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

3

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21

22 **ABSTRACT**

23

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

44

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

47

## 48 **1. Introduction**

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

59 Progress has been made in understanding the pallial organs and ciliation involved in preingestive  
60 selection (Beninger & St-Jean, 1997; Beninger *et al.*, 1997; Ward *et al.*, 1997; Cognie *et al.*,  
61 2003). Different studies have focused on the sorting criteria in bivalves, and many particle  
62 characteristics have been identified as selection cues, namely size (Hughes, 1975; Shumway *et*  
63 *al.*, 1985; Defossez & Hawkins, 1997), organic content (Bacon *et al.*, 1998; Beninger *et al.*,  
64 2008b) or microalgae cell surface properties and compounds (Ward & Targett, 1989; Pales  
65 Espinosa *et al.*, 2007, 2010; Beninger *et al.*, 2008a; Rosa *et al.*, 2013).

66 Particle size was the first criterion used by early researchers examining food selection (Yonge,  
67 1926; Atkins, 1937), however its importance seems variable, with no influence of particle size on  
68 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 confounding 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* (Defosse & 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

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

105 Studying the specific size-dependent sorting mechanism is crucial to understand and model the  
106 transfer of a bioproduct from algal cells to suspension-feeding bivalves. The marine diatom  
107 *Haslea ostrearia* is known to produce marennine, a polyphenolic water-soluble blue pigment  
108 (Pouvreau *et al.*, 2006), responsible for the “greening” of the tissues of numerous marine  
109 invertebrates and the gills of bivalves in particular (Ranson, 1927; Robert, 1983; Turpin *et al.*,  
110 2001; Gastineau *et al.*, 2014b).

111 It has also been shown that the oyster *C. gigas* can feed exclusively on *H. ostrearia* (Barillé *et*  
112 *al.*, 1994) despite of its lower nutritive value compared to other microalgae species (Piveteau,  
113 1999). Although *H. ostrearia* and marennine have long been recognised as the only greening  
114 agent of cultured oysters in France, other species from the same genus and producing  
115 “marennine-like” pigments have been discovered elsewhere in the world (Gastineau *et al.*,  
116 2012a, 2014a, 2015). These authors have also demonstrated that marennine-like pigments have

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

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

130

## 131 **2. Materials and Methods**

### 132 **2.1 Algal culture**

133 The cultures of *Haslea ostrearia* used in this study were obtained from the Nantes  
134 Culture Collection [NCC; temperature: 14°C; light/dark cycle: 14/10 h; light intensity: 100  $\mu\text{mol}$   
135  $\text{photons m}^{-2} \text{s}^{-1}$ ; ES1/3 medium, Provasoli (1968)], Faculté des Sciences et des Techniques,  
136 Université de Nantes (France). Strains with a similar biochemical composition (Joux-Arab *et al.*,  
137 2000) but different cell lengths were selected (Table 1). A particle counter (Coulter Multisizer 3  
138 ®) was used to determine equivalent spherical diameter of the different strains. Sixty-liter mass  
139 cultures were performed in polyethylene bags filled with underground seawater (Cognie, 2001)  
140 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  $3 \cdot 10^6$   
142 cells L<sup>-1</sup> for each cell size, Table 1) for a final total cell concentrations of  $9 \cdot 10^6$  cells L<sup>-1</sup> and  $6.1$   
143  $10^6$  cells L<sup>-1</sup>, 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<sup>-1</sup> 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<sub>2</sub> 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<sup>-1</sup>)  
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

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

191

## 192 **2.6 Data analysis**

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

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

$$199 \quad SI = ([S\% - W\%] / W\%) \times 100$$

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

204

## 205 **3. Results**

### 206 **3.1 Condition A: oysters feeding on three algal cell sizes: particle selection + SEM**

#### 207 **observations**

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

213 principal filaments was approximately 70  $\mu\text{m}$ . In addition, ordinary filaments located in the  
214 apical part of the plicae (or apical filaments) were approximately 45  $\mu\text{m}$  wide. The same  
215 measurements, when performed using SEM, showed that plicae were only 120 to 140  $\mu\text{m}$  wide  
216 (Fig. 1A), ordinary filaments 18 to 26  $\mu\text{m}$  wide (Fig. 1B) and apical filaments 25 and 30  $\mu\text{m}$   
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*

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

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

245

### 246 **3.2 Condition B: oysters' feeding on two algal cell sizes: particle selection + video-** 247 **endoscopy directed sampling**

