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Pheophorbide *a* in *Hydrobia ulvae* faecal pellets as a measure of microphytobenthos ingestion: variation over season and period of day

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ABSTRACT: The microphytobenthos (MPB) – *Hydrobia ulvae* trophic interaction is one of the main channels of material transfer to higher trophic levels in intertidal mudflats. A new non-invasive approach to evaluate the grazing activity of *H. ulvae* on microphytobenthos is proposed. The effects of season and period (combination of tide and day/night) on ingestion rates of *H. ulvae* (using ^{14}C-labeled MPB) and egested pheopigments *a* (using HPLC pigment analysis) were also investigated. *H. ulvae* ingestion rate was found to vary significantly over season and period, being higher in summer and during diurnal low tide periods. This is possibly related to higher growth rates of *H. ulvae* in summer, as well as to an increase in surface MPB biomass during diurnal low tides. A highly significant relationship was found between ingested chl *a* and egested pheophorbide *a*, allowing the estimation of ingestion rate from the amount of egested pheophorbide *a* on *H. ulvae* faecal pellets. This new non-invasive methodology may allow the improvement of long-term studies of consumption rates and the evaluation of grazing of *H. ulvae* on MPB.

KEY WORDS: Pheophorbide *a*/chl *a* ratio · Ingestion rate · Grazing · Microphytobenthos · *Hydrobia ulvae*

INTRODUCTION

The mud snail *Hydrobia ulvae* (Pennant) is one of the most abundant deposit feeders in intertidal mudflats of the North Atlantic coast, forming large populations that can reach densities of up to 10^6 ind. m^{-2} (Barnes 1999). Such high densities may result in heavy grazing pressure on one of the main benthic primary producers of estuarine intertidal mudflats, the microphytobenthos (MPB). The MPB consists of photosynthetic microalgae and cyanobacteria that accumulate in the surface layers of intertidal sediments, forming dense and highly productive biofilms (Underwood & Kromkamp 1999), which are well recognized as the main source of food for *H. ulvae* populations (Bianchi & Levinton 1984, Blanchard et al. 2000, Haubois et al. 2005, Pascal et al. 2008). Intense grazing pressure has been found to cause a significant top-down control on the MPB biomass at the surface of sediments in intertidal areas (Cariou-Le Gall & Blanchard 1995, MacIntyre et al. 1996, Underwood & Kromkamp 1999, Novak et al. 2001, Hagerthey et al. 2002, Hillebrand & Kahlert 2002). Top-down controls also include erosion and several studies have already demonstrated that MPB erosion is controlled by physical variables as well as biological ones, such as bioturbation (Blanchard et al. 1997, Orvain et al. 2003, 2004, 2006, Orvain 2005). In this context, *H. ulvae* also play a role in the dynamics of MBP biomass, facilitating MPB erodability through snail bioturbation activities that occur simultaneously with grazing. This trophic link is one of the main channels of material transfer to higher trophic levels in estuarine food webs since *H. ulvae* is an important prey item for fish, birds and other estuarine invertebrates (Piersma et al. 1993, Aarnio & Mattila 2000).

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The trophic relationship between MPB and mud snails has been mostly investigated through the measurement of the MPB ingestion rate of mud snails, based on the use of ^{14}C-labelled microalgae (Forbes & Lopez 1989, Blanchard et al. 2000, Haubois et al. 2005). The effects of mud snail density (Blanchard et al. 2000) and epipelic diatom cell size (Haubois et al. 2005) have been considered in the estimation of energy flow and grazing rates. However, to our knowledge, the carbon flow through the MPB–*Hydrobia ulvae* link has been quantified only for diurnal low tide periods, and its variability over day/night and tidal cycles has never been characterized.

Several studies have used the pheopigment content of intertidal sediments as an index of grazing activity by zoobenthos (Brotas & Plante-Cuny 1998, Lucas & Holligan 1999, Cartaxana et al. 2003). This is based on the finding that pheophorbide *a* and pheophytin *a* are the main degradation products of chlorophyll (chl) *a* found in sediments (Bianchi et al. 1988, Abele-Oeschger & Theede 1991, Buffan-Dubau et al. 1996) and in the water column (Klein et al. 1986, Strom 1993). The breakdown of chl *a* during digestion by mud snails is known to result in the accumulation of these degradation products in their faecal pellets (Cartaxana et al. 2003).

