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Diet and feeding biology of *Haminoea orbygniana* (Mollusca: Gastropoda: Cephalaspidea)[§]

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The diet and functional biology of the digestive system of the cephalaspidean gastropod *Haminoea orbygniana* was investigated by gut content analysis of animals collected in the Ria Formosa, a coastal lagoon in southern Portugal. The results show that this species is herbivorous, stenophagic and probably non-selective, feeding mainly on diatoms (67.61%) and occasionally on vegetal detritus (4.16%) and Foraminifera (0.04%), but also including sand grains as an important part of the gut contents (28.34%). The role of the gizzard plates in the fragmentation of diatom frustules was demonstrated and is discussed. Data reveal that this process, although possibly important for the digestion and assimilation of food, seems not to be crucial. The role of sand as an additional food source and as an accessory tool complementary to the gizzard plates is discussed. A general review of the diet within the family Haminoeidae is presented, with all recent work indicating that they feed on diatoms and green algae.

INTRODUCTION

Among opisthobranch gastropods the Cephalaspidea show the greatest diversity of feeding strategies, ranging from herbivory (browsing species, microphagus and algae-feeding species) to active predation with some species able to capture prey of higher mobility and larger size (e.g. *Acteon* feeding on polychaetes) (Kohn, 1983).

Several studies have demonstrated that the Cephalaspidea have mostly a carnivorous diet, feeding on a range of organisms such as foraminiferans (*Acteocina*, *Cylichna*, *Philine*, *Retusa* and *Scaphander*), small gastropods (*Navanax*, *Philine*, *Retusa*), bivalves (*Philine*) juvenile sea-urchins, bivalves and polychaetes (*Scaphander*) (Paine, 1965; Hurst, 1965; Shonman & Nybakken, 1978; Morton & Chiu, 1990). The family Bullidae, phylogenetically closely related to Haminoeidae (Mikkelsen, 1996, 2002) is apparently the only omnivorous group (Tchang Si, 1931; Mikkelsen, 1996, 2002).

The family Haminoeidae is cited in the literature as being predominantly herbivorous (Guiart, 1901; Tchang Si, 1931; Rudman, 1971; Boulch-Bleas, 1983; Gibson & Chia, 1989; García et al., 1991; Chester, 1993; Sprung, 1994), although there have been several citations of carnivory, the majority of them in older works (Vayssière, 1880; Berrill, 1931; Macpherson & Gabriel, 1962). Data were found for eight species worldwide studied at different degrees of detail. To those where diet was approached by gut content analysis, diatoms and green algae were always the main food source.

The digestive system of Haminoeidae includes a pair of jaws surrounding the mouth, a radula with a tricuspid rachidian tooth, two hook-shaped inner laterals, and a variable number of outer-laterals per row, and an additional structure restricted to a few groups of cephalspideans, anaspideans and pteropods molluscs—a gizzard between the oesophagus and the stomach (Mikkelsen, 2002), with three corneous ridged plates. According to Rudman (1971) the digestive system in Haminoeidae is functionally adapted to feed on minute food sources.

The aim of this work is to study qualitatively and quantitatively the diet of the species *Haminoea orbygniana* (Férrusac, 1822) an abundant gastropod in coastal lagoons and rivers of southern Portugal. Specific goals are to contribute to a better knowledge of the feeding biology of the digestive system of this species in terms of selectivity, role of gizzard plates and level of assimilation efficiency.

MATERIALS AND METHODS

Sampling

The samples of *Haminoea orbygniana* were collected on 15 March 2001 in Esteiro das Charradas (37°13′56″N–08°03′21″W), a tidal flat covered with the sea grass *Zostera noltii* (typical habitat of this species) in the Ria Formosa a temperate coastal lagoon in southern Portugal. During low tide 70 individuals browsing on the sea grass leaves were collected. To avoid bias resulting from different ontogenic development stages (juveniles vs adults) all collected specimens were adults (total shell length over 10 mm).

Part of the sample (50 individuals) was fixed in neutralized 10% formalin immediately after capture in order to avoid food digestion. The remaining 20 specimens were kept alive for random collection of faecal pellets in the laboratory.

