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Regulation of mucosal lectins in the oyster *Crassostrea virginica* in response to food availability and environmental factors

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ABSTRACT

Lectins are a large and diverse group of sugar-binding proteins involved in nonself recognition and cell-to-cell interactions. Suspension-feeding bivalves, such as the oyster *Crassostrea virginica*, are capable of using these molecules to bind cell surface carbohydrates of food particles, allowing particle capture and selection. The aim of this project was to assess whether the expression of mucosal lectins in *C. virginica* is constant or changes with the season, and to determine whether lectin expression is linked to environmental parameters and/or internal biological factors (gametogenesis). A total of 130 oysters were placed in submerged cages at a tidal estuary and monitored for changes in lectin gene expression over a 1-year period. In parallel, environmental parameters prevailing at the field site, including seawater physicochemical characteristics (temperature, salinity and dissolved oxygen), particulate organic matter and chlorophyll contents, were also monitored. Throughout the study, oysters were dissected and the gills were collected and used for the assessment of the expression of three different lectin genes (CvML, CvML3914 and CvML3912). Remaining tissues were processed for histology and the classification of the gonad development stage. Results showed that when food is abundant, such as during the spring bloom, lectin gene expressions are low, and inversely lectin levels increase with lower food levels. These findings suggest that oysters increase lectin expression to enhance the capture and ingestion of scarce food, while during spring, enough food is already being ingested and lectins are not needed. Furthermore, results showed that as the energy demands of oysters increase (gonad maturation), lectin gene expressions also increase to enhance selective ingestion of nutritious food particles. This study, therefore, demonstrates the seasonality of lectin gene expression in *C. virginica*, and suggests that lectin regulation is related to the reproduction process and abundance of high-quality food.

INTRODUCTION

Among molecules known to be central for nonself recognition and cell-to-cell interactions, lectins are a large and diverse group of sugar-binding proteins that specifically bind to glycans covering living cells (Sharon & Lis, 2004; Vasta, 2009). Lectins are ubiquitously distributed in nature as they are found in viruses, bacteria, fungi, plants, invertebrates and vertebrates (Sharon & Lis, 2004; Vasta & Ahmed, 2008). In bivalve molluscs, lectins have been mostly described in haemolymph (Olafsen, Fletcher & Grant, 1992; Odo *et al.*, 1995), sometimes associated with blood cell (i.e. haemocyte) membranes (Tasumi & Vasta, 2007) or free in the plasma, and mediate the recognition of foreign cells, thereby contributing to self-defence mechanisms (Fisher & Dinuzzo, 1991; Tripp, 1992; Minamikawa *et al.*, 2004; Zhang *et al.*, 2009). In some rare cases, bivalve lectins have been found to be potentially involved in other functions. For example, in *Codakia orbicularis*, a clam known to harbour symbiotic sulfide-oxidizing chemoautotrophic bacteria in its gills (Frenkiel & Moueza, 1995), the lectin 'codakine' has been found to be the predominant protein in the gill (Gourdine & Smith-Ravin, 2002) leading Gourdine *et al.* (2007) to propose its involvement in the mediation of symbiosis by facilitating the attachment and uptake of symbiotic bacteria from the surrounding seawater. In addition to the circulatory system, lectins have been also shown to be present in mucus covering the feeding organs (gills, labial palps) of the American (eastern) oyster *Crassostrea virginica* (Pales Espinosa *et al.*, 2008, 2009; Pales Espinosa & Allam, 2018) and the blue mussel *Mytilus edulis* (Pales Espinosa, Perrigault & Allam, 2010a).

Suspension-feeding organisms such as oysters and mussels remove phytoplankton, produce biodeposits and cycle dissolved nutrients (Prins, Smaal & Dame, 1997; Newell, 2004). These organisms are exposed to a large selection of suspended material present in seawater, including plankton of varying qualities and sizes (Alldredge & Silver, 1988). Instead of indiscriminately ingesting these particles, which may lead to the saturation of their digestive system with poorly nutritious particles (e.g. detritus, silt), suspension-feeding bivalves have developed strategies to select their food to ingest most nutritious particles and reject non-nutritious

ones as pseudofaeces (Newell & Jordan, 1983; Ward *et al.*, 1997; Cognie *et al.*, 2003), therefore enhancing the quality of ingested material and optimizing energy gain (Allen, 1921; Fox, 1936; Ward & Shumway, 2004).