The present work addresses the development of a new non-invasive methodology to evaluate grazing activity of *Hydrobia ulvae* on MPB, based on the relationship between the ingested chl *a* (as a proxy for MPB biomass) and its degradation products, pheophorbide *a* and pheophytin *a.* The ingestion rate of mud snails on microalgae was estimated through the incorporation by *H. ulvae* of benthic microalgae labelled with ^{14}C, and the pigment content of *H. ulvae* faecal pellets was quantified by HPLC. The relationship between egested pheophorbide *a* and ingested chl *a* was characterized by the pheophorbide *a*/chl *a* ratio. This work also investigated how *H. ulvae* ingestion and egestion rates vary over periods of 24 h, comparing different points along the day–night and tidal cycles in spring (April) and summer (July).

MATERIALS AND METHODS

Sampling. Samples of sediment surface containing MPB and *Hydrobia ulvae* were collected from intertidal mudflats in Ria de Aveiro (40° 38′ N, 8° 44′ W), which is a shallow coastal lagoon located on the northwest coast of Portugal. Collection was done during the low tide prior to the experiments. The sediment surface (~top 2 mm) was scraped using a spatula, stored and transported to the laboratory. The sediment samples were sieved through a 1 mm mesh to separate snails from sediment. *H. ulvae* individuals were measured under a stereoscopic microscope and selected according to shell height (apex to aperture). Only individuals considered as adults (>4 mm; Haubois et al. 2002) were used. Selected mud snails were kept in filtered seawater at 20°C and starved for 24 h before the start of the experiments. The sediment was stored until further processing.

Measurement of chl *a* ingestion rate. Measurements of chl *a* ingestion rate in *Hydrobia ulvae* were made in experimentally controlled microcosms (75 cm^2 culture flasks) based on a protocol adapted from Blanchard et al. (2000). This protocol assumes that: (1) labelled microalgae accumulate in the gut of the snails at a constant rate during the experimental period; (2) egestion does not take place during the incubation period when the snails are feeding (2 h, see also Barnes 2001); (3) labelling is a conservative process, i.e. the radioactivity contained in the microalgae does not change with time; and (4) labelled and unlabelled microalgae are grazed at the same rate. Briefly, the protocol involves adding ^{14}C-labelled microalgae to the sediment and then recording the radioactivity incorporated by the snails. The epipelic benthic microalgae were isolated from the sediment using the lens tissue method (Eaton & Moss 1966) and a microalgal suspension was made by rinsing the lens tissue with natural seawater. Na$H^{14}CO_3$ (40 to 50 µCi mmol stock solution; PerkinElmer) was added to 250 ml of the suspension in order to obtain a final concentration of 0.4 µCi ml^{-1}, and left to incubate for 2 h under saturating light at 20°C. The suspension was stirred during incubation to ensure homogeneous labelling. Following incubation, the suspension was centrifuged (3 min at 1500 rpm min^{-1}), the supernatant was discarded, and the remaining material was rinsed in filtered seawater and centrifuged twice to obtain a concentrated suspension of labelled microalgae that is free of non-assimilated Na$H^{14}CO_3$. This suspension was then added to the sediment that was left from the isolation of the microalgae, which had been diluted with filtered sea water (1:1, v/v). The final suspension of sediment and labelled microalgae was then homogenised with a glass rod and 10 ml were introduced to each microcosm, together with 8 snails that were left to feed for 2 h at 20°C (temperature was set to field values). The density of snails in each microcosm (1.4 ind. cm^{-2}) was chosen such that it remained below the density threshold that results in a decrease in ingestion rate (Blanchard et al. 2000). Feeding was interrupted by adding freshwater. The snails were then sieved from the sediment (with a 1 mm mesh sieve), rinsed with filtered seawater and placed in defecating chambers (see below). After defecation, the radioactivity remaining in the snails was measured by pooling all 8 snails from each microcosm and solubilising the soft tissues through the addition of