Eight voucher specimens are placed in the Museo Nacional de Ciencias Naturales de Madrid in Spain (MNCN 15.05/45.840).

Dissection of the digestive tract

Digestive tracts were removed and divided in two parts: (1) the oesophagus, from the buccal mass to posterior of gizzard (hereafter designated as oesophagus); and (2) the stomach and intestine, from the gizzard to the anus (hereafter designated as post-oesophageal tract). Both parts of the digestive tract were opened and the contents removed and preserved in 70% ethanol.

Faecal pellets were obtained on the same day of sampling immediately after specimens arrived in the laboratory and kept in an aquarium. The pellets resultant from the activity of the combined 20 specimens, were randomly sampled from the bottom of the aquaria to fill a 1.5 ml vial, and further preserved in 70% ethanol. This sample was obtained to evaluate the features and composition of excreted material.

Diet

Gut contents were homogenized in a vortex for 30 s at 900 rotations per s and then transferred to 5 ml Utermöhl sedimentation chambers, for a period of 120 min (Utermöhl, 1958; Edler, 1998). Faecal pellets were ultrasonicated for 10 s at 60W, homogenized and transferred to Utermöhl chambers.

For both gut contents and faecal pellets, all items which could be considered potential food (e.g. diatoms, foraminiferans, vegetal detritus, algae, *Zostera*, animal remains, etc.) and inorganic particles (such as sand grains) were identified and counted. Diatoms were distinguished between those with or without chloroplasts, and between those with intact or broken frustules in order to address the role of gizzard plates in the digestion. Quantification of vegetal detritus was performed by counting total number of cells in each piece.

The food items were identified and counted under an inversion microscope equipped with phase contrast. Fifty fields (or fewer, if 100 elements of any species/food item were reached before 50 fields had been searched) were counted (Venrick, 1978). The total number of each food item in the oesophagus, post-oesophageal tract and faecal pellets was calculated following the equation:

$$(E * At)/(Ac * C) \quad (1)$$

where E refers to the number of food items counted, Ac is the area of each field (mm^2), At is the total area of the Utermöhl and C is the number of fields counted (total chamber area=530.93 mm^2 and area of each field=0.1385 mm^2).

Data analysis

The minimum number of specimens required for the characterization of the diet was calculated by graphic analysis considering the cumulative value of specific richness found in each digestive tract against the number of specimens studied. The minimum number of specimens suitable to study the diet refers to the point at which curve stabilization occurs (e.g. Williams, 1987).

Description of diet follows in part the approach used by Megina et al. (2002) and Megina & Cervera (2003), based on the following criteria: (1) qualitative analysis—enumeration of the taxa present in the diet, and determination of diversity and evenness indices to evaluate

Table 1. Haminoea orbygniana *gut and faecal pellet contents (including broken frustules and sand particles) (number of* H. orbygniana *specimens sampled=20; %NI_i, numerical importance index; SD, standard deviation; %FO_i, frequency index).*

	Digestive tract			Faecal pellets
Food item	%NI_i	%SD	%FO_i	%
Total diatoms	67.61	13.76	100	15.42
DIATOMS				
Amphipleura sp.	4.22	4.41	95	—
Bacillaria paxillifer (Müller) Hendey	0.25	0.43	45	—
Cocconeis sp.	10.09	4.45	100	0.86
Coscinodiscus sp.	1.78	1.36	95	—
Cymbella sp.	0.12	0.16	30	—
Diploneis sp. 1	0.53	0.45	75	—
Diploneis sp. 2	0.17	0.28	45	—
Fragillaria sp.	1.11	1.77	65	0.21
Gramatophora undulate (Ehrenberg)	2.03	1.32	95	—
Licmophora flabellate (Carmichael) Agardth	4.75	3.21	100	—
Navicula sp.	1.57	1.76	95	—
Navicula transitrans (Grunow) Cleve	5.43	3.90	100	0.64
Nitzschia distans Gregory	2.11	1.34	95	0.86
Nitzschia longissima (Breb.) Ralfs	0.87	1.23	70	—
Nitzschia sp.	7.13	6.33	100	—
Stauroneis sp.	0.20	0.46	50	—
Broken frustules	25.09	8.29	100	12.85
Sand particles	28.34	10.62	100	84.37
FORAMINIFERA				
Trocommina sp.	0.04	0.09	15	—
VEGETAL DETRITUS	4.16	9.70	25	0.21

