Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. II. Selective rejection

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ABSTRACT: Oysters accumulate relatively low levels of domoic acid (DA) compared to other bivalves. Mafra et al. (2009, in this Theme Section) identified feeding mechanisms of oysters that may lead to low DA accumulation during monospecific blooms of *Pseudo-nitzschia multiseries*. However, several different species of *Pseudo-nitzschia*, as well as other diatoms and flagellates, may co-occur during a bloom. Therefore, the present study investigates pre-ingestive feeding processes that operate when oysters *Crassostrea virginica* are exposed to mixed phytoplankton assemblages containing *P. multiseries* of varying cell length. Guided by video-endoscopy, material transported along the ventral and dorsal gill tracts was sampled and analyzed to determine the site for sorting of microalgae on the pallial organs. There was no preferential rejection of *P. multiseries* in pseudofeces when oysters were exposed to the alga in a mixed suspension with other diatom species (*Thalassiosira weissflogii* or *Chaetoceros muelleri*). In contrast, *P. multiseries* was preferentially rejected when mixed with the flagellates *Isochrysis galbana* or *Rhodomonas lens*, suggesting a qualitative mechanism for particle sorting. This occurred on the gills, followed by further selection on the palps. Oysters also preferentially rejected larger *P. multiseries* cells (82 to 90 µm) relative to smaller ones (24 to 28 µm) on the gills, while no further selection based on size occurred on the palps. This effect is attributed to the fact that *P. multiseries* cells with a length that exceeds the width of the principal filament aperture (ca. 68 µm) are more likely directed to the ventral tract and rejected in pseudofeces. These findings offer an additional explanation for the relatively low DA levels found in oysters during natural *Pseudo-nitzschia* spp. blooms.

KEY WORDS: Oyster · *Crassostrea virginica* · Particle selection · Pseudofeces · Selective ingestion · *Pseudo-nitzschia multiseries* · Domoic acid

INTRODUCTION

Domoic acid (DA), a competitor for glutamic acid receptors in neuronal cells, has been linked to amnesic shellfish poisoning in humans (Wright et al. 1989) and marine animal intoxication outbreaks (e.g. Scholin et al. 2000) in several regions worldwide (reviewed in Hallegraeff 2003). This toxin, initially found in macroalgae (Daigo 1959), is also produced by diatoms, including several species of the genus *Pseudo-nitzschia* (reviewed by Trainer et al. 2008). Although suspension-feeding bivalve molluscs are the main vector for

DA transfer to humans, toxin levels in oysters are often very low, even when DA concentrations exceeding the 20 µg g⁻¹ regulatory level are present in other co-occurring bivalve species (reviewed by Mafra et al. 2009, this Theme Section).

Since DA uptake by bivalves depends on their capture and ingestion of toxic cells, regulation of the suspension-feeding process can noticeably increase or reduce the amount of toxin available for tissue incorporation. In previous laboratory experiments (Mafra et al. 2009), oysters exhibited a relatively low clearance rate (CR) when exposed to unialgal suspensions of

either toxic or non-toxic *Pseudo-nitzschia multiseries* cells, but CR was restored when *P. multiseries* was combined with an alternative, nutritious food source. As other non-toxic phytoplankton species can be present in the water column during *Pseudo-nitzschia* spp. blooms, sometimes representing an equivalent or higher algal biomass (e.g. Fehling et al. 2006, Mafra et al. 2006, Spatharis et al. 2007), the low levels of DA commonly found in oysters could also be the result of an effective mechanism of particle selection. Pre-ingestive selection may occur on the gills and/or labial palps of bivalves (Ward et al. 1994, 1998a) and be affected by seston concentration (Barillé et al. 1993), particle morphology and size (Ward et al. 1998b), particle quality (i.e. organic vs. inorganic; Newell & Jordan 1983, Bayne et al. 1993, Cognie et al. 2003, Beninger et al. 2004) and other characteristics related to the organic content and/or coating of the cells, such as nutritional value (Ward et al. 1997), stickiness (Waite et al. 1995), surface charge (Hernroth et al. 2000) and secreted ectocrines (Ward & Targett 1989).

On the labial palps of bivalves, nutritious organic particles are typically transported to the mouth for ingestion, while undesirable components are rejected as pseudofeces, which provides a mechanism for enrichment of the food supply (Ward & Shumway 2004). In oysters, which are characterized by complex, heterorhabdic (i.e. differentiation of ordinary and principal filaments), pseudo-lamellibranch gills capable of bidirectional particle transport, the particle-sorting process starts earlier on the gills. As a result, oysters can selectively and efficiently ingest a given algal species from a mixed suspension while rejecting others as pseudofeces. Some studies have reported that diatom species are preferentially eliminated as pseudofeces from mixed suspensions with flagellates (e.g. Bougrier et al. 1997). However, diatoms may be an important food item for oysters during seasonal diatom blooms when other microalgae are scarce or a more refractory particle type is present (Decottignies et al. 2007).

The sorting capacity of the gills of oysters is also influenced by particle size and morphology. Using qualitative endoscopic observations, Cognie et al. (2003) showed that diatoms with all dimensional axes ≥70 µm cannot enter the principal filaments and thus cannot be directed efficiently to the dorsal ciliated tracts (dct) of the gills. Instead, such particles are obligatorily transported on the relatively exposed ventral ciliated grooves (vcg), from where a fraction may be lost in pseudofeces. In this case, particle selection can only be performed by the labial palps. Because *Pseudo-nitzschia* spp. cells are long, but narrow (ca. 5 µm wide), some cells can enter the principal filaments if they reach the gills in a dorsoventral orientation. Therefore, *Pseudo-nitzschia* spp. cells can be

transported to both the ventral and dorsal tracts of the gills, as demonstrated by preliminary, qualitative endoscopic observations of the oyster *Crassostrea gigas* exposed to 80 µm long *Pseudo-nitzschia* sp. cells (J. E. Ward et al. unpubl. data). Additionally, the cell size of diatoms is highly variable, as it decreases over time due to successive vegetative divisions (Round et al. 1990), and *Pseudo-nitzschia* spp. of varying cell size, including *P. multiseries* cells as short as 35 µm, can be found during toxic blooms (Bates et al. 1999, Trainer et al. 2007). Thus, we hypothesize that, in oysters, the selective capacity of both the gills and labial palps limits the ingestion of *P. multiseries* cells, and that rejection is enhanced when oysters are exposed to long *P. multiseries* cells (up to 169 µm; Villac 1996).

In this study, a combination of feeding experiments and endoscope-directed *in vivo* sampling was used to investigate the magnitude and site of pre-ingestive selection on the pallial organs of the eastern oysters *Crassostrea virginica* that could lead to preferential rejection of toxic *Pseudo-nitzschia* spp. cells from mixed suspensions. Such a mechanism, combined with reduced CR of *C. virginica* on *Pseudo-nitzschia* spp. cells in a unialgal suspension (Mafra et al. 2009) could partially explain the low levels of DA accumulation in this and other oyster species. Feeding selectivity was examined via qualitative and quantitative analysis of pseudofeces produced from mixed suspensions of a *P. multiseries* clone with flagellates and other diatom species, and by determining the contribution of the gills and labial palps to this process. In addition, mixed suspensions containing *P. multiseries* clones of contrasting size were used to assess the effect of cell size on pre-ingestive selectivity. Post-ingestive mechanisms that could contribute to low accumulation of DA in oysters are the subject of ongoing research.

