Cell size-based, passive selection of the blue diatom Haslea ostrearia by the oyster Crassostrea gigas

Fiddy Prasetiya, Priscilla Decottignies, Laurent Barillé, Romain Gastineau, Boris Jacquette, Amandine Figiel, Michèle Morançais, Réjean Tremblay, Jean-Luc Mouget, Bruno Cognie

Cell size-based, passive selection of the blue diatom *Haslea ostrearia* by the oyster *Crassostrea gigas*

Fiddy S. Prasetiya[a, d], Priscilla Decottignies[b], Laurent Barillé[b], Romain Gastineau[a], Boris Jacquette[a], Amandine Figiel[b], Michèle Morançais[b], Réjean Tremblay[c], Jean-Luc Mouget[a], and Bruno Cognie[b,*]

a. FR CNRS 3473 IUML, Mer-Molécules-Santé (MMS), Université du Maine, Avenue O. Messiaen, 72085 Le Mans cedex 9, France;
b. FR CNRS 3473 IUML, Mer-Molécules-Santé (MMS), Université de Nantes, 2 rue de la Houssinière44322 Nantes cedex 3, France
c. Institut des Sciences de la Mer, 310 allée des Ursulines, Rimouski, Québec, G5L 3A1, Canada
d. Universitas Padjadjaran, Research Center of Biotechnology and Bioinformatics, Jl. Raya Bandung Sumedang KM 21, Jatinangor 40600, Indonesia

* Corresponding author: Bruno.Cognie@univ-nantes.fr; tel. + 33 (0)2 51 12 56 65

ABSTRACT

Preingestive selection has been identified as a feeding mechanisms of oysters that may influence their uptake of particles and microalgal cells. In the last decades, it has been shown that oysters can specifically feed on the pennate diatom *Haslea ostrearia*, which produces the blue pigment marennine, responsible for the greening of oysters. Given that the size of particles or cells plays a significant role in selection process and that diatoms experience a decrease in size as a consequence of their vegetative reproduction, *H. ostrearia* consumption and marennine uptake could be influenced by the preingestive selection. The aim of this work was thus to examine the role of *H. ostrearia* cell size in the selective feeding of *C. gigas*. Individual flow-through chambers were used to deliver mixtures to oysters containing *H. ostrearia* of varying cell length. Inflow, outflow and pseudofaeces samples were collected from the individual chambers during oyster feeding, while video-endoscopy was used to sample material in the dorsal and ventral particle tracts. Diatom cells were counted visually in these samples, which showed that the pseudofaeces contained on average larger cells than the ambient medium. In contrast, the proportions of the different populations of *H. ostrearia* in pseudofaeces were identical to those in ventral tracts, indicating that no selection was performed by the labial palps. In addition, video-endoscopy observations and imaging by scanning electron microscopy of gills and labial palps revealed that only those larger *H. ostrearia* orientated dorso-ventrally could enter the principal filaments and then access the dorsal acceptance tract. These results show that for particles like *Haslea* cells with only one axis exceeding the width of the principal filaments, the selection at the oyster gills is passive and based on cell size.

KEY WORDS: *Crassostrea gigas*, *Haslea ostrearia*, marennine, microalgal cell size, oyster, preingestive selection

1. Introduction

Suspension-feeding bivalves constitute one of the dominant trophic group in estuarine ecosystems, and their feeding mode may strongly affect nutrient recycling, seston dynamics and the benthic food web (Asmus & Asmus, 1991; Prins *et al.*, 1991; Dame, 1993; Ward & Shumway, 2004). In these environments, suspension-feeders have to cope with wide fluctuations in both the quantity and quality of suspended particulate matter (Armstrong, 1958; Berg & Newell, 1986; Fegley *et al.*, 1992; Barillé *et al.*, 1997). It is thus not surprising to find an abundant literature concerning the response of suspension-feeding bivalves to seston fluctuations, in particular the physiological variables related to feeding, such as clearance rate, retention efficiencies and preingestive selection (Jørgensen, 1990; Riisgard, 2001; Riisgård & Larsen, 2001; Ward & Shumway, 2004).

Progress has been made in understanding the pallial organs and ciliation involved in preingestive selection (Beninger & St-Jean, 1997; Beninger *et al.*, 1997; Ward *et al.*, 1997; Cognie *et al.*, 2003). Different studies have focused on the sorting criteria in bivalves, and many particle characteristics have been identified as selection cues, namely size (Hughes, 1975; Shumway *et al.*, 1985; Defossez & Hawkins, 1997), organic content (Bacon *et al.*, 1998; Beninger *et al.*, 2008b) or microalgae cell surface properties and compounds (Ward & Targett, 1989; Pales Espinosa *et al.*, 2007, 2010; Beninger *et al.*, 2008a; Rosa *et al.*, 2013).

Particle size was the first criterion used by early researchers examining food selection (Yonge, 1926; Atkins, 1937), however its importance seems variable, with no influence of particle size on preingestive selection repeatedly reported (Newell & Jordan, 1983; Newell, 1988; Chretiennot-

