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ORIGINAL PAPER

Food quality and season affect gene expression of the mucosal lectin MeML and particle sorting in the blue mussel *Mytilus edulis*

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Abstract The blue mussel, *Mytilus edulis*, is able to use biochemical cues to sort food particles. Recently, a mucosal lectin (MeML) identified in pallial organs of *M. edulis* was suggested to play a role in the capture and sorting of food particles. The current study presents data obtained in 2010 showing that sorting efficiencies and MeML gene expression in *M. edulis* increased when mussels were either fed poor quality food or starved. In addition, results suggest that particle capture and degree of selection increased during the spawning period, although the quality of food delivered to mussels before the experiments seemed to have a stronger impact on particle-sorting efficiency. Overall, the impact of both endogenous (physiological status) and exogenous (food quality) factors were shown to affect the expression of MeML in mussel feeding organs, and the food sorting abilities.

Introduction

In aquatic systems, particle-feeding organisms often dominate the macrobenthos, playing significant roles in ecosystem processes. Suspension-feeding bivalve molluscs, a major subset of benthic particle feeders, are particularly important in benthic-pelagic coupling (Dame 1996). The suspension-feeding activity of dense beds of bivalves is known to reduce turbidity by filtering water and trapping substantial quantities of suspended particles (among them phytoplankton or phytobenthos) (Kautsky and Evans 1987; Prins et al. 1996) which can be transferred to the benthos or cycled into inorganic forms (Baudinet et al. 1990). The disappearance of, or colonization by large populations of suspension feeders has been concomitant with major reorganizations of aquatic communities (Haamer and Rodhe 2000; Norling and Kautsky 2008). The impact of suspension-feeding bivalves on aquatic ecosystems is significantly amplified by their ability, variable among species, to select and ingest specific particles. Indeed, bivalves can select their food from a complex mixture of particles of various qualities in the water column (Newell and Jordan 1983; Ward and Shumway 2004; Pales Espinosa et al. 2008) preferentially ingesting some particle types while rejecting others in pseudofeces (Loosanoff and Engle 1947; Morton 1960).

The blue mussel *Mytilus edulis*, a common epibenthic suspension feeder, is one of the most studied bivalves due to its ecological and economic importance in near-shore ecosystems. As with many mytilid species (Ward et al. 1998; Ke and Wang 2002; Velasco and Navarro 2002), *M. edulis* sorts particles (Cucci et al. 1985; Ward and Targett 1989; Bougrier et al. 1997; Pales Espinosa et al. 2010a, 2011). Although some aspects of the selection process have been elucidated, the mechanism(s) by which particles of poor quality are rejected as pseudofeces while those of higher quality are ingested remains ill-defined, thus limiting the understanding of energy flux through coastal ecosystems. Recently, the involvement of molecular interactions in prey selection has been identified in marine planktonic protozoa (Allen and Dawidowicz 1990; Wootton et al. 2007) and in benthic suspension-feeding organisms. In the latter group, the great scallop, *Pecten maximus*, ingests or rejects *Coscinodiscus perforatus* by detecting the presence of organic components on its frustules (Beninger and Decottignies 2005) and rejecting the

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cleaned frustules of the same diatom species (Beninger et al. 2004). More recently, our results in oysters (Pales Espinosa et al. 2009, 2010b) and mussels (Pales Espinosa et al. 2010a, 2011) showed that particle selection is mediated by interactions between lectins (non-self-recognition proteins that specifically and reversibly bind to sugar residues) present in mucus covering the feeding organs and carbohydrates associated with the surface of suspended food particles. In addition, further investigations showed that the mucosal lectin MeML (for *M. edulis* Mucocyte Lectin) was significantly up-regulated in mussels after several days of starvation, supporting the involvement of this molecule in particle capture (Pales Espinosa et al. 2010a).

The present study investigates factors that may be involved in the modulation of particle selection in *M. edulis*. Interestingly, Bayne and Svensson (2006) reported that feeding in the oyster *Saccostrea glomerata* was highly correlated with both exogenous (seasonal differences in carbon and nitrogen availability) and endogenous (cycles of reproduction and growth) factors. Accordingly, we hypothesized that food quantity and quality and the cycle of gametogenesis and spawning affect mussel feeding behavior, including particle selection and the expression of a mucosal lectin (MeML) involved in particle capture and sorting.

Materials and methods

Organisms

Blue mussels *M. edulis* (52–67 mm SL) were collected from Stony Brook Harbor (Long Island, New York) at different periods of the gametogenic cycle (Newell et al. 1982; Brousseau 1983): May (ripening), July (spawning) and November (somatic growth) 2010. Ten mussels were randomly chosen, dissected and prepared histologically to determine gonad maturity (Brousseau 1983). Mussel shells were cleaned of epibionts, acclimated in the laboratory for a minimum of 1 week (salinity of 28, 15 °C) and fed daily (15 % dry weight) using a mixed diet composed of DT's Live Marine Phytoplankton (Sycamore, Illinois, Pales Espinosa and Allam 2006) supplemented with fresh cultures of the microalgae *Isochrysis galbana* (CCMP1323), *Nitzschia closterium* (D-828), *Rhodomonas salina* (CCMP 1319) and *Tetraselmis maculata* (CCMP897). These species were grown in f/2 media (supplemented with silicate for *N. closterium* and *R. salina*) (Guillard 1982) at 15 °C under a 14-h light:10-h dark photoperiod.

