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

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ABSTRACT

Pre-ingestive selection has been identified as a feeding mechanism of oysters that may influence their uptake of particles and microalgal cells. Oysters can feed specifically on the pennate diatom *Haslea ostrearia*, which produces the blue pigment marennine that is responsible for the greening of oysters. Because the size of particles or cells plays a significant role in the selection process, and given that diatoms experience a decrease in size as a consequence of vegetative reproduction, *H. ostrearia* consumption and marennine uptake might be influenced by pre-ingestive selection. We examined the role of *H. ostrearia* cell size in the selective feeding of *Crassostrea gigas*. Individual flow-through chambers were used to deliver mixtures of *H. ostrearia* of varying cell length to oysters. Inflow, outflow and pseudofaecal samples were collected from chambers during oyster feeding. Video-endoscopy was used to sample material in the dorsal and ventral particle tracts. Diatom cells counts showed that pseudofaeces contained on average larger cells than the ambient medium. However, proportions of the different populations of *H. ostrearia* in pseudofaeces were identical to those in the ventral tracts, indicating that no selection was performed by the labial palps. Video-endoscopy, plus imaging by scanning electron microscopy, of gills and labial palps revealed that only those larger *H. ostrearia* that were orientated dorsoventrally could enter the principal filaments (pfs) and then access the dorsal acceptance tract. These results show that for particles like *Haslea* cells with only one axis exceeding the width of the pfs, the selection on the oyster gills is passive and based on cell size.

INTRODUCTION

Suspension-feeding bivalves form a dominant trophic group in estuarine ecosystems. Their feeding mode affects nutrient recycling, seston dynamics and the benthic food web (Asmus & Asmus, 1991; Prins, Smaal & Pouwer, 1991; Dame, 1993; Ward & Shumway, 2004). Estuarine suspension-feeders have to cope with wide fluctuations in the quantity and quality of suspended particulate matter (Armstrong, 1958; Berg & Newell, 1986; Fegley, MacDonald & Jacobsen, 1992; Barillé *et al.*, 1997). There is an abundant literature concerning the response of suspension-feeding bivalves to seston fluctuations, in particular the physiological variables related to feeding, such as clearance rate, retention efficiencies and pre-ingestive selection (Jørgensen, 1990; Riisgård & Larsen, 2001; Riisgard, 2001; Ward & Shumway, 2004).

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

Particle size was the first criterion used by early researchers examining food selection (Yonge, 1926; Atkins, 1937); however, its importance seems variable, with no influence of particle size on pre-ingestive selection being consistently reported (Newell & Jordan, 1983; Newell, 1988; Chretiennot-Dinet *et al.*, 1991; MacDonald & Ward, 1994; Bougrier, Hawkins & Héral, 1997), while the opposite can also be found in the literature (Ballantine & Morton, 1956; Miura & Yamashiro, 1990; Cognie *et al.*, 2003; Mafra, Bricelj & Ward, 2009).

Nevertheless, the particles used in most of these studies differed in factors other than size, thus generating possible confounding factors. For example, Hughes (1975) observed size-dependent selection of particles by *Abra alba*, but noted a relationship between

particle size and food value. Cognie *et al.* (2003) demonstrated that particle size plays a role in oysters, determining which pallial organs (gills or palps) are involved in sorting. The microalgal species used in that study (*Pleurosigma planctonicum*, *Coscinodiscus perforatus* and *Rhizosolenia setigera*) were too large to have access to principal filaments (pfs) and selection by the gill was therefore not possible. The observed selection was performed by the labial palps and was based not on the particle size, but on their biochemical composition (i.e. intact *vs* empty cells). To our knowledge, few studies have unambiguously supported the idea that bivalves can use a size criterion to discriminate among particles. A preferential size-dependent rejection of larger particles was observed in *Crassostrea virginica* (Tamburri & Zimmer-Faust, 1996) and in *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus* (Defossez & Hawkins, 1997). However, the particles used in these studies were artificial; polystyrene or borosilicated glass particles in the former, silica particles in the latter and these results not completely applicable to natural living microalgal cells. Nevertheless, using natural living cells (i.e. contrasting-sized clones of a toxic diatom), Mafra *et al.* (2009) indisputably demonstrated the role of size in the selection carried out by *C. virginica* gills.

