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Not only greening: The effects of marennine produced by *Haslea ostrearia* on physiological traits of three bivalve species

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ABSTRACT

The marine diatom *Haslea ostrearia* is known for its ability to produce marennine, a blue-green water-soluble pigment, which is responsible for the oyster greening phenomenon in the French Atlantic Coast. Marennine can be considered a bioactive extracellular compound (BEC) as it exhibits activities against detrimental pathogens in bivalve aquaculture. However, marennine could also be toxic to animals. The aim of this experimental study was to assess the effect of marennine produced by *H. ostrearia* upon three commercially important bivalve species, the American oyster *Crassostrea virginica*, the blue mussel *Mytilus edulis* and the giant scallop *Placopecten magellanicus*. Effect of this BEC was also assessed on different ontogenic stages of scallops. The animals were exposed to 3 mg L⁻¹ of marennine and physiological response associated with clearance rates (CR), oxygen consumption and condition index (CI) were measured after 16 h of exposure (day 0), and after one subsequent week of recovery (day 7). In the short term, CR and oxygen consumption on mussels and oysters were significantly altered by marennine but not for scallops. CR recovery was observed in oysters after seven days, but no depuration was observed in mussels. Nevertheless, oxygen consumption in both mussels and oysters recovered on day 7. CI in the marennine-exposed group for both adult oysters and scallops was not significantly different, yet CI of mussels was lowered by 18.2 % compared to control. Scallop adults seemed largely resistant to marennine, but juveniles were more susceptible with a reduced CR and oxygen consumption by 71.9 % and 24.5 %, respectively, and no depuration activity observed until day 7. Additionally, CI in marennine-exposed juvenile scallops was 27 % lower than that of control. This study demonstrates species- and age-specific effect of marennine on bivalves, thus highlighting the importance of further assessment on the utilization of this pigment as a natural product decreasing pathogenicity of bacteria in shellfish aquaculture systems.

1. Introduction

In the aquatic environment, suspension-feeding bivalves are able to adapt to large fluctuations in both quantity and quality of the suspended particulate matter (Dame, 2011; Gosling, 2015). Indeed, they could be exposed to different particle sizes as well as changes in nutritional content, including the possible presence of toxic substances excreted by microscopic organisms (Mafra et al., 2009; Navarro et al., 2018; Prasetya et al., 2017b, 2020). Several studies have reported that many microalgae species are able to accumulate or exudate biotoxins or

biologically active compounds in particular environmental condition. For instance, the dinoflagellates from the genus of *Alexandrium*, such as *A. minutum* or *A. catanella*, are able to produce neurotoxins that include saxitoxin (STX) and its derivatives, also known as paralytic shellfish toxins (PST) (Borcier et al., 2017; Navarro and Contreras, 2010). Other dinoflagellates produce different toxins, as *Azadinium spinosum* with azaspiracid toxins (AZAs), and *Karenia brevis* brevetoxins, a type of neurotoxin that could be lethal to marine organisms, for instance, significant reduction of valve activity, clearance and absorption rate as well as increase of mortality (Basti et al., 2015; Echevarria et al., 2012; Ishida

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et al., 2004; Jauffrais et al., 2012; Manfrin et al., 2012). Numerous studies have also shown the deleterious effects of these compounds upon bivalves when excreted into the water (Hégaret et al., 2011, 2007; Jauffrais et al., 2012; Navarro and Contreras, 2010). To our knowledge, fewer studies have addressed the effects of bioactivity, as well as lower toxic organic compounds on bivalves. Prasetya et al. (2017a) demonstrated that the blue-green, water soluble pigment marennine, an organic compound produced by the non-toxic diatom *Haslea ostrearia* (Gaillon) Simonsen affected behavior (ca. valve closure activity), physiology (ca. clearance rate and oxygen consumption) and biochemical traits (ca. modification of membrane phospholipids in the gills and digestive glands) of juveniles of two cultured species, the blue mussel *Mytilus edulis* and the American oyster *Crassostrea virginica*. Nevertheless, no evidence was documented if this compound could also affect bivalves in the same way at the adult stage or if their sensitivity changes with age. Additionally, the physiological responses of bivalve species to depuration or a bivalve's ability to recover after marennine exposure are also poorly understood, even though blue *Haslea* and the marennine greening effect has long been recognized (e.g., Gaillon, 1820; Lankester, 1886).

Marennine is fixed to the gills and labial palps of bivalves when they filter seawater during *H. ostrearia* blooms where the marennine concentration can be up to 3 mg L⁻¹ (Turpin et al., 2001). In oyster ponds, this is known as oyster greening, a phenomenon that increases their commercial value (Gastineau et al., 2018). This natural greening phenomenon contributes significantly to the economic value of cultured oysters (*Crassostrea gigas*) particularly in the French Atlantic coast due to organoleptic and essential fatty acid modifications in green oysters that result in a more palatable with higher PUFA content (Piveteau, 1999; Prasetya, 2015; Prasetya et al., 2017a; Robert, 1975). Furthermore, recent findings have shown that marennine has antibacterial properties and could act as a natural protective agent against pathogens in aquaculture (Gastineau et al., 2012, 2014; Turcotte et al., 2016). Therefore, marennine could have applications in shellfish aquaculture as a natural antibacterial agent and also immunostimulant. Turcotte et al. (2016) demonstrated that a marennine concentration of 0.1 µg mL⁻¹ in aquaculture tanks enhanced survival and physiological condition of mussels *M. edulis* and scallop *Placopecten magellanicus* larvae. However, at concentrations over 1 µg mL⁻¹ the larval survival is reduced, demonstrating that marennine could be toxic at higher concentrations. Sensitivity of bivalves to marennine seems highly variable in relation to their ontogenic stages, as physiological characteristics of *M. edulis* and the American oyster, *Crassostrea virginica*, juveniles are impacted only when they are exposed to concentrations over 2 µg mL⁻¹ (Prasetya et al., 2017a).