Tetraselmis maculata cultures

Based on previous work by Wikfors et al. (1992), two biochemically distinct populations of *T. maculata* (CCMP897) were produced. Microalgae were grown either in N-replete (8.83×10^{-4} M sodium nitrate) or in N-limited (2.12×10^{-4} M sodium nitrate) f/2 media (Guillard 1982) (Table 1). Cultures were harvested during exponential phase of growth and analyzed for biochemical composition and physical characteristics. Aliquots of each culture were filtered on precombusted (GF/C) filters and analyzed for protein (Pierce BCA protein assay reagent kit, Rockford, IL, USA) and carbohydrate contents (DuBois et al. 1956). Cell size and morphology were examined under an inverted microscope. Digital images of each sample were captured and cell size (μm) of 50 algal cells was measured using image analysis software (ImageJ, National Institutes of Health).

Table 1 Physical and biochemical characteristics of *T. maculata* (Tm–N-replete and Tm–N-limited) grown under different nitrogen conditions (f/2 medium replete (8.83×10^{-4} M sodium nitrate) or limited (2.12×10^{-4} M sodium nitrate), Guillard 1982)

Algae characteristics	Tm–N-replete Mean ± SD	Tm–N-limited Mean ± SD
Diameter (μm)	15.5 ± 0.4*	13.1 ± 0.6
FL3 (fluorescence)	794.2 ± 16.2*	406.2 ± 13.2
SSC (cell complexity)	72.3 ± 2.8*	172 ± 4.8
FSC (equivalent size)	103.8 ± 2.4*	87.5 ± 2.5
Carbohydrate (pg cell^{-1})	22.9 ± 0.85	24.9 ± 1.4
Protein (pg cell^{-1})	14.4 ± 0.99*	3.5 ± 0.6
Carbohydrate/protein ratio	1.6 ± 0.2*	7.1 ± 0.5
Binding of FITC-labeled lectins		
ConA (Concanavalin agglutinin)	2.9 ± 0.1*	3.4 ± 0.3
PNA (Peanut agglutinin)	3.4 ± 0.0*	2.7 ± 0.1
UEA (*U. europaeus* agglutinin)	0.8 ± 0.0*	1.1 ± 0.1
SBA (Soybean agglutinin)	1.0 ± 0.0	1.0 ± 0.0
WGA (Wheat germ agglutinin)	1.2 ± 0.0	1.1 ± 0.0
PEA (*P. sativum* agglutinin)	6.8 ± 0.1*	8.3 ± 0.2

* Indicate significant differences (*t* test and Kolmogorov–Smirnov test, $p < 0.05$)

Binding of FITC-labeled lectins to microalgae

Procedures used to bind lectins to microalgae were adapted from Gauthier et al. (2004) as described by Pales Espinosa et al. (2010b). All lectins used in this study were fluorescently labeled with fluorescein isothiocyanate (FITC, Sigma Aldrich, USA). These included concanavalin agglutinin (ConA, specifity: methyl α-mannopyranoside; D-mannose; D-glucose), *Pisum sativum* agglutinin (PEA, specifity: α-methyl mannoside; α-methyl glucoside), peanut agglutinin (PNA, specifity: α-lactose; D-galactose; *N*-acetylgalactosamine), soybean agglutinin (SBA, specifity: *N*-acetylgalactosamine; D-galactose; methyl α-*D*-galactopyranoside), *Ulex europaeus* agglutinin (UEA I, specifity: L-fucose) and wheat germ agglutinin (WGA, specifity:

N-acetylglucosamine). Briefly, microalgal cultures were centrifuged at 400×*g* for 10 min, washed once and resuspended in 0.22-µm-filtered artificial seawater (FSW, salinity 28). FITC-conjugated lectins were diluted in FSW to 1 mg ml^{-1}. One 50-µl aliquot of lectin (or FSW control) was added to each of the three replicate microcentrifuge tubes containing 1 ml of washed microalgae (10^6 cells). Microalgae were then incubated in the dark at room temperature for 1 h. Each assay was performed in triplicate and analyzed using flow cytometry (see details below).

Feeding experiments

Mussels were randomly divided into four groups and placed in one of the four different feeding regimes (15 % dry weight): mixed diet (DT's and fresh cultures of algae; see above), *T. maculata* (Tm–N-replete), *T. maculata* (Tm–N-limited) and unfed. After 5 days, mussels were transferred to filtered (0.45 µm) seawater for 1 day prior to being used in the sorting experiments. On the following morning, 12 mussels from each treatment were transferred to individual tanks (1 mussel $tank^{-1}$) and submitted to a mixture made with equal concentrations of each microalgal type (i.e., Tm–N-replete and Tm–N-limited, 2×10^5 cells ml^{-1} final concentration). Microalgae were kept in suspension using a micropipette (resuspension every 15 min), and water samples were taken periodically to determine sedimentation from a control tank (i.e., empty shell). Pseudofeces were collected from each mussel 2 h after onset of production. Mussels were then placed in a clean tank filled with filtered seawater and feces were collected after an additional 3 h. Pseudofeces and feces were vortexed or broken using a pipette to disrupt particle aggregates, passed through a 35-µm, nylon-mesh sieve and analyzed using flow cytometry. Mussels were then immediately dissected for gene expression as described below.