69 Dinet *et al.*, 1991; MacDonald & Ward, 1994; Bougrier *et al.*, 1997), while the opposite can also
70 be found in the literature (Ballantine & Morton, 1956; Miura & Yamashiro, 1990; Cognie *et al.*,
71 2003; Mafra *et al.*, 2009).
72 Nevertheless, the particles used in most of these studies differed not only in size, thus generating
73 possible cofounding factors. In that instance, Hughes (1975) observed size-dependent selection
74 of particles by *Abra alba*, but noted a relationship between particle size and food value. Cognie
75 *et al.* (2003) demonstrated that the particle size plays a role in oyster, determining which pallial
76 organs (gills or palps) will be involved in the sorting. The microalgal species used in this study
77 (*Pleurosigma planctonicum*, *Coscinodiscus perforatus* and *Rhizosolenia setigera*) were too large
78 to have access to principal filaments and a selection by the gill was therefore not possible. The
79 observed selection was performed by the labial palps and was based not on the particle size but
80 on their biochemical composition (intact *vs* empty cells). To our knowledge, very few studies
81 have unambiguously supported the idea that bivalves can use a size criterion to discriminate
82 particles. A preferential size-dependent rejection of larger particles was observed in *C. virginica*
83 (Tamburri & Zimmer-Faust, 1996) and in *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes*
84 *decussatus* (Defossez & Hawkins, 1997). However the particles used in these studies were
85 artificial, polystyrene or borosilicated glass particles in the former, silica particles in the latter,
86 and these results not completely transposed to natural living microalgal cells. Nevertheless, using
87 natural living cells, *i.e*. contrasting-sized clones of a toxic diatom, Mafra *et al.* (2009)
88 indisputably demonstrated the role of size in the selection carried out by *C. virginica* gills.
89 A well-known feature of diatom biology that could have a great effect on living cell selection by
90 bivalves,is the MacDonald-Pfitzer rule (Pfitzer, 1869; Macdonald, 1869; Round *et al.*, 1990).
91 During vegetative growth and mitotic divisions, one of the daughter cells is smaller than the
92 mother cell and thus the mean cell size of a population decreases, a phenomenon that usually

leads to the loss of monoclonal cultures in the laboratory. This cell size diminution, unique to diatoms and especially crucial in most pennate species, can be compensated: when a critical threshold size is reached, sexual reproduction and auxosporulation will occur. In diatoms, auxosporulation results in the formation of initial cells, which restore vegetative cells with specific maximum length (Amato, 2010). Due to these biological traits, cells of the same species, but of different sizes, are often found together in the natural environment (Mann, 1988; Potapova & Snoeijs, 1997; Mann *et al.*, 1999). This phenomenon is expected to affect the kinetics of diatom selection and diatom metabolite uptake by bivalves through its effect on their feeding processes, for example clearance rate, preingestive selection and absorption efficiency. In fact, Mafra *et al.* (2009) demonstrated that the ability of *C. virginica* to sort particles according to their size could affect its uptake of the neurotoxin domoic acid from *Pseudo-nitzschia multiseries*.

Studying the specific size-dependent sorting mechanism is crucial to understand and model the transfer of a bioproduct from algal cells to suspension-feeding bivalves. The marine diatom *Haslea ostrearia* is known to produce marennine, a polyphenolic water-soluble blue pigment (Pouvreau *et al.*, 2006), responsible for the "greening" of the tissues of numerous marine invertebrates and the gills of bivalves in particular (Ranson, 1927; Robert, 1983; Turpin *et al.*, 2001; Gastineau *et al.*, 2014b).

It has also been shown that the oyster *C. gigas* can feed exclusively on *H. ostrearia* (Barillé *et al.*, 1994) despite of its lower nutritive value compared to other microalgae species (Piveteau, 1999). Although *H. ostrearia* and marennine have long been recognised as the only greening agent of cultured oysters in France, other species from the same genus and producing "marennine-like" pigments have been discovered elsewhere in the world (Gastineau *et al.*, 2012a, 2014a, 2015). These authors have also demonstrated that marennine-like pigments have

antibacterial, antiviral and antifungal activities, in particular against pathogens of the oyster *C. gigas* (Gastineau *et al.*, 2012b, 2012c). Thus the *Haslea* consortium and its marennine-like pigments have potential application in shellfish aquaculture: used to feed and green oysters, they could sustain growth of bivalves, and protect them against pathogens.

In the present work, cultures of *H. ostrearia*, differing according to their mean cell length, were used to feed the oyster *C. gigas*, to investigate whether cell size affects algal selection by pallial organs (gills and labial palps). For feeding experiments, different methods were used; endoscope-directed *in vivo* sampling and naturally occurring particles (diatom cells) of different sizes, and Scanning Electron Microscopy (SEM) to illustrate the close relationship between the size of the microalgal clones and the structures and ciliation of pallial organs involved in the sorting processes. The ecological implications of preingestive mechanisms by oysters on *H. ostrearia* and based on algal cell length, will be discussed, in particular regarding some biological traits of this diatom species, *e.g.*, the reproductive cycle and population structure.

2. Materials and Methods

2.1 Algal culture

The cultures of *Haslea ostrearia* used in this study were obtained from the Nantes Culture Collection [NCC; temperature: 14°C; light/dark cycle: 14/10 h; light intensity: 100 µmol photons m^{-2} s^{-1}; ES1/3 medium, Provasoli (1968)], Faculté des Sciences et des Techniques, Université de Nantes (France). Strains with a similar biochemical composition (Joux-Arab *et al.*, 2000) but different cell lengths were selected (Table 1). A particle counter (Coulter Multisizer 3 ®) was used to determine equivalent spherical diameter of the different strains. Sixty-liter mass cultures were performed in polyethylene bags filled with underground seawater (Cognie, 2001) supplemented with an enrichment solution containing nitrogen, phosphorus and silicon (Turpin