A well-known feature of diatom biology that could have a great effect on living cell selection by bivalves is the MacDonald-Pfitzer rule (MacDonald, 1869; Pfitzer, 1869; Round, Crawford & Mann, 1990). During vegetative growth and mitotic divisions, one of the daughter cells is smaller than the mother cell, so the mean cell size of a population decreases, a phenomenon that usually leads to the loss of monoclonal cultures in the laboratory. This cell size diminution, unique to diatoms and especially crucial in most pennate species, can be reversed: when a critical threshold size is reached, sexual reproduction and auxosporulation occur. In diatoms, auxosporulation results in the formation of initial cells, which restore vegetative cells to a specific maximum length (Amato, 2010). Consequently, cells of the same species, but of different sizes, are often found together in the natural environment (Mann, 1988; Potapova & Snoeijs, 1997; Mann, Chepurnov & Droop, 1999). This phenomenon is expected to affect the kinetics of diatom selection and diatom-metabolite uptake by bivalves through its effect on their feeding processes, for example clearance rate, pre-ingestive selection and absorption efficiency. In fact, Mafra *et al.* (2009) demonstrated that the ability of *C. virginica* to sort particles according to their size could affect its uptake of the neurotoxin domoic acid from *Pseudo-nitzschia multiseries*.

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

It has also been shown that the oyster *Crassostrea gigas* can feed exclusively on *H. ostrearia* (Barillé *et al.*, 1994), despite its lower nutritive value compared with other microalgal species (Piveteau, 1999). Although *H. ostrearia* and marennine have long been recognized as the only greening agent of cultured oysters in France, other algal species from the same genus and producing 'marennine-like' pigments have been discovered elsewhere in the world (Gastineau *et al.*, 2012a, 2014a, 2016). These authors have also demonstrated that marennine-like pigments have antibacterial, antiviral and antifungal activities, in particular against pathogens of the oyster *C. gigas* (Gastineau *et al.*, 2012b, c). Thus, the *Haslea* consortium and its marennine-like pigments have potential application in shellfish aquaculture: used to feed and green oysters, they could both sustain growth of the bivalves and protect them against pathogens.

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

MATERIAL AND METHODS

Algal culture

Cultures of *Haslea ostrearia* used in this study were obtained from the Nantes Culture Collection (NCC; temperature: 14 °C; light/dark cycle: 14/10 h; light intensity: 100 µmol photons m^{-2} s^{-1}; ES1/3 medium, Provasoli, 1968), Faculté des Sciences et des Techniques, Université de Nantes. Strains with a similar biochemical composition (Joux-Arab, Berthet & Robert, 2000), but different cell lengths, were selected (Table 1). A particle counter (Coulter Multisizer 3) was used to determine equivalent spherical diameter (ESD) of the different strains. Sixty-litre mass cultures were held in polyethylene bags filled with underground seawater (Cognie, 2001), 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 3×10^6 cells l^{-1} for each cell size; Table 1) for a final total cell concentrations of 9×10^6 and 6.1×10^6 cells l^{-1}, delivered to the bivalves under conditions A and B (see below), respectively.

Oyster sampling and maintenance

Adult *Crassostrea gigas* with a mean dry flesh 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 2 min in a 0.1% hypochlorite solution to eliminate parasitic polychetes of the genus *Polydora*. The oysters were then thoroughly washed, maintained for 1 week in filtered (Millipore 0.45 µm) oxygenated seawater.