Although some aspects of the selection process have been elucidated, the actual mechanism(s) by which particles of poor quality are rejected into pseudofaeces, while those of higher quality are ingested, remain unclear. Some studies support the idea that bivalves use chemical cues to discriminate among particles (Kiorboe & Mohlenberg, 1981; Newell & Jordan, 1983; Beninger & Decottignies, 2005; Pales Espinosa, Barille & Allam, 2007). More recently, results in oysters (Pales Espinosa & Allam, 2018) and mussels (Pales Espinosa & Allam, 2013) have shown that particle selection in bivalves is mediated by interactions between lectins present in mucus covering feeding organs and carbohydrates associated with the surface of suspended food particles. Specifically, it was demonstrated that mucus covering the feeding organs of oysters (Pales Espinosa *et al.*, 2009) and mussels (Pales Espinosa *et al.*, 2010a) contains several lectins that differentially bind various microalgal species (Pales Espinosa *et al.*, 2016a; Pales Espinosa, Koller & Allam, 2016b), allowing the capture and ingestion of most nutritious particles. Some of these lectins were further characterized in selected mussels (*M. edulis*: mucosal lectin or MeML; Pales Espinosa *et al.*, 2010a) and oysters (*C. virginica*: mucosal lectin or CvML, CvML3912 and CvML3914; Jing *et al.*, 2011; Pales Espinosa & Allam, 2018). The expression levels of these lectins were shown to increase following starvation during short laboratory studies, suggesting that suspension-feeding bivalves may regulate lectin expression to enhance food particle capture (Pales Espinosa *et al.*, 2010b; Jing *et al.*, 2011).

The aim of this project was to determine whether the expression of mucosal lectins in oysters is constant or varies seasonally, and to evaluate whether lectin expression is linked to food availability, gametogenesis and/or other environmental factors, such as seawater temperature, salinity and dissolved oxygen.

MATERIAL AND METHODS

Organism

One hundred thirty individuals of *Crassostrea virginica* (shell length: 85 ± 15 mm, mean ± SD) were obtained from Atlantic Shellfish Farm (East Islip, New York, USA) and placed in three immersed oyster cages (100 cm × 60 cm × 20 cm) at Flax Pond (a tidal estuary behind a barrier beach in Setauket, New York, USA) for 1 year, starting November 2015.

Water sampling

A total of 23 samplings were scheduled over 1 year including 11 samples, where oysters and water samples were taken, and 12 additional samplings, where only seawater was collected and processed. During February and March 2016, spring bloom was checked on a weekly basis. At each sampling date (on average twice per month, with the exception of the expected time of the spring bloom when there was an average of four field samplings per month), temperature, salinity and dissolved oxygen were recorded using a YSI meter (YSI Instruments, Model No. 85-10FT, Yellow Springs, OH). In addition, 5 l of seawater was collected for chlorophyll analysis and for the assessment of particulate organic matter (POM).

Chlorophyll analysis

Water samples were filtered immediately after sampling using glass microfibre (GF/C) filters (duplicates). The volume of filtered water was 0.5–1 l, depending on the particulate matter in suspension. The filters were then stored at −80 °C until analysis, typically within a month. Chlorophyll was extracted according to the SCOR/UNESCO protocol (SCOR/UNESCO, 1966). Briefly, chlorophyll was extracted using 90% acetone (10% distilled water) for 24 h in the dark at 4 °C. The extract was centrifuged at 2000 × *g* for 10 min at 4 °C. Chlorophyll contents of the extracts were then determined with a spectrophotometer that measures the absorbance at 750, 663, 645 and 630 nm. The absorbance zero was set using blank (90% acetone). Chlorophyll *a* concentration (C_a; units: $mg/m^3 = \mu g/l$) was determined using the equation of SCOR/UNESCO (SU):

$$C_a = (11.64D_{663} - 2.16D_{645} - 0.1D_{630})\, v\, l^{-1}\, V^{-1}$$

where D was the absorbance at a specific wavelength (i.e. indicated by subscript), after correction by the cell-to-cell blank and subtraction of the cell-to-cell blank corrected absorbance at 750 nm; v was the volume of acetone (ml); l was the cell (cuvette) length (cm); and V was the volume of filtered water (l).

POM analysis

GF/C filters were previously burned at 450 °C for 4 h to eliminate organic matter traces. One litre of seawater was then filtered (in duplicates) and rinsed with isotonic ammonium formate (0.5 M). After 24 h desiccation at 60 °C, filters were weighted. This measurement represents the dry particulate matter. Filters were then burned at 450 °C for 4 h and weighed to obtain the particulate mineral matter. POM is the difference between the two measurements.