The aim of the present study was to assess further the effect of marennine excreted in the water column by *H. ostrearia*, on the physiological parameters of three major species of bivalves produced in aquaculture, namely the blue mussel *M. edulis*, the American oyster *C. virginica*, and the giant scallop *P. magellanicus*. Marennine was provided either as culture supernatant of *H. ostrearia* or the blue water (BW), a concentrated supernatant by ultrafiltration process (Turcotte et al., 2016). Here, we test the hypothesis that the effects of marennine on the clearance rate, oxygen consumption and condition index of the tested bivalves species are species-specific. Additionally, we tested the effect of marennine on different ontogenic stages of scallops because this has never been documented in this species. Condition index (CI) of animals was used as indicative physiological traits, generally considered as a good physiological indicator to assess the health status of bivalves (Filgueira et al., 2014; Irisarri et al., 2015). CI has long been applied for biological and commercial purposes (Filgueira et al., 2014) and is also recognized as a useful biomarker to measure the ability of bivalves to withstand both adverse natural and anthropogenic stressors (Anacleto et al., 2015; Castrec et al., 2018; Li et al., 2009; Rouane-hacene et al., 2015). It is known that filter-feeding bivalves, including the commercial species used in this study, accumulate marennine by filtration either of

supernatant (BW) (Prasetya et al., 2017b, 2019) or of purified form (Gastineau et al., 2018), and that the greening of bivalves occurs both in juvenile and adult bivalves (Prasetya et al., 2017a). Therefore, we also aimed to investigate whether the effect of marennine could be observed not only in the short-term (days) but also in the longer-term (weeks) to observe a possible recovery (depuration capacity) of the three different species of bivalves.

2. Materials and methods

2.1. Algal culture

The cultures of *H. ostrearia* (NCC-136) were obtained from the Nantes Culture Collection (NCC), Université de Nantes, France. The stock cultures were maintained in autoclaved F/2 medium at an irradiance of 20 µmol photons m⁻² s⁻¹ in a 14/10 h light/dark cycle. Furthermore, pilot-scale cultures were conducted in two 100 L flat bottom circular photobioreactors (PBRs) as described in Turcotte et al. (2016). The culture supernatant containing marennine (will therefore be referred as Blue Water (BW)) was harvested from the PBR and marennine concentration in BW was estimated on cell-free culture water (syringe-filtered on 0.20 µm, Sterile-R) using the Beer-Lambert law as described in Prasetya et al. (2016). The optical density was measured at 677 nm in a 10 mm cuvette using a spectrophotometer (Cary 100 Bio UV-vis, Agilent Technologies), and the specific extinction coefficient for extracellular marennine ($\epsilon_{677} = 12.13 \text{ L g}^{-1} \text{ cm}^{-1}$), as stated in Pouvreau et al. (2006, 2007). As utilization of purified marennine obtained according to the method from Pouvreau et al. (2006) is considerably costly, the utilization of BW should be considered in bivalve aquaculture. The BW in the PBRs was concentrated until the volumes reached 1 L using two ultrafiltration units (30 and 3 kDa), as described in Pouvreau et al. (2006) and stored for less than one week in high-density polyethylene bottles at 5 °C in the dark.

2.2. Animals and diet preparation

Adult mussels *M. edulis* (size range of length = 41.6–67.7 mm) and oysters *C. virginica* (size range of length = 75.6–85.9 mm) were obtained in June 2015 from shellfish aquaculture leases in St-Pieters Bay, Canada (46°25.963 N; 62°39.914 W). Adult (size range of length = 88.0–96.8 mm) and juveniles (size range of length = 60.1–67.6 mm) of sea scallops, *P. magellanicus*, were collected from the wild in Gaspé Bay, Québec, Canada (48.8316 °N, 64.4869 °W) in late June 2015. Animals were then immediately transported to the Station Aquicole de Pointe-aux-Pères (Institut des Sciences de la Mer, Rimouski, Canada). Upon arrival, a total of 64 individuals of the three species (oysters and mussels, n = 20; large and small scallops, n = 12) were numbered and acclimated to laboratory conditions for 30 days before the experiment was performed. The three species were equally distributed in two 300 L maintenance tanks with continuous aeration, salinity of 29 ppt, temperature of 16 °C and natural photoperiod. Animals were continuously fed with *Pavlova lutheri*, *Tisochrysis lutea* and *Chaetoceros muelleri* at 1:1:1 equivalent dry weight at concentration of 40,000 cells mL⁻¹.

