

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

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4 Fiddy S. Prasetya^{a, d}, Priscilla Decottignies^b, Laurent Barillé^b, Romain Gastineau^a, Boris
5 Jacqueline^a, Amandine Figiel^b, Michèle Morançais^b, Réjean Tremblay^c, Jean-Luc Mouget^a, and
6 Bruno Cognie^{b,*}

7

8

9 a. FR CNRS 3473 IUML, Mer-Molécules-Santé (MMS), Université du Maine, Avenue O.
10 Messiaen, 72085 Le Mans cedex 9, France;

11 b. FR CNRS 3473 IUML, Mer-Molécules-Santé (MMS), Université de Nantes, 2 rue de la
12 Houssinière44322 Nantes cedex 3, France

13 c. Institut des Sciences de la Mer, 310 allée des Ursulines, Rimouski, Québec, G5L 3A1, Canada

14 d. Universitas Padjadjaran, Research Center of Biotechnology and Bioinformatics, Jl. Raya
15 Bandung Sumedang KM 21, Jatinangor 40600, Indonesia

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17

18

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

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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 μmol
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⁻¹ for each cell size, Table 1) for a final total cell concentrations of $9 \cdot 10^6$ cells L⁻¹ and 6.1
143 10^6 cells L⁻¹, delivered to the bivalves in conditions A and B, respectively.

144

145 **2.2 Oyster sampling and maintenance**

146 Adult oysters of *Crassostrea gigas* with a mean dry weight of 0.8 g (SE = 0.1, n = 20)
147 were collected in the intertidal zone of Bourgneuf Bay (France) (46-47° N, 1-2° W). After
148 immediate transfer to the laboratory and removal of shell epibionts, they were placed for two
149 minutes in a 0.1% hypochlorite solution to eliminate parasitic polychetes of the genus *Polydora*.
150 The oysters were then thoroughly washed, maintained during one week in filtered (Millipore
151 0.45 µm) seawater and oxygenated.

152

153 **2.3 Feeding experiments**

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

165

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

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

180

181 **2.5 Video-endoscopy directed sampling**

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

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 μm wide, the ordinary filaments were between 35 and 40 μm , and the opening of the

213 principal filaments was approximately 70 μm . In addition, ordinary filaments located in the
214 apical part of the plicae (or apical filaments) were approximately 45 μm wide. The same
215 measurements, when performed using SEM, showed that plicae were only 120 to 140 μm wide
216 (Fig. 1A), ordinary filaments 18 to 26 μm wide (Fig. 1B) and apical filaments 25 and 30 μm
217 wide (Fig. 1A and D). Principal filaments were no longer visible and too deeply positioned in
218 troughs to allow measurements (Fig. 1B and C). Thus, due to the preparation for SEM, the
219 dimensions of contracted gill plicae correspond to approximately 50% of those observed *in vivo*.

220 Cells of the three distinct algal populations were always observed mixed on all oyster
221 structures involved in the selection process, whether these cells were free or in aggregates of
222 mucus. *Haslea ostrearia* cells were observed on the gill surface without preferential orientation
223 (Fig. 1B, C and D). However, the cells distinguished at the bottom of the principal filaments
224 were always orientated according to the longitudinal axis of the gill filaments (¥ Fig. 1E). *Haslea*
225 *ostrearia* cells transported dorsoventrally along the gill surface reached the ventral grooves (Fig.
226 1E). They were then carried toward the anterior part of the pallial cavity in mucous aggregates
227 (not shown), before being processed on the ridged inner surfaces of the labial palps. Aggregates
228 of mucus (* Fig. 1G) and cells of the three *H. ostrearia* populations were observed on these
229 surfaces (Fig. 1F, G and H).

230 A dimensional gap between the size of the three *H. ostrearia* populations and that of the
231 gill and labial palp structures was apparent. Considering the tissue contraction due to the sample
232 preparation for SEM, an Ho95 cell overlapped more than one apical filaments (Fig. 1D) and
233 more than one and a half ordinary filaments (*Fig. 1E). Regarding labial palps, cells of the
234 largest population were equivalent in size to the width of a plica.

235 SEM studies of the structures and ultrastructures of the pallial organs involved in *C. gigas*
236 feeding were associated with feeding measurements. The mixture of the three *H. ostrearia*

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 *H. ostrearia* 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 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 μm 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 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 ± 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 µm, 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

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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 μm) in the main direction of the filaments. **C.** Ciliation of an ordinary filament
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
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 μm ; **B** = 10 μm ; **C** = 10 μm ; **D** = 10 μm ; **E** = 100 μm ; **F** = 1 mm; **G** = 100 μm ;
537 **H** = 100 μm .

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