MATERIALS AND METHODS

Algal culture. Five toxic *Pseudo-nitzschia multiseries* clones, CLN-20, CLN-30, CLN-46, CLN-50 and CLNN-21, were kindly provided by S. Bates (Department of Fisheries and Oceans, Moncton, Canada) and grown in batch culture. All clones were cultivated in 1.5 l glass Fernbach flasks with autoclaved, 0.22 µm cartridge filtered seawater enriched with *f*/2 medium (Guillard 1975) at 16°C, 30 ppt salinity, a light intensity of 140 µmol quanta m^{-2} s^{-1}, and a 14 h light:10 h dark photoperiod. Five non-toxic algae from the Center for Culture of Marine Phytoplankton (CCMP, West Boothbay Harbor, ME) — the flagellates *Rhodomonas lens* (CCMP Strain 739), *Isochrysis galbana* (T-Iso clone, CCMP1324) and *Pavlova pinguis* (CCMP609), and the diatoms *Thalassiosira weissflogii* (Actin clone, CCMP

1336) and *Chaetoceros muelleri* (CCMP1316) — were also batch-cultured in *f*/2 medium, without added silicate for the flagellates. They were kept under the same culture conditions as *P. multiseries*, except for *I. galbana* and *P. pinguis*, which were cultured in either 20 l, aerated, plastic carboys or in a semi-continuous system (200 l photobioreactors) at 20°C. Cell density of *I. galbana*, *P. pinguis* and *C. muelleri* was determined with a Multisizer 3 particle counter (Beckman-Coulter). Cell density of *R. lens*, *T. weissflogii* and *P. multiseries* clones was measured with a microscope (Leica Model DMLB 100S). Dilutions for microscopy were made to obtain ca. 400 cells per Palmer-Maloney counting chamber.

Cell size (equivalent spherical diameter, ESD) and volume (μm^3) of *Rhodomonas lens*, *Isochrysis galbana*, *Pavlova pinguis* and *Chaetoceros muelleri* were obtained using the particle counter. For both *Thalassiosira weissflogii* and *Pseudo-nitzschia multiseries* clones, cell length and width (n = 30) were measured prior to each experiment using a microscope with a coupled Pulnix camera (Model TMC-7DSP) and image analysis software (Image Pro Plus Version 4.5, Media Cybernetics). Only cell length of *P. multiseries* clones is reported in Table 1, as width was similar among clones and over time (4.3 to 5.2 μm). Cellular volume of *T. weissflogii* was calculated by assuming a cylindrical shape, and that of *P. multiseries* using the formula described by Hillebrand et al. (1999) and modified by Lundholm et al. (2004) for *Pseudo-nitzschia* spp.: cell volume (μm^3) = $(0.6 \times L \times W^2) + (0.2 \times L \times W^2)$, where *L* and *W* are maximum length and width in micrometers, respectively.

DA concentration in *Pseudo-nitzschia multiseries* cultures was determined in triplicate by gently filtering 15 ml aliquots through Whatman GF/F glass microfiber filters (25 mm diameter, 0.7 μm minimum particle retention) followed by analysis of the fluorenylmethoxycarbonyl (FMOC) derivative in cellular and dissolved fractions by high performance liquid chromatography (HPLC) following the methods of Pocklington et al. (1990). All *P. multiseries* clones were harvested at the stationary phase of growth, when cultures exhibited the maximum intra-cellular toxicity throughout the growth cycle. Only cellular toxicities are reported in Table 1, as particulate DA is known to be the primary route for toxin accumulation (Novaczek et al. 1991). The clones were predominantly single-celled in the stationary phase, with <5 % of the cells forming short chains of ≤4 cells, such that chain-formation was not a confounding variable in these experiments. Processing of *P. multiseries* chains by *Crassostrea virginica* is the subject of future investigation.

Selective feeding experiments. Experiments were conducted at the Marine Research Station, Institute for Marine Biosciences (MRS/IMB), National Research Council of Canada (NRC), Halifax, Nova Scotia. Juvenile eastern oysters *Crassostrea virginica* (first year cohort, mean shell height [SH] ± standard error [SE]: 21.4 ± 0.3 mm), were acquired from growers in Prince Edward Island, Canada, in October 2005 and kept in 1000 l insulated tanks containing active upwellers at ca. 1500 oysters per tank, 12°C and 30 ppt salinity. Shell height represented the maximum dimension from the umbo to the ventral margin of the shell. Oysters were fed *Pavlova pinguis* and *Isochrysis galbana*

Table 1. Characteristics of diets exposed to the eastern oyster *Crassostrea virginica* in endoscopic observations and feeding rate experiments. Diet composition and cell density, toxicity, cell size and total biomass concentration are presented. CLN and CLNN: *Pseudo-nitzschia multiseries* (*Ps-m*) clones; *C. muelleri*: *Chaetoceros muelleri*; *I. galbana*: *Isochrysis galbana*; *R. lens*: *Rhodomonas lens*; nt: non-toxic

Experiment	Diet	Composition (cell density in cells ml^{-1})	Toxicity (pg DA cell^{-1}) *Ps-m* /other spp.	Mean cell size (μm)	Total biomass concentration (mg l^{-1})
Feeding Mixed diets	M1	CLN-20 (9100) + *I. galbana* (87 000)	0.7 / nt	28[a] / 4.5[b]	7.4
	M2	CLN-46 (2000) + *I. galbana* (81 000)	0.8 / nt	82[a] / 4.5[b]	6.2
	M3	CLN-50 (1800) + *I. galbana* (70 000)	0.7 / nt	100[a] / 4.5[b]	6.7
	M4	CLN-50 (2400) + *R. lens* (9800)	5.9 / nt	68[a] / 7.6[b]	5.8
	M5	CLN-50 (2800) + *C. muelleri* (34 000)	5.9 / nt	68[a] / 5.4[b]	6.7
	M6	CLN-50 (2200) + *T. weissflogii* (3500)	0.7 / nt	100[a] / 23[a]	8.1
	M7	CLN-46 (4200) + CLN-20 (11 300)	1.2 / 0.6	82[a] / 28[a]	11.1
	M8	CLN-50 (1300) + CLN-20 (7300)	0.5 / 0.4	90[a] / 25[a]	5.7
Video-endoscopy	E1	CLNN-21 (2000) + CLN-30 (7000) + *I. galbana* (40 000)	0.2 / 0.03 / nt	99[a] / 35[a] / 4.5[b]	10.8
	E2	CLN-46 (3700) + *R. lens* (12 000)	0.05 / nt	46[a] / 7.6[b]	6.8
	E3	CLN-46 (1400) + *T. weissflogii* (1500) + *I. galbana* (40 000)	0.07 / nt / nt	69[a] / 23[a] / 4.5[b]	4.6

[a]Cell length; [b]ESD: equivalent spherical diameter

at a total cell density equivalent to 30 000 *I. galbana* cells ml^{-1} and acclimated to the experimental diet ca. 18 h before each trial. After being used in the selectivity experiments, oysters were stored frozen and then oven-dried at 80°C for 24 h to obtain the dry weight (DW) of soft tissue.

Clearance rate (CR, i.e. the volume of water cleared of particles per unit time, in ml min^{-1}) of oysters was measured in five 400 ml acrylic chambers (6 to 8 oysters per chamber), plus 1 chamber without oysters as a control for phytoplankton settlement. Mixing of the suspension was achieved with a motor-driven magnetic stirrer held on the top of the chamber, which prevented disturbance and re-suspension of oyster biodeposits. The experimental diet (Table 1) was gravity-fed to the chambers from a common 60 l header tank, and a peristaltic pump re-circulated the pooled outflow water from the chambers back to the header tank. Following a flow-through acclimation period, flow was interrupted and samples were taken from each chamber before and after a period that allowed oysters to deplete 15 to 30% of the cells in suspension.

Pseudofeces production rate (Pf, i.e. number of cells rejected as pseudofeces per unit time, in cells min^{-1}) was measured in a similar experimental system, as described in Mafra et al. (2009). CR, filtration and ingestion rates (FR and IR, i.e. number of cells filtered and ingested per unit time; in cells min^{-1}) were calculated from the following equations:

$$\text{CR (ml min}^{-1}) = [(\log_e C_i - \log_e C_f) - (\log_e Cc_i - \log_e Cc_f)] \times (V/t) \quad \text{(Coughlan 1969)} \tag{1}$$

$$\text{FR (cells min}^{-1}) = \text{CR} \times \text{geomean} (C_i, C_f) \tag{2}$$

$$\text{IR (cells min}^{-1}) = \text{FR} - \text{Pf} \tag{3}$$

where C_i and C_f are initial and final particle concentrations (cells ml^{-1}), Cc_i and Cc_f are the initial and final particle concentrations in the control chamber, V is the volume of the chamber (in ml) corrected for the volume occupied by the oysters, and t is the incubation time (in min). The geometric means of C_i and C_f were used in the calculation of FR. All measured feeding rates (CR, FR, IR and Pf) were weight-standardized following the general allometric equation for suspension-feeding bivalves as reviewed by Bayne & Newell (1983):

$$\text{FdR}_{std} = (W_{avg} / W_{exp})^{0.616} \times \text{FdR}_{exp} \tag{4}$$

where FdR$_{std}$ is the weight-standardized feeding rate, W_{avg} is the soft tissue DW of an average oyster (0.02 g in our experiments), FdR$_{exp}$ and W_{exp} are the experimental (i.e. measured) FR and soft tissue DW (in g), respectively.