Flow cytometry analysis of phytoplankton

A 2-ml subsample of raw seawater was frozen at −80 °C after the addition of 37% formaldehyde (2% final concentration) for phytoplankton analysis and counts. This was performed using a FACSCalibur flow cytometer (Becton–Dickinson Biosciences, CA, USA). A minimum of 10^5 particles were analysed. The 488-nm argon and the 635-nm red diode lasers were used for excitation, and phytoplankton were characterized based on one or more of the following parameters: forward (FSC; particle size) and side (SSC; intracellular complexity) light scatters, and photosynthetic pigments' autofluorescence (FL2 at 585 nm for phycoerythrin, FL3 at 675 nm for chlorophyll *a* and FL4 at 695 nm for phycocyanin). The determination of the major algal line was based on particle size and pigment composition (Jeffrey & Vesk, 1997), relying on the comprehensive work by Read *et al.* (2014). First, particles with no fluorescence in FL3 (e.g. debris, bacteria) were gated and removed from the analysis. Regions were then positioned to identify and count the small blue-green algal line (cyanophytes, FSC and FL2), the golden-brown (e.g. cryptophytes) and green algal lines (e.g. chlorophytes), the large diatoms (bacillariophytes) and remaining microalgae that are not covered in these categories (FL4 and FL2). To evaluate the importance of the cell biovolumes as opposed to cell counts, the estimated cell biovolume was calculated for each of the five groups assuming that each cell is a sphere (to simplify the calculation) and using the following cell sizes for each group: 20 µm diameter for large diatoms, 8 µm for green algal line, 15 µm for golden-brown algal line, 3 µm for blue-green algal line and 5 µm for other unclassified microalgae. The approximate biovolume of each algal group (µl/ml) was then deducted from the cell concentrations (cells/ml).

Oyster sampling

Ten randomly picked oysters from the three cages were collected and measured on specific sampling dates (every other seawater sampling date). Gill tissues were dissected, immediately flash frozen and stored at −80 °C until processed, typically within 10 days. In addition, a 1–2-mm-wide cross-section of each animal across the digestive gland, gonad and gills was collected, placed into 10% neutral buffered formalin for 1 week and processed for histological

Table 1. Primers and real-time PCR thermal profiles used in this study.

Gene	Forward sequence (5′–3′)	Reverse sequence (3′ –5′)	Denaturation (temperature, time)	Annealing (temperature, time)	Extension (temperature, time)	Cycles (*n*)
18S rRNA	CGCCGGCGACGTATCTTTCAA	CTGATTCCCCGTTACCCGTTA	Same as for each target gene			
CvML	ATGACTACATCAAGGAGGGC	CGCAGATGTACTACTGTCCG	95 °C, 30 s	55 °C, 30 s	72 °C, 60 s	35
CvML3914	CCACATAGCAAACCTCATTAAAC	AATCTGAAGCACATGGGTC	95 °C, 15 s	62 °C, 20 s	72 °C, 18 s	40
CvML3912	GTTCCTGGCAAATTTTATGCGAA	AATGAAAGCCGCAGAATCGG	95 °C, 15 s	62 °C, 25 s	72 °C, 20 s	35

Transcripts of three lectin genes (CvML, CvML3914 and CvML3912) and one housekeeping gene (18S rRNA) were measured.

evaluation. One histological section (5 µm thickness) was generated from each oyster. Sections were mounted on glass slides, stained with haematoxylin-eosin and then examined microscopically for the assessment of gonad development. The oysters were assigned to one of five gonad developmental stages (Loosanoff, 1942), with 0 being reproductively resting (undifferentiated/inactive reproductive tissue) and 4 being fully mature (gonad follicles full or nearly full of mature gametes). For statistical analysis, gonad stages were ranked as advanced (partially or fully mature), intermediate (early gametogenic stages) or low (post-spawning or resting).

Real-time PCR analysis

Three mucosal lectins were chosen based on previous characterization, available specific primers and the confirmed presence of these lectins in *C. virginica* pallial mucus (Jing *et al.*, 2011; Pales Espinosa *et al.*, 2016a, b; Pales Espinosa & Allam, 2018). These were CvML (LOC11101515), CvML3912 (LOC111112474) and CvML3914 (LOC111115169). For each sampling date, RNA was extracted from eight frozen gill tissues using the NucleoSpin® RNA kit (Macherey-Nagel) following the manufacturer's protocol. RNA concentrations were then measured with a nanodrop spectrophotometer. We generated cDNA from extracted mRNA using M-MLV reverse transcriptase (Promega) with poly-T primers, and the cDNA was used as template with the sets of primers and thermal profiles indicated in Table 1.