180 µl of Soluene-350 (Packard) tissue solubilizer for 72 h at 50°C in a water bath (Selecta Frigiterm-10). A volume of 1.8 ml Hionic-fluor (Packard) scintillation cocktail was then added to the solution and radioactivity was measured in a liquid scintillation counter (Beckman LS 6000 IC). The above procedure was repeated in 4 experiments made within 2 different 24 h periods — one in April (spring) and the other in July (summer) 2009. The 4 experiments within each 24 h period coincided with and replicated 4 different combinations of day and tidal phases (day/high water, day/low water, night/high water and night/low water) to which the snails would be subjected if they were in the field. Day and night conditions were simulated by turning lights on and off, respectively, and tide conditions were reproduced by adding a relatively higher level of water on high tide microcosms. All the 4 experiments were conducted precisely at the same time as high and low tide occured in the field. In each experiment, 5 microcosms were used, 3 being used to determine ingestion rate and 2 used to measure pheopigments.

Ingestion rates (*IR*, µg chl *a* ind.$^{-1}$ h^{-1}) of *Hydrobia ulvae* were calculated from the total radioactivity ingested by each snail (*IRad*, dpm ind.$^{-1}$ h^{-1}) using:

$$IR = IRad \times CR \quad (1)$$

where *CR* (µg chl *a* dpm^{-1}) is a conversion factor to transform radioactivity counts into chl *a* biomass (dpm: disintegrations per minute). *CR* was calculated from measurements of radioactivity and chl *a* that were made in a 1 ml suspension of sediment set aside before inoculation of the microcosms. Radioactivity was read in the liquid scintillation counter (Beckman LS 6000 IC) after addition of 1 ml Hionic-fluor (Packard) scintillation cocktail. Chl *a* was extracted in 90% aqueous acetone and quantified spectrophotometrically (Genesys 6, Thermo Spectronic) following the method of Lorenzen (1967).

HPLC pigment analysis of faecal pellets. In order to collect the faecal pellets, the mud snails were placed in defecating chambers without food after being removed from the microcosms. The defecating chambers consisted of plastic flasks with net (500 µm) bottom partitions to ensure separation between individuals and faecal pellets. The snails were left to defecate for 48 h and the faecal pellets were then retrieved from the bottom compartment, frozen in liquid nitrogen and stored at −80°C until freeze-drying. After freeze-drying, the pellets were weighed and pigments were extracted in 95% cold buffered methanol (2% ammonium acetate) for 15 min, with 30 s sonication (Brotas & Plante-Cuny 1998). The solution was then filtered using 0.2 µm pore filters (Fluoropore PTFE filter membranes) and extracts were immediately injected into an HPLC system (Shimadzu) with a photodiode array (SPD-M10ADVP) and fluorescence (RF-10AXL) detectors. Chromatographic separation was achieved using a C18 column for reverse phase chromatography (Supelcosil, 25 cm length, 4.6 mm diameter, 5 µm particles) and a 35 min elution programme. The solvent gradient followed that of Kraay et al. (1992), with a flow rate of 0.6 ml min^{-1} and an injection volume of 100 µl. Pigments were identified from absorbance spectra and retention times, and concentrations calculated from signals in the photodiode array and fluorescence detectors. Identification and calibration of the HPLC peaks were done using commercial standards: chl *a* standard from Sigma-Aldrich, and pheophytin and pheophorbide *a* standards from DHI (Institute for Water and Environment, Hørsholm, Denmark).

