

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

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- 22 ABSTRACT
- 23

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

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

47

48 **1. Introduction**

Suspension-feeding bivalves constitute one of the dominant trophic group in estuarine 49 ecosystems, and their feeding mode may strongly affect nutrient recycling, seston dynamics and 50 the benthic food web (Asmus & Asmus, 1991; Prins et al., 1991; Dame, 1993; Ward & 51 Shumway, 2004). In these environments, suspension-feeders have to cope with wide fluctuations 52 in both the quantity and quality of suspended particulate matter (Armstrong, 1958; Berg & 53 Newell, 1986; Fegley et al., 1992; Barillé et al., 1997). It is thus not surprising to find an 54 abundant literature concerning the response of suspension-feeding bivalves to seston 55 56 fluctuations, in particular the physiological variables related to feeding, such as clearance rate, retention efficiencies and preingestive selection (Jørgensen, 1990; Riisgard, 2001; Riisgård & 57 Larsen, 2001; Ward & Shumway, 2004). 58 Progress has been made in understanding the pallial organs and ciliation involved in preingestive 59 selection (Beninger & St-Jean, 1997; Beninger et al., 1997; Ward et al., 1997; Cognie et al., 60 2003). Different studies have focused on the sorting criteria in bivalves, and many particle 61 characteristics have been identified as selection cues, namely size (Hughes, 1975; Shumway et 62 al., 1985; Defossez & Hawkins, 1997), organic content (Bacon et al., 1998; Beninger et al., 63 2008b) or microalgae cell surface properties and compounds (Ward & Targett, 1989; Pales 64 Espinosa et al., 2007, 2010; Beninger et al., 2008a; Rosa et al., 2013). 65 Particle size was the first criterion used by early researchers examining food selection (Yonge, 66 1926; Atkins, 1937), however its importance seems variable, with no influence of particle size on 67 preingestive selection repeatedly reported (Newell & Jordan, 1983; Newell, 1988; Chretiennot-68

Dinet *et al.*, 1991; MacDonald & Ward, 1994; Bougrier *et al.*, 1997), while the opposite can also
be found in the literature (Ballantine & Morton, 1956; Miura & Yamashiro, 1990; Cognie *et al.*,
2003; Mafra *et al.*, 2009).

72 Nevertheless, the particles used in most of these studies differed not only in size, thus generating possible cofounding factors. In that instance, Hughes (1975) observed size-dependent selection 73 of particles by Abra alba, but noted a relationship between particle size and food value. Cognie 74 et al. (2003) demonstrated that the particle size plays a role in oyster, determining which pallial 75 organs (gills or palps) will be involved in the sorting. The microalgal species used in this study 76 (Pleurosigma planctonicum, Coscinodiscus perforatus and Rhizosolenia setigera) were too large 77 to have access to principal filaments and a selection by the gill was therefore not possible. The 78 observed selection was performed by the labial palps and was based not on the particle size but 79 80 on their biochemical composition (intact vs empty cells). To our knowledge, very few studies have unambiguously supported the idea that bivalves can use a size criterion to discriminate 81 particles. A preferential size-dependent rejection of larger particles was observed in C. virginica 82 (Tamburri & Zimmer-Faust, 1996) and in Mytilus edulis, Ruditapes philippinarum and Tapes 83 decussatus (Defossez & Hawkins, 1997). However the particles used in these studies were 84 artificial, polystyrene or borosilicated glass particles in the former, silica particles in the latter, 85 and these results not completely transposed to natural living microalgal cells. Nevertheless, using 86 natural living cells, *i.e.* contrasting-sized clones of a toxic diatom, Mafra *et al.* (2009) 87 indisputably demonstrated the role of size in the selection carried out by C. virginica gills. 88 A well-known feature of diatom biology that could have a great effect on living cell selection by 89 bivalves, is the MacDonald-Pfitzer rule (Pfitzer, 1869; Macdonald, 1869; Round et al., 1990). 90 During vegetative growth and mitotic divisions, one of the daughter cells is smaller than the 91 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 diatoms and especially crucial in most pennate species, can be compensated: when a critical 94 threshold size is reached, sexual reproduction and auxosporulation will occur. In diatoms, 95 96 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, 97 but of different sizes, are often found together in the natural environment (Mann, 1988; Potapova 98 & Snoeijs, 1997; Mann et al., 1999). This phenomenon is expected to affect the kinetics of 99 100 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, 101 Mafra et al. (2009) demonstrated that the ability of C. virginica to sort particles according to 102 their size could affect its uptake of the neurotoxin domoic acid from Pseudo-nitzschia 103 104 multiseries. 105 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 106 107 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 108 invertebrates and the gills of bivalves in particular (Ranson, 1927; Robert, 1983; Turpin et al., 109 110 2001; Gastineau et al., 2014b). It has also been shown that the oyster C. gigas can feed exclusively on H. ostrearia (Barillé et 111 al., 1994) despite of its lower nutritive value compared to other microalgae species (Piveteau, 112 1999). Although *H. ostrearia* and marennine have long been recognised as the only greening 113

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

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.