Relative quantification of transcripts from each target gene was carried out in 10-μl reactions with Takyon No Rox SYBR 2X MasterMix blue dTTP (Eurogentec), 100 nM final primer concentration and 5 ng of RNA-equivalent cDNA. The PCR reactions were performed using a Mastercycler ep realplex PCR machine (Eppendorf). Each run was followed by a melting curve analysis for quality control. PCR efficiency (E) was determined for each primer pair by determining the slopes of standard curves obtained from serial dilutions of cDNA. The correct amplification products were confirmed using gel electrophoresis. Expression levels of each lectin were normalized to the 18S rRNA gene (ΔCt; calculated as Ct of target gene − Ct of housekeeping gene) and relative transcript levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Data analysis

One-way analysis of variance (ANOVA) was used to compare lectin expression data between different sampling dates after checking for data normality and homoscedasticity using SigmaStat v. 3.1 (https://systatsoftware.com). Lectin expression data were also used in a principal component analysis (PCA) aimed at exploring the major drivers affecting lectin transcription in individual oysters. In parallel, a series of stepwise regression analyses were performed to identify the environmental drivers that best predict lectin expression results. In this case, lectin expression results from individual oysters were averaged for each sampling date and arithmetic means used in stepwise regression analyses; we used Minitab v. 18 for this. Stepwise regression removes and adds terms to the model for identifying the most informative subset of the terms. Default settings of Minitab were used (alpha to enter or exit = 0.15).

Table 2. Environmental parameters (temperature, salinity and dissolved oxygen) during the 1-year experiment.

Date	Temperature (°C)	Salinity (PSU)	Dissolved oxygen (mg/l)
10 October 2015	11.8	29	8.60
26 December 2015	9.0	33	9.31
16 January 2016	3.0	31	13.95
29 January 2016	-0.5	31	12.20
4 February 2016	3.5	30	12.50
6 February 2016	2.5	30	NA
20 February 2016	5.5	28	13.80
12 March 2016	2.5	31	13.30
27 March 2016	3.0	31	12.51
15 April 2016	14.0	30	11.80
5 May 2016	8.0	30	9.70
20 May 2016	15.0	29	9.14
3 June 2016	16.5	29	7.25
17 June 2016	21.0	28	7.61
9 July 2016	19.0	30	6.66
23 July 2016	22.0	30	6.05
6 August 2016	20.5	30	5.14
28 August 2016	25.0	30	3.77
10 September 2016	25.0	30	8.68
24 September 2016	18.0	32	7.95
10 October 2016	12.0	31	7.50
5 November 2016	8.0	32	9.06

RESULTS

Environmental parameters

Throughout the study, the temperature varied from −0.5 °C in late January to 25 °C in late August and early September (Table 2). Salinity varied between 28 and 33 PSU, while dissolved oxygen fluctuated from 3.77 mg/l in late August to 13.95 mg/l in mid-January. Dissolved oxygen peaked in mid-January (16 January 2016) reaching 13.95 mg/l and was minimal in late August (28 August 2016) at 3.77 mg/l (Table 2).

POM

There was a peak in POM in early February (4 February 2016) with a maximum of 4.6 ± 0.3 mg/l, followed by a drop to 2.7 ± 0.2 mg/l in March (12 March 2016) (Fig. 1). From mid-March to early May, POM stayed between 2.9 ± 0.3 mg/l (12 March 2016) and 2.5 ± 0.4 mg/l (5 May 2016). The values increased from mid-May to late July, reaching 4.2 ± 0.1 mg/l (23 July 2016), and decreased