141 *et al.*, 2001). The different populations were then mixed at equivalent cell densities (around 3 10^6
142 cells L^{-1} for each cell size, Table 1) for a final total cell concentrations of 9 10^6 cells L^{-1} and 6.1
143 10^6 cells L^{-1}, delivered to the bivalves in conditions A and B, respectively.
144
145 **2.2 Oyster sampling and maintenance**
146 Adult oysters of *Crassostrea gigas* with a mean dry weight of 0.8 g (SE = 0.1, n = 20)
147 were collected in the intertidal zone of Bourgneuf Bay (France) (46-47° N, 1-2° W). After
148 immediate transfer to the laboratory and removal of shell epibionts, they were placed for two
149 minutes in a 0.1% hypochlorite solution to eliminate parasitic polychetes of the genus *Polydora*.
150 The oysters were then thoroughly washed, maintained during one week in filtered (Millipore
151 0.45 µm) seawater and oxygenated.
152
153 **2.3 Feeding experiments**
154 Two series of experiments were run over different periods; one with oysters feeding on
155 three algal cell sizes (condition A), the other, on two algal cell sizes (condition B). After 24
156 hours of acclimation prior to experimental conditions (A or B, Table 1), oysters were randomly
157 chosen and placed in a flow-through experimental system, as described in Cognie et al. (2003).
158 The mean flow rate for each individual tray was 8 L h^{-1} at 16°C. Two trays containing an empty
159 shell were used as sedimentation controls. After one hour of oyster acclimation, seawater
160 samples (50 mL) were collected at the outflow of the experimental individual trays every 15
161 minutes for one hour. Pseudofaeces were collected at the end of the observations. The individual
162 samples (outflow seawater and pseudofaeces) were fixed with acetic Lugol's solution and
163 analysed separately as replicates. Cells were counted by means of light microscopy using
164 "Nageotte" type hematimetric units. For each sample, a minimum of 300 cells was counted.

165

166 **2.4 Sample preparation for Scanning Electron Microscopy (SEM)**

167 Oysters intended for SEM observations were placed in flow-through trays and fed the
168 same mixture as in experimental condition A (Table 1). Once pseudofaeces appeared, the oysters
169 were collected, shucked and immediately fixed in a 2.5% hypertonic glutaraldehyde solution in
170 sodium cacodylate buffer 0.1 M (Beninger *et al.*, 1995). The collection-fixation step was
171 performed within 30 s to limit stress-related mucus production and preserve the functional stage
172 of the animal. After fixation (for at least 48 h), individuals were partially dehydrated in
173 successive alcohol baths at increasing concentrations (up to 70%). At this stage, the oysters were
174 dissected using a dissection microscope and microsurgical instruments. The samples were
175 subsequently totally dehydrated in a 100% anhydrous alcohol bath and dried with CO_2 using a
176 critical point apparatus. The samples were then plated with gold-palladium alloy and observed
177 under a scanning electron microscope with field effect (JEOL 5400). To estimate the tissue
178 contraction of the samples during SEM preparation, additional measurements were made with a
179 dissecting microscope on live individuals still on the half shell.

180

181 **2.5 Video-endoscopy directed sampling**

182 This sampling was conducted concomitantly with the feeding measurements as described
183 in section 2.3, following the procedure reported in Cognie et al. (2003). At least 24 h before the
184 sampling, a small aperture was milled in the shells to prevent damage of the optical insertion
185 tube (OIT) when the oyster valves closed. Specimens were placed in flow-through trays (8 $L.h^{-1}$)
186 and fed the same mixture as in experimental condition A (Table 1). Sampling was performed
187 every 15 min for 1 h in both the dorsal and ventral particle grooves (1 mL using a micropipette)
188 and at the inflow and outflow of the trays (50 mL). After one hour of observation, pseudofaeces

were collected. The samples (outflow seawater, pseudofaeces, and from ventral and dorsal grooves) were treated and analysed as described in section 2.3.

2.6 Data analysis

All statistical analyses were performed using XLSTAT 2014 software. The percentages of the different cell sizes were compared in the various samples using the Kruskal-Wallis test (Conover, 1999). The hypotheses tested in experimental condition B are described in Table 2.

For condition B, a selection index (SI, Cognie *et al.*, 2003) was calculated for the various pallial sites (dorsal and ventral gill tracts, labial palps) to determine the degree and direction of selection at each site:

$$SI = ([S\% - W\%] / W\%) \times 100$$

where S% is the percentage of small *H. ostrearia* cells in the sample (dorsal or ventral tracts, pseudofaeces) and W% the percentage of small *H. ostrearia* cells in the water. Calculated SI values were arcsine transformed and compared to zero or between them using a one-sample t-test.

3. Results

3.1 Condition A: oysters feeding on three algal cell sizes: particle selection + SEM observations

The heterorhabdic gill of *C. gigas* is composed of plicae and troughs corresponding to the locations of ordinary and principal filaments, respectively (Fig. 1A and B). The latter were difficult to observe due to their position deep between each plica and the contraction effect induced by the preparation for SEM study. *In vivo* observations showed that the plicae were 200 to 250 µm wide, the ordinary filaments were between 35 and 40 µm, and the opening of the

213 principal filaments was approximately 70 µm. In addition, ordinary filaments located in the
214 apical part of the plicae (or apical filaments) were approximately 45 µm wide. The same
215 measurements, when performed using SEM, showed that plicae were only 120 to 140 µm wide
216 (Fig. 1A), ordinary filaments 18 to 26 µm wide (Fig. 1B) and apical filaments 25 and 30 µm
217 wide (Fig. 1A and D). Principal filaments were no longer visible and too deeply positioned in
218 troughs to allow measurements (Fig. 1B and C). Thus, due to the preparation for SEM, the
219 dimensions of contracted gill plicae correspond to approximately 50% of those observed *in vivo*.
220 Cells of the three distinct algal populations were always observed mixed on all oyster
221 structures involved in the selection process, whether these cells were free or in aggregates of
222 mucus. *Haslea ostrearia* cells were observed on the gill surface without preferential orientation
223 (Fig. 1B, C and D). However, the cells distinguished at the bottom of the principal filaments
224 were always orientated according to the longitudinal axis of the gill filaments (¥ Fig. 1E). *Haslea*
225 *ostrearia* cells transported dorsoventrally along the gill surface reached the ventral grooves (Fig.
226 1E). They were then carried toward the anterior part of the pallial cavity in mucous aggregates
227 (not shown), before being processed on the ridged inner surfaces of the labial palps. Aggregates
228 of mucus (* Fig. 1G) and cells of the three *H. ostrearia* populations were observed on these
229 surfaces (Fig. 1F, G and H).
230 A dimensional gap between the size of the three *H. ostrearia* populations and that of the
231 gill and labial palp structures was apparent. Considering the tissue contraction due to the sample
232 preparation for SEM, an Ho95 cell overlapped more than one apical filaments (Fig. 1D) and
233 more than one and a half ordinary filaments (*Fig. 1E). Regarding labial palps, cells of the
234 largest population were equivalent in size to the width of a plica.
235 SEM studies of the structures and ultrastructures of the pallial organs involved in *C. gigas*
236 feeding were associated with feeding measurements. The mixture of the three *H. ostrearia*