Statistical analysis. The existence of a linear relationship between pheopigments and chl *a* was tested using linear regression analysis, and regression equations (slope and intercept) were compared using Analysis of Covariance (ANCOVA). A 2-way ANOVA with blocking was carried out to test the effect of season (April and July) and period (combination of tidal stage and day–night cycle) on *Hydrobia ulvae* ingestion rate and on the egestion of pheopigments. Prior to analysis, assumptions were verified and data transformed when necessary. However, variances in *H. ulvae* ingestion rates were heterogeneous and this condition could not be corrected by any of the usual transformations. Therefore, the ANOVAs were performed on the ranks of the observations (Zar 1996). This procedure homogenized variances and corresponds to a nonparametric ANOVA. Tukey's test was used for post hoc comparison of the main effects when significant differences were found. A 1-way ANOVA was performed to test the effect of season on the amount of chl *a* available and to test for differences in *H. ulvae* ingestion rates between microcosms under identical conditions. All statistical analysis was carried out following Zar (1996) and using STATISTICA v.8 (StatSoft).

RESULTS

Variation in *Hydrobia ulvae* ingestion rate

The amount of ^{14}C accumulated in labelled microalgae was monitored during the experiments, confirming that labelling is a conservative process ($n = 5$, $p = 0.741$, dpm ml^{-1} algal suspension = 59 866 × hours after labelling + 44 119). *Hydrobia ulvae* ingestion rate did not change significantly between microcosms under identical conditions (e.g. ANOVA; April LT day: $p = 0.933$ and April HT night: $p = 0.995$).

Fig. 1 shows the variation in ingestion rates over the periods monitored around diurnal and nocturnal tides in April and July. A significant effect of season on *Hydrobia ulvae* ingestion rate was observed (Table 1; $p < 0.001$). Mean ingestion rate increased significantly from April to July (Fig. 1), despite the fact that the chl *a* contents available in the sediment in April and July were not significantly different (10.93 ± 1.65 and 14.06 ± 4.60 µg chl *a* g dry wt, respectively; 1-way ANOVA, $p = 0.529$). Effects of period were also noticeable, suggesting that the ingestion of microalgae by mud snails depended on the combination of day/night and tide, being higher during daytime low tides (Table 1, Fig. 1). Interaction between both factors was not significant (Table 1; $p = 0.479$).

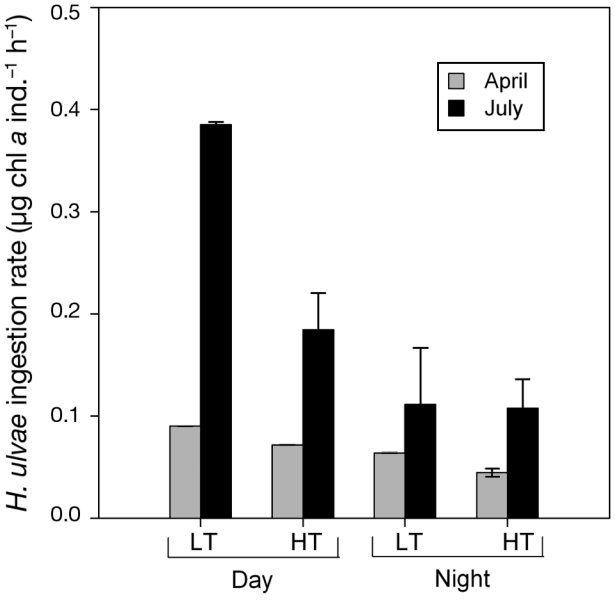

Fig. 1. *Hydrobia ulvae*. Individual mean (3 replicates) ingestion rate for different periods of 24 h (combination of day/night and low/high tide) in April and July. Error bars: ±SE. LT: low tide, HT: high tide

Variation in egested pheopigments

Fig. 2 shows the variation in egested pheopigments per *Hydrobia ulvae* adult. Pheophorbide *a* content (µg pheophorbide *a* ind.$^{-1}$; Fig. 2A) was always higher than pheophytin *a* (µg pheophytin *a* ind.$^{-1}$; Fig. 2B) in *H. ulvae* faecal pellets. There was a significant effect of season and of the period of day on the production of both pheopigments (Table 1). Moreover, interaction between season and period was significant for the egestion of pheopigments by mud snails (Table 1). The egestion of pheophorbide *a* during the day in July was significantly higher under low tide than under high tide, while these differences between tides were not observed in April (Fig. 2A, Table 2). During the night, the egestion of pheophorbide *a* did not vary with season or tide (Fig. 2A, Table 2). The egestion of pheophytin *a* during diurnal low tide as well as under nocturnal high tide did not show significant differences conditioned by season (Fig. 2B, Table 2). However, under diurnal high tide, the levels of pheophytin *a* egested were relatively higher in April and lower in July (Fig. 2B, Table 2) when compared to the other situations.