To investigate selective ingestion/rejection of *Pseudonitzschia multiseries* cells, clones varying in cell size were tested in mixed suspensions with a second species, either a flagellate (Diets M1 to M4) or another diatom species (Diets M5 and M6; Table 1). All cellular dimensions (length, width, height) of the species used were >4.3 μm, which allows a minimum of ca. 80 to 87% retention by the oyster gill (Riisgård 1988, Ward & Shumway 2004). Prior to every trial, cellular volume was calculated for both algal species and the suspension was prepared by adding equivalent cellular volumes of each alga to the 60 l header tank. Suspensions were monitored over time to ensure an approximate 50:50 volume ratio of both species throughout the trial, and total concentrations were sufficiently high (≥4.2 mg DW l^{-1}) to assure that pseudofeces were produced. Because *P. multiseries* cells are long but narrow (width = 4.3 to 5.2 μm), differential retention by the gills was investigated in mixed suspensions with a flagellate or other diatom of a different cell size. For each mixed suspension, CR measurements were taken from a different group of oysters over an interval that was adjusted to limit cell depletion to only 15 to 30% (ranging from 14 to 19 min for Diets M3, M4 and M5, to 35 to 70 min for M1, M2 and M6). Reduced retention of *P. multiseries* cells by the gills (H$_1$; Table 2) is thus verified if the CR for *P. multiseries* is significantly lower than that for the second alga within a mixed suspension (paired *t*-test; α = 0.05). Pseudofeces production was also measured for each component of the binary diets and used to calculate the IR from Eq. (3). Finally, FR (Eq. 2) was expressed in terms of total cell volume and then partitioned into IR and Pf. The proportion (*p*) of each algal clone rejected in pseudofeces was compared within each diet, and the hypothesis of selective rejection of *P. multiseries* cells was assessed by *t*-test (α = 0.05), following an arcsine transformation.

In addition, cell-volume-based ratios of *Pseudonitzschia multiseries* to a second species were calculated for the suspension and pseudofeces. A greater ratio in pseudofeces than that offered in the suspension, as revealed by 1-tailed *t*-test (α = 0.05), confirms preferential rejection of *P. multiseries* cells by oysters, as a result of selective filtration and/or rejection (H$_2$; Table 2).

Two additional suspensions (Diets M7 and M8; Table 1) were prepared by mixing 2 *Pseudo-nitzschia multiseries* clones with similar toxicity but different cell sizes. They were used to test the effect of *P. multiseries* size alone on selective feeding of oysters. Feeding trials were conducted as before, and ratios of large clone to small clone were calculated in the suspension and pseudofeces. Therefore, if the ratio in pseudofeces differs significantly from that in the offered suspension (2-tailed *t*-test; α = 0.05), cell size can be considered as a factor affecting particle selectivity (H$_{2.1}$; Table 2).

Table 2. *Crassostrea virginica*. Possible pre-ingestive feeding mechanisms resulting in reduced filtration and/or ingestion of *Pseudo-nitzschia multiseries* cells, and hypotheses tested in each experiment. dct: dorsal ciliated tracts; EI: electivity index; CR_{Ps-m}: clearance rate of oysters on *Pseudo-nitzschia multiseries* (*Ps-m*) cells; $CR_{2nd\ sp.}$: clearance rate of oysters on a second species in mixed suspension with *Ps-m*; PF: pseudofeces; Susp: suspension; vcg: ventral ciliated grooves

Feeding process	Avoidance mechanism	Diets tested (experiment)	Underlying hypotheses
Filtration by the gills	1. Reduced retention of narrow *Ps-m* cells on the gills	M1 to M6 (feeding)	H_0 (no reduction): $CR_{Ps-m} \geq CR_{2nd\ sp.}$ H_1 (reduced retention): $CR_{Ps-m} < CR_{2nd\ sp.}$
Ingestion	2. Selective rejection of *Ps-m* cells in PF	M1 to M6 (feeding)	H_0 (no selection): ratio $[Ps\text{-}m/2\text{nd sp.}]_{PF}$ = ratio $[Ps\text{-}m/2\text{nd sp.}]_{Susp}$ H_2 (selective rejection): ratio $[Ps\text{-}m/2^{nd}\ sp.]_{PF}$ > ratio $[Ps\text{-}m/2^{nd}\ sp.]_{Susp}$
	2.1 Selective rejection of large cells in PF	M7 and M8 (feeding)	H_0 (no selection): ratio $[large/small\ cell]_{Pf}$ = ratio $[large/small\ cell]_{Susp}$ $H_{2.1}$ (size selection): ratio $[large/small\ cell]_{PF} \neq$ ratio $[large/small\ cell]_{Susp}$
	2.2 Particle sorting on the gills	E1 to E3 (endoscopy)	H_0 (no sorting): EI in the dct = EI in the vcg $H_{2.2}$ (sorting on the gills): EI in the dct $\neq$ EI in the vcg
	2.3 Particle sorting on the labial palps	E1 to E3 (endoscopy)	H_0 (no sorting): EI in pseudofeces = EI in the vcg $H_{2.3}$ (sorting on the palps): EI in pseudofeces $\neq$ EI in the vcg

Because all *Pseudo-nitzschia multiseries* clones were harvested in the stationary phase and mixed with another species harvested at the exponential phase, an additional experiment was performed to compare the relative rejection of exponential versus stationary *P. multiseries* cells. CLN-20 cells were harvested after 11 and 41 d of culture, representing mid-exponential and mid-stationary phases, respectively. Oysters were fed similar cell densities of unialgal CLN-20 diets at both growth phases. CR was measured (see Mafra et al. 2009), and pseudofeces were collected to quantify Pf. The proportion of cells rejected as pseudofeces was calculated in both treatments, and the hypothesis that *P. multiseries* cells in exponential and stationary phases are differentially rejected was tested by a *t*-test ($\alpha = 0.05$), following arcsine transformation of the proportions.

Video-endoscopic observations. The sites on the pallial feeding organs where selection of *Pseudo-nitzschia multiseries* cells could potentially take place were investigated *in vivo* by means of video-endoscopic techniques. Adult *Crassostrea virginica* (mean SH ± SE: 116 ± 2 mm) were acquired from AquaDelights Seafoods, NS, Canada, and kept in the laboratory under the same conditions as described for juveniles. Two weeks before the experiment, oysters were prepared by carefully trimming the antero-ventral, outer edge of the shell with an electric saw to allow insertion and prevent breakage of the optical insertion tube of the endoscope (Bricelj et al. 1998). Experiments followed methods described in Ward et al. (1991), using an endoscope mounted on a micromanipulator and attached to a colour video camera with an optical-zoom adapter.

Oysters were acclimated to the experimental temperature (18 to 20°C) for 2 wk and then transferred to 1 l containers. They were fed a diet of 40 000 *Isochrysis*

galbana cells ml^{-1} for 30 min, a sufficient time to ascertain that they had opened their valves and were feeding normally. After this acclimation period, different mixed diets (Table 1) were offered in a flow-through system with a peristaltic pump at 160 ml min^{-1} for ca. 2 h. The first diet was composed of 2 *Pseudo-nitzschia multiseries* clones differing in cell size (Diet E1), the second of a *P. multiseries* clone mixed with the flagellate *Rhodomonas lens* (Diet E2), and the third diet was composed of a *P. multiseries* clone mixed with an equivalent cell volume of the diatom *Thalassiosira weissflogii* (Diet E3). In order to stimulate oyster feeding, 40 000 *I. galbana* cells ml^{-1} were added to Diets E1 and E3 only, as preliminary observations indicated that overall feeding activity was reduced in the presence of a suspension consisting exclusively of diatoms. Duplicate samples of the processed particles were collected from the dorsal ciliated tracts (dct) and ventral ciliated grooves (vcg) with a micropipette connected to a micro-peristaltic pump at a suction rate of 0.55 ml min^{-1} for ca. 5 min. The material in suspension was sampled every 5 to 10 min to obtain a time-integrated sample. Duplicate samples of pseudofeces, when available, were also recovered after rejection by the labial palps using a pipette. Electivity indices (EI), based on cell volume, were calculated for pseudofeces, dct and vcg samples using the formula of Jacobs (1974), modified by Ward et al. (1998b):

$$EI = \frac{r - p}{(r + p) - (2rp)} \qquad (5)$$

where *r* is the proportion of 'Alga B' in the post-capture samples (dct, vcg, pseudofeces), and *p* is the proportion of the same cells in suspension (food supply). A positive EI indicates enrichment of 'Alga B' in the

sample, whereas a negative EI indicates enrichment of 'Alga A', the first component of the binary mixed diet.