Feeding experiments

Two series of experiments were run over different periods; one with oysters feeding on three algal cell sizes (condition A), the other on two algal cell sizes (condition B). After 24 h of acclimation prior to experimental conditions (A or B; Table 1), oysters were randomly chosen and placed in a flow-through experimental system, as described by Cognie *et al.* (2003). The mean flow rate for each individual tray was 8 l h^{-1} at 16 °C. Two trays containing

Table 1. Experimental conditions (mean ± SE).

	POM (mg l^{-1})	Cell concentration (10^6 cells l^{-1})	Cell length (µm)
Condition A	2.5 ± 0.1	3.1 ± 0.2	Ho50: 50.2 ± 1.4
		3.2 ± 0.3	Ho75: 74.9 ± 2.2
		2.9 ± 0.3	Ho95: 95.2 ± 1.8
Condition B	1.9 ± 0.1	3.0 ± 0.3	Ho-small: 37.5 ± 5.2
		3.1 ± 0.3	Ho-large: 99.4 ± 2.2

Ho, *Haslea ostrearia*; POM, particulate organic matter.

an empty shell were used as sedimentation controls. After 1 h of oyster acclimation, seawater samples (50 ml) were collected at the outflow of the experimental individual trays every 15 min for 1 h. Pseudofaeces were collected at the end of the observations. The individual samples (outflow seawater and pseudofaeces) were fixed with acetic Lugol's solution and analysed separately as replicates. Cells were counted by means of light microscopy using 'Nageotte'-type haematimetric units. For each sample, a minimum of 300 cells was counted.

Sample preparation for SEM

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

Video-endoscopy directed sampling

This sampling was conducted concomitantly with the feeding measurements described above, following Cognie *et al.* (2003). At least 24 h before sampling, a small aperture was milled in the shell margins to prevent damage of the optical insertion tube 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 1 h of observation, pseudofaeces were collected. The samples (outflow seawater, pseudofaeces and from ventral and dorsal grooves) were treated and analysed as described above for the feeding experiments.

Data analysis

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

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

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

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

RESULTS

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

The heterorhabdic gill of *Crassostrea gigas* is composed of plicae and troughs corresponding to the locations of ordinary and pfs, respectively (Fig. 1A, 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 200–250 µm wide, the ordinary filaments 35–40 µm and the opening of the pfs was *c.* 70 µm wide. In addition, ordinary filaments located in the apical part of the plicae (or apical filaments) were *c.* 45 µm wide. The same measurements, when performed using SEM, showed that plicae were only 120–140 µm wide (Fig. 1A), ordinary filaments 18–26 µm wide (Fig. 1B) and apical filaments 25–30 µm wide (Fig. 1A, D). Pfs were no longer visible and too deeply positioned in troughs to allow measurements (Fig. 1B, C). Thus, due to the preparation for SEM, the dimensions of contracted gill plicae correspond to *c.* 50% of those observed *in vivo*.

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

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.

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

Table 2. Experimental hypotheses tested in condition B.

Hypothesis	Test of hypothesis	Conclusion if hypothesis accepted
H_0	Proportions not significantly different at sampling sites	No selection by the pallial organs
H_1	Proportions significantly different at sampling sites	Selection by the pallial organs
H_{1a}	Proportions significantly different in water and at ventral and/or dorsal tracts	Selection at gills
H_{1b}	Proportions significantly different in ventral tracts and pseudofaeces	Selection at labial palps
H_{1c}	H_{1a} and H_{1b} accepted	Selection at gills and labial palps