Relationship between egested pheopigments and ingested chl *a*

Ingested chl *a* was found to vary linearly with egested pheophorbide *a* (Fig. 3A; $n = 24$, $r^2 = 0.937$, $p < 0.001$), but not with egested pheophytin *a* (Fig. 3B; $n = 24$, $r^2 = 0.011$, $p = 0.628$). A highly significant correlation was also found with the whole pheopigment content (pheophorbide *a* + pheophytin *a*) (data not shown; $n = 24$, $r^2 = 0.934$, $p < 0.001$). The relationship between pheophorbide *a* and chl *a* did not vary substantially between April and July, as no significant differences were found between the slopes or intercepts of the linear regression equations (ANCOVA, $p = 0.837$ and $p = 0.418$, respectively). Regarding the relationship

Table 1. Non-parametric ANOVA and 2-way ANOVA of the effects of season (S) and period (P) on *Hydrobia ulvae* ingestion rate (chl *a*) and on the egestion of pheopigments (pheophorbide *a* and pheophytin *a*). ns: $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Source of variation	Ingestion rate (chl *a*)				Pheophorbide *a*				Pheophytin *a*			
	df	MS	*F*	p	df	MS	*F*	p	df	MS	*F*	p
Effect												
S	1	662	116	***	1	0.003	67.6	***	1	5.57	36.0	***
P	3	127	22	***	3	0.001	25.0	***	3	0.55	3.53	*
Interaction												
S × P	3	4.94	3	ns	3	0.001	19.1	***	3	3.10	20.1	***
Error	16	5.71	–	–	16	0.000	–		16	0.16	–	

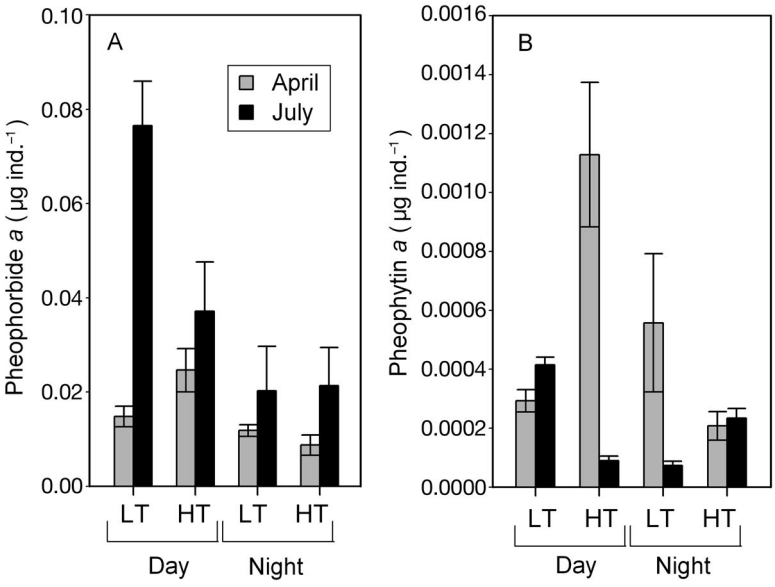

Fig. 2. *Hydrobia ulvae*. Mean (3 replicates) egested pheophorbide *a* (A) and pheophytin *a* (B) for different periods of day in April and July. Error bars: ±SE. LT: low tide, HT: high tide

between pheophytin *a* and chl *a*, significant differences were found between slopes (ANCOVA, $p < 0.05$), but not between intercepts (ANCOVA, $p = 0.556$). Considering the stronger correlation found between egested pheophorbide *a* and ingested chl *a*, the ingestion rate of *Hydrobia ulvae* adults could be determined by estimating the ingested chl *a* using Eq. (2) from the linear regression equation in Fig. 3A.