Flow cytometry analysis

Microalgae were analyzed using a FACSCalibur flow cytometer (Becton–Dickinson Biosciences, California, USA). A minimum of 10^4 events were analyzed. The 488-nm argon laser was used for excitation, and microalgae were characterized based on one or more of the following parameters: forward (FSC; particle size) and side (SSC; intracellular complexity) light scatters, FITC fluorescence (FL1, 535 nm), and chlorophyll auto-fluorescence (FL3, 675 nm).

Effect of four feeding treatments on *M. edulis* mucosal lectin (MeML)—mRNA transcription

After being fed with specific diet for 5 days and submitted to particle selection experiments, the expression of MeML transcripts (see the full sequence and characteristics in Pales Espinosa et al. 2010a) in pallial tissues of 12 mussels was measured using real-time PCR. Gills and palps were dissected and immediately flash-frozen and stored at −80 °C until processed as described by Pales Espinosa et al. (2010a). RNA extraction was performed using TRI Reagent® (MRC, Cincinnati, Ohio). cDNA amplification was generated from extracted RNA using M-MLV RT (Promega, Madison, Wisconsin). One set of gene-specific primers for MeML (forward 5′-ATGCTCAATTGGCTGG CATCATGG-3′ and reverse 5′-ATCCGGGAATCTTCGA TGTCTTGC-3′) was used to amplify a 181-bp product, and 18S ribosomal RNA was used as the housekeeping gene (forward 5′-CTGGTTAATTCCGATAACGAACGAGAC TCTA-3′ and reverse 5′-TGCTCAATCTCGTGTGGCTA AACGCCACTTG-3′). Real-time PCR assay was carried out in an Eppendorf RealPlex cycler with 6 µl of 1:15 diluted cDNA. The amplifications were performed in a 20-µl reaction volume containing 1× Brilliant II SYBR green qPCR Master mix (Stratagene) and 100 nM of each primer. The thermal profile for real-time PCR assay was an initial denaturation step at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 30 s, annealing and extension at 60 °C for 1 min. Each run was followed by a melting curve program for quality control. PCR efficiency (E) was determined for each primer pair by determining the slopes of standard curves obtained from serial dilution analysis of cDNA. The comparative CT method was used in conjunction with 18S as the endogenous reference gene to determine the expression level of MeML among tissues (Livak and Schmittgen 2001).

Data treatment and statistical analysis

All data are presented as mean ± SD, and differences were considered significant at an alpha level of 0.05. Results obtained for microalgae treated with FITC-labeled lectins are presented as ratios between fluorescent intensities (geometric mean of channel number) of treated cells and the baseline auto-fluorescence in FL1 (control). Additionally, the geometric mean fluorescence values were divided by the FITC/protein ratio determined for each lectin (relative fluorescence intensity) to allow comparisons between different lectins. For all flow cytometry data, statistical analyses between *T. maculata* grown under limiting or normal nitrogen levels were performed on raw flow cytometric histograms using a Kolmogorov–Smirnov (K–S) package in the CellQuest Pro software (Becton–Dickinson Immunocytometry Systems). The K–S test compares the distributions of histograms, calculates a *D* value which represents the greatest difference between the two curves and establishes the probability (*p*) that the two histograms are different. Differences in size and biochemical

composition of the two *T. maculata* populations were assessed using a Student's *t*-test.

Data obtained from the feeding experiments were analyzed using goodness-of-fit test (*G* test). Two series of tests were performed comparing the proportion of each type of particle in samples of the diet and pseudofeces collected from the oysters. The first series of tests ensured that within each treatment, replicate samples of the diet and pseudofeces were not significantly different. The second series tested the null hypothesis that the proportion of each particle type (i.e., Tm–N-Replete and Tm–N-limited) in the diet or in the pseudofeces was not different between treatments. In addition to the comparison of raw counts, a sorting efficiency (SE) index was calculated in order to examine the particle selection (Iglesias et al. 1992). This index was defined as:

$$SE = 1 - (P/D),$$

where P and D represent the proportion of the particle of interest in the pseudofeces and diet, respectively. A positive SE for a given particle type indicates that it is preferentially ingested (particle type is depleted in the pseudofeces, compared to diet), a negative SE indicates rejection (particle type is enriched in the pseudofeces compared to diet), and zero indicates the absence of active selection. After checking their normal distributions and variance homogeneity, calculated SE values obtained for each of the two particles in each treatment were compared to zero using a one sample *t* test (two-tailed). The null hypothesis was that the selection efficiencies were equal to zero (i.e., no selection). Sorting efficiency values were also submitted to two-way ANOVA (followed with a Holm–Sidak post hoc test when needed) using SigmaStat (version 3.1) to assess the effect of sampling season and preconditioning diets on particle sorting. Real-time PCR data (delta Ct) were also analyzed using a two-way ANOVA and are presented as relative fold expression ($2^{-\Delta\Delta}$Ct) using the arithmetic mean as a reference. Correlation analyses (Spearman rank) were performed using SigmaStat to evaluate the relationships between MeML expression in gills and labial palps and between MeML expression and sorting efficiencies.