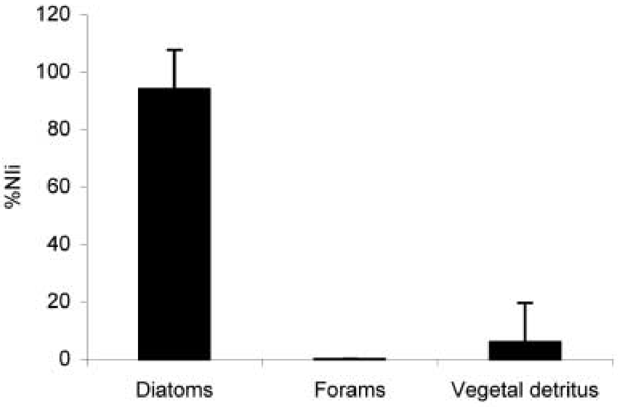

Figure 1. *Haminoea orbygniana*: numerical importance index of the food items (excluding sand particles) (bars=standard deviation).

the trophic strategy and dietary specialization. For this purpose, Shannon diversity index (H′) and Pielou evenness index (J) were calculated (Krebs, 1989); and (2) quantitative analysis—the numerical importance of each prey item ($\%NI_i$=proportion of the total food item attributable to food item *i*) $\%NI_i=(N_i/N)*100$, where Ni is the total number of food *i* and N is the total number of all food items, and the frequency of different items ($\%FO_i$=proportion of *H. orbygniana* for which item *i* was recorded) $\%FO_i=(N_{ei}/N_e)*100$, where N_{ei} is the number of *H. orbygniana* specimens from which the prey *i* was recorded, and N_e is the total number of *H. orbygniana* specimens studied.

The percentage and digestive condition of each food item in the faecal pellets was calculated, with the purpose of evaluating the level of diet assimilation.

The role of the gizzard plates was evaluated by testing the following four null hypotheses: (1) the number of diatoms with chloroplasts in the oesophagus is less than or the same as the number of diatoms without chloroplasts in the oesophagus; (2) the number of diatoms without chloroplasts in the post-oesophagus tract is less than or the same as the number of diatoms with chloroplasts in the post-oesophagus tract; (3) the number of diatoms with unbroken frustules in the oesophagus is less than or the

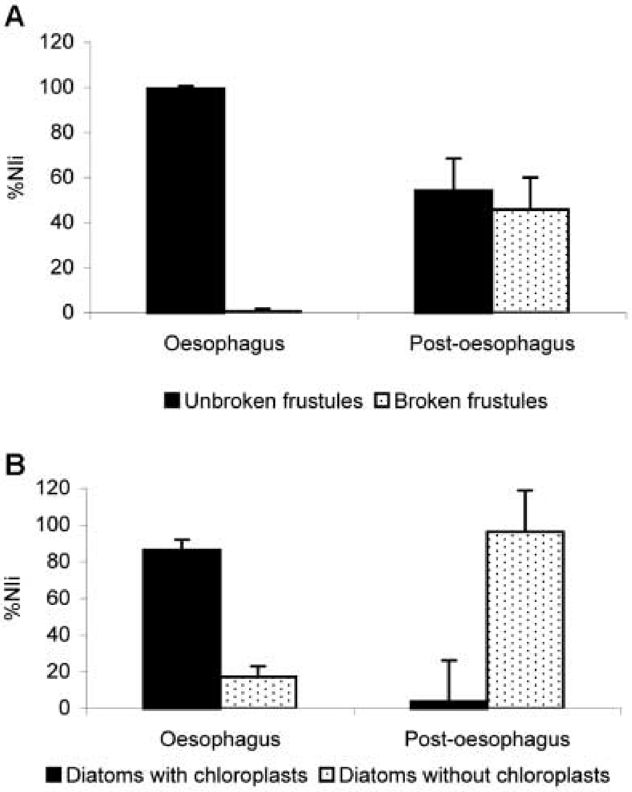

Figure 2. *Haminoea orbygniana*: (A) numerical importance index of intact and broken diatoms in the oesophagus and post-oesophageal tract; and (B) numerical importance index of diatoms with and without chloroplasts in the oesophageal and post-oesophagus tract (bars=standard deviation).

