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Effect of marennine produced by the blue diatom *Haslea ostrearia* on behavioral, physiological and biochemical traits of juvenile *Mytilus edulis* and *Crassostrea virginica*

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ABSTRACT

Haslea ostrearia is a marine diatom that synthesizes and releases marennine, a water-soluble blue-green pigment responsible for the greening of the gills and labial palps of bivalves. The present study evaluated the effect of different marennine concentrations (0, 0.5, 1.0 and 2.0 mg l^{-1}) on the behavior (valve opening), physiology (clearance rates, oxygen consumption, assimilation efficiency and scope for growth) and biochemistry (fatty acid composition of neutral and polar lipids) of two commercially important bivalves, the blue mussel *Mytilus edulis* and the eastern oyster *Crassostrea virginica*. Under short-term (<1 day) exposure, the concentration of marennine found on the gills of both species was positively correlated to the concentration of dissolved marennine in the water medium. However, a behavioral response was detected at the higher marennine concentration (2.0 mg l^{-1}) and both species displayed curtailed valve opening compared to control groups. Under longer-term (8 weeks) exposure, marennine (at 2 mg l^{-1}) significantly decreased scope for growth by 58% and 85% (ANOVA; $F_{3, 31} = 3.39$, $P = 0.034$ and $F_{3, 31} = 3.08$, $P = 0.044$) for *M. edulis* and *C. virginica* respectively. The greening process had an effect on total fatty acids contained in the digestive gland of mussels only, suggesting that marennine interferes with the accumulation of energy reserves in this bivalve. In the polar lipids of the gills, greening increased the concentration of unsaturated fatty acids of *C. virginica* but not *M. edulis*, suggesting a possible regulatory mechanism counteracting the marennine effect in oysters. In conclusion, marennine is currently viewed as a non-toxic compound produced by a non-toxic algae species, yet our results show that the substance impairs the biology of bivalves. Such effects should therefore be taken into consideration before the application of mareninne in aquaculture settings.

1. Introduction

Molluscan bivalves acquire food and oxygen by filtering water across their gills. Water movement inside the shell is ensured by gill ciliary motion, triggered by an active mechanism responding to the presence of a dissolved chemical or particulate matter in water. The most visible behavior associated to filtration or ventilation is the opening of the valves, whereas the closing of valves may signal satiation (Morton, 1973; Bayne, 1998; Cranford, 2001) or protection of soft tissues (Gainey and Shumway, 1988; Katticaran and Salih, 1992; Ait Fdil et al., 2006; Hégaret et al., 2007; Ait Ayad et al., 2011). For example, previous work has shown that toxic microalgae producing the paralytic shellfish toxin (PST) can alter bivalve behavior, physiology, and cellular activity (Bricelj et al., 2005). Toxic dinoflagellates in particular can result in valve closure and lowered filtration rate, oxygen consumption, absorption efficiency and Scope for Growth (SFG) (Bardouil et al., 1993; Bricelj et al., 1996; Hégaret et al., 2007, 2011; Navarro and Contreras, 2010). However, little attention has been given to the effects of non-toxic organic compounds on bivalves. Moreover, the few studies on this topic focus exclusively on biotoxin compounds produced by dinoflagellates and the effect that these compounds have on bivalve hemocytes (Hégaret and Wikfors, 2005; Ford et al., 2008).

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The aim of the present study was to assess whether marennine as a non-toxic bioactive compound can affect behavioral, physiological and biochemical traits of bivalves. Specifically, the study focuses on the effects of marennine, a water-soluble blue pigment that is excreted by the non-toxic pennate diatom *Haslea ostrearia* (Gaillon) Simonsen. Marennine binds preferentially to the gills and labial palps of bivalves (Robert, 1975) and is responsible for the greening of Pacific oysters *Crassostrea gigas*. This natural phenomenon, which occurs erratically in oyster ponds on the French Atlantic Coast, increases the economic value of cultured oysters due to the organoleptic modification in green oysters. During its proliferation, marennine concentration in oyster ponds can reach up to 3.4 mg l^{-1} (Turpin et al., 2001). Moreover, marennine has antibacterial and antiviral properties and therefore acts as a natural protective agent against pathogens (Pouvreau et al., 2007, 2008; Gastineau et al., 2012a,b, 2014). For instance, in vitro studies by Gastineau et al. (2012b) demonstrated that marennine at 1 mg l^{-1} was able to inhibit the growth of pathogenic bacteria *Vibrio aesturianus*. Interestingly, several species from the same genus *Haslea* produce "marennine-like" pigments in other parts of the world (Gastineau et al., 2012a, 2014). Hence, there are potential worldwide applications of marennine in shellfish aquaculture.