2.3. Greening of animals

In the present work, the animals were greened (n = 32) in individual chamber (i.e., 1 L cylindrical glass jars – see Prasetya et al., 2017a) by using BW (equivalent to aquaculture relevant concentration of marennine, 3 mg L⁻¹) for 16-h and fed with a mixture (3% of mean mussel dry weight) of three species of microalgae as already described in the section “Animals and diet preparation.”. Individuals used for control (n = 32) were kept under similar condition without marennine exposure. Greened and control animals were then transferred to the maintenance tank for 7 days as already described during acclimatization period to estimated depuration capacity.

2.4. Physiological response to greening

Physiological measurements were performed on 10 individuals by species (mean shell length, SL \pm SE: 57 \pm 1.7 mm and 80 \pm 0.9 mm for mussels and oysters, respectively) and also by size for scallops (63 \pm 0.8 mm and 91.5 \pm 1.4 mm, for juvenile and adult scallops, respectively) immediately at the end of 16-h exposure (T0) and 7 days later (T7) on individuals randomly sampled. Ten metabolic chambers (see Prasetya et al., 2017a) filled with 1 μ m-filtered and UV treated seawater (temperature of 16 °C and salinity of 29 ppt) were used simultaneously to measure physiological condition of 10 animals (5 greened and 5 non-greened) and two reference chamber (empty shell). Reference chambers were used in clearance rate measures to estimate sedimentation constant of food and for oxygen consumption as blank reference and subtracted for animals values from same sequence. Animals that remained closed in the chamber during the measurement were excluded from physiological analysis. Physiological rates were standardized to a mass-specific rate equivalent for individuals of 1 g dry mass using weight exponents as previously described in Bayne et al. (1987).

Clearance rate (CR), which is the volume of water removed of suspended particles per unit time, was measured using a static system as described in Prasetya et al. (2017a). Briefly, animals were placed in 1.3 L metabolic chambers 1 h prior to measurements, then fed with *T. lutea* at an initial concentration of 100 000 cells mL⁻¹. Circulation in metabolic chamber was generated by aeration around the wall of the chamber to avoid the re-suspension of biodeposits. Food particles were counted every 15 min during 60 min of measurement using a particle counter machine (Beckman Coulter Counter Z2, Mississauga, ON, Canada). The instrument was installed with a 70 μ m aperture tube and was adjusted to measure *T. lutea* (size range = 4–8 μ m), which is known to be retained efficiently by the three bivalve species (Prasetya et al., 2017a; Ward and Shumway, 2004). The utmost difference between two consecutive measurements was used to calculate CR as described in Gilek et al. (1992).

Oxygen consumption (VO₂) was measured on the same individuals in 0.85 L metabolic chambers following Prasetya et al. (2017a). The measurement was performed 24 h after CR measurement. Briefly, the rate of oxygen uptake was determined by sealing the metabolic chambers and measuring the decrease in dissolved oxygen using an YSI (5331) polarographic electrode coupled to a YSI micro-oxymeter (Yellow Springs, OH, USA). The output signal was monitored continuously on a chart recorder until there was a 20 % decrease in oxygen saturation. VO₂ was then expressed as ml O₂ g⁻¹ tissue dry weight h⁻¹.

Calculation of the Condition Index (CI, %), shell length (L, mm), tissue (DWt, g) and shells (DWs, g) dry weight were conducted on a total of 64 individuals after the 7 days of depuration following marennine exposure. Body tissues of animals were dissected from the shell valves. Samples were dried separately in pre-weighed aluminum containers at 110 °C for 24 h to determine the dry shell weight (DWs) and the dry tissue weight (DWt). The CI was calculated according to (Freeman, 1974) as follows:

$$CI = (\text{tissue dry weight} / \text{shell dry weight}) \times 100.$$

2.5. Data analysis

Two-way ANOVA was performed to examine main and interactive effects of marennine exposure (2 levels, 0 and 3 mg L⁻¹) and bivalve species on CR, VO₂ and CI. Additionally, another two-way ANOVA was carried out to observe the difference on measured physiological parameters (CR, VO₂ and CI) in different ontogenic stages of *P. magellanicus*. Before application of ANOVA, homoscedasticity was graphically verified by confirming the spread residuals and normality was tested by Saphiro-Wilk. When necessary, the data was square root

transformed. Bonferroni post hoc tests were applied when significance of factors or interaction was observed by ANOVA. The depuration activity was tested statistically using a *t*-test between the CR, VO₂ or CI values obtained immediately and 7 days after marennine exposure. All statistical analyses were conducted at a significance level at $p < 0.05$ and performed using Graphpad Prism 5 for Mac OS X software.

3. Results

3.1. Effect of marennine on physiological traits of different species of adult bivalves

The two-way ANOVA confirmed that at T0 both main factors (marennine exposure and bivalve species) had a significant effect on CR (Table 1; Fig. 1A). Immediately after the greening process (T0), the CR of adult mussels, oysters and scallops exposed to marennine was reduced by 76, 57 and 84 %, respectively (Fig. 1A; $P < 0.05$) compared to controls not exposed to marennine. In the control, mussels and oysters CRs were significantly different from scallops, with values 57 and 55 % higher, respectively ($P < 0.05$). The initial reduction of CR in oysters exposed to marennine was almost similar to that of control after one week (Fig. 1B, $t = 0.375$; $P > 0.05$). However, no recovery (depuration) appeared for both mussels and scallops for which the CR at the end of the experiment (T7) was still significantly lower than the controls (Fig. 1B; $t_m = 0.010$ and $t_s = 0.042$; $P < 0.05$ for mussels and scallops, respectively).