Table 2. Haminoea orbygniana. *Percentage of different food items found in the faecal pellets.*

Food item	%
Total of diatoms	15.42
Diatoms with chloroplasts	7.20
Diatoms without chloroplasts	92.80
Diatoms with intact frustules	76.47
Diatoms with broken frustules	23.53
Total of vegetal detritus	0.21
Total of sand particles	84.37

Table 3. *Review of the diet in the family Haminoeidae.*

Species	Food	Data source	Reference
Haminoea brevis	Bivalves	?	Macpherson & Gabriel (1962)
Haminoea callidegenita	Diatoms, detritus, pieces of *Ulva*	Gut contents and faecal pellets	Gibson & Chia (1989)
Haminoea hydatis	Diatoms, algae and hydroids	Gut contents	Berrill (1931)
Haminoea hydatis	*Cladophora* and diatoms	Gut contents	Boulch-Bleas (1983)
Haminoea navicula (as *H. hydatis*)	Foraminiferans, diatoms, sponges, hydroids and bryozoa	Gut contents	Vayssière (1880)
Haminoea navicula	Herbivora	?	Guiart (1901)
Haminoea orbygniana (as *H. hydatis*)	Microphytobenthos and detritus	Field observations	Sprung (1994)
Haminoea solitaria	Diatoms, detritus and algae	Gut contents	Chester (1993)
Haminoea vesicula	Diatoms and periphyton of *Zostera* sp.	?Field observations	Gibson & Chia (1991)
Haminoea zelandiae	*Ulva*, *Enteromorpha*, diatoms	Gut contents and field observations	Rudman (1971)

same as the number of diatoms with broken frustules in the oesophagus; and (4) the number of diatoms with broken frustules in the post-oesophagus tract is less than or the same as the number of diatoms with unbroken frustules in the post-oesophagus tract. The hypotheses were tested using a two-tailed Student *t*-test at 95% (Zar, 1996).

RESULTS

Quantitative and qualitative analyses of the diet

After evaluating the gut contents of 20 specimens we found that beyond 11 specimens stabilization of the curve occurs, meaning that any addition to the number of specimens studied does not add new food items.

Nineteen food items were found (including sand particles) (Table 1). Diet was dominated largely by diatoms ($\%NI_i=67.61\pm13.76$ and $\%FO_i=100$), with 16 species represented. Vegetal detritus ($NI_i=4.16\pm9.79$ and $\%FO_i=25$), foraminiferans of the genus *Trocommina* ($NI_i=0.04\pm0.09$ and $FO_i=15$) and sand particles ($\%NI_i=28.34\pm10.62$ and $\%FO_i=100$) were also present (Table 1).

The relative importance of diatoms in the general context of diet ($\%NI_i=93.98\pm13.64$), contrasting with the foraminiferans ($\%NI_i=0.05\pm0.12$) and vegetal detritus ($\%NI_i=5.97\pm13.60$) is shown in Figure 1 (after excluding the sand grains from the analysis).

Diatoms in the diet are dominated by a small group of species, five of them making up 75% of the total of diatoms cells identified. Species with higher numerical importance indices are *Cocconeis* sp. (23.81%), *Nitzschia longissima* (12.82%), *Licmophora flabellata* (11.21%), *Amphipleura* sp. (9.97%) and *Navicula transitrans* (4.98%).

Trophic specialization

The diversity and evenness values obtained (18 prey items considered after exclusion of broken frustules and sand particles) were $H'=2.36$ and $J=0.82$. Use of both indices considering only the diet in terms of major prey/taxa groups (diatoms, foraminiferans and algae/vegetal detritus) leads to considerable lower values namely $H'=0.23$ and $J=0.21$.