With respect to response variables, our study focused on valve movement, scope for growth (SFG) and the buildup of energy reserves. SFG represents the energy absorbed during feeding subtracted by the energy lost through respiration and excretion (Widdows and Johnson, 1988; Gilek et al., 1992). SFG is sensitive to environmental stressor such as salinity, temperature and toxic substances (Widdows and Johnson, 1988; Widdows et al., 2002; Pernet et al., 2008, 2007; Navarro and Contreras, 2010). Regarding energy, ectotherm animals such as bivalves have the capacity to remodel their lipid membranes to maintain fluidity in response to temperature or environmental changes (Hulbert and Else, 1999; Hulbert, 2007; Pernet et al., 2007; Parent et al., 2008; Parrish, 2013). Recent works (Pernet et al., 2007, 2008; Parent et al., 2008; Parrish, 2013) suggest that the fatty acid composition of neutral (NL) and polar (PL) lipids is indicative of acclimation. In particular NL represent energetic reserves that support metabolism and growth of organisms, while PL constitute membrane structure where its fluidity may change due to the enlargement or reduction in unsaturated fatty acid content (Bergé and Barnathan, 2005; Fokina et al., 2014, 2015). Responses to marennine were investigated using two commercially important bivalves, the eastern oyster *Crassostrea virginica* and the blue mussel *Mytilus edulis*. First, we tested the hypothesis that dissolved extracellular marennine affects the valve opening of bivalves. We then examined whether greened oysters and mussels had different physiological (clearance rates, oxygen consumption, assimilation efficiency and scope for growth) and biochemical characteristics (tissues fatty acids composition).

2. Materials and methods

2.1. Animals and diet preparation

Juveniles (aquaculture spat) of mussels (*M. edulis)* and oysters (*C. virginica)* were obtained from shellfish aquaculture leases in Prince Edwards Island, Canada (46°25.963 N; 62°39.914 W). Water temperature at the time of collection (end of August 2014) was 16 °C and salinity was 29 ppt. Animals were immediately transported to the Station Aquicole de Pointe-aux-Pères (Institut des Sciences de la Mer, Rimouski, Canada). Upon arrival, 128 individuals of both species (mean shell length = 28.8 ± 0.7 mm for mussels and 25.9 ± 0.9 mm for oysters) were numbered with bee tags, and were then acclimated to laboratory conditions for 30 days before experiment was conducted. An idependent-samples paired *t*-test was conducted to compare the growth (shell length) of both species before and after acclimation period. No significant difference was observed in shell-length for both species, indicating an absence of growth during acclimation (mussels: $t(31) = 2.81$, $p = 0.50$ and oysters: $t(31) = 2.87$, $p = 0.50$). The two species were equally distributed in two 300 l maintenance tanks with light 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 volume and a daily ration of 40 cells μl^{-1}. Algae were sampled two times during the experiment for fatty acid analysis (Table 1).

2.2. Marennine extraction and purification

H. ostrearia was produced in 100 l photobioreactors as described in Gastineau et al. (2014) and extracellular marennine was extracted and purified as described in Pouvreau et al. (2006). Briefly, 1 μm-filtered culture supernatant of *H. ostrearia* was passed through a two-step ultrafiltration process (30 and 3 kDa) with cartridges fitted with regenerated cellulose spiral membranes (Prep/Scale Spiral Wound TFF-6 0.54 m^2 Emd Millipore). At the end of filtration, the retentate was collected and the pigment was then separated by anion-exchange chromatography on a 100 ml column of DEAE sepharose fast flow media using an ÄKTAFPLC system and a XK50 column (GE Lifesciences). Finally, the EMn collected was then dialyzed, freeze-dried and stored in the dark at −20 °C until use.