$$\text{Ingested chl } a \text{ (µg ind.}^{-1}) = 9.58 \text{ Pheophorbide (µg ind.}^{-1}) + 0.003 \quad (2)$$

DISCUSSION

Relationship between egested pheopigments and ingested chl *a*

The major aim of this study was to investigate the possibility of establishing a new non-invasive methodology to estimate the ingestion rate as chl *a* flux from benthic microalgae to *Hydrobia ulvae*, based on the relationship between ingested chl *a* and egested degradation products. Pheopigments appeared as the main degradation products of chl *a* (Buffan-Dubau et al. 1996, Cartaxana et al. 2003). Clear peaks of pheophorbide *a* and pheophytin *a* were detected in the HPLC analysis of *H. ulvae* faecal pellets as already described by Cartaxana et al. (2003), in contrast with the absence of pheopigments reported by Ford & Honeywill (2002) for mud snails from the Eden Estuary (Scotland). These differences could be related to re-ingestion of faecal pellets by snails, as degradation of pheophorbide to colourless residues (undetected) as a result of re-ingestion was already pointed out (Cartaxana et al. 2003 and references therein). Pheophorbide *a* was found in high concentrations in faecal pellets, being significantly correlated with the amount of microalgae (as chl *a*) previously ingested by the same individuals. This suggests a close link between the pheophorbide *a* produced by *H. ulvae* adults and the ingested chl *a*, allowing the establishment of a direct relation between them. This relationship can thus be used to non-invasively estimate the chl *a* ingested by snails (see next section).

Pheophorbide *a* was the major pheopigment found in faecal pellets of *Hydrobia ulvae*. It is formed by the removal of both the Mg atom and the phytol chain from the chl *a* molecule. In contrast to pheophorbide *a*, pheophythin *a* showed no correlation with ingested chl *a*. The extent of pigment digestion should vary as a function of the composition of the grazed community as well as the gut chemistry

Table 2. Tukey's test of the interaction between season (S) and period (P) on the egestion of pheopigments (pheophorbide *a* and pheophytin *a*) by *Hydrobia ulvae*. LTd – Diurnal low tide, HTd – Diurnal high tide, LTn – Nocturnal low tide, HTn – Nocturnal high tide. ns: $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Season	Period	April				July			
		LTd	HTd	LTn	HTn	LTd	HTd	LTn	HTn
Pheophorbide *a*									
April	LTd	–	ns	ns	ns	***	*	ns	ns
	HTd	ns	–	ns	ns	***	ns	ns	ns
	LTn	ns	ns	–	ns	***	**	ns	ns
	HTn	ns	ns	ns	–	***	*	ns	ns
July	LTd	***	***	***	***	–	***	***	***
	HTd	*	ns	**	**	***	–	ns	ns
	LTn	ns	ns	ns	ns	***	ns	–	ns
	HTn	ns	ns	ns	ns	***	ns	ns	–
Pheophytin *a*									
April	LTd	–	*	ns	ns	ns	*	**	ns
	HTd	*	–	ns	*	ns	***	***	**
	LTn	ns	ns	–	ns	ns	**	***	ns
	HTn	ns	**	ns	–	ns	ns	ns	ns
July	LTd	ns	ns	ns	ns	–	**	**	ns
	HTd	*	***	**	ns	**	–	ns	ns
	LTn	***	***	***	ns	*	ns	–	*
	HTn	ns	*	ns	ns	ns	ns	*	–