Functional biology of the digestive system

The percentage of unbroken frustules ($\%NI_i=99.45\pm1.24$) in the oesophagus was significantly higher than that of broken frustules ($\%NI_i=0.55\pm1.24$) (df=38; $t=2.02$; $P<0.01$). In the post-oesophageal tract, the percentage of broken frustules ($\%NI_i=45.74\pm14.22$) was not significantly different from unbroken ($\%NI_i=54.21\pm14.22$), (df=38; $t=2.02$; $P=0.07$). A clear trend to a convergence in both values is notable, as a result of a considerable rise in the number of broken frustules, which do not exceed the number of unbroken diatoms (Figure 2A).

Differences in the percentages of diatoms with and without chloroplasts between both parts of the digestive system are highlighted in Figure 2B. In the oesophagus there is a significantly higher percentage of diatoms with chloroplasts ($\%NI_i=86.30\pm22.59$) than those without ($\%NI_i=13.70\pm22.59$) (df=38; $t=2.02$; $P<0.01$). A significant change in this value in the post-oesophagael tract was observed with the percentage of diatoms without chloroplasts rising considerably ($\%NI_i=96.29\pm5.68$) with a consequent drop in the number of diatoms with chloroplasts ($\%NI_i=3.71\pm5.68$) (df=38; $t=2.02$; $P<0.01$).

For the faecal pellets (Table 2) the food items found were the same as in the gut except for foraminiferans. The percentage of diatoms without chloroplasts is very much higher (92.80%) than those with chloroplasts (7.23%). A similar situation is found in the percentage of diatoms with intact frustules (76.47%) compared with broken (23.53%).

DISCUSSION

Diet

Rudman (1971) demonstrated how the structure and function of the buccal apparatus in the family Haminoeidae constrains available food to items of small dimensions which can be ingested entire (e.g. diatoms) or to algae with free tips that fit within the mouth (e.g. filamentous algae of the genus *Enteromorpha*). Apparently algae such as *Ulva* spp. (or similar morphotypes) seem to be extremely difficult to eat since they do not have free tips upon which the buccal apparatus can act.

The results obtained for *Haminoea orbygniana* in the Ria Formosa where it feeds almost exclusively upon epiphytic diatoms, consuming accidentally other items such as foraminiferans and vegetal detritus supports Rudman's idea. Despite several authors pointing out that *Haminoea* spp. can feed on sea grass leaves (e.g. García et al., 1991) our results reinforce the view that although sea grasses are the main habitat of these molluscs, they do not feed directly on the plant but in the periphyton. Other authors claimed that Haminoeidae are omnivorous (Vayssière, 1880; Berrill, 1931; Macpherson & Gabriel, 1962), however all recent studies based on gut content analysis describe this family as being exclusively herbivorous (Rudman, 1971; Boulch-Bleas, 1983; Gibson & Chia, 1989; Chester, 1993), and this conclusion is supported by our results (Table 3).

Trophic specialization and selectivity

Trophic specialization can be examined in two different ways; either considering the whole assemblage of food items (species richness) or the major taxonomic groups to which the prey belong (i.e. diatoms, foraminiferans, algae, etc.). The results from this study show that although *Haminoea orbygniana* feeds upon 18 species, diatoms largely dominate the diet, and five species only compose 75% of the entire diatom assemblage. If we compare *H. orbygniana* with other genera of cephalaspidean molluscs such as *Philine* or *Scaphander* which feed upon a broad range of items (foraminiferans, small gastropods and bivalves, juvenile sea-urchins and polychaetes) (Hurst, 1965; Shonman & Nybakken, 1978; Morton & Chiu, 1990), *H. orbygniana* should be regarded as a stenophagic species, with a very specialized diet.

Haminoea orbygniana is likely to feed randomly from the available diatoms existing on the substrate, with selection of species not a consequence of any particular preference but merely the result of the natural diatom abundances. Chester (1993) found seasonal changes of *Haminoea solitaria* diet resulting from the annual dynamics of the food items in the natural environment.