2.3. Behavioral response to marennine

Valve opening was closely monitored during a short-term (17.5 h) exposure to marennine. The experiment simultaneously tested four

Table 1
Total fatty acid composition of microalgal species used in the diet for mussels and oysters. Values are mean ± Standard Error.

Variable	*Chaetoceros gracilis* (CHGRA)	*Tisochrysis lutea* (TISO)	*Pavlova lutheri* (PLUTH)
Fatty acid composition (mol %)			
14:0	0.00 ± 0.00	15.74 ± 0.96	9.93 ± 0.48
15:0	0.82 ± 0.07	0.58 ± 0.02	0.45 ± 0.01
16:0	29.37 ± 1.31	18.63 ± 0.32	19.87 ± 0.40
17:0	0.25 ± 0.02	0.59 ± 0.05	0.44 ± 0.02
18:0	2.02 ± 0.02	2.86 ± 0.18	2.71 ± 0.10
20:0	0.14 ± 0.00	0.87 ± 0.17	0.59 ± 0.04
21:0	0.00 ± 0.00	0.28 ± 0.27	0.28 ± 0.27
22:0	0.21 ± 0.01	1.60 ± 0.38	0.96 ± 0.07
24:0	0.17 ± 0.07	1.26 ± 0.28	0.49 ± 0.48
Σ SFA	33.11 ± 1.42	42.40 ± 0.81	35.71 ± 0.12
14:1	0.07 ± 0.00	0.54 ± 0.09	0.00 ± 0.00
16:1	29.81 ± 0.35	7.19 ± 0.89	20.21 ± 0.40
17:1	0.00 ± 0.00	0.81 ± 0.05	0.00 ± 0.00
18:1 n-9c	2.69 ± 0.27	12.60 ± 0.06	2.31 ± 0.16
18:1 n-9 t	2.40 ± 0.28	0.00 ± 0.00	0.00 ± 0.00
18:1 n-7	0.03 ± 0.00	3.08 ± 0.04	2.94 ± 0.03
20:1	0.22 ± 0.01	0.82 ± 0.14	0.64 ± 0.02
22:1 n-9	0.08 ± 0.00	0.76 ± 0.13	0.51 ± 0.03
22:1 n-11	0.02 ± 0.00	0.77 ± 0.14	0.53 ± 0.04
24:1	0.15 ± 0.00	0.55 ± 0.10	0.43 ± 0.02
Σ MUFA	35.48 ± 0.19	27.14 ± 0.33	27.56 ± 0.41
18:3 n-3	0.53 ± 0.05	3.93 ± 0.21	1.14 ± 0.01
18:3 n-6	2.72 ± 0.15	1.57 ± 0.09	0.55 ± 0.50
18:4 n-3	3.01 ± 0.32	0.00 ± 0.00	0.00 ± 0.00
18:2 n-6c	2.50 ± 0.09	2.47 ± 2.40	1.16 ± 0.04
18:2 n-6 t	0.48 ± 0.05	1.04 ± 0.34	0.61 ± 0.05
20:4 n-6	5.84 ± 0.07	0.93 ± 0.09	0.90 ± 0.02
20:5 n-3	9.67 ± 0.30	4.51 ± 0.41	20.36 ± 0.48
20:3 n-6	0.67 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
20:3 n-3	0.11 ± 0.01	0.80 ± 0.16	0.00 ± 0.00
20:2	0.13 ± 0.02	0.71 ± 0.15	0.60 ± 0.03
22:6 n-3	4.34 ± 0.31	12.77 ± 1.53	9.92 ± 0.05
22:2	0.15 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:5 n-3	1.27 ± 0.01	1.72 ± 0.03	1.49 ± 0.02
Σ PUFA	31.41 ± 1.23	30.46 ± 1.15	36.72 ± 0.28
Total fatty acids ($\mu g\ g^{-1}$)	185.11 ± 15.70	36.56 ± 10.75	34.40 ± 5.42