ANOVA for VO₂ showed a significant difference for both factors at T0 (Table 1). Immediately after greening, mean VO₂ of adult mussels and oysters was significantly lower (30 and 48 %, respectively) than the controls (Fig. 1C; $P < 0.05$). However, we observed an absence of response for scallops, as the values were similar between the control and animals exposed to marennine ($t = 0.266$, $P > 0.05$; Fig. 1C). Furthermore, adult mussels had higher VO₂ than oysters and scallops (Fig. 1C; $P < 0.05$). Interestingly, after 7-days of marennine exposure (T7), VO₂ in mussels and oysters returned to the normal state (no difference with the control, Fig. 1D, $P > 0.05$), indicating the ability of these bivalves to depurate sufficiently to recuperate their VO₂ level.

The two-way ANOVA demonstrated a significant interaction

Table 1

Results of the two-way ANOVA on the interactive effects of marennine exposure and bivalve species on the clearance rate (CR) and oxygen consumption (VO₂). Significant differences at $P < 0.05$ are in bold and indicated by *.

Effect	SS	MS	F-value	P-value
T0				
CR				
Marennine exposure	8.999	8.999	9.215	0.004*
Species	2.949	1.474	1.510	0.028*
Marennine x Species	0.863	0.432	0.442	0.646
Error	36.14	0.977		
VO₂				
Marennine exposure	2.799	2.799	8.467	0.006*
Species	17.72	8.861	26.80	0.001*
Marennine x Species	0.579	0.289	0.875	0.424
Error	13.88	0.331		
T7				
CR				
Marennine exposure	3.648	3.648	3.719	0.062
Species	5.912	2.956	3.014	0.063
Marennine x Species	3.858	1.929	1.966	0.157
Error	31.39	0.980		
VO₂				
Marennine exposure	0.133	0.133	0.379	0.541
Species	21.07	10.54	29.99	0.001*
Marennine x Species	0.014	0.007	0.020	0.979
Error	15.11	0.351		