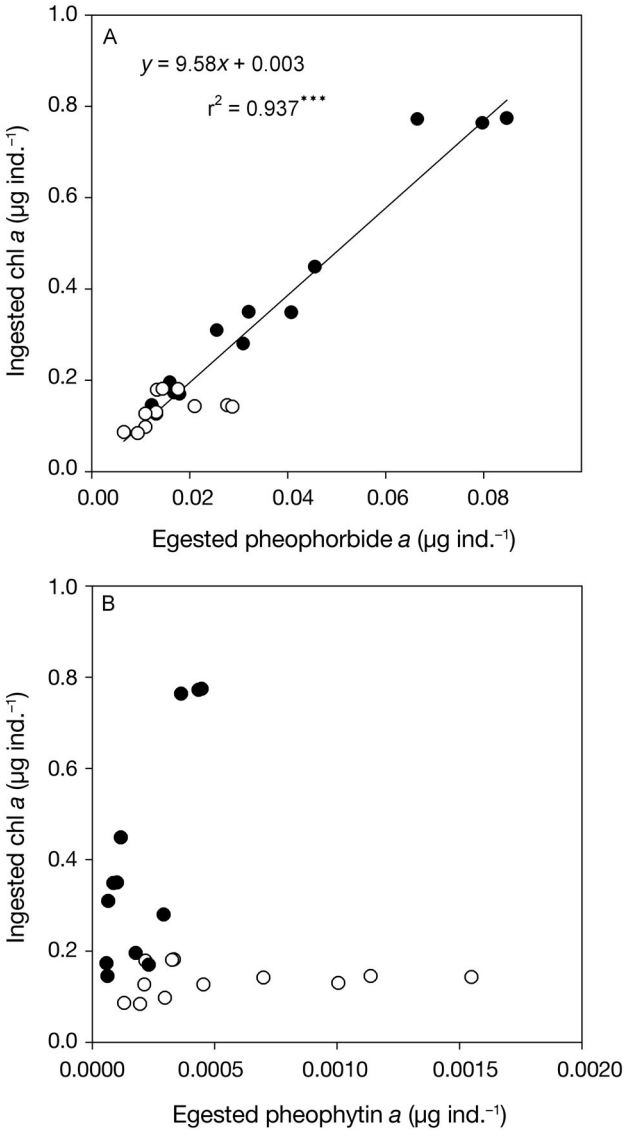

Fig. 3. *Hydrobia ulvae*. Relationship between egested pheophorbide *a* (A) and pheophytin *a* (B) and the previously ingested chl *a* in April (○) and July (●). ***$p < 0.001$

and gut residence time of the grazer (Penry & Frost 1991, Cartaxana et al. 2003). Penry & Frost (1991) and Cartaxana et al. (2003) showed 16× higher accumulation of pheophorbide *a* relative to pheophythin *a* in faecal pellets of *H. ulvae* feeding on MPB.

Pheopigments as consumption markers: methodological aspects

Photosynthetic pigments have been widely used as taxonomic biomarkers and, more recently, as grazing markers in planktonic and benthic photosynthetic communities (Roy et al. 1996, Barranguet et al. 1997, Brotas & Plante-Cuny 2003, Cartaxana et al. 2003). The results of the present work support the use of pheophorbide *a* content of *Hydrobia ulvae* faecal pellets as a potential marker for ingested chl *a* (see Eq. 2). The methodology proposed in this study for estimating the MPB biomass ingested by mud snails has several advantages over methodologies based on the use of pre-labelled food sources. One main advantage is that it allows estimates to be made non-invasively, avoiding the sacrifice of the animals. It also contributes to improved long-term studies of consumption rates, which is impossible to achieve using labelling techniques. The traditional labelling approach implies that the label should be homogenously distributed within the available food and the specific activity should remain constant over the experiment (Lopez & Cheng 1983). This requires continuous control of the application, impeding long-term monitoring in the field. Furthermore, the new method allows the characterization of MPB consumption in natural conditions, allowing a better assessment of net primary production through consideration of losses due to grazing pressure.