Isochrysis galbana, offered exclusively to stimulate feeding, was not taken into account for EI calculation. Particle selection on the gills ($H_{2.2}$; Table 2) is consequently confirmed if EI in the dct is statistically different from that in the vcg. Similarly, if EI of pseudofeces and vcg samples differ significantly, then further particle selection takes place on the labial palps ($H_{2.3}$; Table 2). EI was expressed as an average of 3 to 8 oysters, and the values were compared by 1-way repeated-measures analysis of variance (ANOVAR; $\alpha = 0.05$).

After the experiment, the gills of 5 oysters were removed and left in filtered seawater at 4°C for 2 h. Gills were then observed under a Wild Heerbrugg stereoscope (Model M5-48357) and 5 to 12 principal filament apertures (pfa) were measured from the 2 central demibranchs of each oyster. The pfa is defined as the space between adjacent plicae, which allows access to the principal filament (see Fig. 5). Measurements were taken at 32× magnification using a coupled camera and image analysis software as described pre-

viously for algal measurements. Since the aperture tends to be wider toward the ventral region, each pfa width was expressed as a mean of 7 to 12 measurements taken along the filament. In addition, comparative *in vivo* measurements were taken from the video images recorded during sampling. In this case, the ordinary filament width, a fairly constant dimension throughout the oyster gills, was measured from dissected oysters and used as a reference to calibrate the video images.

RESULTS

Oysters cleared *Pseudo-nitzschia multiseries* cells (i.e. removed from suspension by retaining on the gills) at comparable or higher rates than flagellates and other diatom species offered in mixed, binary suspensions (Fig. 1). The hypothesis that oysters could remove the narrow *P. multiseries* cells from suspension with lower efficiency than other species mixed in a binary diet (H_1; Table 2) was thus rejected. Surprisingly, when
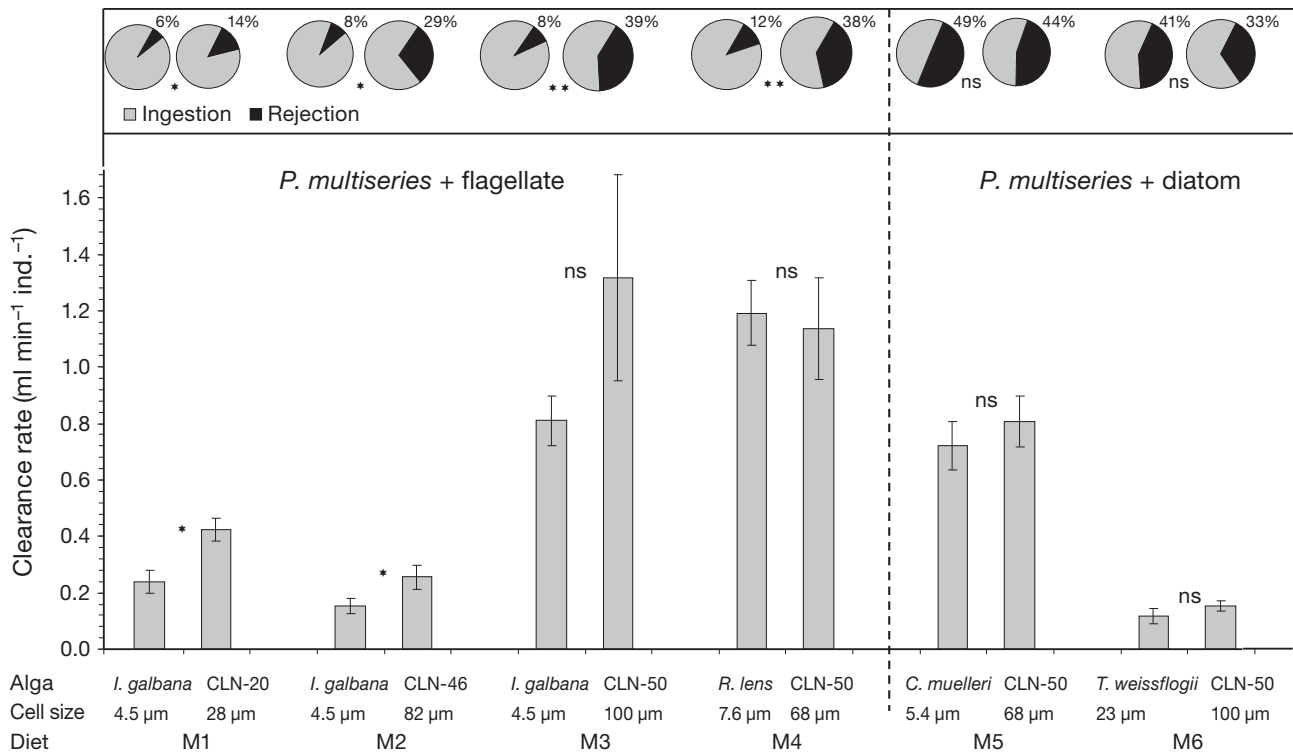

Fig. 1. *Crassostrea virginica* juveniles (mean shell height ± SE: 21.4 ± 0.3 mm). Clearance rate (mean ± SE, n = 5 chambers, 8 to 12 oysters per chamber) of juvenile oysters fed *Pseudo-nitzschia multiseries* clones (0.7 to 0.8 pg domoic acid [DA] cell[-1]) in various mixed suspensions. Each binary diet was composed of equivalent cell volumes of 2 algae, with cell sizes (length for *P. multiseries* and *Thalassiosira weissflogii*, and equivalent spherical diameter for *Isochrysis galbana*, *Rhodomonas lens*, *Chaetoceros muelleri*) shown in the graph. Clearance rates for each pair of algae were statistically compared within every diet and results are shown above bars. Pie charts indicate the percentage of filtered cells that were rejected as pseudofeces (in black) in each diet, with statistical results shown between the pairs of charts. ns: non-significant difference; *p < 0.05, **p < 0.01

3 different *P. multiseries* clones, ranging from 28 to 100 µm in cell length and 4.6 to 5.0 µm in cell width, were mixed with the small and nearly spherical flagellate *Isochrysis galbana* (ESD = 4.5 µm) in Diets M1 to M3, CR of the latter was significantly lower than that of the *P. multiseries* clone in 2 out of 3 diets tested (M1 and M2, p = 0.02 and 0.04, respectively), indicating a lower retention efficiency of *I. galbana* cells by oyster gills. In the third diet (M3), clearance of *I. galbana* was also lower than that of *P. multiseries*, but because of the high variability of the data the difference was not statistically significant (p = 0.12). Differential retention efficiency, as reflected in CR values, was not observed in Diets M4 to M6, in which *P. multiseries* clone CLN-50 was mixed with *Rhodomonas lens*, *Chaetoceros muelleri*, or *Thalassiosira weissflogii*, species with a cell size greater than that of *I. galbana* (p = 0.78, 0.22 and 0.58, respectively). Additionally, there was no relationship between CR and cell length of *P. multiseries*.