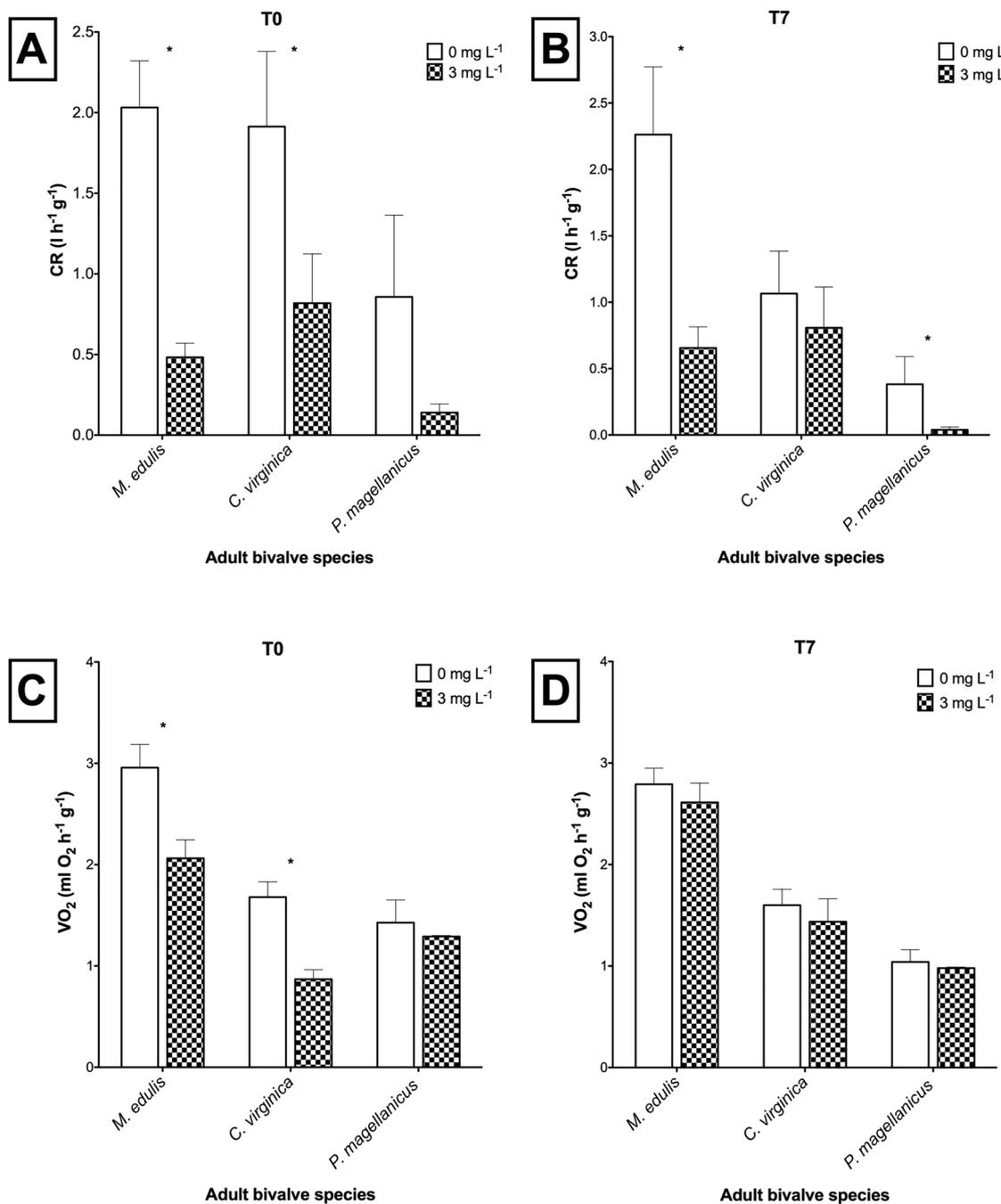


Fig. 1. Clearance rate (CR) and consumption of oxygen (VO₂) of adult mussels, oysters and scallops for a standard animal 1 g in whole body dry mass (mean ± standard error of mean (SEM), N = 64). Measurements were conducted immediately after marennine exposure (A & C) and 7 days later (B & D) for unexposed (control) and exposed individuals to BW marennine (3 mg L⁻¹). Significant differences (P < 0.05) indicated by *.

between marennine and species (Table 2; Fig. 2). The interaction between marennine exposure and species indicated that marennine

Table 2

Results of the two-way ANOVA on the interactive effects of marennine exposure and bivalve species on the condition index (CI). Significant differences (P < 0.05) indicated in bold and by *.

Effect	SS	MS	F-value	P-value
CI				
Marennine exposure	23.71	23.71	11.38	0.001*
Species	370.1	185.1	88.82	0.001*
Marennine x species	14.10	7.05	3.385	0.042*
Error	95.84	2.084		

affected differentially CI depending on species. For instance, mean CI did not vary across marennine exposure for adults of *C. virginica* and *P. magellanicus*. However, for *M. edulis*, a 3 mg L⁻¹ exposure of marennine reduced mean CI by ~ 18 % compared to the control (Fig. 2). Additionally, Pearson correlation revealed that the CI was significantly correlated to the consumption of oxygen (VO₂) but only in mussels (r = 0.418, P = 0.042). No significant correlation was observed between CI and CR for the three species as well as VO₂ for both oysters and scallops (P > 0.05).

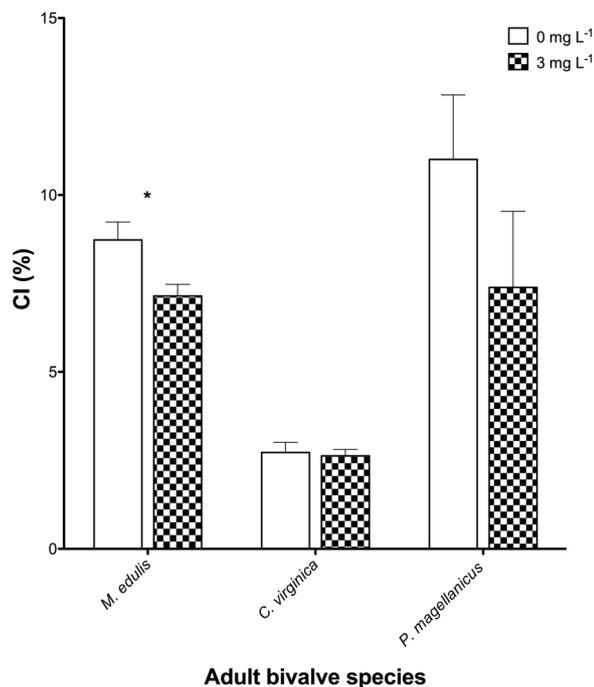


Fig. 2. Condition index (CI) of adult mussels, oysters and scallops 7 days after exposure or not to BW containing 3 mg L⁻¹ marennine. Values are mean \pm standard error (SE). Significant differences ($P < 0.05$) indicated by *.

3.2. Effect of marennine on physiological traits of different size in *P. magellanicus*

At T0, both bivalve size and marennine exposure had a significant negative effect on CR (Table 3), and the pattern between CR and exposure was similar for both sizes of *P. magellanicus*. That is, mean CR was reduced by $\sim 75\%$ for both large and small individuals (Fig. 3A), although the smaller scallops had significantly higher CR than larger

Table 3

Results of the two-way ANOVA on the interactive effects of marennine exposure and size of *Placopecten magellanicus* on the clearance rate (CR) and oxygen consumption (VO_2). Significant differences ($P < 0.05$) are indicated in bold and by *.