Results

Physical and biochemical characteristics of the two *T. maculata* populations

The cells of the two *T. maculata* populations (Tm–N-replete and Tm–N-limited) differed in several physical and biochemical criteria (Table 1; Fig. 1) which allowed them to be distinguished using flow cytometry (Fig. 1b). Microscopic observations and flow cytometry analysis showed that Tm–N-replete cells were larger (15.5 ± 0.4 μm), their chlorophyll auto-fluorescence (FL3, arbitrary units) was higher (794.2 ± 16.2), and their intracellular complexity (SSC, arbitrary units) was lower (72.3 ± 2.8) compared to Tm–N-limited cells (13.1 ± 0.6, 406.2 ± 13.2 and 172.0 ± 4.8 μm, respectively). Tm–N-replete cells contained significantly more protein (14.4 ± 0.99; $p < 0.01$) but slightly less carbohydrate (22.9 ± 0.85; $p = 0.22$) than Tm–N-limited cells (3.5 ± 0.6 and 24.9 ± 1.35 pg cell^{-1}, respectively), resulting in a significantly lower carbohydrate/protein ratio (1.6 ± 0.2 vs. 7.1 ± 0.5, $p < 0.01$).

Fluorescence intensity ratios showed significant binding of three out of the six tested lectins to the surface of cells of the two *T. maculata* populations (Table 1): PEA, PNA, Con A. The binding of UEA I, WGA and SBA to the surface of *T. maculata* was very weak or absent. More specifically, comparison between the two *T. maculata* populations showed that the binding of PEA (Table 1, $D = 0.17$, K–S test, $p < 0.001$), Con A ($D = 0.11$, $p < 0.001$) and UEA I ($D = 0.08$, $p < 0.001$) was significantly higher in Tm–N-limited indicating higher abundance of α-methyl mannoside and/or α-methyl glucoside residues (PEA), methyl α-mannopyranoside, D-mannose and/or D-glucose (ConA) and of L-fucose (UEA I). In contrast, the binding of PNA ($D = 0.12$, $p < 0.001$) was significantly higher in Tm–N-replete as compared to Tm–N-limited, suggesting a higher abundance of α-lactose, D-galactose, *N*-acetylgalactosamine (GalNAc).

Particle selection experiments

Total microalgal concentration given to each mussel in the different treatments was 2×10^5 cells ml^{-1}. Although the targeted proportion of each particle (Tm–N-limited and Tm–N-replete) in the food supply was 50 %, small non-significant variations were observed (47.6–52.4 %, data not shown, goodness-of-fit tests). More importantly, the initial proportions remained stable during the experiment indicating that differential settling of microalgal species had not occurred. The percentage of each particle type (Tm–N-limited and Tm–N-replete) in the food supply, pseudofeces and feces was recorded for the different treatments, and data are presented as sorting efficiencies (SEs, Fig. 2).

Mixed diet

In all experiments, sorting efficiencies (Fig. 2) revealed that mussel pseudofeces were depleted of Tm–N-replete cells compared to the food supply. These results indicate that mussels preferentially ingested Tm–N-replete cells while they rejected Tm–N-limited cells, especially in July where SEs for both *T. maculata* populations were

