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Phytoplankton Profitability and Use as Organic Matter Source by *Pinna nobilis*

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ABSTRACT

We studied the retention of organic matter (OM) from phytoplankton by the bivalve *Pinna nobilis* Linnaeus, 1758. Three replicates of identical doses of 1.8 L (~25 g dry weight) of three species of microalgae (*Pavlova lutheri*, *Isochrysis galbana* and *Chaetoceros calcitrans*) were given to 6 fan mussel placed in individual tanks with gentle aeration at 20ºC. The animals were let to filter the water for 8 hours. After the 8 hours biodeposits were recovered and the bulk of water was passed through a 35µm mesh to recover the remaining unfiltered particles. Thus, known the original amount of OM present in phytoplankton added and both in rejected and remaining material, the retained OM was calculated. *P. nobilis* filtered most of the phytoplankton (23.30 ± 3.49 g (95.0 ± 0.93% of total phytoplankton added) and retained 0.80 ± 0.07 g of OM (84.44 ± 4.17% of total OM added). These values are similar to those of retained OM from 20g of muddy detritus. Comparing the two food sources, we hypothesize that *P. nobilis* would get most of the OM from other food sources such as muddy detritus and that microalgae would provide complements such as mono- and polyunsaturated fatty acids.

KEYWORDS: *bivalve, diet, phytoplankton, organic matter, food acceptance.*

RESUMEN

Hemos estudiado la retención de materia orgánica (MO) procedente de fitoplancton por el bivalvo *Pinna nobilis* Linnaeus, 1758. Dosis idénticas de 1.8 L (~25 g peso seco) de tres especies diferentes de microalgas (*Pavlova lutheri*, *Isochrysis galbana* y *Chaetoceros calcitrans*) se suministraron por triplicado a 6 individuos mantenidos en tanques individuales con flujo suave de aireación y temperatura constante de 20 °C. La duración de la experiencia fue de 8 horas. Tras este periodo los biodepósitos se recuperaron, filtrándose además la totalidad del volumen de agua empleado mediante una malla de 35 µm, recuperando así cualquier partícula no filtrada. De esta forma, conocida por una parte la MO inicial presente tanto en el fitoplancton suministrado como en las cantidades expulsadas y no consumidas, se calculó la proporción de MO retenida. *P. nobilis* filtró casi la totalidad del fitoplancton (23.30 ± 3.49 g (95.0 ± 0.93% del total de fitoplancton añadido) de los cuales contenían (0.80 ± 0.07 g, de MO del fitoplancton (84.44 ± 4.17% del total de MO añadida). Estos valores son similares a los de MO retenida procedente de 20 g de detritus fangoso. Comparando ambas fuentes de alimentación, nuestra hipótesis sugiere que *P. nobilis* podría alimentarse de detritus para obtener elevados porcentajes de MO mientras que las microalgas aportarían complementos como ácidos grasos mono- y poliinsaturados.

PALABRAS CLAVE: *bivalvo, dieta, fitoplancton, material orgánica, aceptación de alimento.*

INTRODUCTION

The fan mussel *Pinna nobilis* Linnaeus, 1758 is an endangered bivalve endemic to the Mediterranean Sea. It is the largest bivalve in the Mediterranean (up to 1 m) and it shows the fastest growth of bivalves (up to 10 cm/year in young specimens) (Moreteau

& Vicente, 1982; Richardson et al., 1999; Siletic & Peharda, 2003). The large size and fast growth suggest that the species must have a particular feeding strategy facilitating food input and assimilation. The particularities of this strategy are, however, presently unknown. Feeding ecology of this species has been subject to experimental studies only during the last years. In fact, the first literature related to this subject was purely descriptive without experimental work. Stenta (1901) described the waste channels through which *P. nobilis* ejected the undigested particles. Yonge (1953) observed that these particles, denominated pseudofeces, were ejected continuously by the waste channels during the process of suspension feeding. It was not until 48 years later when an isotopic analysis of the organic matter (OM) (δ^{13} C and δ^{15} N) from the pseudofeces collected from two individuals was carried out. Thus Kennedy et al. (2001) was the first who suggested that macroalgal epiphytes would contribute a significant component in the diet of *P. nobilis*. However, Box et al. (2009) demonstrated how individuals colonized by the invasive macroalgae *Lophocladia lallemandii* presented high levels of Glutathione S-transferase (GST) in their digestive glands. The presence of this enzyme associated to detoxification processes indicate that the ingestion of cytotoxic compounds such as the lophocladine affect negatively this species. On the contrary a positive effect is produced by the epiphytes of *Posidonia oceanic* leaves. Cabanellas et al. (2010) confirmed that they represent the highest contribution to the δ^{13} C signatures of *P. nobilis.*

To this moment phytoplankton was still considered the main component in the diet of this species. Davenport et al. (2011), however, observed that detritus represented more than 95% of the stomach content in fan mussels. Subsequently, Nadjek et al. (2013) confirmed that *P. nobilis* ingests and assimilates predominantly detritus in its diet. However, the author concludes that the species also ingest much higher quality items such as phyto- and zooplankton which provide the necessary mono- and polyunsaturated fatty acids (MUFA and PUFA respectively). This idea was also supported by Trigos et al. (2014) who observed that large amounts of OM from muddy detritus were retained by *P. nobilis* (47.50 ± 11.23% of the filtered OM).