populations represented a food ration of 2.5 mg L^{-1} of total seston containing an organic fraction of around 50% (Table 1). Microscopic measurements of frustule lengths showed that the three *H. ostrearia* populations were significantly different in size (Table 1; ANOVA, $P < 0.001$).

The proportions of the three populations were the same at the inflow and outflow of individual tanks (Table 3; χ^2 test, $P > 0.05$), indicating that they were retained with the same efficiency on gills. The proportions of the three populations at the inflow, however, were significantly different from those in the pseudofaeces (Table 3; χ^2 test, $P \leq 0.05$), indicating preferential preingestive rejection of the two larger cell populations.

3.2 Condition B: oysters' feeding on two algal cell sizes: particle selection + video-endoscopy directed sampling

The mixture of the two *H. ostrearia* populations represented a food ration of 1.9 mg L^{-1} of total seston containing an organic fraction of around 50% (Table 1). Biometry applied to frustule lengths showed that the two *H. ostrearia* populations were significantly different (ANOVA, $P < 0.001$). During the video-endoscopy directed sampling oysters showed no disruption of their feeding behaviour.

The value of the selection index (SI) at outflow was not significantly different from zero (t-test, $P > 0.05$; Fig. 2), indicating that both populations were retained with the same efficiency on gills. However, the SI for pseudofaeces was significantly lower than zero (t-test, $P < 0.001$), clearly allowing the rejection of the null hypothesis *Ho* and acceptance of *H1*: selection occurred on the pallial organs. The mean SI for ventral groove and dorsal tract were significantly different from zero (t-test, $P < 0.001$), thus allowing acceptance of *H1a*: selection occurred on the gills ($P < 0.05$). There was no significant difference between the mean SI for ventral groove and

pseudofaeces (t-test, $P > 0.05$). The experimental hypotheses *H1b* and *H1c* may therefore be rejected: no selection was performed on the labial palps. Selection indices clearly showed that dorsal tracts were enriched with small *H. ostrearia* cells whereas ventral grooves and pseudofaeces were enriched in large *H. ostrearia* cells (Fig. 2).

4. Discussion

In the present study, cells were retained on the gill with the same efficiency when oysters were fed mixed suspensions containing *H. ostrearia* of varying cell length. Previous studies demonstrated that, in bivalves, retention efficiency varies with particle size and concentration. In *C. gigas*, Barillé et al. (1993) showed that particles above 6 µm ESD (equivalent spherical diameter) were retained with 100 % efficiency at the total seston concentration used in our study (3.8 or 4.9 mg L^{-1}). This maximal retention efficiency threshold is slightly less than the size of the smallest *H. ostrearia* cells fed to the oysters (Ho-small, 6.1 ± 0.1 mean ESD ± SE), indicating that the cells of the three populations were retained with 100 % efficiency.

From both experiments, we demonstrated that the size of *H. ostrearia* cells is an essential criterion influencing preingestive selection in *C. gigas*. Oysters preferentially rejected in pseudofaeces the larger *H. ostrearia* cells (Ho-large or Ho75 and Ho 95) when offered in mixed suspensions with the smaller ones (Ho-small or Ho 50). In oysters, preingestive sorting ability has been related to the presence of antagonistic ciliary tracts on the surface of the ordinary filaments composing plicae (Atkins, 1937; Ribelin & Collier, 1977; Ward *et al.*, 1994, 1998b). Particles transported by the median frontal cilia of ordinary filaments are directed towards the ventral grooves in which they are conveyed within an aggregate of mucus for a further preferential rejection. Conversely, particles transported by the marginal frontal cilia on both sides of the median frontal cilia are directed towards the dorsal grooves and then conveyed to the

mouth as a mucus suspension. Figures 1B-E show the dimensional relationship between the size of the three *H. ostrearia* populations and that of the gill structures likely to perform particle sorting. Thus, for large cells with a naviculoid shape, such as those used in our study, bidirectional particle transport by ordinary filaments would seem difficult to achieve, even if particles were directed according to the longitudinal axis of the filaments.

Another mechanism that could account for selection at the gill level is related to the differentiation of heterorhabdic gills into principal and ordinary filaments (Atkins, 1937; Ward *et al.*, 1994, 1998a; Beninger & St-Jean, 1997). In bivalves possessing such a gill type, preingestive sorting may be performed using the principal filaments for the material to be ingested and the ordinary filaments for the material to be rejected. Our SEM and video-endoscopy observations showed that the cells of the larger populations were present in the troughs containing principal filaments, but were always orientated dorso-ventrally. Smaller cells were observed accessing principal filaments with no preferential orientation. These qualitative data confirm that passive selection related to particle size may operate on gills, as suggested by Ward and Shumway (2004) and previously observed in *C. virginica* by Mafra et al. (2009).