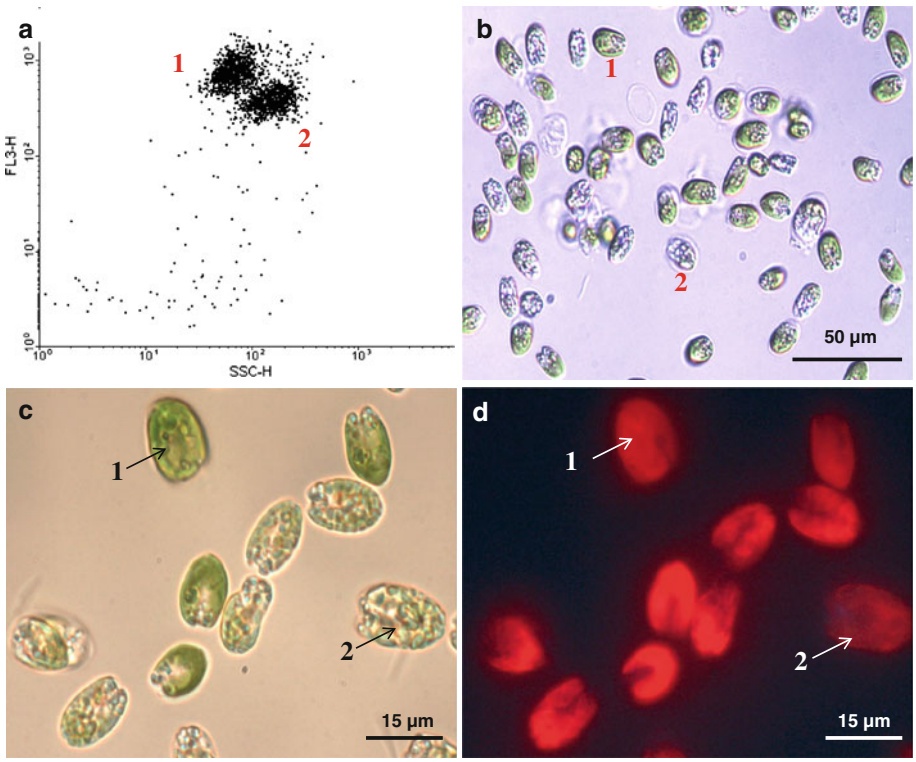

Fig. 1 Cytogram (**a**) and bright field (**b**, **c**) and fluorescent (**d**) micrographs of (1) *T. maculata* grown under normal (Tm–N-replete) and (2) limited (Tm–N-limited) nitrogen conditions. **c**, **d** Same microscopic field

significantly different (0.04 ± 0.03 and -0.04 ± 0.03, respectively, $n = 12$, Holm–Sidak post hoc test, $p < 0.01$).

Tm–N-replete

Sorting efficiencies in mussels fed with Tm–N-replete for 5 days prior to the beginning of the sorting experiment showed that pseudofeces were depleted of Tm–N-limited cells compared to the food supply, indicating that mussels preferentially ingested Tm–N-limited cells while they rejected Tm–N-replete cells. Differences were significant in July and November ($n = 11$, 9, Holm–Sidak post hoc test, $p < 0.01$, Fig. 2).

Tm–N-limited

For mussels fed for 5 days with Tm–N-limited cells prior to the sorting experiment, SEs were not significantly different and were close to zero in July and November, revealing that mussels did not select one microalgal population over the other (Fig. 2). In May, positive and negative SEs were measured for Tm–N-replete and Tm–N-limited cells, respectively, although differences were not statistically significant.

Unfed

In May and November, SEs were significantly positive ($n = 12$, t test, $p < 0.001$) for Tm–N-replete (0.06 ± 0.04 and 0.05 ± 0.03, respectively, Fig. 2), whereas they were negative for Tm–N-limited cells (-0.05 ± 0.04 and -0.04 ± 0.03, respectively). In July, the results were reversed and SEs were significantly positive for Tm–N-limited (0.12 ± 0.06, $n = 11$, Holm–Sidak post hoc test, $p < 0.01$), whereas they were negative for Tm–N-replete cells (-0.12 ± 0.07). These results indicate that mussels preferentially ingested Tm–N-replete cells in May and November while they preferentially ingested Tm–N-limited cells in July.

Effect of diet quality on MeML–mRNA transcription levels

Changes in MeML transcription in mussel gills and labial palps were investigated in response to the four diets (mixed diet, Tm–N-replete, Tm–N-limited and unfed). Two-way ANOVA results showed a significant effect of preconditioning diet on MeML transcript levels in gills and labial palps (Table 2; Fig. 3). Transcripts levels of MeML were generally higher in both gills and labial palps among mussels starved for 5 days (Fig. 3) compared to other treatments. This was the case for samples in May (110- and 142-fold compared to overall average for gills and palps, respectively), July (919- and 96-fold) and November (853- and 427-fold). Mussels fed Tm–N-limited cells (Fig. 3) for 5 days also showed a general increase in MeML expression in gills and labial palps. This induction, however, was only