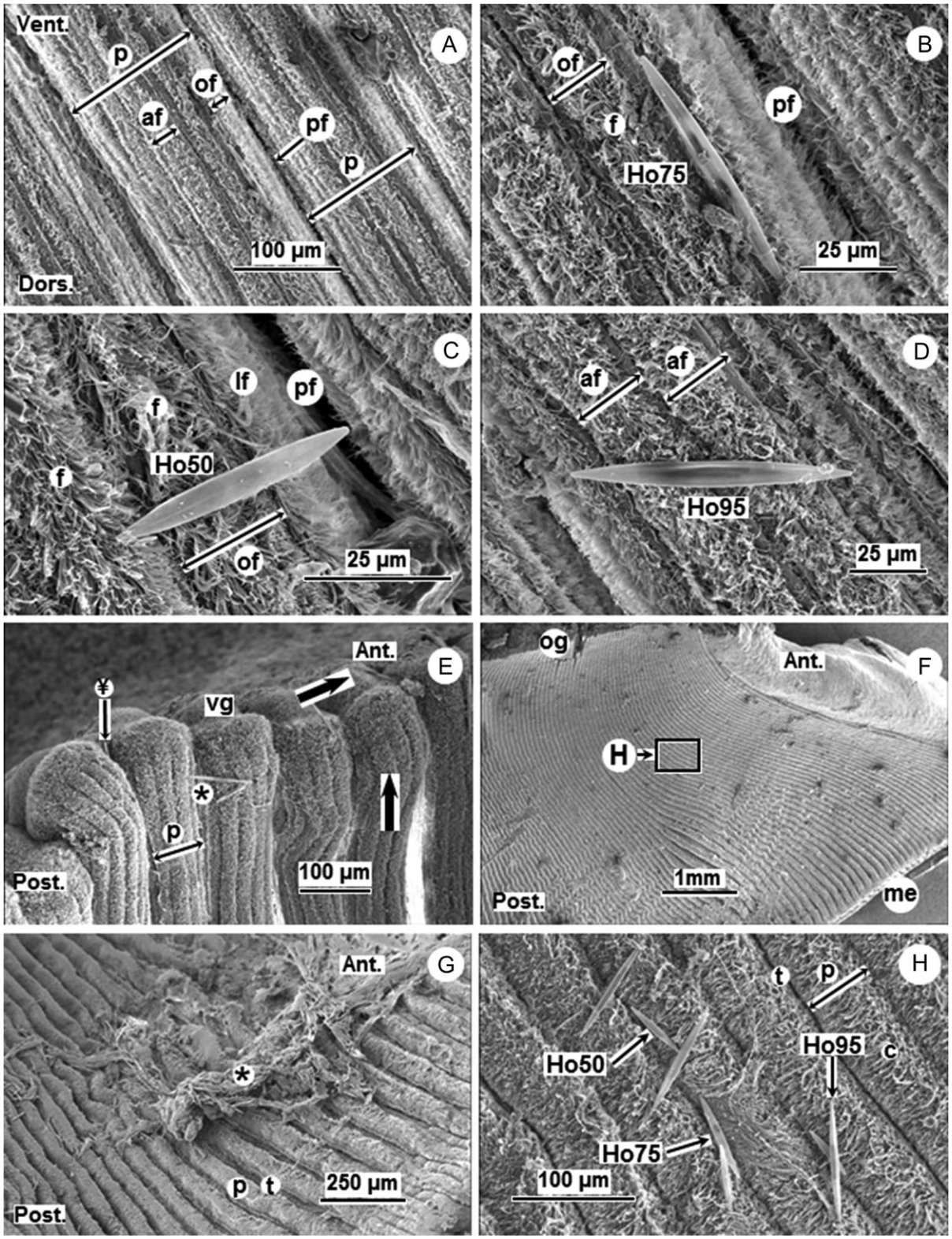

Figure 1. SEM studies of the gill and labial palps of *Crassostrea gigas* fed with three different-sized populations of *Haslea ostrearia*. **A.** Frontal view of gill showing differentiation between principal filaments and plicae constituted of ordinary and apical filaments. **B.** Detail of plicae showing a *H. ostrearia* cell (length 75 µm) in main direction of filaments. **C.** Ciliation of an ordinary filament conveying a *H. ostrearia* cell (length 50 µm). **D.** Detail of plicae showing a *H. ostrearia* cell (length 95 µm) lying across more than one apical filament (af). **E.** Side view of gill and ventral groove (vg). Arrows indicate direction of main particle transport. **F.** Ridged surface of labial palp. **G.** Detail of ridged labial palp surface covered with aggregate of mucus (*) containing the three cell sizes of *H. ostrearia*. **H.** Detail of ridged labial palp surface (area indicated in **F**), with cells of the three different-sized populations of *H. ostrearia*. Abbreviations: af, apical filament; Ant., anterior part; c, cilia; Dors., dorsal part; f, frontal cilia; g, grooves; lf, laterofrontal cirri; me, marginal edge; of, ordinary filament; og, oral groove; p, plicae; pf, principal filament; Post., posterior part; Vent., ventral part; vg, ventral groove. Scale bars: **A, E, G, H =** 100 µm; **B–D =** 10 µm; **F =** 1 mm.

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

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

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

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