Particles transported in dorsal grooves and those conveyed with mucus aggregates in ventral grooves are directed towards the ridged surfaces of the labial palps (Ward, 1996). In bivalves with homorhabdic gills, the labial palps are considered to be the main site for sorting and regulating the quantity of particles ingested. In bivalves with heterorhabdic gills such as oysters of the genus *Crassostrea*, the palps are thought to play a secondary role in these two functions and to serve mainly to reject the non-ingested material as pseudofaeces (Beninger & St-Jean, 1997; Ward *et al.*, 1998a). The differentiation of heterorhabdic gills into two types of filaments enables these two functions to be performed before the material reaches the palps. In addition, recent studies have demonstrated that labial palps may also sort algal cells on the basis

of their chemical properties (Cognie *et al.*, 2003; Beninger *et al.*, 2008a, 2008b; Mafra *et al.*, 2009). In the present study, using *H. ostrearia* cells of contrasting size, we observed selection by oyster gills but not by labial palps, suggesting that particle size did not affect the selective ability of these two pallial organs in the same way.

The state of knowledge concerning the size criterion in selection by oyster pallial organs is clarified and summarized in Table 4. Particle dimensions (length, width, height) should be considered a physical constraint affecting access to principal filaments (pfs) and consequently the selective ability of the oyster gill. Particles with all dimensions smaller than the pf width can access principal filament troughs freely and their selection may occur at gills and/or labial palps. In contrast, particles with all dimensions greater than the pf width are unable to enter the principal filaments and selection at gills is not possible. For particles with only one dimension greater than the pf width, their access to principal filaments is limited to particles orientated dorso-ventrally. The selection at the gills is passive or mechanical and a secondary selection at labial palps is possible.

The demonstration that *C. gigas* selectively rejects larger cells of *H. ostrearia* could have some ecological significance, both in natural environments and in aquaculture, especially in oyster ponds. For example, in estuarine and coastal waters, oysters feed selectively on pennate diatoms, which constitute an important food source in intertidal areas with large mud flats (Cognie *et al.*, 2003). Considering the stock of cultivated and wild oysters, the sorting process evidenced in the present work may affect the structure of the microalgal populations in the vicinity of oysterbeds. Our study suggests that the preferential rejection of large-sized cells in pseudofaeces could favour the development and/or maintenance of sub-populations of large-sized *Haslea*, given that *H. ostrearia* cells rejected in pseudofaeces have a revival capacity that is not altered by preingestive processing (Barillé & Cognie, 2000). Furthermore, like many pennate

diatoms, sexually competent cells of *H. ostrearia* can carry out auxosporulation to compensate for the size reduction associated with vegetative divisions, but only when cell size decreases to 65-70 µm, which represents 50% of the maximum cell length of the species (Neuville & Daste, 1979; Davidovich *et al.*, 2009; Mouget *et al.*, 2009). Therefore, this study shows that *C. gigas* preferentially ingests sexually mature cells and rejects immature ones, which could modify *H. ostrearia* cell size distribution and population dynamics in oyster ponds. Given the crucial role of cell size in the life cycle of diatoms, the impact of this predation pressure on the population dynamics of *H. ostrearia* in oyster ponds remains to be assessed, especially considering the importance of marennine in the greening process, but also regarding its many biological activities (*e.g.*, antibacterial) that could be exploited in aquaculture.

Acknowledgements

The authors are grateful to Pierre Gaudin, Denise Jahan and Philippe Rosa for their assistance with algal cultures and oysters preparation. Carol Robins is acknowledged for linguistic revision. We would also like to thank the Syndicat Mixte pour le Développement de l'Aquaculture et de la Pêche en Pays de Loire and the Région Pays de la Loire for their financial support (programme PANDHA). This publication also benefited from funding from the European Commission under the Community's Seventh Framework Programme BIOVADIA (contract No. FP7-PEOPLE-2010-IRSES-269294, Biodiversity and Valorisation of Blue Diatoms).

References

AMATO A. 2010. Diatom reproductive biology: living in a crystal cage. The International Journal of Plant Reproductive Biology, **2**: 1–10.

ARMSTRONG F. A. J. 1958. Inorganic suspended matter in sea water. Journal of Marine Research, **17**: 23–34.

ASMUS R. M. & ASMUS H. 1991. Mussel beds: limiting or promoting phytoplankton?. Journal of Experimental Marine Biology and Ecology, **148**: 215–232.

ATKINS D. 1937. On the Ciliary Mechanisms and Interrelationships of Lamellibranchs Part II: Sorting Devices on the Gills. Quarterly Journal of Microscopical Science, **s2-79**: 339–373.

BACON G. S., MACDONALD B. A. & WARD J. E. 1998. Physiological responses of infaunal (Mya arenaria) and epifaunal (Placopecten magellanicus) bivalves to variations in the concentration and quality of suspended particles: I. Feeding activity and selection. Journal of Experimental Marine Biology and Ecology, **219**: 105–125.

BALLANTINE D. & MORTON J. E. 1956. Filtering, feeding, and digestion in the lamellibranch Lasaea rubra. Journal of the Marine Biological Association of the United Kingdom, **35**: 241–274.

BARILLE L., BOUGRIER S., GEAIRON P. & ROBERT J.-M. 1994. Alimentation expérimentale de l'huître Crassostrea gigas à l'aide de navicules bleus Haslea ostrearia (Simonsen) de différentes tailles. Oceanologica Acta, **17**: 201–210.

BARILLÉ L. & COGNIE B. 2000. Revival capacity of diatoms in bivalve pseudofaeces and faeces. Diatom Research, **15**: 11–17.

BARILLÉ L., PROU J., HÉRAL M. & BOUGRIER S. 1993. No influence of food quality, but ration-dependent retention efficiencies in the Japanese oyster Crassostrea gigas. Journal of Experimental Marine Biology and Ecology, **171**: 91–106.