According to these results, phytoplankton would be a secondary source of OM behind muddy detritus. However, phytoplankton is known as a direct source of OM in estuarine and marine environments (Rochelle-Newall & Fischer, 2002). Thus, could the differences in ingestion between muddy detritus and phytoplankton be a question of availability in *P. nobilis*? Could phytoplankton alone sustain a population of *P. nobilis* in natural conditions or is muddy detritus required for the survival of the individuals? In these terms, the main purpose of the present study is i) evaluate the degree of acceptance of phytoplankton and of profitability of the OM present in a microalgae diet by *P. nobilis* when high doses are provided, ii) evaluate the profitability of phytoplankton as OM source and iii) estimate whether a diet mainly composed by phytoplankton could sustain a population of *P. nobilis* in natural conditions.

MATERIALS AND METHODS

The experiments were carried out on 6 fan mussels between 34.6 and 57.2 cm total size (Ht). Individuals were collected manually by SCUBA diving in different surveys between July and September 2012, in Embiez archipelago, South East of France (43°4′25" N; 5°46′7" E) (Fig. 1). Sampling was carried out at 19 meters depth within a *Posidonia oceanica* meadow. The individuals were transported to the laboratory where fan mussels were maintained in experimental conditions and subjected to different experiments such as evaluating detritus acceptance (Trigos *et al*, 2014) or collecting gametes to study the species hatchery in laboratory (Trigos *et al*, 2013). The rule followed to select the animals was collecting individuals of different sizes in order to avoid biases in the experiments (in this case phytoplankton assimilation) related to animal size. The byssus was carefully extracted avoiding tearing the tissues during collection. By keeping this proteinic structure in perfect conditions we reduce stress on animals and facilitate future re-settlement in the field after finishing the experiments. The valves were brushed removing all epibiontic biomass to reduce interference in the experiments. For long-time storage the animals were kept in groups of three individuals in 300 l tanks with open water circuits. For the feeding experiments each animal was placed for 8 h in a 60 litre closed circuit-tank and was not fed the day before experiments. Water temperature was kept constant (20°C) during the whole process. Soft and constant aeration was used to provide oxygen and to keep food particles in suspension.

When doses of microalgae were prepared, food rations of the flagellates *Pavlova lutheri* and *Isochryisis galbana* and diatom *Chaetoceros calcitrans* were adjusted according to the clearance rates observed in the tanks the days before experiments started. In this way we avoided the threshold of digestive system collapse by providing an excess of food (Bayne et al., 1989; Velasco & Navarro, 2003). Consequently, phytoplankton rations for each experiment were finally adjusted to 1.9 L (1.8 L + 0.1 L aliquot) of the following cell concentrations (x10^6 cells·mL^{-1} ± SE): 4.6 ± 0.8 (*I. galbana*); 3.4 ± 0.6 (*P. lutheri*) and 3.5 ± 0.2 (*C. calcitrans*). Average cell concentration was determined with a 0.0025 mm^2 Malassez counting chamber. Once we had established the cell concentrations to be used in the experiments, we studied the quantity of organic matter (OM) present in our microalgae cultures, in order to ensure that we could extrapolate total organic content of the total volume. For this we prepared three 0.1 L mixtures with the

three species of microalgae (Helm et al. 2006). After filtering these mixtures with Whatman® glass fibber filters (0.7µm GF/F) and ashing according to Conover (1966) method, we observed no significant difference in OM present in the three aliquots. Therefore, we used one aliquot to extrapolate total organic content of the 1.8 l of microalgae mixture provided to the individuals.

To determine the total retention of phytoplankton and the profitability by *P. nobilis* of the OM contained in phytoplankton, we considered as retained phytoplankton (RP) the amount of material that a specimen may hold inside its body after the 8 h of duration of the experiment. After the 8 hours, biodeposits (B) were recovered and filtered with GF/F fiber filters. The bulk of water from the tanks was also filtered through a 35 µm mesh to determine the remaining fraction of suspended material. The Conover method was also used to determine by weight difference how much OM was consumed. Three replicates were carried out with each individual. The animals were left to rest for three days between replications. Once all experiments were finished, individuals were introduced back in their original collection places.

