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# Does allelopathy affect co-culturing *Haslea ostrearia* with other microalgae relevant to aquaculture?

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**Abstract** *Haslea ostrearia* is a marine diatom known to produce marennine, a water-soluble blue-green pigment responsible for the greening of oysters in ponds along the French Atlantic coast. This phenomenon occurs seasonally when *H. ostrearia* blooms in oyster ponds, and it increases the economic value of cultured oysters. From an ecological perspective, *H. ostrearia* blooms are accompanied by a decrease in the abundance of other microalgae, suggesting that this diatom produces allelochemicals. Recent studies showed that purified marennine has other biological activities, for instance antioxidant, antibacterial, and antiviral activities, which could be used in aquaculture to promote this pigment as a natural antipathogen agent. One important issue regarding the possible use of *H. ostrearia* in aquaculture as a mixed algal diet, however, is the importance of marennine allelopathy. In this study, we investigated the allelopathic effect of *H. ostrearia* on the growth of five microalgal species relevant to aquaculture: *Chaetoceros calcitrans*, *Skeletonema costatum*, *Phaeodactylum tricornutum*, *Tetraselmis suecica*, and *Tisochrysis lutea*. Allelopathic tests were realized by co-culturing these microalgae with *H. ostrearia* in batch and in semi-continuous mode, based on initial biovolume ratios. Our findings showed that inhibition of the growth of microalgae due to the presence of *H. ostrearia* and marennine was species dependent. *Skeletonema costatum*, *C. calcitrans*, and *T. lutea* were significantly more sensitive, whereas *T. suecica* and *P. tricornutum* appeared to be more resistant. Growth irradiance significantly influenced the allelopathic effect against the sensitive species *S. costatum*, and the *H. ostrearia* production of marennine increases with irradiance. Data presented in this study partly support the hypothesis that marennine released into the culture medium possibly acts as an allelochemical compound, thus explaining the dominance of *H. ostrearia* and the loss of sensitive algae in oyster ponds, but also that some species are insensitive, which allows co-culturing and use in a mixed algal diet in aquaculture.

**Keywords** Allelopathy · Aquaculture · *Haslea ostrearia* · Marennine · Microalgae

Fiddy S. Prasetiya and Ikha Safitri contributed equally to this work.

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## Introduction

Autotrophic microorganisms compete for resources, such as light and nutrients, which can involve chemical interactions with toxins or allelopathic compounds (e.g., Legrand et al. 2003; Leflaive and Ten-Hage 2007). In general, allelopathy is defined as a toxicological interaction between an "emitter" and its direct competitors or predators, the "target organisms" (Leflaive and Ten-Hage 2007). The emitter organism produces and releases metabolites that cause a variety of negative effects, for instance growth inhibition, cell lysis, loss of motility, and even death of the target organisms (Arzul et al. 1999; Inderjit and Duke 2003; Tillmann et al. 2007; Tang

and Gobler 2011). In an aquatic ecological context, allelopathic interactions play important roles in species successions and occurrence of blooms (Keating 1977; Takano et al. 2003). Like many phytoplankters, diatoms produce allelochemical and toxigenic compounds (e.g., Sharp et al. 1979; Yamasaki et al. 2007; Ianora and Miralto 2010), including polyunsaturated fatty acids (PUFAs) (Jüttner et al. 2001) and polyunsaturated aldehydes (PUAs) (Adolph et al. 2003; Jüttner 2005; Ribalet et al. 2007, 2009). These compounds can have negative effects on bacteria (Desbois et al. 2008, 2009), phytoplankton competitors from different taxonomic groups, including diatoms (Sharp et al. 1979; Casotti et al. 2005; Yamasaki et al. 2007) and also grazers (Jüttner et al. 2001; Jüttner 2005; Pohnert et al. 2002).