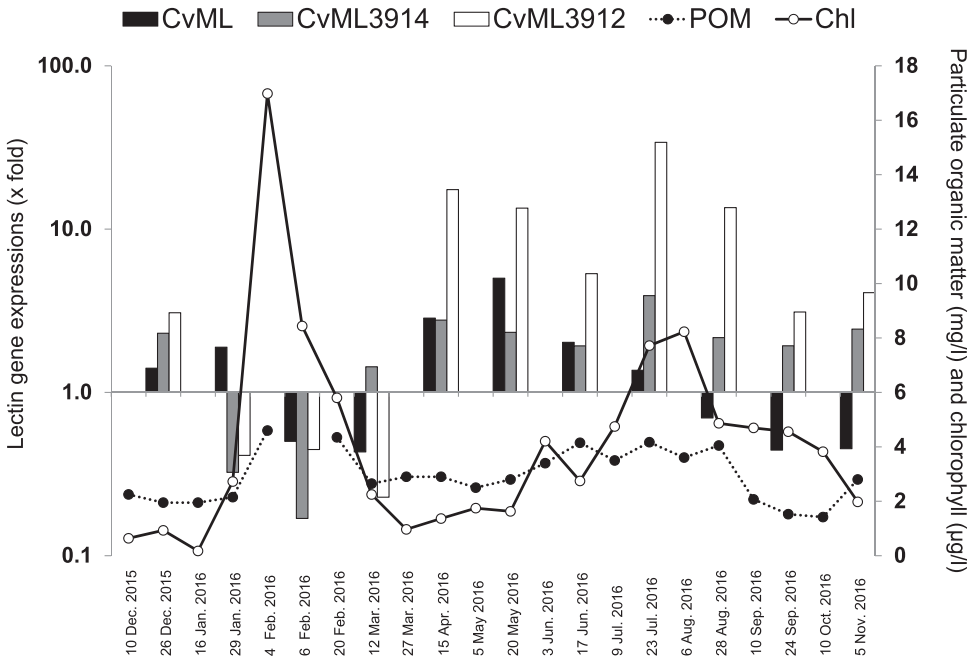

Figure 1. Expression of the three lectin genes CvML, CvML3914 and CvML3912 (referenced to the left *y*-axis; $n = 8$ oysters per data point) in oysters and POM and chlorophyll *a* (Chl) (both referenced to the right *y*-axis) in seawater sampled at different dates. Error bars and statistical differences are not shown to simplify the graph but are presented in Table 3 and Supplementary Material Table S1.

from late August to early October when POM reached 1.4 ± 0.1 mg/l (10 October 2016). POM then increased to 2.8 ± 0.1 mg/l in early November (5 November 2016).

Chlorophyll

The sampling captured the spring bloom when chlorophyll levels in seawater increased from 0.2 ± 0.1 µg/l in mid-January (16 January 2016) to a maximum of 17.0 ± 0.7 µg/l on 4 February 2016 (Fig. 1). It dropped to a minimum of 1.0 ± 0.1 µg/l in late March (27 March 2016), followed by a gradual increase until late July (23 July 2016), when chlorophyll reached 8.3 ± 0.1 µg/l before decreasing to 2.0 ± 0.1 µg/l at the end of the experiment.

Cytometry analysis

During the spring bloom captured in early February (4 February 2016), the green algal line represented about 50% of the cell counts of all microalgae, followed by the blue-green algal line at 11%, the golden-brown algal line at 4% and the large diatoms at 2%; undetermined microalgae represented 25% (Fig. 2). The large diatoms represented 67% of the microalgal population biovolume, while the green algal line and golden-brown algal line represented 20% and 10%, respectively. The blue-green algal line and the undetermined microalgae represented less than 3%. In spring and summer, the biovolume of the microalgal population was mostly dominated by the green algal line and the large diatoms. Later in the year (10 September 2016), the cell counts of the blue-green algal line represented 41% of the microalgal population, followed by the green algal line at 37% and undetermined microalgae at 20%. The large diatoms and the golden-brown algal line represented less than 2%. At that date, the green algal line represented 57% of the microalgal population biovolume, while the large diatoms, the golden-brown algal line, the undetermined microalgae and the blue-green algal line represented 25%, 8%, 7% and 3%, respectively.

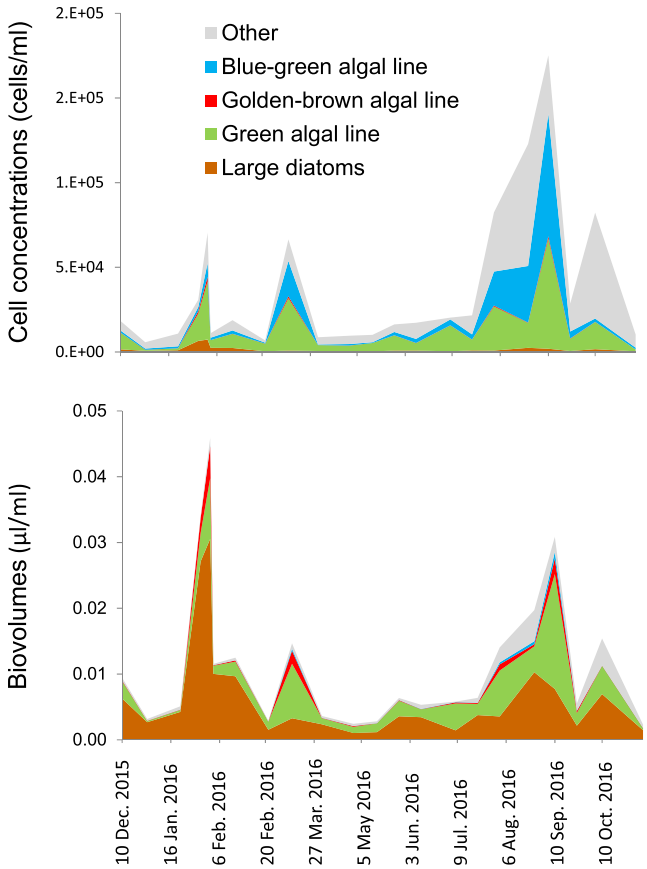

Figure 2. Relative abundance of the major microalgal groups in seawater sampled at different dates. Note the high abundance of diatoms during the spring bloom sample collected on 29 January 2016 compared to the summer bloom on 28 August 2016.