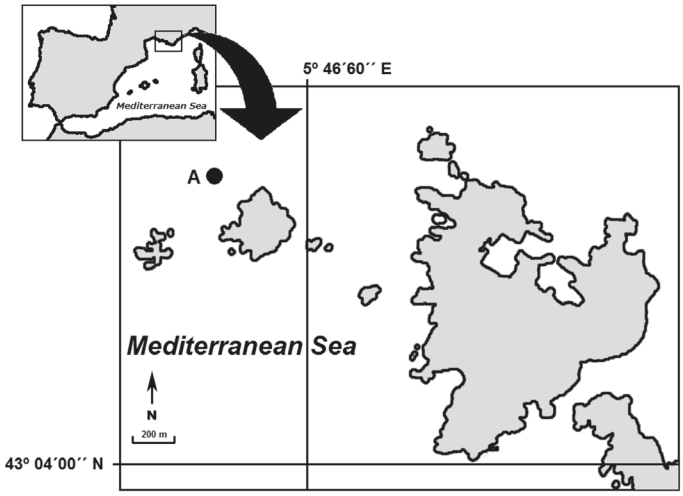

Figure 1. Map of Embiez island (South eastern France). A - site where individuals were collected for this study.

Statistical analysis

The data were analyzed by SPSS® program. The normality and homoscedasticity of variables were confirmed by Kolmogorov-Smirnov (K-S) test, P-P / Q-Q plots and the Levene´s test respectively. To estimate the hypothesis of the possible relationship between microalgae retention and biodeposits produced with the size of animals, the Pearson correlation coefficient was computed.

RESULTS

The 1.8 L volume of microalgae mixture that was provided to the individuals included a mean value of (average ± SE, N = 6) 24.50 ± 3.49 g of phytoplankton (dry weight) and contained 0.94 ± 0.08 g of OM (3.90 ± 0.44% of phytoplankton added) (Table 1). Biodeposits recovered weighted 1.20 ± 0.13 g (5.0 ± 0.93% of total). The filtration of the bulk of water and subsequent application of Conover method showed that the remaining content of OM was negligible. Thus, the only considered fraction of recovered OM was the B (mostly pseudofeces and some feces). Therefore, the experimental activity showed that the RP was 23.30 ± 3.49 g, N = 6 (95.0 ± 0.93% of total phytoplankton added) and that 0.80 ± 0.07 g, N = 6 of OM (84.44 ± 4.17% of total OM added) was retained (Fig. 2). The Pearson correlation showed no significant relation between the size of the fan mussel and microalgae

retention ($F_{(1,4)}$ = 0.196, P > 0.05) and between animal size and B production ($F_{(1,4)}$ = -0.467, P > 0.05). The OM in B (0.14 ± 0.04 g) represents a 15.56 ± 4.15% of total OM added (Fig. 2).

Table 1. Individual balance of material registered in the phytoplankton feeding experiments. The different fractions observed in the process and their respective organic matter (OM) content are expressed in grams (g) and percentage (%) with respect to the total added. (B) biodeposits (faeces and pseudofaeces). (P) phytoplankton added. (RP) retained phytoplankton after 8 hours.

	INPUTS				OUTPUTS				RETAINED			
	P		POM		B		BOM		RP		RPOM	
Individual	(ml)	(g)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
12/001	1800	25.14	1.08	4.30	1.28	5.10	0.17	16.08	23.90	94.90	0.91	83.92
12/005	1800	26.46	0.88	3.30	1.07	4.00	0.08	9.86	25.40	96.90	0.79	90.14
12/006	1800	23.70	0.95	4.00	1.04	4.40	0.13	14.79	22.70	95.60	0.81	85.21
12/007	1800	18.96	0.83	4.40	1.19	6.30	0.14	17.39	17.80	93.70	0.68	82.61
12/008	1800	29.46	1.00	3.40	1.24	4.20	0.22	22.18	28.20	95.80	0.77	77.82
12/010	1800	23.58	0.93	3.90	1.39	5.90	0.12	13.15	22.20	94.10	0.81	86.95
Average (±SD)	1800 ±0.00	24.50 ±3.49	0.94 ±0.08	3.90 ±0.44	1.20 ±0.13	5.00 ±0.93	0.14 ±0.04	15.56 ±4.15	23.30 ±3.49	95.00 ±0.93	0.80 ±0.07	84.44 ±4.17