The value of the SI at outflow was not significantly different from zero (*t*-test, $P > 0.05$; Fig. 2), indicating that both populations were retained with the same efficiency on gills. However, the SI for pseudofaeces was significantly lower than zero (*t*-test, $P < 0.001$), clearly allowing the rejection of the null hypothesis H_0 and acceptance of H_1: selection occurred on the pallial organs. The mean SI for ventral groove and dorsal tract were significantly different from zero (*t*-test, $P < 0.001$), thus allowing acceptance of H_{1a}: selection occurred on the gills ($P < 0.05$). There was no significant difference between the mean SI for ventral groove and pseudofaeces (*t*-test, $P > 0.05$). The experimental hypotheses H_{1b} and H_{1c} may therefore be rejected: no selection was performed on the labial palps. Selection indices clearly showed that dorsal tracts were enriched with small *H. ostrearia* cells, whereas ventral grooves and pseudofaeces were enriched in large *H. ostrearia* cells (Fig. 2).

Table 3. Comparison of the proportions of the three *Haslea ostrearia* populations at sampling sites in condition A.

Mean proportions of the three populations (% ±SE)				χ^2 test (*vs* inflow)	
	Ho50	Ho75	Ho95	χ^2 observed	*P*
Inflow	40 (0.2)	31 (0.1)	29 (0.1)	–	–
Outflow	38 (0.1)	32 (0.1)	30 (0.1)	0.09	NS
Pseudofaeces	21 (0.1)	40 (0.2)	39 (0.1)	8.95	*

*$P \leq 0.05$; NS, not significant; Ho, *Haslea ostrearia*.

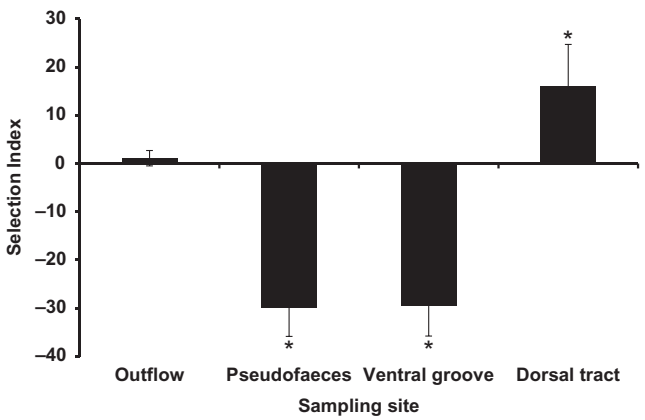

Figure 2. Selection indices for small *Haslea ostrearia* cells of samples taken *in vivo* from outflow, gills (dorsal and ventral tracts) and pseudofaeces of *Crassostrea gigas*. Error bars represent 95% confidence interval of the mean. Asterisks denote indices significantly different from 0 ($P < 0.001$).