BARILLÉ L., PROU J., HÉRAL M. & RAZET D. 1997. Effects of high natural seston concentrations on the feeding, selection, and absorption of the oyster Crassostrea gigas (Thunberg). Journal of Experimental Marine Biology and Ecology, **212**: 149–172.

BENINGER P., DUFOUR S. & BOURQUE J. 1997. Particle processing mechanisms of the eulamellibranch bivalves Spisula solidissima and Mya arenaria. Marine Ecology Progress Series, **150**: 157–169.

BENINGER P. G. & ST-JEAN S. D. 1997. Particle processing on the labial palps of Mytilus edulis and Placopecten magellanicus (Mollusca: Bivalvia). Marine ecology progress series. Oldendorf, **147**: 117–127.

BENINGER P. G., ST-JEAN S. D. & POUSSART Y. 1995. Labial palps of the blue mussel Mytilus edulis (Bivalvia: Mytilidae). Marine biology, **123**: 293–303.

384 BENINGER P. G., VALDIZAN A., COGNIE B., GUIHENEUF F. & DECOTTIGNIES P. 2008a. Wanted: alive and not dead:
385 functioning diatom status is a quality cue for the suspension-feeder Crassostrea gigas. Journal of Plankton
386 Research, **30**: 689–697.
387 BENINGER P. G., VALDIZAN A., DECOTTIGNIES P. & COGNIE B. 2008b. Impact of seston characteristics on qualitative
388 particle selection sites and efficiencies in the pseudolamellibranch bivalve Crassostrea gigas. Journal of
389 Experimental Marine Biology and Ecology, **360**: 9–14.
390 BERG J. A. & NEWELL R. I. E. 1986. Temporal and spatial variations in the composition of seston available to the
391 suspension feeder Crassostrea virginica. Estuarine, Coastal and Shelf Science, **23**: 375–386.
392 BOUGRIER S., HAWKINS A. J. S. & HÉRAL M. 1997. Preingestive selection of different microalgal mixtures in
393 Crassostrea gigas and Mytilus edulis, analysed by flow cytometry. Aquaculture, **150**: 123–134.
394 CHRETIENNOT-DINET M.-J., VAULOT D., GALOIS R., SPANO A.-M. & ROBERT R. 1991. Analysis of larval oyster
395 grazing by flow cytometry. Journal of Shellfish Research, **10**: 457–463.
396 COGNIE B. 2001. Alimentation de l'huître Crassostrea gigas (Thunberg): étude des mécanismes de sélection des
397 particules et des processus rétroactifs entre le bivalve et les microalgues. PhD thesis, University of Nantes.
398 COGNIE B., BARILLÉ L., MASSÉ G. & BENINGER P. G. 2003. Selection and processing of large suspended algae in the
399 oyster Crassostrea gigas. Marine Ecology Progress Series, **250**: 145–152.
400 CONOVER W. J. 1999. Practical Non parametric Statistics. 3rd edition. John Wiley and Sons, New York.
401 DAME R. F. 1993. The Role of Bivalve Filter Feeder Material Fluxes in Estuarine Ecosystems. In: Bivalve Filter
402 Feeders (R. F. Dame, ed.), pp. 245–269. Springer Berlin Heidelberg.
403 DAVIDOVICH N. A., MOUGET J.-L. & GAUDIN P. 2009. Heterothallism in the pennate diatom Haslea ostrearia
404 (Bacillariophyta). European Journal of Phycology, **44**: 251–261.
405 DEFOSSEZ J.-M. & HAWKINS A. J. S. 1997. Selective feeding in shellfish: size-dependent rejection of large particles
406 within pseudofaeces from Mytilus edulis, Ruditapes philippinarum and Tapes decussatus. Marine Biology,
407 **129**: 139–147.
408 FEGLEY S. R., MACDONALD B. A. & JACOBSEN T. R. 1992. Short-term variation in the quantity and quality of seston
409 available to benthic suspension feeders. Estuarine, Coastal and Shelf Science, **34**: 393–412.
410 GASTINEAU R., DAVIDOVICH N. A., BARDEAU J.-F., CARUSO A., LEIGNEL V., HARDIVILLIER Y., JACQUETTE B.,
411 DAVIDOVICH O. I., RINCÉ Y., GAUDIN P., COX E. J. & MOUGET J.-L. 2012a. Haslea karadagensis