Table 3. Statistical comparisons of lectin expression between different dates (one-way ANOVA followed by Student–Newman–Keuls pairwise multiple comparisons).

Date	CvML	CvML3914	CvML3912
10 December 2015	ab	ac	abc
29 January 2016	ac	abc	ab
6 February 2016	abc	b	ab
12 March 2016	bc	abc	b
15 April 2016	ac	ab	ac
20 May 2016	c	c	c
17 June 2016	ac	ac	ac
23 July 2016	ac	ac	c
28 August 2016	a	ac	ac
24 September 2016	a	c	ac
5 November 2016	a	ac	ac

For each lectin, sampling dates not sharing similar letters (a, b or c) have significantly different expression levels ($P < 0.05$). See Figure 1 for expression levels.

Lectins

For the lectin CvML3914, the minimum expression level occurred in early February (6 February 2016) at a value of 0.17-fold (±0.15) (Fig. 1). The two other lectins, CvML and CvML3912, had their minimum expression levels in early March (12 March 2016), at a value of 0.43-fold (±0.54) and 0.22-fold (±0.14), respectively. Expression levels then increased and CvML reached a maximum of 5.00-fold (±1.70) in mid-May (20 May 2016), while CvML3914 and CvML3912 continued increasing until late July (23 July 2016), reaching a maximum of 3.91-fold (±4.02) and 34.00-fold (±7.80), respectively. All three lectins then decreased into late September (24 September 2016), with CvML reaching 0.44-fold (±0.20), CvML3914 at 1.93-fold (±1.29) and CvML3912 at 3.11-fold (±2.08). Despite some variability between individual oysters collected at each time interval, statistical comparisons highlighted that all three lectins had their minimal expression levels during or right after the spring bloom (Fig. 1, Table 3 and Supplementary Material Table S1).

Gonad development

The majority of oysters (70–100%) displayed stage 1 (early gametogenic development) from early December to late April (Fig. 3). Starting mid-April until mid-June, the percentage of stage 1 decreased (i.e. from 100% to 0%), while stage 2 (medium gametogenic development) and stage 3 (advanced gametogenic development) increased (from 0% to 40% and from 0% to 60%, respectively). From mid-June to late August, the percentages of stages 2 and 3 decreased (from 40% to 0% and from 60% to 0%, respectively), as stage 4 (fully mature) increased (from 0% to 80%). From late August to early November, the percentage of stage 0 (resting) increased (from 20% to 100%) as stage 4 decreased (from 80% to 0%).

Integrative data analysis

PCA showed that lectin expression profiles (combining the expression of all three lectins) clustered based on gonad maturation status, suggesting an important effect of gonad condition on lectin gene expression (Fig. 4). Stepwise regression analyses further highlighted the influence of gonad condition and food availability on lectin expression (summary presented in Table 4). For instance, the best model explaining the expression of CvML included gonad condition as the only explanatory variable: ΔCt CvML = 25.565 − 0.620 × gonad maturation ($R^2 = 45.68\%$, adjusted $R^2 = 39.64\%$). In this case, ΔCt decreased (CvML expression increased) with the maturation of the gonad. In addition to gonad maturation, the second-best model explaining CvML expression included chlorophyll concentrations in seawater, with decreasing lectin expression as chlorophyll concentrations in seawater increased (adjusted $R^2 = 35.5\%$). The addition of microalgae cell counts and biovolumes derived from flow cytometry analysis did not improve the regression models; hence, only chlorophyll *a* was used as it has been more widely investigated during environmental surveys. Similarly, the expression of CvML3914 was also shown to