The pennate diatom *Haslea ostrearia* can co-occur with other phytoplankton in oyster-fattening ponds along the west coast of France. This diatom has the particular feature of synthesizing and excreting the water-soluble blue pigment marennine, responsible for the greening of oysters, an erratic and little-controlled phenomenon, which gives an added value to the bivalve *Crassostrea gigas* in the French oyster industry. Previous works have hypothesized that marennine accumulation in cells was correlated with an unfavorable environment, such as nutrient deficiency (Neuville and Daste 1978; Robert 1983). This could be, however, the transient result of cell division slowdown, as it also has been observed that light was an important factor for both growth and marennine production (Mouget et al. 1999, 2004, 2005; Rech et al. 2008). The presence of *H. ostrearia* and marennine in oyster ponds may have an impact on co-occurring phytoplankton. Indeed, during the greening process in oyster ponds, a bloom of *H. ostrearia* is observed, concomitant with a significant decrease in phytoplankton, particularly *Skeletonema costatum* and *Nitzschia closterium* populations, and some authors have suggested that the dominancy of *H. ostrearia* could occur by allelopathic interactions (Moreau 1970; Neuville and Daste 1978; Turpin et al. 1999). This was confirmed by Pouvreau et al. (2007), who showed that the purified form of marennine could act as an allelopathic compound, affecting the growth and development of some diatom species, not only directly by contact but also indirectly through a shading effect in the water column (absorption in the red part of the spectrum). Thus, the persistency as well as the erratic dominance of *H. ostrearia* in oyster ponds could be explained by possible allelopathic interactions with co-occurring microalgae.

Apart from allelopathic property, it has been demonstrated that purified marennine has other biological activities, for instance antioxidant (Pouvreau et al. 2008), antibacterial, antiviral, and antiproliferative activities (Gastineau et al. 2012c). Some of these biological activities could be of great importance in the field of aquaculture. For instance, in vitro study showed that purified forms of marennine significantly inhibited the development of *Vibrio splendidus* and *Vibrio aestuarianus*, which are important pathogens contributing to summer mass mortality of oysters in European region and worldwide (Gastineau et al. 2012c, 2014). Both antibacterial and antiviral activities observed in vitro could lead to the potential development of *H. ostrearia* and marennine with maximum benefit to the oyster industry and, more widely, to sustainable aquaculture. Indeed, conventional methods for controlling microbial pathogens in aquaculture by the use of chemical disinfectants and antimicrobial drugs have led to antibiotic-resistant bacterial strains that may cause a significant decrease in animal production (Alderman and Hastings 1998; Cabello 2006). Therefore, in recent aquaculture development, utilization of natural antibiotics is becoming progressively favored as a feasible method in management practices for disease prevention in bivalve hatcheries (Van den Bogaard and Stobberingh 2000; De et al. 2014). Hence, *H. ostrearia* and marennine could be good candidates for use as a natural protector in shellfish larviculture, a very susceptible stage to pathogenic bacteria and viruses in mass mortality events (Paillard et al. 2004).

The present study focuses on the assessment of the allelopathic effect of *H. ostrearia* in realistic conditions of microalgal co-cultures (batch and semi-continuous mode, low cell density), to identify which algal species relevant for aquaculture are either sensitive or resistant to *H. ostrearia* and its supernatant containing marennine. The hypothesized source of allelopathy, marennine in solution in the growth medium, was measured throughout the body of *H. ostrearia* cultures and filtered culture supernatants. Growth kinetics and allelopathic tests were conducted on several microalgae species commonly used in aquaculture as feed of *C. gigas*, or in hatcheries, and possible diet combinations are discussed. To circumvent any bias on allelopathic effect resulting from possible competition or nutrient and light limitations, cultures were maintained at low cell density using a semi-continuous mode.

## Materials and methods

### Culture conditions

Six marine microalgal strains relevant for aquaculture were used in this work: *Haslea ostrearia* (NCC-148.7), *Skeletonema costatum* (NCC-53), and *Phaeodactylum tricornutum* (NCC-18), obtained from the Nantes Culture Collection (NCC), and *Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Tisochrysis lutea*, provided by Ifremer, Nantes. All species were cultured under non-axenic conditions in sterilized 500-mL Erlenmeyer flasks, containing 250 mL of artificial seawater medium (Mouget et al. 2009) at 16±1 °C. Cultures were grown at irradiance of 100 μmol photons $m^{-2}$ $s^{-1}$ provided by Philips TLD 36 W/965

fluorescent tubes or a high-intensity discharge lamp (Osram HQI T, 400 W). Irradiance was measured with a Li-Cor LI-189 quantum meter and a 2π Li-Cor Q21284 quantum sensor, in a 14:10 h light/dark cycle.