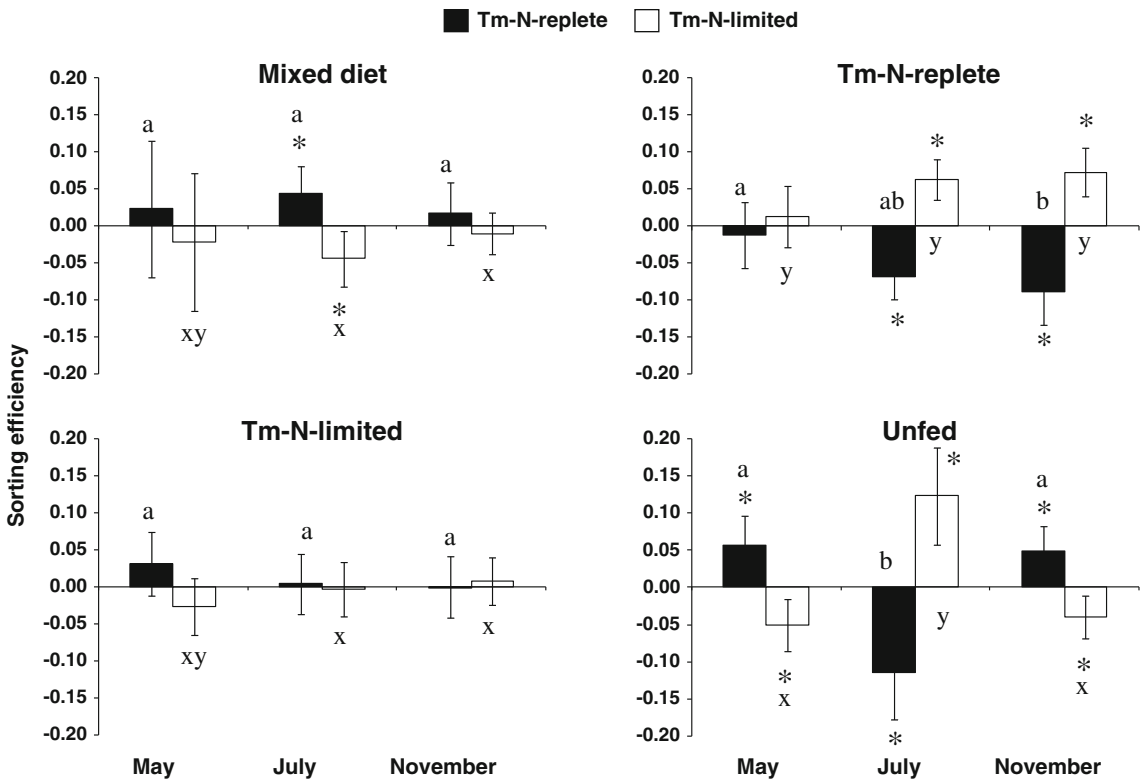

Fig. 2 Sorting efficiencies (mean ± SD) of *M. edulis* exposed to 1 of 4 treatments before being fed a diet made with equal proportions of Tm–N-replete and Tm–N-limited. Positive or negative values indicate preferential ingestion or rejection, respectively. *Asterisk* indicates a SE that is significantly different from 0 (one sample *t* test, $n = 12$, $p < 0.001$). *Different letters* (*above and below bars*) denote significant differences (Holm–Sidak post hoc test, $p < 0.05$) in SE (for both Tm–N-replete and Tm–N-limited) between mussels collected at different months within each treatment (*a* and *b*) or those from each sampling date but submitted to different treatments (*x* and *y*)

Table 2 Summary of two-way ANOVA results assessing the effect of sampling date and preconditioning diets on MeML transcript levels in labial palps and gills in *M. edulis*

	Sampling date	Conditions	Sampling date versus condition
MeML labial palps	$p < 0.001$	$p < 0.001$	$p = 0.004$
	Nov > May = Jul	UN > TL > TR = MD	(see Fig. 3 for details)
MeML gills	$p < 0.001$	$p < 0.001$	$p > 0.05$
	Nov > Jul > May	UN > TL = MD = TR	(see Fig. 3 for details)

MD mixed diet, *TL* Tm–N-limited, *TR* Tm–N-replete, *UN* unfed

significant in labial palps in May (12-fold) and July (19-fold) compared to mussels fed the mixed diet or Tm–N-replete cells. MeML transcript levels tended to be similar in gills and labial palps of mussels fed mixed diet and Tm–N-replete cells except for gills in May and July.

Impact of sampling season on MeML–mRNA expression

MeML transcript levels varied seasonally in both gills and labial palps (Table 2; Fig. 3). In gills, the transcription level of MeML was generally lowest in May and highest in November regardless of the preconditioning diet. Trends in the transcription level of MeML in labial palps were different than in gills and seemed to be more influenced by the quality of microalgae in the diet than by the season. When mussels were fed higher-quality diets (i.e., mixed diet and Tm–N-replete), the relative transcription levels of MeML in labial palps were highest in November and lowest in May (Holm–Sidak post hoc test, $p < 0.05$). In contrast, this seasonal trend was not observed when mussels were poorly fed (i.e., fed Tm–N-limited or unfed, Fig. 3).