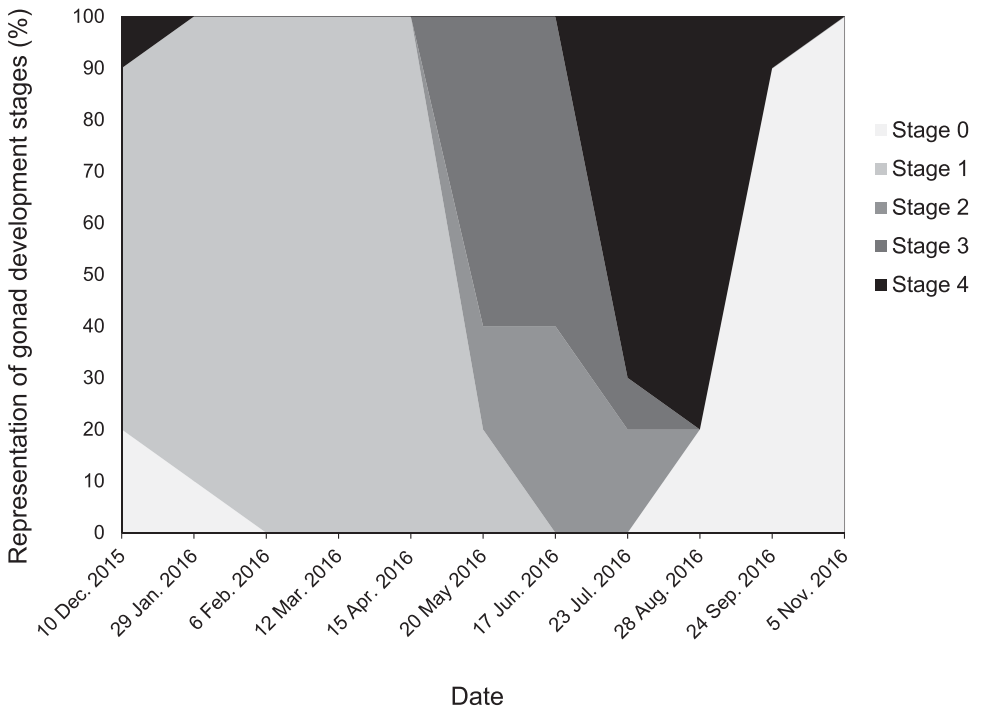

Figure 3. Percentage distribution of the different developmental stages of oyster gonads. The stages are as follows: 0, resting; 1, early gametogenic development; 2, medium gametogenic development; 3, advanced gametogenic development; and 4, fully mature.

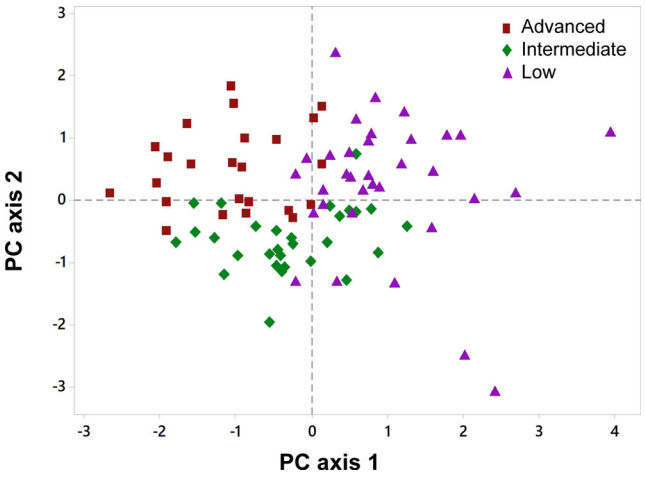

Figure 4. PCA showing the clustering of lectin expression based on gonad maturation stages. Gonad stages were ranked as advanced (partially or fully mature; corresponding to stages 3 and 4 in Figure 3), intermediate (early gametogenic stages; corresponding to stages 1 and 2 in Figure 3) or low (post-spawning or resting).

Table 4. Summary of regression analyses estimating relationships between lectins and independent variables.

	Temperature	Gonad maturation	Chlorophyll
CvML	NA	+	–
CvML3914	NA	NA	–
CvML3912	+	+	NA

Positive (+), negative (–) and absence (NA) of correlation are indicated.

decrease with increasing chlorophyll concentrations. In this case, the best model explaining the expression of CvML3914 included two explanatory variables: ΔCt CvML3914 = 4.782 + 0.2130 × chlorophyll + 0.4235 × O_2 (R^2 = 77.77%, adjusted R^2 = 72.22%). Finally, the expression of CvML3912 was best explained by seawater temperature (ΔCt CvML3912 = 18.485 − 0.1760 × temperature) as lectin expression increased (smaller ΔCt) with increasing temperatures (R^2 = 66.43%, adjusted R^2 = 62.70%). In addition to temperature, the second-best explanatory model explaining CvML3912 expression was gonad maturation, with lectin expression increasing as gonad maturation progressed (adjusted R^2 = 60.5%).

DISCUSSION

In aquatic (marine) environments, organisms commonly rely on molecular sensing and communication to perform essential biological interactions, because the physical properties of water are conducive to this signal transmission process. These various interactions involve different types of molecules, among them lectins, which are defined as carbohydrate-binding proteins of non-immune origin that are able to agglutinate cells through interaction with carbohydrates associated with cell surface (Vasta & Ahmed, 2008). Lectins are widely distributed throughout living organisms from viruses to invertebrates and vertebrates. They play multiple biological functions involving self and nonself recognition, such as symbiosis (Bulgheresi *et al.*, 2006), defence against pathogens (Tasumi & Vasta, 2007), reproduction (Springer *et al.*, 2008) and predation (Griffiths & Richardson, 2006). Lectins have also been found to participate in the capture and ingestion of microalga by members of the Dinophyceae (Ucko *et al.*, 1999). More specifically, the marine dinoflagellate *Oxyrrhis marina* uses a mannose-binding lectin as a feeding receptor for recognizing its prey *Isochrysis galbana* (Wootton *et al.*, 2007).