412 (Bacillariophyta): a second blue diatom, recorded from the Black Sea and producing a novel blue pigment.
413 European Journal of Phycology, **47**: 469–479.
414 GASTINEAU R., DAVIDOVICH N. A., HALLEGRAEFF G. M., PROBERT I. & MOUGET J.-L. 2014a. Reproduction in
415 microalgae. In: Reproduction Biology of Plants (K. G. Ramawat, J. M. Mérillon, & K. R. Shivanna, eds.),
416 pp. 1–28. CRC Press.
417 GASTINEAU R., HANSEN G., DAVIDOVICH N. A., DAVIDOVICH O. I., BARDEAU J.-F., KACZMARSKA I., EHRMAN J. M.,
418 LEIGNEL V., HARDIVILLIER Y., JACQUETTE B., POULIN M., MORANÇAIS M., FLEURENCE J. & MOUGET J.-L.
419 2015. A new blue-pigmented hasleoid diatom, Haslea provincialis, from the Mediterranean sea. European
420 Journal of Phycology, **accepted**.
421 GASTINEAU R., HARDIVILLIER Y., LEIGNEL V., TEKAYA N., MORANÇAIS M., FLEURENCE J., DAVIDOVICH N.,
422 JACQUETTE B., GAUDIN P. & HELLIO C. 2012b. Greening effect on oysters and biological activities of the
423 blue pigments produced by the diatom Haslea karadagensis (Naviculaceae). Aquaculture, **368**: 61–67.
424 GASTINEAU R., POUVREAU J.-B., HELLIO C., MORANÇAIS M., FLEURENCE J., GAUDIN P., BOURGOUGNON N. &
425 MOUGET J.-L. 2012c. Biological Activities of Purified Marennine, the Blue Pigment Responsible for the
426 Greening of Oysters. Journal of Agricultural and Food Chemistry, **60**: 3599–3605.
427 GASTINEAU R., TURCOTTE F., POUVREAU J.-B., MORANÇAIS M., FLEURENCE J., WINDARTO E., PRASETIYA F. S.,
428 ARSAD S., JAOUEN P., BABIN M., COIFFARD L., COUTEAU C., BARDEAU J.-F., JACQUETTE B., LEIGNEL V.,
429 HARDIVILLIER Y., MARCOTTE I., BOURGOUGNON N., TREMBLAY R., DESCHÊNES J.-S., BADAWY H.,
430 PASETTO P., DAVIDOVICH N., HANSEN G., DITTMER J. & MOUGET J.-L. 2014b. Marennine, Promising Blue
431 Pigments from a Widespread Haslea Diatom Species Complex. Marine Drugs, **12**: 3161–3189.
432 HUGHES T. G. 1975. The sorting of food particles by Abra sp. (bivalvia: tellinacea). Journal of Experimental Marine
433 Biology and Ecology, **20**: 137–156.
434 JØRGENSEN C. B. 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. Olsen &
435 Olsen.
436 JOUX-ARAB L., BERTHET B. & ROBERT J.-M. 2000. Do toxicity and accumulation of copper change during size
437 reduction in the marine pennate diatom Haslea ostrearia?. Marine Biology, **136**: 323–330.
438 MACDONALD B. A. & WARD J. E. 1994. Variation in food quality and particle selectivity in the sea scallop
439 Placopecten magellanicus (Mollusca: Bivalvia). Marine Ecology-Progress Series, **108**: 251–251.

MACDONALD J. D. 1869. I.—On the structure of the Diatomaceous frustule, and its genetic cycle. Annals and Magazine of Natural History, **3**: 1–8.

MAFRA L. L., BRICELJ V. M. & WARD J. E. 2009. Mechanisms contributing to low domoic acid uptake by oysters feeding on Pseudo-nitzschia cells. II. Selective rejection. Aquatic Biology, **6**: 213–226.

MANN D. G. 1988. Why didn't Lund see sex in Asterionella? A discussion of the diatom life cycle in nature. Algae and the aquatic environment, **29**: 385–412.

MANN D. G., CHEPURNOV V. A. & DROOP S. J. M. 1999. Sexuality, Incompatibility, Size Variation, and Preferential Polyandry in Natural Populations and Clones of Sellaphora Pupula (bacillariophyceae). Journal of Phycology, **35**: 152–170.

MIURA T. & YAMASHIRO T. 1990. Size Selective Feeding of Anodonta calipygos, a Phytoplanktivorous Freshwater Bivalve, and Viability of Egested Algae. Japanese Journal of Limnology (Rikusuigaku Zasshi), **51**: 73–78.

MOUGET J.-L., GASTINEAU R., DAVIDOVICH O., GAUDIN P. & DAVIDOVICH N. A. 2009. Light is a key factor in triggering sexual reproduction in the pennate diatom Haslea ostrearia. FEMS Microbiology Ecology, **69**: 194–201.

NEUVILLE D. & DASTE P. 1979. Observations concernant les phases de l'auxosporulation chez la Diatomée Navicula ostrearia (Gaillon) Bory en culture in vitro. Comptes rendus hebdomadaires des séances de l'Académie des sciences. Série D, Sciences naturelles, **288**: 1497–1499.

NEWELL R. I. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, Crassostrea virginica. Understanding the estuary: advances in Chesapeake Bay research, **129**: 536–546.

NEWELL R. I. E. & JORDAN S. J. 1983. Preferential ingestion of organic material by the American oyster Crassostrea virginica.. Marine ecology progress series. Oldendorf, **13**: 47–53.

PALES ESPINOSA E., BARILLÉ L. & ALLAM B. 2007. Use of encapsulated live microalgae to investigate pre-ingestive selection in the oyster Crassostrea gigas. Journal of Experimental Marine Biology and Ecology, **343**: 118–126.

PALES ESPINOSA E., HASSAN D., WARD J. E., SHUMWAY S. E. & ALLAM B. 2010. Role of Epicellular Molecules in the Selection of Particles by the Blue Mussel, Mytilus edulis. The Biological Bulletin, **219**: 50–60.

PFITZER E. 1869. Uber den Bau und die Zellteilung der Diatomeen. Bot. Zeitung, **27**: 774–776.

PIVETEAU F. 1999. Étude des arômes de l'huître creuse Crassostrea gigas : conséquences d'un affinage à l'aide des microalgues Skeletonema costatum et Haslea ostrearia = Aroma of oyster Crassostrea gigas: effect of supplemementation with the microalgae Skeletonema costatum and Haslea ostrearia.. Doctoral dissertation, Nantes.

POTAPOVA M. & SNOEIJS P. 1997. The natural life cycle in wild populations of Diatoma moniliformis (Bacillariophyceae) and its disruption in an aberrant environment. Journal of Phycology, **33**: 924–937.

POUVREAU J.-B., MORANÇAIS M., FLEURY F., ROSA P., THION L., CAHINGT B., ZAL F., FLEURENCE J. & PONDAVEN P. 2006. Preliminary characterisation of the blue-green pigment "marennine" from the marine tychopelagic diatom Haslea ostrearia (Gaillon/Bory) Simonsen. Journal of applied phycology, **18**: 757–767.

PRINS T. C., SMAAL A. C. & POUWER A. J. 1991. Selective ingestion of phytoplankton by the bivalvesMytilus edulis L. andCerastoderma edule (L.). Hydrobiological Bulletin, **25**: 93–100.