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

253 The value of the selection index (SI) at outflow was not significantly different from zero  
254 (t-test,  $P > 0.05$ ; Fig. 2), indicating that both populations were retained with the same efficiency  
255 on gills. However, the SI for pseudofaeces was significantly lower than zero (t-test,  $P < 0.001$ ),  
256 clearly allowing the rejection of the null hypothesis  $H_0$  and acceptance of  $H_1$ : selection occurred  
257 on the pallial organs. The mean SI for ventral groove and dorsal tract were significantly different  
258 from zero (t-test,  $P < 0.001$ ), thus allowing acceptance of  $H_{1a}$ : selection occurred on the gills ( $P$   
259  $< 0.05$ ). There was no significant difference between the mean SI for ventral groove and

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

264

#### 265 **4. Discussion**

266 In the present study, cells were retained on the gill with the same efficiency when oysters  
267 were fed mixed suspensions containing *H. ostrearia* of varying cell length. Previous studies  
268 demonstrated that, in bivalves, retention efficiency varies with particle size and concentration. In  
269 *C. gigas*, Barillé et al. (1993) showed that particles above 6  $\mu\text{m}$  ESD (equivalent spherical  
270 diameter) were retained with 100 % efficiency at the total seston concentration used in our study  
271 (3.8 or 4.9  $\text{mg L}^{-1}$ ). This maximal retention efficiency threshold is slightly less than the size of  
272 the smallest *H. ostrearia* cells fed to the oysters (Ho-small,  $6.1 \pm 0.1$  mean ESD  $\pm$  SE),  
273 indicating that the cells of the three populations were retained with 100 % efficiency.

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

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

289 Another mechanism that could account for selection at the gill level is related to the  
290 differentiation of heterorhabdic gills into principal and ordinary filaments (Atkins, 1937; Ward *et*  
291 *al.*, 1994, 1998a; Beninger & St-Jean, 1997). In bivalves possessing such a gill type, preingestive  
292 sorting may be performed using the principal filaments for the material to be ingested and the  
293 ordinary filaments for the material to be rejected. Our SEM and video-endoscopy observations  
294 showed that the cells of the larger populations were present in the troughs containing principal  
295 filaments, but were always orientated dorso-ventrally. Smaller cells were observed accessing  
296 principal filaments with no preferential orientation. These qualitative data confirm that passive  
297 selection related to particle size may operate on gills, as suggested by Ward and Shumway  
298 (2004) and previously observed in *C. virginica* by Mafra *et al.* (2009).

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

308 of their chemical properties (Cognie *et al.*, 2003; Beninger *et al.*, 2008a, 2008b; Mafra *et al.*,  
309 2009). In the present study, using *H. ostrearia* cells of contrasting size, we observed selection by  
310 oyster gills but not by labial palps, suggesting that particle size did not affect the selective ability  
311 of these two pallial organs in the same way.

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

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

332 diatoms, sexually competent cells of *H. ostrearia* can carry out auxosporulation to compensate  
333 for the size reduction associated with vegetative divisions, but only when cell size decreases to  
334 65-70  $\mu\text{m}$ , which represents 50% of the maximum cell length of the species (Neuville & Daste,  
335 1979; Davidovich *et al.*, 2009; Mouget *et al.*, 2009). Therefore, this study shows that *C. gigas*  
336 preferentially ingests sexually mature cells and rejects immature ones, which could modify *H.*  
337 *ostrearia* cell size distribution and population dynamics in oyster ponds. Given the crucial role of  
338 cell size in the life cycle of diatoms, the impact of this predation pressure on the population  
339 dynamics of *H. ostrearia* in oyster ponds remains to be assessed, especially considering the  
340 importance of marennine in the greening process, but also regarding its many biological  
341 activities (*e.g.*, antibacterial) that could be exploited in aquaculture.

342

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352

### 353 **References**

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



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

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

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

361 BACON G. S., MACDONALD B. A. & WARD J. E. 1998. Physiological responses of infaunal (*Mya arenaria*) and  
362 epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended  
363 particles: I. Feeding activity and selection. *Journal of Experimental Marine Biology and Ecology*, **219**:  
364 105–125.

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

367 BARILLE L., BOUGRIER S., GEAIRON P. & ROBERT J.-M. 1994. Alimentation expérimentale de l'huître *Crassostrea*  
368 *gigas* à l'aide de navicules bleus *Haslea ostrearia* (Simonsen) de différentes tailles. *Oceanologica Acta*, **17**:  
369 201–210.