After retention on the gills, particles were either ingested or rejected in pseudofeces. In mixed suspensions, the percentage of particles that were rejected by the oysters varied as a function of particle size and

algal species. When mixed with a flagellate (Diets M1 to M4), 14 to 39% of the filtered *Pseudo-nitzschia multiseries* cells were rejected as pseudofeces prior to ingestion (Fig. 1), with the greatest percent rejection for the larger clones, CLN-46 in Diet M2 and CLN-50 in Diets M3 and M4. In contrast, rejection of the flagellates *Isochrysis galbana* (4.5 µm ESD) and *Rhodomonas lens* (7.6 µm ESD) from the same suspensions was consistently low, ranging from only 6 to 12% of the filtered cells (Fig. 1). Percent rejection of *P. multiseries* cells was significantly higher than that of the flagellates in all diets (p = 0.004 to 0.037). However, when *P. multiseries* was offered in suspension with the smaller diatoms *Chaetoceros muelleri* (5.4 µm ESD; Diet M5) and *Thalassiosira weissflogii* (23 µm; Diet M6), both components of the binary diets were rejected in similar proportions, regardless of cell size and species (p = 0.75 and 0.10, respectively).

Due to both differential retention on the gills and rejection in the pseudofeces, the ratio of *Pseudo-nitzschia multiseries* to *Isochrysis galbana*, expressed in terms of cell volume (Fig. 2; Diets M1 to M3) increased substantially from ca. 1 in the mixed suspension to 3.6, 4.8 and 7.3 in pseudofeces (p = 0.0002,
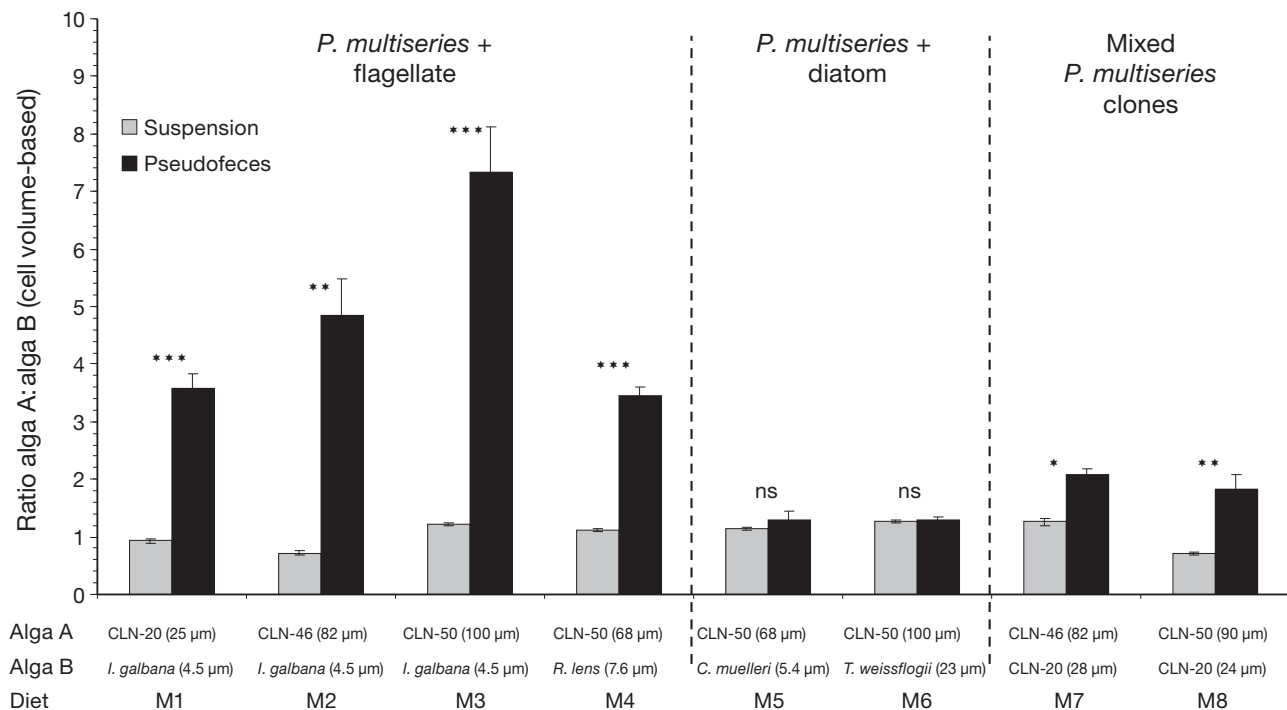

Fig. 2. *Crassostrea virginica* juveniles (mean shell height ± SE: 21.4 ± 0.3 mm). Ratio of 2 algae (Alga A:Alga B) in the mixed suspension and in pseudofeces produced by oysters (mean ± SE; n = 5 chambers, 6 to 9 oysters per chamber). Oysters were offered various *Pseudo-nitzschia multiseries* clones in a mixed diet with *Isochrysis galbana*, *Rhodomonas lens*, *Chaetoceros muelleri*, *Thalassiosira weissflogii*, or another *P. multiseries* clone of contrasting cell length. Each binary diet was composed of equivalent cell volumes of 2 algae (cell size as defined in Fig. 1 shown in parentheses). Statistical results of within-diet comparisons are shown above bars. ns: non-significant difference; *p < 0.05, **p < 0.01, ***p < 0.001

0.001 and 0.0005, respectively). In addition, the ratio of *P. multiseries* to *Rhodomonas lens* increased significantly from 1.1 in suspension to 3.5 in pseudofeces (Diet M4; p = 0.009), which can only be explained by selective rejection, as the 2 algal species were removed from the mixed suspension at comparable rates. In contrast, no selectivity was observed when oysters were offered CLN-50 in mixed suspensions with the smaller diatom species *Chaetoceros muelleri* (Diet M5; p = 0.17) and *Thalassiosira weissflogii* (Diet M6; p = 0.29). Overall, the hypothesis of preferential rejection of *P. multiseries* cells in pseudofeces (H$_2$; Table 2) was only confirmed in diets where *P. multiseries* was mixed with a flagellate (Figs. 1 & 2).

When a large *Pseudo-nitzschia multiseries* clone was combined with the small clone CLN-20 (Diets M7 and M8; Fig. 2), oysters preferentially rejected the larger cells. The ratio of large to small *P. multiseries* cells increased to a lesser degree compared to the diets containing flagellates, from 1.2 in the suspension to 2.1 in pseudofeces for Diet M7 and from 0.7 to 1.8 for Diet M8 (Fig. 2). The differences, however, were statistically significant (p = 0.014 and 0.004, respectively), confirming the hypothesis that cell size affects the selective rejection of *P. multiseries* cells by the oysters (H$_{2.1}$; Table 2).

Oysters removed comparable amounts of *Pseudo-nitzschia multiseries* cells in exponential and stationary phases from unialgal suspensions, as indicated by the similar FR (Fig. 3). However, because a greater number of cells in the exponential phase was rejected in pseudofeces, the proportion of cells that were actually ingested by the oysters was lower for cultures in exponential than for those in stationary phases (p = 0.027).

The video-endoscopy experiments allowed sampling of particles transported on the ventral and dorsal gill tracts, which further elucidated the site of particle selection. Adult oysters (mean pfa width ± SE: 68.2 ± 0.6 μm), exhibited negative EI values in the vcg when fed a mixed diet containing *Pseudo-nitzschia multiseries* clones of contrasting cell length, indicating considerable enrichment in larger cells (CLNN-21, 'Alga A' = 99 μm) relative to the suspension (Diet E1; Fig. 4). As CLNN-21 cells were preferentially directed to the ventral groove relative to the smaller cells of clone CLN-30 ('Alga B' = 35 μm), the dct were enriched in small CLN-30 cells, as reflected by positive EI values. The hypo-
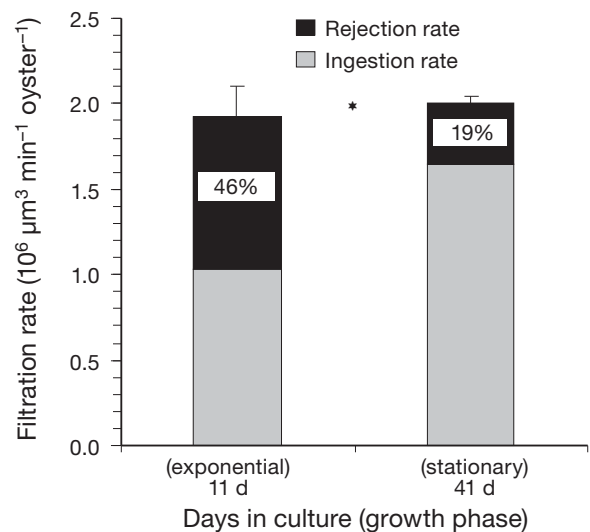