## Experimental setup

All series of experiments encompassed the measurement of the cellular volume of the different species of microalgae. The rationale was to consider possible allelopathic effects in a function of both cell volume ratios and cell concentrations. For each target species, allelopathic effects were inferred from the difference in growth rates and kinetics between monospecific cultures (controls) and co-cultures with *H. ostrearia* and from marennine concentration in the culture medium.

### *Biovolume measurement of microalgae*

The aim of this measurement was to estimate the cell volume of each microalgal species and to start allelopathic experiments (co-cultures) using the same volume of biomass (see Table 1). The purpose of using the same volume of biomass was to remove bias resulting from differences in biomass initial value (inoculum) when species were co-cultured (i.e., the same initial biovolume, but different initial cell concentrations). Calculations of surface area and biovolume of the different species of microalgae were performed according to the method of Hillebrand et al. (1999). The ratios between the biovolumes of *H. ostrearia* and those of other microalgae tested are presented in Table 1. Biovolume measurement was conducted when cells were in the exponential phase of growth. A light microscope (Zeiss Axiostar Plus) with a magnification of ×400–1000 depending on the species was directly connected to a camera (AxioCam iCc 1) and computer. ImageJ software was used to measure the length, width, and height of microalgal cells.

### *Allelopathic test of H. ostrearia on microalgae tested*

Algal cultures were grown in batch mode to identify the beginning and the range of the exponential phase for all species tested and in semi-continuous mode to maintain the culture in exponential growth at low cell density. The semi-continuous mode of culture minimizes competition for nutrients and light, which possibly occurred when cells are maintained in batch culture. For each species, cells were acclimated to their growth conditions for at least 1 week and maintained in exponential growth phase by dilution with fresh medium every 4 days. Approximately 75 % of culture total volume at the 4th day was discarded, and the fresh medium was added to the remaining 25 % to complete to the initial volume (250 mL). The cell concentration and the cell biovolume for each microalga were determined every 2 days in batch mode or every 4 days in semi-continuous mode, for 12 to 16 days (at least three consecutive dilution cycles). On each sampling day, the samples were gently stirred in the Erlenmeyer flask prior to cell counting to avoid aggregates. Furthermore, cell growth was monitored by measuring cell densities with Nageotte or Neubauer counting chambers. Growth rate was calculated as $\mu$ (day$^{-1}$) using Eq. 1:

$$\mu = \frac{\ln N_2 - \ln N_1}{d_2 - d_1} \quad (1)$$

where $N_1$ and $N_2$ represent the cell density at the start and the end of each growth period and $d_1$ and $d_2$ are the time of measurement.

Tests for the allelopathic effect were performed in sterile Erlenmeyer flasks containing *H. ostrearia* (final cell density, $5 \times 10^3$ cells mL$^{-1}$) and *T. lutea*, *T. suecica*, *C. calcitrans*, *S. costatum*, or *P. tricornutum* (final cell density varying according to the species, to reach the same total biovolume as *H. ostrearia*; see biovolume ratio, Table 1). To estimate the effect of allelopathy of *H. ostrearia* and supernatant

**Table 1** Mean cell growth rate (day$^{-1}$), cell biovolume (μm$^3$), and cell surface area (μm$^2$) during the exponential growth phase of several microalgae in semi-continuously cultivated monospecific culture

| Species | Exponential growth rate (day$^{-1}$) | Cell biovolume (μm$^3$) | Cell surface area (μm$^2$) | Shape model of biovolume used | Biovolume ratio |
|---|---|---|---|---|---|
| *H. ostrearia* | 0.51 ± 0.03 | 758 ± 11 | 765 ± 7 | Prism on elliptic base | 1 |
| *T. suecica* | 0.44 ± 0.02 | 382 ± 17 | 155 ± 6 | Prolate spheroid | 2 |
| *P. tricornutum* | 0.41 ± 0.01 | 80 ± 2 | 117 ± 2 | Half-elliptic prism | 9 |
| *T. lutea* | 0.75 ± 0.04 | 58 ± 5 | 64 ± 4 | Prolate spheroid | 13 |
| *S. costatum* | 0.29 ± 0.02 | 29 ± 0 | 81 ± 1 | Cylinder + 2 half spheres | 27 |
| *C. calcitrans* | 0.22 ± 0.01 | 17 ± 1 | 40 ± 1 | Half-elliptic prism | 44 |