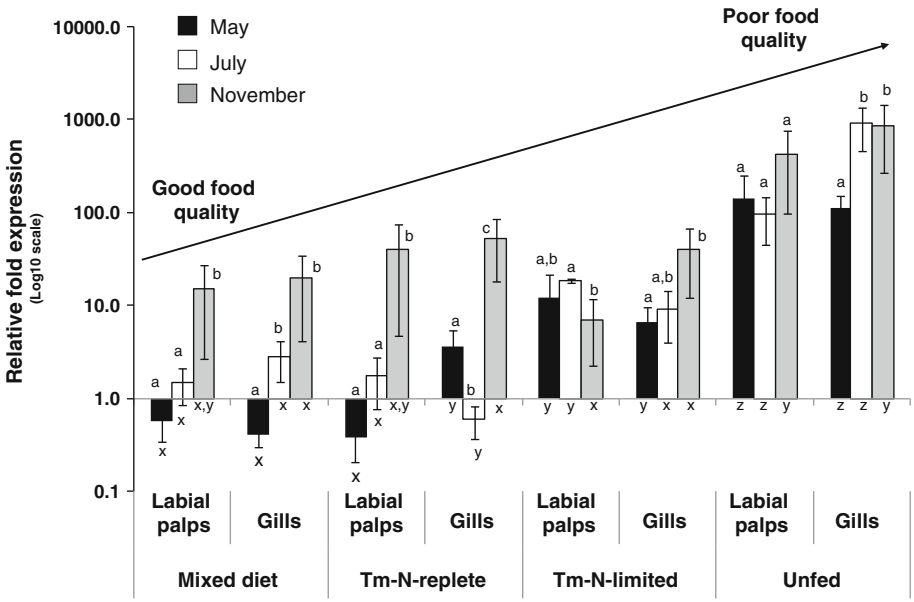

Fig. 3 MeML transcript levels in labial palps and gills of *M. edulis* exposed to 1 of 4 treatments. Levels are presented as relative expression to the mean of the four treatments ($n = 12$ mussels/treatment). For each organ, *different letters* denote significant differences (Holm–Sidak post hoc test, $p < 0.05$) between mussels collected at different months within each treatment (*a*, *b* and *c*) or those from each sampling date but submitted to different treatments (*x*, *y* and *z*)

Relationship between sorting efficiencies and MeML–mRNA transcription levels

MeML transcript levels in gills and palps were correlated (Spearman rank correlation, $r = 0.51$, $p < 0.001$, $n = 144$). More interesting, sorting efficiencies were significantly correlated with MeML expression in palps (Spearman rank correlation against Tm–N-replete, $r = -0.19$ and $p = 0.028$) but not in gills ($p = 0.6$). This correlation was markedly higher among unfed mussels ($r = -0.51$ and $p = 0.002$).

Discussion

This study evaluated the effect of seasonal variation and food quality on the selection of food particles in the mussel *M. edulis*. The results show that mussels can differentiate between cells of the same algal species with different biochemical compositions (total carbohydrate and protein). Furthermore, the results also show that the preferential ingestion of food particles can be modulated in mussels collected at different times of the year or in mussels maintained with different quality diets. Finally, the ability of mussels to sort food particles was correlated with the transcription levels of a mucosal lectin believed to be involved in particle capture and processing.

The alga *T. maculata* was grown on different media, and the resulting cells were different enough to be discriminated microscopically or by means of flow cytometry. When grown on a nitrogen-poor media, *T. maculata* cells (Tm–N-limited) were depleted in protein, but not in carbohydrate (slightly higher), and the resulting carbohydrate/protein ratio significantly increased, corroborating the results of Wikfors et al. (1992) for the same alga and those of Baldwin (1995) on the diatom *Thalassiosira pseudonana*. Additionally, the analysis of carbohydrates covering the surface of *T. maculata* cells, using FITC-labeled lectins, revealed significant changes in the carbohydrate make-up depending on culture conditions. In particular, there was an increase in several carbohydrates (glucose and mannose residues) on the cell surface of nitrogen-limited microalgae. This result was used in designing of the particle-sorting experiments since it was previously observed that carbohydrates on the surface of cells play a major role in particle sorting (Pales Espinosa et al. 2010b). As a consequence, the two populations of *T. maculata* were very useful in evaluating the effect of diet quality/quantity on mussel feeding behavior.

Results demonstrated that particle selection in *M. edulis* was significantly affected by the quality of diet received several days preceding the experiment. Interestingly, the sorting efficiencies in mussels fed a diversified and rich diet were low compared to starved mussels. In addition, both types of *T. maculata* (Tm–N-replete or Tm–N-limited) were preferentially ingested or rejected as pseudofeces depending on the previous treatment. Thus, when fed a mixed diet, mussels preferentially selected *T. maculata* N-replete while they rejected *T. maculata* N-limited cells. The sorting behavior was completely opposite in mussels previously fed *T. maculata* N-replete where *T. maculata* N-limited cells were preferentially ingested. These results suggest that mussels adapt their feeding strategy to sort different food particles to fulfill their metabolic requirements and compensate for the lack of specific biochemical compounds (e.g., carbohydrates or proteins). Bayne and

Svensson (2006) suggested such a mechanism in their study of the seasonality of particle sorting in the Sydney oyster *S. glomerata*. These authors proposed that "when nitrogen was potentially limiting to growth and/or maintenance (….) pre-ingestive selection ensured nitrogen enrichment of ingested matter."

In parallel, the present study shows that the expression of the mucosal lectin MeML in mussel feeding organs significantly increased in relation to the poor food quality consumed before the analysis. The highest up-regulation of this gene was observed when mussels were starved for 5 days, and these mussels, interestingly, displayed the highest sorting efficiencies. In addition, the sorting efficiencies and MeML expression were among the lowest observed in mussels fed the rich mixed diet. This result is intriguing because one would expect it to be disadvantageous to select among particles when the diet is limited or mussels are starved, and in contrast, bivalves would increase selection when they were "well fed." In fact, several studies support the idea that selection efficiency in bivalves increases with the quality of the seston (Newell et al. 1989; Macdonald and Ward 1994; Bacon et al. 1998) even though other reports found the opposite. For example, when fed a high concentration of seston of low organic content, *M. edulis* increased the efficiency with which filtered matter of higher organic content was selected for ingestion (Bayne et al. 1993). This disparity may reflect differences in behavior in bivalve species or within species subject to different experimental conditions as discussed by Bacon et al. (1998).