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

130

131 **2. Materials and Methods**

132 **2.1 Algal culture**

The cultures of *Haslea ostrearia* used in this study were obtained from the Nantes 133 134 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, 135 Université de Nantes (France). Strains with a similar biochemical composition (Joux-Arab et al., 136 2000) but different cell lengths were selected (Table 1). A particle counter (Coulter Multisizer 3 137 (R) was used to determine equivalent spherical diameter of the different strains. Sixty-liter mass 138 cultures were performed in polyethylene bags filled with underground seawater (Cognie, 2001) 139 140 supplemented with an enrichment solution containing nitrogen, phosphorus and silicon (Turpin

et al., 2001). The different populations were then mixed at equivalent cell densities (around 3 10⁶
cells L⁻¹ for each cell size, Table 1) for a final total cell concentrations of 9 10⁶ cells L⁻¹ and 6.1
10⁶ cells L⁻¹, delivered to the bivalves in conditions A and B, respectively.

- 144
- 145 **2.2 Oyster sampling and maintenance**

Adult oysters of *Crassostrea gigas* with a mean dry weight of 0.8 g (SE = 0.1, n = 20)
were collected in the intertidal zone of Bourgneuf Bay (France) (46-47° N, 1-2° W). After
immediate transfer to the laboratory and removal of shell epibionts, they were placed for two
minutes in a 0.1% hypochlorite solution to eliminate parasitic polychetes of the genus *Polydora*.
The oysters were then thoroughly washed, maintained during one week in filtered (Millipore
0.45 µm) seawater and oxygenated.

152

153 **2.3 Feeding experiments**

Two series of experiments were run over different periods; one with oysters feeding on 154 three algal cell sizes (condition A), the other, on two algal cell sizes (condition B). After 24 155 hours of acclimation prior to experimental conditions (A or B, Table 1), oysters were randomly 156 chosen and placed in a flow-through experimental system, as described in Cognie et al. (2003). 157 The mean flow rate for each individual tray was 8 L h⁻¹ at 16°C. Two trays containing an empty 158 shell were used as sedimentation controls. After one hour of oyster acclimation, seawater 159 samples (50 mL) were collected at the outflow of the experimental individual trays every 15 160 minutes for one hour. Pseudofaeces were collected at the end of the observations. The individual 161 samples (outflow seawater and pseudofaeces) were fixed with acetic Lugol's solution and 162 analysed separately as replicates. Cells were counted by means of light microscopy using 163 "Nageotte" type hematimetric units. For each sample, a minimum of 300 cells was counted. 164

165

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

Oysters intended for SEM observations were placed in flow-through trays and fed the 167 same mixture as in experimental condition A (Table 1). Once pseudofaeces appeared, the oysters 168 were collected, shucked and immediately fixed in a 2.5% hypertonic glutaraldehyde solution in 169 sodium cacodylate buffer 0.1 M (Beninger et al., 1995). The collection-fixation step was 170 performed within 30 s to limit stress-related mucus production and preserve the functional stage 171 of the animal. After fixation (for at least 48 h), individuals were partially dehydrated in 172 successive alcohol baths at increasing concentrations (up to 70%). At this stage, the oysters were 173 174 dissected using a dissection microscope and microsurgical instruments. The samples were subsequently totally dehydrated in a 100% anhydrous alcohol bath and dried with CO₂ using a 175 critical point apparatus. The samples were then plated with gold-palladium alloy and observed 176 under a scanning electron microscope with field effect (JEOL 5400). To estimate the tissue 177 contraction of the samples during SEM preparation, additional measurements were made with a 178 dissecting microscope on live individuals still on the half shell. 179

- 180
- 181 **2.5 Video-endoscopy directed sampling**

This sampling was conducted concomitantly with the feeding measurements as described in section 2.3, following the procedure reported in Cognie et al. (2003). At least 24 h before the sampling, a small aperture was milled in the shells to prevent damage of the optical insertion tube (OIT) when the oyster valves closed. Specimens were placed in flow-through trays (8 L.h⁻¹) and fed the same mixture as in experimental condition A (Table 1). Sampling was performed every 15 min for 1 h in both the dorsal and ventral particle grooves (1 mL using a micropipette) 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 dorsalgrooves) were treated and analysed as described in section 2.3.