Fig. 3. *Crassostrea virginica* juveniles (mean shell height ± SE: 21.4 ± 0.3 mm). Filtration rate (composite bars, mean ± SE) and the relative allocation between ingestion rate and rejection, i.e. pseudofeces production rate (n = 5 chambers, 8 oysters per chamber) of oysters fed *Pseudo-nitzschia multiseries* clone CLN-20 at exponential and stationary phases (0.07 and 0.6 pg DA cell^{-1}, respectively). Star between bars indicates a significant difference in the proportion of cells rejected in pseudofeces (p < 0.05). Cell length was equivalent in both growth stages (25 μm)
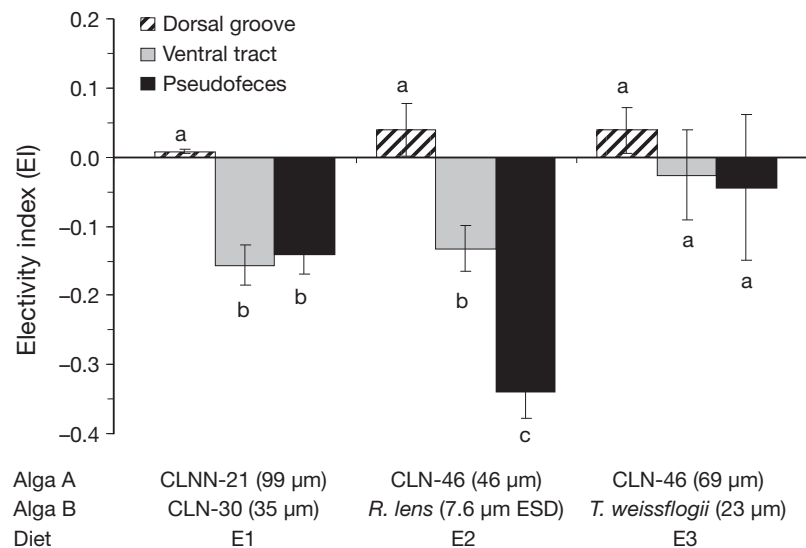

Fig. 4. *Crassostrea virginica* adults (mean shell height ± SE: 116 ± 2 mm). Electivity indices (EI, mean ± SE) in the dorsal ciliated tracts, ventral ciliated grooves and in pseudofeces of oysters fed *Pseudo-nitzschia multiseries* clones in 3 mixed suspensions: Diet E1: CLNN-21 + CLN-30; Diet E2: CLN-46 + *Rhodomonas lens*; and Diet E3: CLN-46 + *Thalassiosira weissflogii*. Diets 'E1' and 'E3' were enriched with *Isochrysis galbana* (40 000 cells ml^{-1}) to stimulate feeding. For each diet, a negative EI value indicates enrichment in 'Alga A', with a positive EI indicating enrichment in 'Alga B' at a given sampling site. Different letters indicate statistical difference in EI for each diet (α = 0.05). Electivity indices represent the average of 8, 6 and 3 oysters in Diets 'E1', 'E2' and 'E3', respectively
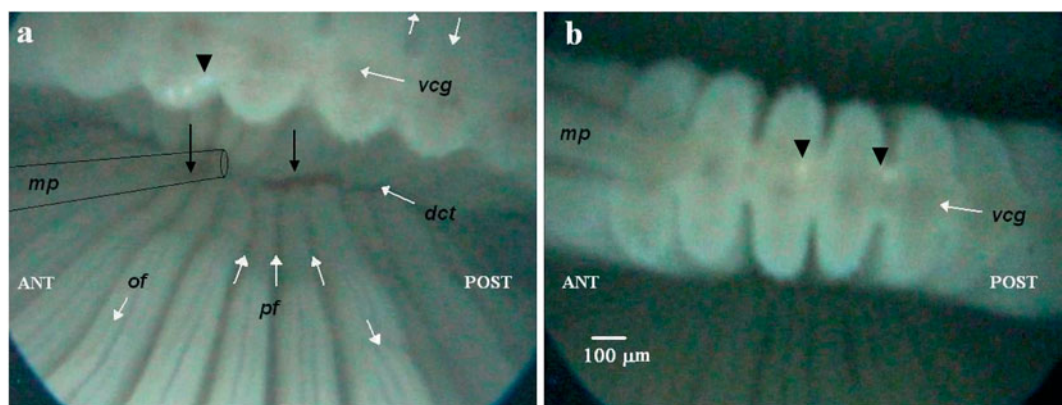
Fig. 5. *Crassostrea virginica.* Diet E2 (*Rhodomonas lens + Pseudo-nitzschia multiseries* clone CLN-46); video micrograph taken during endoscope-directed sampling of processed particles on the: (a) dorsal ciliated tract (dct) and (b) ventral ciliated groove (vcg). Note the dominance of reddish particles (*R. lens*; black arrows) carried on the dct contrasting to the more transparent, bright particles (*P. multiseries*; black arrowheads) transported on the vcg. White arrows indicate direction of particle transport on the dct, vcg, principal filaments (pf) and on the ordinary filaments (of) that compose the plicae. Samples were taken with a micropipette (mp, outlined in Panel a) connected to a micro-peristaltic pump (see 'Materials and methods'). ANT: anterior; POST: posterior

thesis of particle selection on the gills (H$_{2.2}$; Table 2) was strongly supported by the markedly different EI values between the dct and vcg (p = 0.001). In addition, the EI in pseudofeces did not differ from that in the vcg of the gills (p = 0.99), showing that no further sorting based on particle size took place on the labial palps.

Particle sorting also occurred on the gills of oysters fed the flagellate *Rhodomonas lens* in a mixed suspension with a relatively small (45 μm) *Pseudo-nitzschia multiseries* clone, CLN-46 (Diet E2; Figs. 4 & 5). Similarly to the first diet, the negative EI measured in the vcg differed significantly from the positive EI found in the dct (p = 0.018), indicating selective rejection of *P. multiseries* on the gills. This time, however, additional rejection of *P. multiseries* occurred on the labial palps, as indicated by the significantly greater negative EI of pseudofeces samples compared to the ventral groove samples (p = 0.021). This result shows that both the gills and labial palps can be active sites for particle selection in the oysters *Crassostrea virginica*. In contrast, no particle selectivity was found on the gills or on the labial palps of oysters fed *P. multiseries* clone CLN-46 (69 μm) in a mixed suspension with another diatom, *Thalassiosira weissflogii* (Diet E3; Fig. 4).

DISCUSSION

In a companion study (Mafra et al. 2009), we found that a combination of low CR and increasing pseudofeces production at higher cell densities led to reduced ingestion of *Pseudo-nitzschia multiseries* from unialgal suspensions by *Crassostrea virginica*. In the same study, oysters were found to exhibit a higher overall CR

when *P. multiseries* clones were offered in a mixed suspension with the flagellate *Isochrysis galbana.* Since toxic *Pseudo-nitzschia* spp. blooms in the natural environment are often associated with high abundances of other algal species (Fehling et al. 2006, Mafra et al. 2006, Spatharis et al. 2007), including multiple *Pseudonitzschia* spp. (Rines et al. 2002, Kaczmarska et al. 2007), this finding suggests that DA intake by oysters could be facilitated in mixed suspensions with a more palatable food source. In the present study, oyster gills were shown to clear *P. multiseries* cells at similar or higher rates than flagellate species in a mixed suspension. However, after capture on the gills, *P. multiseries* cells were preferentially rejected by both juvenile and adult oysters in pseudofeces, leading to enrichment of the ingested food in flagellates and potentially reducing the DA intake by the shellfish. When *P. multiseries* clones were mixed in suspension with another diatom species, no selective feeding occurred and both algae were similarly rejected in pseudofeces.