PROVASOLI L. 1968. Media and prospects for the cultivation of marine algae. In: Cultures and Collections of Algae. Proceedings of the US-Japan Conference, Hakone, September 1966pp. 63–75. Japanese Society for Plant Physiology.

RANSON G. 1927. L'absorption de matières organiques dissoutes par la surface expérieure du corps chez les animaux aquatiques. Annales de l'Institut Océanographique, **IV**: 49–174.

RIBELIN B. W. & COLLIER A. 1977. Studies on the gill ciliation of the American oyster Crassostrea virginica (Gmelin). Journal of Morphology, **151**: 439–449.

RIISGARD H. U. 2001. On measurement of filtration rates in bivalves : the stony road to reliable data : review and interpretation. Mar Ecol Prog Ser, **211**: 275–291.

RIISGÅRD H. U. & LARSEN P. S. 2001. Minireview: Ciliary filter feeding and bio-fluid mechanics—present understanding and unsolved problems. Limnology and Oceanography, **46**: 882–891.

ROBERT J.-M. 1983. Fertilité des eaux des claires ostréicoles et verdissement: utilisation de l'azote par les diatomées dominantes. Doctoral dissertation, Nantes.

ROSA M., WARD J. E., SHUMWAY S. E., WIKFORS G. H., PALES-ESPINOSA E. & ALLAM B. 2013. Effects of particle surface properties on feeding selectivity in the eastern oyster Crassostrea virginica and the blue mussel Mytilus edulis. Journal of Experimental Marine Biology and Ecology, **446**: 320–327.

ROUND F. E., CRAWFORD R. M. & MANN D. G. 1990. The diatoms: biology & morphology of the genera. Cambridge University Press.

SHUMWAY S. E., CUCCI T. L., NEWELL R. C. & YENTSCH C. M. 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. Journal of Experimental Marine Biology and Ecology, **91**: 77–92.

TAMBURRI M. N. & ZIMMER-FAUST R. K. 1996. Suspension feeding: Basic mechanisms controlling recognition and ingestion of larvae. Limnology and Oceanography, **41**: 1188–1197.

TURPIN V., ROBERT J.-M., GOULLETQUER P., MASSÉ G. & ROSA P. 2001. Oyster greening by outdoor mass culture of the diatom Haslea ostrearia Simonsen in enriched seawater. Aquaculture Research, **32**: 801–809.

WARD J. E. 1996. Biodynamics of suspension-feeding in adult bivalve molluscs: particle capture, processing, and fate. Invertebrate Biology,: 218–231.

WARD J. E., LEVINTON J. S., SHUMWAY S. E. & CUCCI T. 1997. Site of particle selection in a bivalve mollusc. Nature, **390**: 131–132.

WARD J. E., LEVINTON J. S., SHUMWAY S. E. & CUCCI T. 1998a. Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Marine Biology, **131**: 283–292.

WARD J. E., NEWELL R. I., THOMPSON R. J. & MACDONALD B. A. 1994. In vivo studies of suspension-feeding processes in the eastern oyster, Crassostrea virginica (Gmelin). The Biological Bulletin, **186**: 221–240.

WARD J. E., SANFORD L. P., NEWELL R. I. E. & MACDONALD B. A. 1998b. A new explanation of particle capture in suspension- feeding bivalve molluscs. Limnology and Oceanography, **43**: 741–752.

WARD J. E. & SHUMWAY S. E. 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. Journal of Experimental Marine Biology and Ecology, **300**: 83–130.

WARD J. E. & TARGETT N. M. 1989. Influence of marine microalgal metabolites on the feeding behavior of the blue mussel Mytilus edulis. Marine Biology, **101**: 313–321.

YONGE C. M. 1926. Structure and physiology of the organs of feeding and digestion in Ostrea edulis. Journal of the Marine Biological Association of the United Kingdom (New Series), **14**: 295–386.

Figure 1. Scanning electron microscopy studies of the gill and labial palps of *Crassostrea gigas* fed with three different-sized populations of *Haslea ostrearia.* **A.** Frontal view of the gill showing differentiation between the principal filaments (pf) and the plicae constituted of ordinary (of) and apical (af) filaments. **B.** Detail of plicae showing a *Haslea ostrearia* cell (length, 75 µm) in the main direction of the filaments. **C.** Ciliation of an ordinary filament conveying a *Haslea ostrearia* cell (length, 50 µm). **D.** Detail of plicae showing a *Haslea ostrearia* cell (length, 95 µm) lying across more than one apical filament (af). **E.** Side view of the gill and ventral groove (vg). Arrows indicate main particle transport. **F.** Ridged surface of a labial palp. H, area corresponding to figures H. **G.** Detail of a ridged labial palp surface covered with an aggregate of mucus (∗) containing the three cell sizes of *Haslea ostrearia*. **H.** Detail of the ridged labial palp surface, with cells of the three different-sized populations of *Haslea ostrearia*.

Abbreviations: af, apical filament; Ant., anterior part; c, cilia; Dors., dorsal part; f, frontal cilia; g, grooves; lf, laterofrontal cirri; me, marginal edge; of, ordinary filament; og, oral groove; p, plicae; pf, principal filament; Post., posterior part; Vent., ventral part; vg, ventral groove. Scale bars: **A** = 100 µm; **B** = 10 µm; **C** = 10 µm; **D** = 10 µm; **E** = 100 µm; **F** = 1 mm; **G** = 100 µm; **H** = 100 µm.

Figure 2. Selection indices for small *Haslea ostrearia* cells of samples taken *in vivo* from the outflow, gills (dorsal and ventral tracts) and pseudofaeces. Error bars represent the 95 % confidence interval of the mean. * denote indices significantly different from zero ($P < 0.001$).