In suspension-feeding bivalves (e.g. oysters), lectins have been previously shown to be involved in food particle selection (Pales Espinosa *et al.*, 2009). Because live phytoplankton cells are covered with carbohydrates (glycocalyx), lectins present in mucus covering the feeding organs allow suspension-feeding bivalves to capture and ingest most nutritious particles while particles lacking surface carbohydrates (mineral particles, detritus) are rejected before ingestion as pseudofaeces (Pales Espinosa *et al.*, 2010b, 2011). Interestingly, previous studies also showed that bivalves are able to adjust food sorting throughout the growing season to satisfy physiological demands (Bayne & Svensson, 2006; Pales Espinosa & Allam, 2013), although the precise mechanism for this regulation remains unclear.

In this study, results show that the gene expression of three mucosal lectins (i.e. CvML, CvML3914 and CvML3912) in *Crassostrea virginica* is related to exogenous (chlorophyll *a*) and endogenous (gametogenesis) cues. The three lectins showed an inverse relationship between their expression levels and chlorophyll and POM during the spring bloom (Fig. 1). In other words, whenever the 'food' is abundant (spring bloom), the gene expression of lectins was low, and inversely lectin levels were higher with lower food levels (early summer). This result is in agreement with a previous laboratory study reporting that the expression of the mucosal lectin MeML in the feeding organs of the mussel *Mytilus edulis* significantly increased when the bivalve was maintained under poor feeding conditions (Pales Espinosa & Allam, 2013). The highest upregulation of this gene was observed when mussels were starved for 5 days and, interestingly, these mussels displayed the highest sorting efficiencies. In contrast, the sorting efficiencies and MeML expression were among the lowest observed in mussels fed a rich mixed diet. This may indicate that while oysters, like mussels, increase lectin expression to enhance the capture and ingestion of scarce food, during spring bloom, enough food is already being ingested and lectins are not needed.

In addition to the lack of food, the expression of lectins can also be linked to internal biological processes, such as reproduction (Pales Espinosa & Allam, 2013). With the comparison of Figures 1 and 3, it can be clearly seen that as more oysters transitioned from stage 1 into stages 2 and 3, the lectin gene expression increases dramatically. Throughout the months where the oysters were building gametes, lectin gene expressions stayed high. As the oysters started to spawn in August, lectin gene expressions dropped. These trends may be linked to internal energy demands that increase as the oysters go through their reproduction period. Therefore, high lectin levels can facilitate the capture and ingestion of more microalgal particles when internal energy demands are high. Finally, the high level of mucosal lectin expression during summer may also be linked to the increased abundance of microbial pathogens (bacteria, protists) in seawater at this time. As a matter of fact, an earlier study has shown a significant upregulation of CvML and CvML12 following bath exposure to the opportunistic pathogen *Vibrio alginolyticus*, but not to the injection in the adductor muscle (Jing *et al.*, 2011; Pales Espinosa & Allam, 2018), highlighting the potential role of these mucosal lectins in the interactions between oyster and waterborne microorganisms at the pallial interfaces.

This study also showed that the regulation of each of the three mucosal lectins is unique. For instance, if the expression of the three mucosal lectins was low from January to March, and high from April to July, the expression of CvML starts to drop in June while the expression of CvML3914 and CvML3912 stays at a high level, suggesting that the three molecules are involved in multiple functions. If the expression of all of these lectins is upregulated while the bivalves experience energy deprivation (e.g. absence of food or physiological need such as gametogenesis; Jing *et al.*, 2011; Pales Espinosa & Allam, 2018), the best predictor was different for each of these lectins: gonad maturation (followed by chlorophyll levels in seawater) for CvML, chlorophyll (followed by dissolved

oxygen) for CvML3914 and temperature (followed by chlorophyll) for CvML3912. Interestingly, the use of microalgae biovolume (derived from flow cytometry) in the analysis did not improve the regression models and the overall chlorophyll content represented a stronger explanatory variable driving lectin regulation. This is not surprising since food selection (i.e. ingestion) in suspension-feeding bivalves is not solely linked to particle size, and microalgae within a size range that can trigger capture may still be rejected in pseudofaeces (Pales Espinosa & Allam, 2013).

Overall, this study demonstrates that the expression of several mucosal lectins in *C. virginica* varies seasonally and suggests that lectin regulation in oyster gills is related to the reproduction process and abundance of food. Although not tested in this study, we cannot rule out that the expression of some of these lectins is also regulated by the abundance of pathogens in seawater, including infectious bacteria and protists. These findings allow a better understanding of factors regulating oyster growth and benthic–pelagic coupling in coastal systems.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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