In the present study, differential clearance of cells did not occur when juvenile oysters were fed the relatively long and narrow *Pseudo-nitzschia multiseries* in mixed suspensions with another relatively large but more spherical diatom species or the flagellate *Rhodomonas lens* (Diets M4 to M6; Fig. 1). However, when different *P. multiseries* clones, ranging in cell length from 28 to 100 μm, were mixed in a binary diet with the smaller flagellate *Isochrysis galbana* (4.5 ± 0.6 μm, mean ESD ± SD), the latter was removed from suspension at a consistently lower rate than *P. multiseries*, irrespective of clone size (Diets M1 to M3; Fig. 1). Since both algal particles theoretically approach the gills at the same velocity, the result suggests that *Crassostrea*

virginica gills may have retained *I. galbana* with lower efficiency than *P. multiseries* cells in the present study. For *C. virginica*, the reported size threshold at which particles are filtered with 100% efficiency varies somewhat among individual studies, but averaged data suggest that only particles ≥5 to 6 µm, slightly larger than *I. galbana* cells, can be fully retained on the gills (Ward & Shumway 2004). Moreover, exposure to high particle concentrations may reduce bivalve retention efficiency by changing the interfilamentary distance of the gills. For example, Palmer & Williams (1980) showed that retention efficiency of *C. virginica* for particles >4.35 µm dropped from 100% at 1.4 mg DW seston l^{-1} to 86% at concentrations as high as 6.5 mg l^{-1}. Because the total biomass concentrations of Diets M1 to M3 ranged from 6.2 to 7.4 mg l^{-1} in the present study, the lower clearance of *I. galbana* in mixed suspensions with *P. multiseries* is attributed to <100% retention efficiency of the former alga. Periodic variations in gill porosity were also observed by Haven & Morales-Alamo (1970) in *C. virginica* feeding at high seston concentrations (ca. 10 mg l^{-1}), and helped to explain how oysters can vary their CR while keeping the valves open (Palmer 1980). Variations of the oysters' retention efficiency would not affect filtration of *P. multiseries* cells at high cell densities because of their large size, and this is not expected to be a mechanism contributing to reduce DA intake from toxic cells at bloom densities. In fact, unialgal suspensions of 2 *Pseudo-nitzschia* species, *P. multiseries* and *P. pseudodelicatissima*, at common bloom concentrations (ca. 10^6 cells l^{-1}) were filtered by *C. virginica* at rates comparable to 2 other diatoms, *Thalassiosira weissflogii* and *Ditylum brightwellii* (Thessen et al. 2002).

Although the presence of a flagellate in mixed suspensions with *Pseudo-nitzschia multiseries* was shown to increase the overall CR in *Crassostrea virginica* (Mafra et al. 2009), some CR values reported in the present study were unexpectedly low, namely for Diets M1 and M2 (Fig. 1). High particle concentrations, such as those used in these diets, may lead to CR reduction in bivalves (Riisgård 2001). This was unlikely the case in the present study, however, as oysters exposed to other mixed suspensions at comparable concentrations (Diets M3 to M4; Table 2) showed much higher CR (Fig. 1). It is also improbable that the lower CR values observed in Diets M1 and M2 were due to artefacts in the measurement of clearance, since the method employed was the same for all diets and precautions to limit recirculation in the chambers by maintaining ≤30% depletion were taken. As Riisgård (2001) pointed out, even when suitable methods are applied, disparity in CR values between different experiments may reflect differences in bivalve condition and/or the influence of environmental factors.

We provide independent evidence from both feeding rate experiments with juvenile oysters and quantitative video-endoscopic observations of particle transport on the gills of adult oysters that the size of *Pseudo-nitzschia multiseries* cells is a critical factor influencing oyster pre-ingestive selection. The percentage of *P. multiseries* cells rejected in pseudofeces from mixed suspensions with *Isochrysis galbana* increased with an increase in cell length from 23 to 100 µm (Diets M1 to M4; Fig. 1). In addition, oysters preferentially rejected the larger *P. multiseries* clones CLN-46 (82 µm) and CLN-50 (90 µm) when offered in mixed suspensions with the smaller (24 to 28 µm) and similarly toxic clone CLN-20 (Diets M7 and M8; Fig. 2). Video-endoscopy-assisted sampling confirmed that the selective ability of oyster gills was affected when large cells were filtered. At least a portion of the larger *P. multiseries* cells, likely those not dorsoventrally oriented, was unable to enter the principal filaments, and was thus carried along the ventral grooves of *Crassostrea virginica* (Diet E1; Fig. 4). This confirms that a single algal species, *P. multiseries*, can be selected by *C. virginica* based exclusively on cell size, as previously demonstrated with polystyrene beads of contrasting diameters (40 and 275 µm) by Tamburry & Zimmer-Faust (1996). The gills were the only possible site for such selection, as we found no further selectivity on the labial palps (Diet E1; Fig. 4).

The present study also provides quantitative data that support the qualitative video-endoscopy observations made by Cognie et al. (2003) in the Pacific oyster *Crassostrea gigas*. Using the large, non-toxic pennate diatoms *Pleurosigma planctonicum* and *Rhizosolenia setigera*, and the centric diatom *Coscinodiscus perforatus*, these researchers found that cells with all axes greater than the pfa width (69 µm; Cognie et al. 2003) and some cells with 1 axis exceeding this width were unable to enter the principal filaments. Because the pfa width in our study (68.2 ± 0.6 µm, mean ± SE) was measured from relaxed, dissected gills, this measurement cannot be considered as a fixed threshold. During our video-endoscopic observations, this gap appeared to be variable and even disappeared as oysters stretched and contracted their gills. Our *in vivo* measurements of the pfa width from actively feeding *Crassostrea virginica*, however, were on average 66.7 ± 1.3 µm, mean ± SE, very similar to the values we obtained from dissected gills and those reported earlier for *C. gigas*.

Pseudo-nitzschia spp. cells are noticeably smaller and narrower (ca. 5 µm width) than the diatoms used by Cognie et al. (2003), in which the 2 pennate species were characterized by a length of 350 to 700 µm and a width of 35 to 40 µm. In the present study, we used *P. multiseries* clones of contrasting sizes to obtain a mixed suspension of cells either shorter or longer than the pfa

width, and confirmed that cells with 1 axis longer than this critical size are selectively rejected by oysters. Furthermore, our quantitative measures of feeding selection were obtained for a planktonic, toxic alga of public health relevance. Confirmation that oysters selectively reject high proportions of *P. multiseries* cells >70 µm in length is particularly important, given that at least 92 % of cells in the natural environment exceed this size threshold (Bates et al. 1999), and that *Pseudo-nitzschia* spp. of highly varying cell size have been associated with shellfish closures during toxic blooms (Trainer et al. 2007). In addition, this finding has major ecological significance as *Pseudo-nitzschia* spp. are a ubiquitous component of the phytoplankton in coastal waters worldwide (Hasle 2002). Although cell toxicity may be directly related to the size of a given *P. multiseries* clone, we demonstrated that CR inhibition of oysters fed *Pseudo-nitzschia* spp. cells could not be attributed to DA toxicity (Mafra et al. 2009). Therefore, it is improbable that DA toxicity was the cause of the selective feeding of oysters exposed to *P. multiseries* in the present study. Furthermore, in previous endoscopic observations (L. L. Mafra et al. unpubl. data), larger *P. multiseries* cells (100 µm; 0.6 pg DA cell⁻¹) were preferentially rejected by adult *C. virginica*, even when mixed in suspension with a smaller but more toxic clone (24 µm; 1.1 pg DA cell⁻¹).