A potential disadvantage of the proposed methodology is the possible pheopigment conversion into colourless products, which could not be quantified by HPLC. This limitation, however, is shared by all methods based on the use of pheopigments as grazing markers (Buffan-Dubau et al. 1996, Cartaxana et al. 2003). The hypotheses that mud snails re-ingest their own faecal pellets (Lopez-Figueroa & Niell 1978) might contribute to the degradation of pheophorbide *a* to colourless residues (Cartaxana et al. 2003). The application of a previous starvation period was necessary to ensure that all pheopigments accumulated in faecal pellets resulted from the degradation of the labelled chl *a*. However, the use of this approach would require the test to be conducted under natural conditions to address the effects of non-starvation and re-ingestion of own pellets. Preliminary results of the application of the proposed methodology under natural conditions have shown that under non-limiting food (opposite to starvation), *Hydrobia ulvae* faecal pellets contained similar levels of pheophorbide *a* and higher values of chl *a* (Coelho 2010). This suggests the incomplete digestion of ingested microalgae under microcosm conditions.

The constancy of the relationship between pheophorbide *a* and chl *a* over the range of different combinations of seasons and periods strongly suggests the general applicability of the method for studying the trophic link between primary producers and consumers in aquatic environments. Furthermore, the important role that MPB and *Hydrobia ulvae* play in estuarine ecosystems, as well as the relevance of this link in the structure of benthic food chains, reinforce the potential applicability of the results of this study.

Daily variation in *Hydrobia ulvae* ingestion rate

The linear accumulation of labelled benthic microalgae by *Hydrobia ulvae* adults over periods of 2 h has been previously recorded (Blanchard et al. 2000, Haubois et al. 2005) and similar results on the accumulation of bacteria by *H. ulvae* were registered (Pascal et al. 2008). The absence of egestion over the 2 h of feeding may be associated with the complexity of the digestive tracts of molluscs, which allows partitioning of food particles within the gut. Nutritious particles from microalgae or bacteria are usually diverted to the digestive gland for intracellular digestion (Pascal et al. 2008), resulting in higher residence times (Kofoed et al. 1989, Pascal et al. 2008).

The individual ingestion rates of *Hydrobia ulvae* adults varied from 0.044 to 0.090 µg chl *a* ind.$^{-1}$ h^{-1} (from HT to LT) in April and from 0.107 to 0.385 µg chl *a* ind.$^{-1}$ h^{-1} (from HT to LT) in July. Considering a C/chl *a* ratio of 40 (de Jonge 1980), this is equivalent to 1.6 to 3.6 µg C ind.$^{-1}$ h^{-1} in April and to 4.3 to 15.4 µg C ind.$^{-1}$ h^{-1} in July. Thus, the results of the present study on the ingestion rate of MPB during night periods (1.6 and 4.3 µg C ind.$^{-1}$ h^{-1} in April and July, respectively) are consistent with those previously reported for the ingestion rate of benthic microalgae (mostly diatoms) (Forbes & Lopez 1989, Haubois et al. 2005, Pascal et al. 2008). However, the values found for diurnal simulations were higher than the ones described in the literature, reaching almost 3 × more in summer (3.6 and 15.4 µg C ind.$^{-1}$ h^{-1} in April and July, respectively). The remarkably high ingestion rates of microalgae by mud snails in July might be associated with the life cycle of *H. ulvae*. Maximum growth rates have been found to occur during the summer in European estuaries (Sola 1996, Haubois et al. 2002) and some studies have also shown the positive relationship between growth rate and ingestion rate for other *Hydrobia* species (Bianchi & Levinton 1984) as well as for other molluscs (Sommer et al. 1999).

Natural seasonal changes in gastropod physiology or in sediment characteristics might be responsible for some of the observed variability in ingestion rates. Our results showed that *Hydrobia ulvae* ingestion rates were always higher during the day, indicating an important role of the day–night cycle in the ingestion activity of mud snails. This agrees with an observed increase in *H. ulvae* crawling activity with light (Orvain & Sauriau 2002). However, it contrasts with the results of Pascal et al. (2008) showing similar ingestion rates under light and dark conditions. This work thus provides essential new information on the effects of light (day) on the ingestion rate of *H. ulvae*. However, we should point out that during the day, higher values of ingested chl *a* were found during low tide, as significant differences were established by the effect of tide. This suggests that an increase in grazing by *H. ulvae* adults under natural conditions could be synchronized with an increase in MPB biomass at the sediment surface during diurnal low tides.

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