191

192 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:

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

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 ttest.

204

205 **3. Results**

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

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207 observations
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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 μ m wide. The same measurements, when performed using SEM, showed that plicae were only 120 to 140 µm wide 215 216 (Fig. 1A), ordinary filaments 18 to 26 µm wide (Fig. 1B) and apical filaments 25 and 30 µm wide (Fig. 1A and D). Principal filaments were no longer visible and too deeply positioned in 217 troughs to allow measurements (Fig. 1B and C). Thus, due to the preparation for SEM, the 218 dimensions of contracted gill plicae correspond to approximately 50% of those observed in vivo. 219 Cells of the three distinct algal populations were always observed mixed on all oyster 220 structures involved in the selection process, whether these cells were free or in aggregates of 221 mucus. Haslea ostrearia cells were observed on the gill surface without preferential orientation 222 (Fig. 1B, C and D). However, the cells distinguished at the bottom of the principal filaments 223 224 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. 1E). They were then carried toward the anterior part of the pallial cavity in mucous aggregates 226 (not shown), before being processed on the ridged inner surfaces of the labial palps. Aggregates 227 of mucus (* Fig. 1G) and cells of the three *H. ostrearia* populations were observed on these 228 surfaces (Fig. 1F, G and H). 229

A dimensional gap between the size of the three *H. ostrearia* populations and that of the gill and labial palp structures was apparent. Considering the tissue contraction due to the sample preparation for SEM, an Ho95 cell overlapped more than one apical filaments (Fig. 1D) and more than one and a half ordinary filaments (*Fig. 1E). Regarding labial palps, cells of the 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 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; χ^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; χ^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 <i>H. ostrearia</i> populations represented a food ration of 1.9 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 Ho and acceptance of H1: 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 <i>H1a</i> : 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 <i>H1b</i> and <i>H1c</i> 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 µm ESD (equivalent spherical |
| 270 | diameter) were retained with 100 % efficiency at the total seston concentration used in our study |
| 271 | $(3.8 \text{ or } 4.9 \text{ mg } \text{L}^{-1})$. This maximal retention efficiency threshold is slightly less than the size of |
| 272 | the smallest <i>H. ostrearia</i> cells fed to the oysters (Ho-small, 6.1 ± 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 <i>H. ostrearia</i> 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 |
| | 12 |

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 289 differentiation of heterorhabdic gills into principal and ordinary filaments (Atkins, 1937; Ward et 290 al., 1994, 1998a; Beninger & St-Jean, 1997). In bivalves possessing such a gill type, preingestive 291 sorting may be performed using the principal filaments for the material to be ingested and the 292 ordinary filaments for the material to be rejected. Our SEM and video-endoscopy observations 293 showed that the cells of the larger populations were present in the troughs containing principal 294 295 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 selection related to particle size may operate on gills, as suggested by Ward and Shumway 297 (2004) and previously observed in C. virginica by Mafra et al. (2009). 298

Particles transported in dorsal grooves and those conveyed with mucus aggregates in 299 ventral grooves are directed towards the ridged surfaces of the labial palps (Ward, 1996). In 300 301 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 302 oysters of the genus Crassostrea, the palps are thought to play a secondary role in these two 303 functions and to serve mainly to reject the non-ingested material as pseudofaeces (Beninger & 304 St-Jean, 1997; Ward et al., 1998a). The differentiation of heterorhabdic gills into two types of 305 306 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 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 312 is clarified and summarized in Table 4. Particle dimensions (length, width, height) should be 313 considered a physical constraint affecting access to principal filaments (pfs) and consequently 314 the selective ability of the oyster gill. Particles with all dimensions smaller than the pf width can 315 access principal filament troughs freely and their selection may occur at gills and/or labial palps. 316 In contrast, particles with all dimensions greater than the pf width are unable to enter the 317 principal filaments and selection at gills is not possible. For particles with only one dimension 318 319 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 labial palps is possible. 321

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

332 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 333 65-70 µm, which represents 50% of the maximum cell length of the species (Neuville & Daste, 334 335 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. 336 ostrearia cell size distribution and population dynamics in oyster ponds. Given the crucial role of 337 cell size in the life cycle of diatoms, the impact of this predation pressure on the population 338 dynamics of *H. ostrearia* in oyster ponds remains to be assessed, especially considering the 339 importance of marennine in the greening process, but also regarding its many biological 340 activities (e.g., antibacterial) that could be exploited in aquaculture. 341 342 Acknowledgements 343

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353 **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.
- 361 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
- 377 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.
- 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.