Results of our study suggest that factors other than cell size are involved in particle selection by the gills and labial palps of *Crassostrea virginica*. This conclusion is based on the fact that relatively small flagellates and diatoms (4.5 to 28 µm) were differentially rejected in pseudofeces when offered in a mixed suspension with a *Pseudo-nitzschia multiseries* clone, some of which had cell sizes smaller than the pfa width (<68 µm). Similar findings have been reported previously for *C. gigas* when delivered a mixed suspension of 2 qualitatively different particle types of comparable size, e.g. *Spartina* spp. detrital particles and *Rhodomonas lens* (Ward et al. 1997) and, more recently, when delivered a mixed suspension of the live diatoms *Actinoptychus senarius* and artificially cleaned, empty frustules (Beninger et al. 2008a). In the present study, we show conclusively that *C. virginica* was able to sort among algae of similar size from different taxa (i.e. diatoms vs. flagellates). While flagellated cells were mostly ingested (Fig. 1), up to 49 % of the small diatoms (cell size <68 µm) were rejected in pseudofeces of oysters when offered in mixed suspensions with either *Isochrysis galbana* or a larger *P. multiseries* clone. Similarly, in a previous study, the diatom *Phaeodactylum tricornutum* was preferentially rejected in the pseudofeces of 5 other bivalve species when delivered in a mixed suspension with the dinoflagellate *Prorocentrum minimum* and the naked flagellate *Chroomonas*

salina (Shumway et al. 1985). Prior studies have suggested that bivalves make use of chemical cues to discriminate among particles (Newell & Jordan 1983, Shumway et al. 1985, Ward & Targett 1989). Contact with the extracellular organic envelope, whose composition varies from diatoms to flagellates (Ward & Targett 1989) and even among diatom species (Volcani 1981), may be a major cue for selection by bivalve pallial organs. Alternatively, substances released from inside the cell or from its organic covering may be recognized by some bivalves, since *Crassostrea gigas* was able to selectively reject permeable microcapsules enclosing the diatom *Nitzschia closterium* while preferentially ingesting those containing the green alga *Tetraselmis suecica* (Espinosa et al. 2007).

Diatoms may contain metabolites that cause inhibitory effects on feeding and fitness parameters of suspension-feeding grazers (Shaw et al. 1995, Ianora et al. 2003). Whether such metabolites affect particle selection in bivalves, however, is not known. When Bougrier et al. (1997) offered 5 diets composed of a combination of 3 to 4 species from different taxa, *Crassostrea gigas* preferentially rejected in pseudofeces 3 relatively small diatom species (*Skeletonema costatum*, *Chaetoceros calcitrans* and *Nitzschia closterium*) compared with 3 similarly sized flagellate species (*Pavlova lutheri*, *Tetraselmis suecica* and *I. galbana*). In a study on the European oyster *Ostrea edulis*, Bricelj et al. (1998) reported that, compared to several dinoflagellates, the diatom *Thalassiosira weissflogii* was mainly transported along the ventral grooves of the gill where material is more likely to be rejected in pseudofeces. The present study shows that *Crassostrea virginica* were unable to sort between 2 diatom species, when *Pseudo-nitzschia multiseries* clones were mixed in a suspension with *T. weissflogii* or *Chaetoceros muelleri* (Diets M5 and M6; Fig. 2). This contrasts with the ability of *C. gigas* to preferentially ingest 2 out of 4 benthic diatom species from the Naviculaceae family, ranging in cell length from 22 to 60 µm (Cognie et al. 2001).

The present study provides the first evidence that selection of different microalgal species may occur concurrently in 2 distinct pallial organs of a heterorhabdic bivalve. In a mixed suspension with the flagellate *Rhodomonas lens*, selective rejection of *Pseudo-nitzschia multiseries* clone CLN-46 occurred simultaneously on the gills and labial palps of *Crassostrea virginica* (Diet E2; Figs. 4 & 5). This dual-site sorting capacity, suggested to be a general ability of heterorhabdic bivalves, has been confirmed in the scallop *Pecten maximus* (Beninger et al. 2004) and the oyster *Crassostrea gigas* (Beninger et al. 2008a), which were fed live and artificially cleaned, dead diatoms. However, oysters seem to have a more refined particle

selection mechanism than scallops. This idea was recently supported by the work of Beninger et al. (2008b), who found that, contrary to the scallop *P. maximus* (Beninger & Decottignies 2005), the oyster *C. gigas* was able to distinguish between live and naturally dead diatoms in a mixed suspension, the latter still covered by a peri-frustular envelope.

The chemical mechanisms involved in particle selection have not been elucidated, but the mapping of mucocytes on the labial palps and gill epithelium of different bivalve species is consistent with the use of mucus during the entire particle handling process (Beninger et al. 1993, 2005, Beninger & Dufour 1996). Mucocytes of the pallial organs secrete mucus of varying viscosity for different functions (Beninger & Dufour 1996, Beninger & St.-Jean 1997, Beninger et al. 2005). Except for the dorsal tracts and principal filaments, transport of particles occurs on relatively exposed sites of the oyster gills in a perpendicular or opposite direction to the prevailing inhalant current (Ward et al. 1994); thus, transport is only possible if particles strongly adhere to the ciliated epithelium by mucus (Beninger et al. 2005). Of all components of bivalve mucus, lectins (Fisher 1992) are the most probable to act as a particle agglutinant. Lectins are glycoproteins of non-immune origin that specifically and reversibly conjugate with sugars (Goldstein et al. 1980), including those covering algal cells (Waite et al. 1995, Cho 2003). A mannose-binding lectin has been shown to act as a feeding receptor for prey recognition by the heterotrophic dinoflagellate *Oxyrrhis marina* (Wootton et al. 2007), and recent studies suggest that lectins in mucus secreted by the feeding organs of *Crassostrea virginica* may be involved in particle selection (Espinosa et al. 2008). Affinity for different lectins varies among diatom species (Waite et al. 1995) and between diatoms and dinoflagellates, with the latter binding to a greater variety of lectin types (Cho 2003). Thus, if lectins associated with the feeding structures bind more effectively to certain groups of microalgae, this might explain why *C. virginica* showed no pre-ingestive selection among diatom species, but demonstrated selection between *Pseudo-nitzschia multiseries* and 2 flagellate species.

Oysters in the present study rejected greater amounts of exponentially growing *Pseudo-nitzschia multiseries* cells than those in the stationary phase. This finding could be explained by the fact that accumulation of cell-surface carbohydrates is lower in diatoms undergoing faster growth (Waite et al. 1995). Cell stickiness also tends to be lower in the exponential phase, which may influence adhesion between the cells and surfaces of the feeding structures. These factors may help to explain the preferential rejection of cleaned, empty frustules over intact diatom cells by *Pecten*

maximus (Beninger et al. 2004) and *Crassostrea gigas* (Cognie et al. 2003), and rejection of naturally dead over live cells by *C. gigas* (Beninger et al. 2008b), assuming that the composition of the peri-frustular envelope changes after cell death. Therefore, it is unlikely that the use of *P. multiseries* cells in the stationary phase in our mixed diets caused their preferential rejection over flagellates by the oysters, since cells growing exponentially were rejected to an even greater degree in both unialgal (Fig. 3) and mixed diets (data not shown). Additionally, the similar feeding selectivity found in both juvenile (21.4 ± 0.3 mm, mean SH ± SE) and adult (116 ± 2 mm, mean SH ± SE) *Crassostrea virginica* confirms that the pallial organs were fully developed in the 1 to 1.5 yr old juveniles used in the present study. In fact, after 16 to 22 wk of rearing at 22 to 25°C, the complete mantle rejection system of *C. gigas* was functional in juveniles between 10 and 24 mm in shell height (Beninger & Cannuel 2006).

In conclusion, the relatively low DA levels found in *Crassostrea virginica* when exposed to toxic *Pseudo-nitzschia* spp. blooms can be explained at least partially by 2 processes: (1) pre-ingestive rejection of *P. multiseries* in mixed suspensions, which is more pronounced for larger cells (present study), and (2) reduced CR elicited in unialgal suspensions (Mafra et al. 2009). Indeed, 2 wk contamination experiments in the laboratory confirmed that *C. virginica* accumulates much less DA from *P. multiseries* than the mussel *Mytilus edulis* under the same experimental conditions, and that the difference was much greater when larger cells (>68 µm) were offered (L. L. Mafra et al. unpubl. data). In our study, *P. multiseries* occurred mostly as single cells (>95%); thus, the role of chain formation by *P. multiseries* on the selective ability of oysters requires further investigation. Nevertheless, we suggest that rejection of this toxic diatom by oysters will be more pronounced during natural blooms, when large cells are growing exponentially and favourable conditions trigger the formation of long, stepped chains. As a result, oysters in contact with multi-specific blooms, dominated by toxic *Pseudo-nitzschia* spp. and other non-toxic species, may filter a high biomass of phytoplankton without necessarily accumulating the levels of DA predicted from their clearance rates.

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