The principle of increased selection when high-quality food is abundant is not applicable here, and the selection of a specific type of particle rather than others seems to reflect internal demands as discussed by Bayne and Svensson (2006). Therefore, it could be hypothesized that when *M. edulis* are starved and need food, lectin production increases with the primary goal of capturing as much "good food" as possible. If a specific lectin with a particular affinity for a given carbohydrate is produced, then selection occurs for algal cells covered with that carbohydrate. Positive selection for cells with carbohydrate coating would reduce the ingestion of less nutritive particles, such as detritus, damaged cells or inorganic particles.

Previous modeling work by Willows (1992) showed that bivalves can modulate food particle sorting and/or enhance their filtration rates in order to improve net energy gain and maintain a constant metabolic digestive investment. Increasing the production of lectins in order to select good quality particles and maximize energy gain in mussels with a poor food supply would represent a strategy explaining how this bivalve is well adapted to and grows rapidly in turbid, near shore waters (Hawkins et al. 1996). Assuming that the energetic cost of producing lectin is lower than that of behavioral changes such as increasing the filtration rate, lectin regulation would represent an efficient strategy for the capture of high-quality particles when food (and consequently energy gain) is limited. Cost-efficient energetic strategies such as the one we describe here fit well in the general functional model of bivalve feeding proposed by Willows (1992).

Interestingly, particle selection in mussels fed the same diet varied quantitatively (either positive or negative SEs but with different intensities; mixed diet, Tm–N-replete and Tm–N-limited) and qualitatively (inverted SEs; unfed) in relation to the month of sampling, suggesting a relationship between feeding behavior and the changes in energy requirements over the gametogenic cycle as proposed by Bayne and Svensson (2006). In May, SEs calculated for mussels from all treatments excluding "unfed" were not significant reflecting very limited or no particle sorting. In contrast, SEs calculated in July (mixed diet, unfed and Tm–N-replete) and to a lesser extent November (unfed, Tm–N-replete) were significant demonstrating particle selection. Taken together, these results could indicate that endogenous requirements in mussels (loss of energy due to spawning in June/July) affect particle selection/capture. Summer spawning in mussels, and more generally in bivalves, is a stressful event requiring a large amount of energy (Seed and Suchanek 1992), which weakens individuals and sometimes leads to high mortalities (Myrand et al. 2000). Previous studies highlighted the importance of diet quality/quantity during gametogenesis and spawning in mussels (Newell et al. 1982; Fearman et al. 2009). Increasing particle capture and/or particle selection to enhance energy gains would help bivalve recovery following spawning.

Bivalves with homorhabdic ctenidia (gill with a single filament type) such as *M. edulis* are usually incapable of qualitative selection on the gills, and labial palps often represent the selection site for food particles (Beninger and St-Jean 1997; Ward et al. 1998). This particularity was underlined by Ward et al. (1997, 1998) who used video endoscopy to show that selection in *M. edulis* is restricted to the labial palps, with ctenidia only involved in the transport of particles to the palps for further processing. In the present study, we added to this information by showing the important role of mucosal lectin in particle sorting. In fact, while both gills and labials palps contain the mucosal lectin MeML (Pales Espinosa et al. 2010a), sorting efficiencies are only correlated with MeML mRNA transcription in labial palps. Obviously, given the overall low correlation coefficient ($r = -0.19$ for Tm–N-replete), it is likely that factors other than MeML transcription levels affect particle sorting, including the expression of other mucosal lectins involved in particle sorting (Pales Espinosa et al. 2011). Overall, the regulation of mucosal lectins has

previously been shown to be rapid in mussels (MeML) and oysters (CvML). For instance, the expression of MeML and CvML was significantly up-regulated after 3–5 days of starvation (Pales Espinosa et al. 2010a; Jing et al. 2011) suggesting that the capture and/or sorting of particles can be rapidly modulated in response to stress or a specific requirement. In the present study, the high correlation ($r = -0.51$ for Tm–N-replete) between lectin expression in the labial palps and SE further supports this scenario. If true, sorting regulation via modulation of lectin expression provides suspension-feeding bivalves with a flexible means to qualitatively and quantitatively control particle uptake to satisfy internal requirements.

In conclusion, this study showed significant changes in lectin-binding profiles among microalgae grown in N-replete or N-limited media, highlighting modification in cell surface carbohydrate signatures. These alterations caused changes in the way microalgae are processed by the feeding organs of mussels leading to their ingestion or rejection in pseudofeces, possibly through specific interactions between cell surface carbohydrates and mucosal lectins associated with the feeding organs. The ability of mussels to sort these particles varied seasonally and according to preconditioning diets. Sorting efficiencies were correlated with expression of a mucosal lectin (MeML) in labial palps, which also varied with sampling season and precondition of the mussels. Although the role of lectins in food particle selection has been previously demonstrated in other marine organisms (Allen and Dawidowicz 1990; Wootton et al. 2007), our understanding of the role of these molecules in particle processing by suspension-feeding bivalves remains in its infancy (Pales Espinosa et al. 2009, 2010b). These findings warrant further clarification of the role of MeML (and other) mucosal lectin(s) in particle capture and sorting mechanisms in mussels.

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