- BENINGER P. G., VALDIZAN A., COGNIE B., GUIHENEUF F. & DECOTTIGNIES P. 2008a. Wanted: alive and not dead:
 functioning diatom status is a quality cue for the suspension-feeder Crassostrea gigas. Journal of Plankton
 Research, 30: 689–697.
- BENINGER P. G., VALDIZAN A., DECOTTIGNIES P. & COGNIE B. 2008b. Impact of seston characteristics on qualitative
 particle selection sites and efficiencies in the pseudolamellibranch bivalve Crassostrea gigas. Journal of
 Experimental Marine Biology and Ecology, 360: 9–14.
- BERG J. A. & NEWELL R. I. E. 1986. Temporal and spatial variations in the composition of seston available to the
 suspension feeder Crassostrea virginica. Estuarine, Coastal and Shelf Science, 23: 375–386.
- BOUGRIER S., HAWKINS A. J. S. & HÉRAL M. 1997. Preingestive selection of different microalgal mixtures in
 Crassostrea gigas and Mytilus edulis, analysed by flow cytometry. Aquaculture, 150: 123–134.
- CHRETIENNOT-DINET M.-J., VAULOT D., GALOIS R., SPANO A.-M. & ROBERT R. 1991. Analysis of larval oyster
 grazing by flow cytometry. Journal of Shellfish Research, 10: 457–463.
- COGNIE B. 2001. Alimentation de l'huître Crassostrea gigas (Thunberg): étude des mécanismes de sélection des
 particules et des processus rétroactifs entre le bivalve et les microalgues. PhD thesis, University of Nantes.
- COGNIE B., BARILLÉ L., MASSÉ G. & BENINGER P. G. 2003. Selection and processing of large suspended algae in the
 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.
- DAME R. F. 1993. The Role of Bivalve Filter Feeder Material Fluxes in Estuarine Ecosystems. In: Bivalve Filter
 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.
- DEFOSSEZ J.-M. & HAWKINS A. J. S. 1997. Selective feeding in shellfish: size-dependent rejection of large particles
 within pseudofaeces from Mytilus edulis, Ruditapes philippinarum and Tapes decussatus. Marine Biology,
 129: 139–147.
- FEGLEY S. R., MACDONALD B. A. & JACOBSEN T. R. 1992. Short-term variation in the quantity and quality of seston
 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.
- GASTINEAU R., DAVIDOVICH N. A., HALLEGRAEFF G. M., PROBERT I. & MOUGET J.-L. 2014a. Reproduction in
 microalgae. In: Reproduction Biology of Plants (K. G. Ramawat, J. M. Mérillon, & K. R. Shivanna, eds.),
 pp. 1–28. CRC Press.
- GASTINEAU R., HANSEN G., DAVIDOVICH N. A., DAVIDOVICH O. I., BARDEAU J.-F., KACZMARSKA I., EHRMAN J. M.,
 LEIGNEL V., HARDIVILLIER Y., JACQUETTE B., POULIN M., MORANÇAIS M., FLEURENCE J. & MOUGET J.-L.
 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.
- HUGHES T. G. 1975. The sorting of food particles by Abra sp. (bivalvia: tellinacea). Journal of Experimental Marine
 Biology and Ecology, 20: 137–156.
- JØRGENSEN C. B. 1990. Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology. Olsen &
 Olsen.
- JOUX-ARAB L., BERTHET B. & ROBERT J.-M. 2000. Do toxicity and accumulation of copper change during size
 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.
- 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.
- 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.
- 467 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,

471 Nantes.

- 472 POTAPOVA M. & SNOEIJS 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
- 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.
- 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.
- 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.
- 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.

- 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.
- 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.

- 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.
- 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.
- WARD J. E., SANFORD L. P., NEWELL R. I. E. & MACDONALD B. A. 1998b. A new explanation of particle capture in
 suspension- feeding bivalve molluses. 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.
- 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.

519

- 521 **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
- 526 conveying a *Haslea ostrearia* cell (length, 50 μm). **D.** Detail of plicae showing a *Haslea*
- 527 *ostrearia* cell (length, 95 μm) 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
- 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: $\mathbf{A} = 100 \ \mu\text{m}$; $\mathbf{B} = 10 \ \mu\text{m}$; $\mathbf{C} = 10 \ \mu\text{m}$; $\mathbf{D} = 10 \ \mu\text{m}$; $\mathbf{E} = 100 \ \mu\text{m}$; $\mathbf{F} = 1 \ \text{mm}$; $\mathbf{G} = 100 \ \mu\text{m}$; 537 $\mathbf{H} = 100 \ \mu\text{m}$.
- 538
- 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).