370 BARILLÉ L. & COGNIE B. 2000. Revival capacity of diatoms in bivalve pseudofaeces and faeces. *Diatom Research*,  
371 **15**: 11–17.

372 BARILLÉ L., PROU J., HÉRAL M. & BOUGRIER S. 1993. No influence of food quality, but ration-dependent retention  
373 efficiencies in the Japanese oyster *Crassostrea gigas*. *Journal of Experimental Marine Biology and Ecology*,  
374 **171**: 91–106.

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

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

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

382 BENINGER P. G., ST-JEAN S. D. & POUSSART Y. 1995. Labial palps of the blue mussel *Mytilus edulis* (Bivalvia:  
383 *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., BOURGOUNGON 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., BOURGOUNGON 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 *Placocopecten magellanicus* (Mollusca: Bivalvia). *Marine Ecology-Progress Series*, **108**: 251–251.

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

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

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

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

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

451 MOUGET J.-L., GASTINEAU R., DAVIDOVICH O., GAUDIN P. & DAVIDOVICH N. A. 2009. Light is a key factor in  
452 triggering sexual reproduction in the pennate diatom Haslea ostrearia. *FEMS Microbiology Ecology*, **69**:  
453 194–201.

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

457 NEWELL R. I. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American  
458 oyster, Crassostrea virginica. *Understanding the estuary: advances in Chesapeake Bay research*, **129**: 536–  
459 546.

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

462 PALES ESPINOSA E., BARILLÉ L. & ALLAM B. 2007. Use of encapsulated live microalgae to investigate pre-ingestive  
463 selection in the oyster Crassostrea gigas. *Journal of Experimental Marine Biology and Ecology*, **343**: 118–  
464 126.

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

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

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

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

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

477 PRINS T. C., SMAAL A. C. & POWWER A. J. 1991. Selective ingestion of phytoplankton by the bivalves *Mytilus edulis*  
478 L. and *Cerastoderma edule* (L.). *Hydrobiological Bulletin*, **25**: 93–100.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

519

520

521 **Figure 1.** Scanning electron microscopy studies of the gill and labial palps of *Crassostrea gigas*  
522 fed with three different-sized populations of *Haslea ostrearia*. **A.** Frontal view of the gill  
523 showing differentiation between the principal filaments (pf) and the plicae constituted of  
524 ordinary (of) and apical (af) filaments. **B.** Detail of plicae showing a *Haslea ostrearia* cell  
525 (length, 75  $\mu\text{m}$ ) in the main direction of the filaments. **C.** Ciliation of an ordinary filament  
526 conveying a *Haslea ostrearia* cell (length, 50  $\mu\text{m}$ ). **D.** Detail of plicae showing a *Haslea*  
527 *ostrearia* cell (length, 95  $\mu\text{m}$ ) lying across more than one apical filament (af). **E.** Side view of  
528 the gill and ventral groove (vg). Arrows indicate main particle transport. **F.** Ridged surface of a  
529 labial palp. H, area corresponding to figures H. **G.** Detail of a ridged labial palp surface covered  
530 with an aggregate of mucus (\*) containing the three cell sizes of *Haslea ostrearia*. **H.** Detail of  
531 the ridged labial palp surface, with cells of the three different-sized populations of *Haslea*  
532 *ostrearia*.  
533 Abbreviations: af, apical filament; Ant., anterior part; c, cilia; Dors., dorsal part; f, frontal cilia;  
534 g, grooves; lf, laterofrontal cirri; me, marginal edge; of, ordinary filament; og, oral groove; p,  
535 plicae; pf, principal filament; Post., posterior part; Vent., ventral part; vg, ventral groove. Scale  
536 bars: **A** = 100  $\mu\text{m}$ ; **B** = 10  $\mu\text{m}$ ; **C** = 10  $\mu\text{m}$ ; **D** = 10  $\mu\text{m}$ ; **E** = 100  $\mu\text{m}$ ; **F** = 1 mm; **G** = 100  $\mu\text{m}$ ;  
537 **H** = 100  $\mu\text{m}$ .

538

539 **Figure 2.** Selection indices for small *Haslea ostrearia* cells of samples taken *in vivo* from the  
540 outflow, gills (dorsal and ventral tracts) and pseudofaeces. Error bars represent the 95 %  
541 confidence interval of the mean. \* denote indices significantly different from zero ( $P < 0.001$ ).  
542