DISCUSSION

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

From both experiments, we demonstrated that the size of *H. ostrearia* cells is an essential criterion influencing pre-ingestive selection in *C. gigas*. Oysters preferentially rejected in pseudofaeces the larger *H. ostrearia* cells (Ho-large or Ho75 and Ho95) when offered in mixed suspensions with the smaller ones (Ho-small or Ho50). In oysters, pre-ingestive sorting ability has been related to the presence of antagonistic ciliary tracts on the surface of the ordinary filaments composing plicae (Atkins, 1937; Ribelin & Collier, 1977; Ward *et al.*, 1994, 1998b). Particles transported by the median frontal cilia of ordinary filaments are directed towards the ventral grooves in which they are conveyed within an aggregate of mucus for a further preferential rejection. Conversely, particles transported by the marginal frontal cilia on both sides of the median frontal cilia are directed towards the dorsal grooves and then conveyed to the mouth as a mucous suspension. Figure 1B–E show the dimensional relationship between the size of the three *H. ostrearia* populations and that of the gill structures likely to perform particle sorting. Thus, for large cells with a naviculoid shape, such as those used in our study, bidirectional particle transport by ordinary filaments would seem difficult to achieve, even if particles were directed according to the longitudinal axis of the filaments.

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

Particles transported in dorsal grooves and those conveyed with mucus aggregates in ventral grooves are directed towards the ridged surfaces of the labial palps (Ward, 1996). In bivalves with homorhabdic gills, the labial palps are considered to be the main site for sorting and regulating the quantity of particles ingested. In bivalves with heterorhabdic gills such as oysters of the genus *Crassostrea*, the palps are thought to play a secondary role in these two functions and to serve mainly to reject the noningested material as pseudofaeces (Beninger & St-Jean, 1997; Ward *et al.*, 1998a). The differentiation of heterorhabdic gills into two types of filaments enables these two functions to be performed before the material reaches the palps. In addition, recent studies have demonstrated that labial palps may also sort algal cells on the basis of their chemical properties (Cognie *et al.*, 2003; Beninger *et al.*,

Table 4. Role of particle size in selection at oyster pallial organs.

Particle dimensions	Access to pf	Selection	
		At gill	At labial palps
All axes < pf width	Free	Possible	Possible
All axes > pf width	Not possible	Not possible	Possible
1 axis > pf width	Limited	Passive	Possible

pf, principal filament.

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

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

The demonstration that *C. gigas* selectively rejects larger cells of *H. ostrearia* could have some ecological significance, both in natural environments and in aquaculture, especially in oyster ponds. For example, in estuarine and coastal waters, oysters feed selectively on pennate diatoms, which constitute an important food source in intertidal areas with large mud flats (Cognie *et al.*, 2003). Considering the stock of cultivated and wild oysters, the sorting process evidenced in the present work may affect the structure of the microalgal populations in the vicinity of oyster beds. Our study suggests that the preferential rejection of large-sized cells in pseudofaeces could favour the development and/or maintenance of subpopulations of large-sized *Haslea*, given that *H. ostrearia* cells rejected in pseudofaeces have a revival capacity that is not altered by pre-ingestive processing (Barillé & Cognie, 2000). Furthermore, like many pennate diatoms, sexually competent cells of *H. ostrearia* can carry out auxosporulation to compensate for the size reduction associated with vegetative divisions, but only when cell size decreases to 65–70 µm, which represents 50% of the maximum cell length of the species (Neuville & Daste, 1979; Davidovich, Mouget & Gaudin, 2009; Mouget *et al.*, 2009). Therefore, this study shows that *C. gigas* preferentially ingests sexually mature cells and rejects immature ones, which could modify *H. ostrearia* cell size distribution and population dynamics in oyster ponds. Given the crucial role of cell size in the life cycle of diatoms, the impact of this predation pressure on the population dynamics of *H. ostrearia* in oyster ponds remains to be assessed, especially considering the importance of marennine in the greening process, but also regarding its many biological activities (e.g. antibacterial) that could be exploited in aquaculture.

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