Effect	SS	MS	F-value	P-value
T0				
CR				
Marennine exposure	7.543	7.543	6.662	0.027*
Size	8.408	8.408	7.426	0.021*
Marennine x Size	2.189	2.189	1.934	0.195
Error	11.32	1.132		
VO₂				
Marennine exposure	0.344	0.342	5.666	0.039*
Size	0.901	0.901	14.83	0.003*
Marennine x Size	0.119	0.119	1.964	0.019*
Error	0.608	0.061		
T7				
CR				
Marennine exposure	3.127	3.127	7.755	0.019*
Size	9.006	9.007	22.34	0.008*
Marennine x Size	1.350	1.351	3.350	0.097
Error	4.302	0.403		
VO₂				
Marennine exposure	0.321	0.322	4.976	0.049*
Size	2.140	2.142	33.19	0.002*
Marennine x Size	0.213	0.212	3.289	0.009*
Error	0.654	0.064		

ones. Similar results were observed at T7 (Table 3; Fig. 3B). For VO_2 , effects of marennine exposure differed between size classes at both times (Table 3; Fig. 3C and D). Oxygen consumption was higher in small scallops, and was reduced by $\sim 25\%$ with exposure to marennine. Larger scallops showed no significant effect due to marennine exposure. At T7, the CR of small and large scallops exposed to marennine 7 days before were similar to the CR at T0 (Fig. 3A; $P > 0.05$), suggesting that the juvenile scallops were not able to depurate from the marennine fixed on their tissues. At the end of the experiment (day 7), the VO_2 in small scallops did not recover to the normal state (Fig. 3C, $P < 0.05$). This suggests that smaller scallops did not recover completely after marennine fixation. For large scallops, no significant effect of marennine was observed immediately after marennine exposure or 7 days later ($P > 0.05$).

Mean CI varied significantly with marennine exposure in different size scallops ($P = 0.02$; Table 4). The CI of small scallops in control was 27% higher than the small scallop exposed to marennine (Fig. 4; $P = 0.020$). Moreover, Pearson correlation analysis revealed that the CI in juvenile scallops was significantly correlated to CR ($r = 0.735$, $P = 0.024$) but not with the consumption of oxygen ($r = 0.551$, $P = 0.124$).

4. Discussion

4.1. Physiological responses of adult bivalves resulting from exposure to marennine

Fixation of marennine in tissues can be evidenced by the greening of gills and a concomitant reduction of marennine concentration in the medium after 16 h when the treatment of greening of animals started (Prasetya et al., 2017a). In this study, we observed that fixation of marennine on the gills significantly decreased the filtration activity in adults of all 3 bivalve species. Reduction in feeding activity is supported by the reduction of microalgae consumption observed for adult bivalves exposed to marennine compared to that of control. However, the magnitude of marennine impact on both parameters appeared to be species-specific. Thus, generalizations about the feeding activity response of bivalves to non-PST or BEC compound could not easily be established. Indeed, similar results have been demonstrated in other studies regarding the effect of non-PST compounds produced by the microalgae on bivalves CR. For instance, Castrec et al. (2018) demonstrated that CR of Pacific oysters *C. gigas* exposed to BEC from the dinoflagellate *Alexandrium minutum* is significantly reduced by approximately 46%, indicating that the effect of BECs could be detrimental for oysters. Borcier et al. (2017) showed that similar BEC excreted from *A. minutum* had negative effects on filtration activity of the giant scallop *P. magellanicus* with a CR reduction of 49%. Interestingly, same authors also observed that reduction in CR by BEC has larger magnitude than PST compounds, since the CR of scallops exposed to PST was 86% higher than that of individuals exposed to BEC. Additionally, negative effects of the non-PST compound marennine has been demonstrated by Prasetya et al. (2017a), where marennine (2 mg L⁻¹) can reduce the consumption of oxygen in juvenile oysters *C. virginica*. For oxygen consumption, the trends were analogous, except for *P. magellanicus* that showed higher resistance, as the VO_2 values immediately after marennine exposure were similar to the non-exposed individuals. Thus, the response to marennine exposure also seems species-specific for this physiological trait.

Several hypotheses have been proposed to explain the reduction in filtration activity and oxygen consumption due to the fixation of BEC in bivalves. First, the bioactive compounds exuded by certain microalgae may have allelopathic effect upon food cells given to bivalves, either by affecting their nutritional value or provoking the formation of aggregates (such as algal-derived organic matter (AOM) and transparent exopolymeric particles (TEPs)), that may clog bivalve gills (Prasetya et al., 2016; Tillmann et al., 2008, 2007, Villacorte et al., 2015, 2013). However, in the present study, fixation of marennine by the bivalves