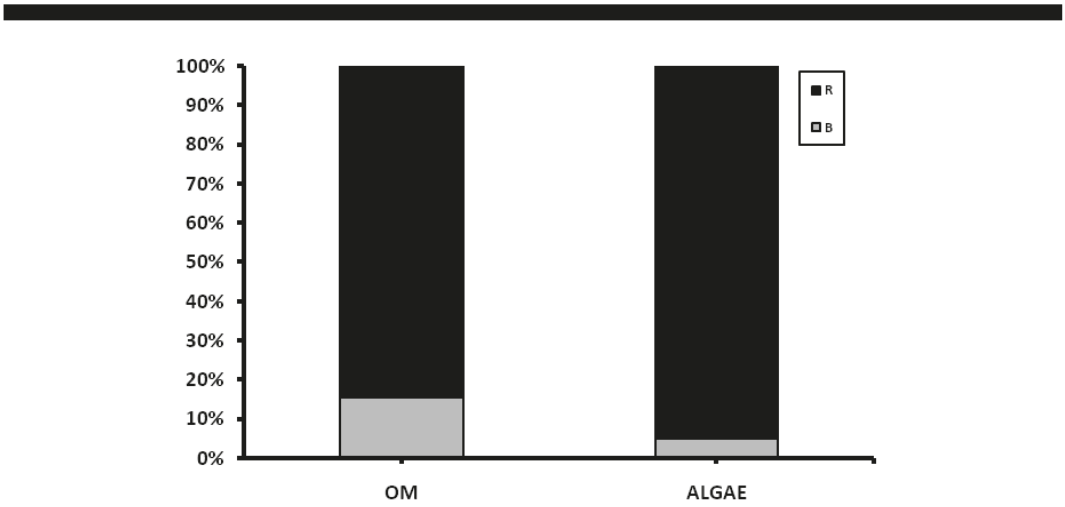

Figure 2. Mean rejection and retention percentages of phytoplankton and organic matter (OM) registered in microalgae feeding experiments; (**OM**) organic matter percentages (**ALGAE**) microalgae mixture percentages. *B biodeposits* rejected by animals after 8 hours. *R retained* fraction by fan mussels during experiments.

DISCUSSION

The data reveal great percentages of phytoplankton retention by *P. nobilis*. These retained amounts of phytoplankton were proportionally higher than those observed for other food sources such as detritus (95.0 ± 0.93% of total filtered phytoplankton

(present study) *vs*. 65.42± 8.65% of filtered detritus (Trigos *et al.*, 2014)). However, the proportion of retained OM from filtered muddy detritus is c. 3 times higher than that from phytoplankton (0.47±0.25 g of retained OM from 7.30±0.85 g of filtered muddy detritus vs. 0.80 ± 0.07 g of retained OM from 24.50 g of filtered phytoplankton). This indicates that muddy detritus provides proportionally more OM than phytoplankton per unit weight. Furthermore, the phytoplankton concentration in the doses provided in the present experiment ranged from 3.4 to 4.6 x10^6 cells·mL^{-1}, much above normal cell concentrations for coastal areas, which usually are between 10^3 and 10^5 cells·mL^{-1} (Moncheva *et al.,* 2001). This suggests that even in the periods when high availability of phytoplankton exists, proportionally a much greater amount of OM matter would be available from richer food sources such as resuspended detritus. A little water movement would provide much more OM from sediment resuspension than the phytoplankton present in the water column. Probably a population of *Pinna nobilis* would not be able to survive feeding only on phytoplankton, and ingesting OM from detritus is a necessity. As indicated by Nadjek et al. (2013) phytoplankton would provide the necessary complements such as mono- and polyunsaturated fatty acids (MUFA and PUFA respectively) to *P. nobilis* diet.

The statistical analysis shows that there is not enough evidence to assume a relationship between body size and the B production. According to Grizzle et al. (2001), several filter feeding bivalves such as scallops, clams or oysters, regulate an excess of particles reducing consumption rates and not by rejecting more pseudofaeces. In case of *P. nobilis,* however, elevated concentrations of microalgae rations appear not to affect the ingestion behaviour, but the animals seemed to increase the amount of B production in comparison with normal daily feeding (*pers. obs.*).

In any case, in this experiment the B and the filtration of the bulk of water were the two only fractions studied to quantify the non-retained OM. It is possible that some material was excreted in other ways (i.e. dissolved material). This could be an alternative explanation to the high values of retained phytoplankton observed. Thus, future programs may address this issue in order to complete this gap of knowledge.

Some authors recommended maintaining other bivalves for 5 hours in similar experiments with closed circuits (Griffiths, 1980; Berry & Schleyer, 1983; Vincendeau & Robert, 1987). Our previous observations indicated that all animals had eliminated most of the digested food in 24 h. Moreover, our previous observations indicated that the individuals were comfortable in the 60 l tanks for long periods, thus the duration of our experiments was set to 8 hours according to the observed rhythms of faeces and peseudofaeces production. With this setting, food rejection from previous meals was negligible at the beginning of the experiments and we could recover most of faeces and pseudofaeces.

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