The techniques used in this study to identify the diet of *H. orbygniana* did not permit any estimate of the role of fungi and bacteria (except for cyanobacteria) which might lead to an underestimation of the diet quality and quantity. However, Jensen & Siegismund (1980) after evaluating the relative importance of diatoms and bacteria in the general context of the diet of the prosobranch gastropod *Hydrobia ulvae*, stressed that the former had a major role in the growth and production of the snail, with bacteria showing a minor importance as a consequence of a low efficiency in terms of both digestion and assimilation.

According to Kohn (1983) feeding activities in the family Haminoeidae are induced by the contact of food with chemoreceptor structures particularly the Hancock organ and sensory palps which lie adjacently to the mouth. Nevertheless, this statement might result from a generalization based on descriptions from other better studied cephalaspid gastropods such as *Acteocina*, *Retusa* and *Philine* (Fretter, 1939; Burn & Bell, 1974; Chester, 1993; Berry, 1994; Cedhagen, 1996). Chester (1993) was the first author to claim the absence of food selection in the genus *Haminoea*, denying any function for the sensory structures.

The large number of sand particles found in the digestive tract and faecal pellets might be interpreted as an indication of the non-selective feeding behaviour of *H. orbygniana* as claimed by Chester (1993) [the importance index for sand particles contained in the diet of *H. orbygniana* (28%) is very similar to that calculated by Chester (1993) for *H. solitaria* (26%)]. Our results support the idea that the sensory structures are not involved in strict prey selection, but are possibly involved in localization of areas with high concentration of food, as previously suggested by Chester (1993).

On the other hand, if we assume that sand-coating bacteria might act as an important food resource in *Haminoea* diet, a different explanation to the large amount of sand can be raised. The presence of such a large quantity of sand both in gut and faecal contents could be explained as the result of a feeding strategy in order to use the bacterial film as a food source. If this proves to be true, then sand ingestion will switch from a passive act to an active process, and therefore the Haminoeidae should be regarded as a feeding selective group (as previously stated by Kohn, 1983), with sensory structures playing their own role in food localization. An additional scenario that can not be discarded is the role in which sand together with the gizzard plates might play in crushing diatoms and vegetal tissue, helping to improve the efficiency of the digestive process. Further research is required to test these hypotheses.

The role of the gizzard plates

Although in the post-oesophageal tract the number of broken diatoms is less frequent than unbroken, the rise in broken diatoms in the posterior digestive tract is unequivocal evidence that something is occurring inside the gizzard. Crushing of diatoms by the gizzard is believed to facilitate the release of cell contents for stomach digestion and digestive gland assimilation. According to Fretter (1939) and Rudman (1971) the three gizzard plates act as a millstone, which under normal conditions almost totally crush ingested food items. The cell walls are totally or partially destroyed and the cell contents are released. The food is digested in the stomach (post-oesophageal tract) aided by enzymes produced in the digestive gland and released throughout ducts that connect both structures.

Rudman (1971) observed that when a large amount of food is ingested, a fraction can pass undamaged through the gizzard. This is because the overload did not allow the gizzard plates to crush the food, and since the stomach does not have the ability to digest undamaged food items, they pass directly to the intestine being excreted in the form of faecal pellets full of undigested material. However, our data demonstrate that despite the gizzard plates crushing a considerable number of diatoms, a large majority passes apparently undamaged through the gizzard even though digested. Chester (1993) obtained similar results for *H. solitaria*.

In our opinion several explanations can be suggested to account for these observations. Firstly, we admit that this could be an artefact resulting from the methodology followed during this work. Some rupture in the diatoms might indeed occur at a micro-scale level, resulting from a combined action of the gizzard plates and sand grains in a similar process to that in bird gizzards. These micro-ruptures might not be discernible under the inversion microscope, but could be large enough to allow release of cell contents. Or secondly, the majority of diatoms do not suffer indeed any kind of rupture, which leads to the possibility of a re-evaluation of the function of the gizzard.

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