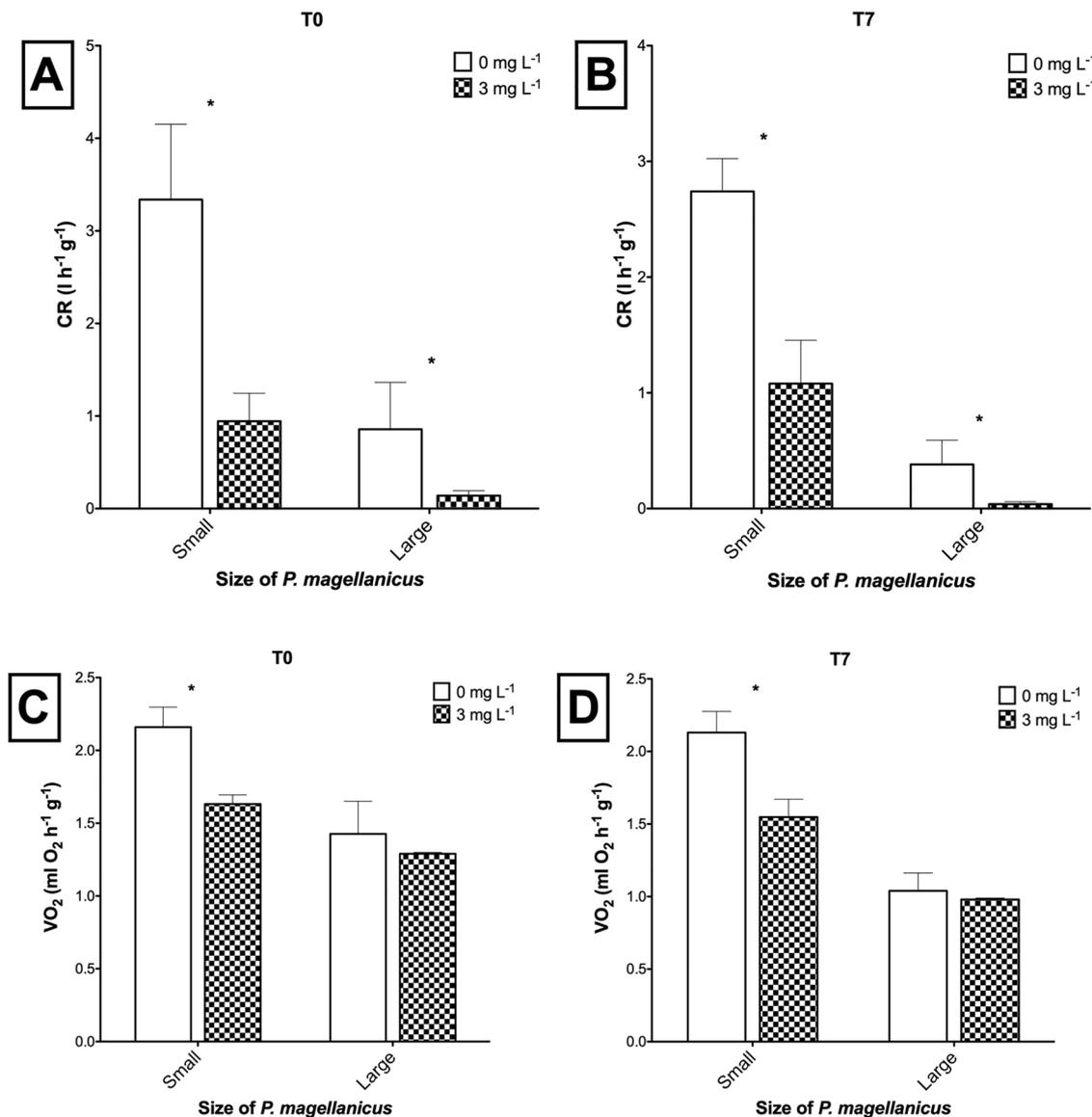


Fig. 3. Clearance rate (CR) and consumption of oxygen (VO₂) of different sizes of scallops for a standard animal 1 g in whole body dry mass (mean ± standard error of mean (SEM), N = 28). Measurements were conducted immediately after marennine exposure (T0: A & C) and 7 days later (T7: B & D) for each group of treatment (control) and exposed to BW marennine (3 mg L⁻¹). Significant differences (P < 0.05) indicated by *.

Table 4

Results of two-way ANOVA on the interactive effects of marennine exposure and sea scallop size on the condition index (CI). Significant differences (P < 0.05) are indicated in bold and by *.

Effect	SS	MS	F-value	P-value
<i>CI</i>				
Marennine exposure	28.10	28.10	7.605	0.020*
Size	7.297	7.290	1.975	0.192
Marennine x Size	1.175	1.175	0.318	0.582
Error	36.95	3.697		

occurred prior to filtration measurement, avoiding any possibility of interaction between marennine and microalgae cells use to feed bivalves. The second hypothesis is that the detrimental effect could alter bivalve tissues directly through interactions between marennine and gill cell membranes. This assumption is supported by the finding from Prasetya et al. (2017a) who found that purified marennine (2 mg L⁻¹) affects the filtration activity and oxygen consumption in juvenile mussels *M. edulis* and oysters *C. virginica*. These authors also observed that

the alteration in CR and VO₂ is correlated significantly to valve gaping as short-behavioral response indicators and also marennine accumulation in bivalve gills.

The present study demonstrated that the impact of marennine on condition index (CI) of adult bivalves 7 days after exposure also appeared to be species specific with the greatest impact observed only in adult mussels, and not in adult oysters and scallops. A CI reduction in bivalves due to exposure to microalgae BEC also has been reported in Castrec et al. (2018), with CI in oyster *C. gigas* significantly lowered due to exposure to non-PST BEC produced by *A. minutum*. In contrast, Haberkorn et al. (2010) showed no significant difference in CI measured in *C. gigas* PST-exposed to *A. minutum* in comparison with the control. This variability in CI impact between species and studies could be related to differential susceptibility and depuration capacity of different organisms, but also to BEC composition and the use of different levels of "contamination".

