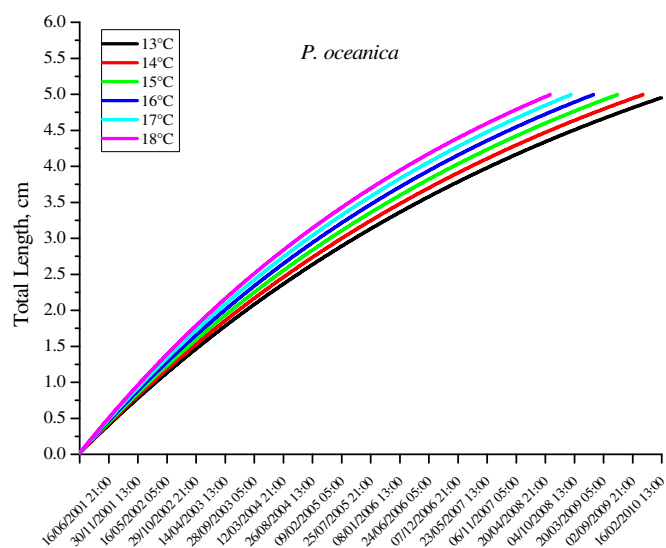


Fig. 4. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with *P. oceanica*.

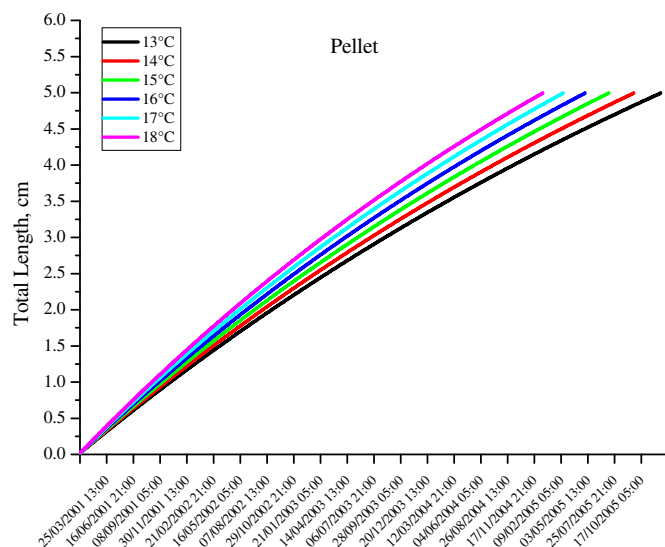


Fig. 5. Simulated growth performances resulting from intake energetic strategies adopted by individuals fed exclusively with pellets.

supplied with pelleted food, *M. galloprovincialis* was able to modify feeding strategies by meeting the type I functional response, which is conventionally thought to be the more frequent model adopted among filter feeders (Bontes et al., 2007). According to the categorization provided by Holling (1966) such response is described by a region of linear increase up to a certain threshold of food abundance which is determined by the incipient limiting level; therefore compared to the type II response, the expression of the type I response gave the advantage of increasing the consumption rates at intermediate food availability and not showing a saturation plateau of digestive processes.

However, a recent review revealed that the majority of filter feeders did not show the type I functional response and the reasons were likely to be found in the conditions that mussels must fulfill in order to show this type of response (Jeschke et al., 2004). Anyway, our results suggested that all the conditions have been satisfied in organisms fed with fish-farm food pellets. Certainly, the digestibility of dietary components being improved (Khater et al., 2014), the presence of pellets would guarantee both the handling and satiation conditions (sensu Jeschke et al., 2004), while the dimension of the detrital particles was homogenized during their preparation, so that the sizes were such that they allowed organisms to meet the digestion condition. Results of the feeding strategies also seemed to be mirrored by the biometric data collected during the growth experiments and by the modeling exercise carried out within this study. Our feeding trials showed significant increases in biomass of organisms fed with pellets and monoalgal diet compared to those fed seagrass diets and although not statistically different better growth performances in presence of pellets, confirming the nature of bivalves to be generalists (e.g., Dame, 1996; Lehane and Davenport, 2006). The DEB models allowed to demonstrate that growth trajectories could vary among diets and under our simulated body temperature (BT) conditions. Comparisons based on different food sources revealed that regardless of the simulated BT, the microalgae and pellet diets generated the best growth performances, compared to those that resulted from models simulating *P. oceanica* as a food proxy. Indeed, as shown in Table 2, in an hypothetical culture system placed close to seagrass meadows, the amount of time estimated for an individual to reach the commercial size was more than twice than that spent in areas where the main food proxy was represented by both phytoplankton and pellets (as in IMTA). Also, our results were consistent with other studies where mussel growth was related to the organic content of particulate matter rather than the phytoplankton abundance or chlorophyll-a. Results from those studies indicated that

chlorophyll-a was part of bivalves food in most coastal areas worldwide, although they were usually able to rely on detritus and particulate matter, such as, organic waste from uneaten pelleted feed, fish, and bivalve faeces in fish-farming impacted areas (Mazzola and Sarà, 2001; Saraiva et al., 2011). Apart from the sensitivity of the individual's growth and environment interactions to global changes, a vein of sustainability of IMTA is crucial to understand how environmental conditions combine to drive the ultimate fitness of the cultivated species. In this context, the DEB model helps in estimating the fecundity potential of cultivated organisms. With few exceptions (i.e., simulated BT of 18 °C), the results showed that *M. galloprovincialis* was able to allocate energy in a reproductive buffer, which was converted to eggs at the time of reproduction, under every simulated body temperature and every food source. The presence of gametes, as part of the somatic weight, and the magnitude of each spawning event, could have a significant impact on the success of aquaculture practices being responsible for both the price of the harvested shellfish as well as the negative impacts associated with gamete release (sensu Sarà et al., 2007). Our results suggested that the energetic balance of organisms maintained under feeding regimes based on microalgae and pellets showed lower reproductive outputs (MT, TRO, and RE) than those obtained with simulations carried out with feeding performances of mussels relying on a diet exclusively of seagrass detritus, probably as a result of a longer simulated time period to reach the size of 5 cm, which was two-fold in case of growth trajectories performed in the presence of one among the two other diets. Indeed, with the exception of feeding performances, the same model and parameter set were used to predict mussel growth under different food and temperature scenarios, underlying the idea that a generic mussel model should represent an average (growth) performance of mussels under given environmental conditions. However, further research on the optimal feeding and digestive behaviour obtained for mussels fed with mixed diets as proxies of natural environments, might provide a better coverage for the changes in an individual's metabolic requirement during growth, which depending on the feeding capacity of mussels might vary between species and life stages, larvae to adults (Fernandez-Reiriz et al., 2011).

The need for adopting an ecosystem approach to site selection and framing the allocation of areas dedicated to aquaculture activities within the broader context of the Marine Spatial Planning requires the use of modeling tools as a support for decision-making in aquaculture. The integrated model described in this study can provide a useful means to design responsible aquaculture production systems for tomorrow. The mechanistic nature of such models combined with broad applications to other species, allows the consideration of the effects of different environmental drivers such as water temperature and food availability inexplicitly calculating the metabolism of the cultivated species, increasing our ability to prevent impacts and to assist with site selection, moving toward the sustainability of integrated multitrophic aquaculture (Diana et al., 2013; Klinger and Naylor, 2012).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.aquaculture.2016.10.030>.

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