Deleterious effects of extracellular compounds on bivalve tissues have already been reported to validate a stronger decrease of CR, VO₂ and CI (Borcier et al., 2017; Castrec et al., 2018). Given that the unknown molecular structure of marennine may hamper the explanation

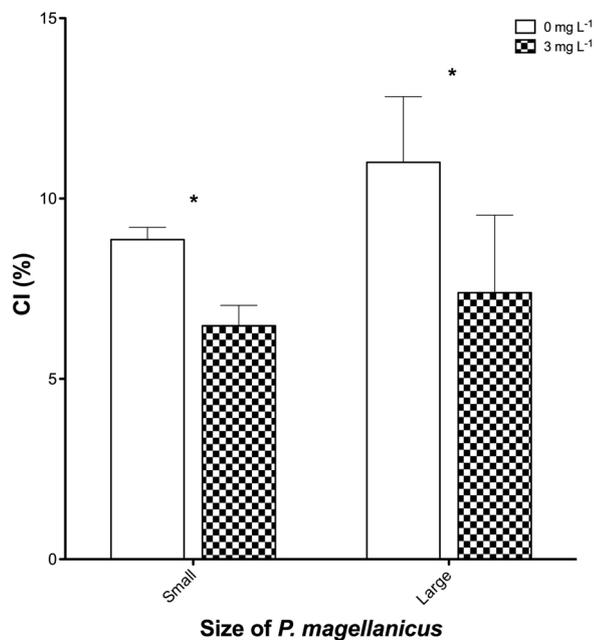


Fig. 4. Condition index (CI) of small and large scallops for individuals 7 days after exposure or not to BW containing 3 mg L⁻¹ of marennine. Values are mean \pm standard error (SEM). Significant differences ($P < 0.05$) indicated by *.

concerning the mechanism of action of this pigment in altering bivalve's physiological rates, the interaction between this pigment and bivalves' pallial organs may enlighten our results. Previous findings from Prasetya et al. (2017a) and Gastineau et al. (2018) revealed that marennine is mainly fixed on the gills of bivalves, but it can also be fixed in the labial palps and mantle cavity (e.g. in *C. gigas*, *C. virginica*, *M. edulis* and *P. magellanicus*). Marennine could thus stimulate the chemoreceptors of the mantle of bivalves, or directly alter gill integrity, thus being responsible for direct effects observed in these animals.

4.2. Marennine effect on scallops: the physiological response depends on the size

In the present work, a significant decrease in CR, VO₂ and CI was detected in young scallops exposed to marennine, but this alteration was visible in the adult scallops only for CR. Thus, the deleterious sensitivity to marennine seems related to the bivalve ontogenic stage. The sensitivity of juvenile scallops to BECs has been demonstrated in Borcier et al. (2017), where the filtration activity of BEC-exposed scallops was significantly reduced. Additionally, differences in the sensitivity to the biotoxin in function of bivalve age have also been demonstrated in the Manila clam *Ruditapes philippinarum*, with the juveniles more vulnerable than the adults when they are exposed to biotoxin from *Alexandrium tamarense* (Bricelj et al., 2011). However, the molecules used in these studies are not similar to marennine and general comparison may be problematic. Gastineau et al. (2018) observed that the juvenile *C. gigas* exposed to a *H. ostrearia* culture supernatant containing marennine (BW) exhibits lower filtration activity than the control group, however, studies on the effect of such BECs produced by non-dinoflagellate species on bivalves are still scarce, which limits the comparisons between species.

4.3. Marennine depuration

In this study, no depuration effect was observed in juvenile scallops, both CR and consumption of oxygen being lower in marennine than in control treatment. These results are perhaps the consequence of an almost irreversible fixation of marennine on gills, which may affect their

functioning, or of a period too short for depuration to happen in only 7 days. Prasetya et al. (2017a), showed that marennine could be fixed in the gills of juvenile *C. virginica* and *M. edulis* for a long period of time, with gills remaining green for up to 8 weeks after marennine exposure. Moreover, marennine may induce stress and depletion in energetic reserves accumulated in the digestive glands of bivalves, indicated by the strong decrease in total fatty acids (FAs) in neutral lipids (Prasetya et al., 2017a). This study also demonstrated that marennine can affect the fluidity of gills' membrane, causing a depletion in oxygen consumption as stress response, which explains its negative impact observed in the condition index of small scallops after only 7 days. Adults were less susceptible to marennine on the long term as the three species showed an increase of the VO₂, 7 days after marennine exposure comparatively to small scallops. Thus, adults seem to present a better depuration process for marennine. In oysters, a similar efficiency can be hypothesized but not in mussels as their CI after 7 days are affected by marennine exposure. Thus, more work, particularly on the depuration duration are needed before the use of this pigment as a natural product decreasing pathogenicity of bacteria in shellfish aquaculture systems to avoid important negative impact on bivalves productivity.

5. Conclusion

The present work demonstrates how the bioactive extracellular compound such as marennine, not known to be harmful, affects the physiological rates of bivalves. Indeed, it has been observed that the supernatant of the diatom *H. ostrearia* culture reduces the clearance rate, oxygen consumption and condition index of bivalves. The alteration in these physiological parameters appeared to be species- and age-specific. These findings improve our understanding of marennine and its possible effect on bivalves, and will allow us to obtain the maximum benefits of this microalgae-derived bioactive compound in shellfish aquaculture.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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