Cell biovolume for each species was calculated based on the geometric shape for cell biovolume in Hillebrand et al. (1999). Values are means ± standard error. Mean growth rates are the average of the rates in exponential phase in each dilution cycles ($n = 4$) at each replicate of species ($n = 3$). Cell biovolume for each species was calculated at the exponential phase ($n = 40$)

containing marennine, the percent inhibition (I%) was calculated as the percent difference in growth rate of the treatment relative to control (Eq. 2):

$$\text{Percent inhibition (I\%)} = \frac{\mu_c - \mu_t}{\mu_c} \quad (2)$$

where $\mu_c$ and $\mu_t$ represent the growth rate controls (monospecific culture) and treatments (co-culture) at the exponential phase, respectively.

*Effect of light intensity on marennine production and allelopathic activity*

This series of experiments was run using one sensitive microalgal species, *S. costatum*, evidenced as previously described. Co-cultures of microalgae (*H. ostrearia* and the target species) were prepared according to the biovolume ratio method and grown at different irradiances, 20, 100, and 500 μmol photons $m^{-2}$ $s^{-1}$, representing low (limiting), medium, and high (saturating) light, respectively. For each co-culture, marennine concentration was determined as described below. For each algal species, cell density, growth rate, and percent inhibition were determined as described above.

*Estimation of marennine concentration*

The concentration of extracellular marennine (EMn) present in each sample was calculated at the end of the growth period (at $t = 12$th day and at each dilution cycle or 4th day, for batch and semi-continuous culture, respectively). Co-culture supernatants were filtered on a Millipore filter (0.22 μm) prior to the measurement. Afterwards, the absorbance of the supernatant was measured by UV-visible spectrophotometry (Perkin Elmer Lambda 25) and the concentration was determined as described in Robert et al. (2002). The concentration ($C$) of EMn (g $L^{-1}$) was calculated according to the following formula (Eq. 3):

$$[C] = \frac{A\lambda_{max}}{\varepsilon\lambda_{max} \times \ l} \quad (3)$$

where $A\lambda_{max}$ is the absorbance at the peak wavelength in the red region (674 nm), $\varepsilon\lambda_{max}$ is the specific extinction coefficient at the peak wavelength, and $l$ is the cuvette path length.

*Statistical analyses*

All data were analyzed using SigmaPlot version 12.0 for Windows. Prior to statistical analyses, normality and homogeneity of data were checked using the Shapiro-Wilk and Kolmogorov-Smirnov test, respectively. All statistical analyses were performed at a maximum significance level of 5 % by one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) post hoc multicomparison test. In addition, analysis of covariance (ANCOVA) was performed to test the effect of different light levels and marennine concentrations on the allelopathic activity of *H. ostrearia* on the most sensitive target microalgal species.

## Results

### Biovolume and growth kinetics of *H. ostrearia* and microalgae tested

Microalgal biovolume allows estimation of the relative importance of co-cultured algae in a global biomass. For a single cell, the variation in cell biovolume corresponds to the different shapes and sizes (length, width, and depth) (Table 1). Based on the calculation of the length of transapical axis of the microalgae tested, they can be classified into two classes, *P. tricornutum* and *T. suecica*, representing the large class (mean transapical axis = 22.3 ± 0.3 and 12.6 ± 0.2 μm, respectively), whereas *S. costatum* (4.4 ± 0.1 μm), *C. calcitrans* (4.5 ± 0.1 μm), and *T. lutea* (5.1 ± 0.1 μm) represent the small class. The determination of cell biovolumes, using the standard geometric shapes of Hillebrand et al. (1999), showed that *H. ostrearia* had the highest average cell biovolume followed by *T. suecica*, *P. tricornutum*, *T. lutea*, *S. costatum*, and *C. calcitrans* (Table 1). For the allelopathy tests with co-cultures in batch mode and in semi-continuous mode, experiments were run, taking into account the cell biovolume ratios, to set the same initial total biovolume for each species.

During growth kinetics in batch mode, *P. tricornutum* and *T. lutea* showed the highest cell concentration ($702 \times 10^4$ and $650 \times 10^4$ cells $mL^{-1}$, respectively) by the end of the growth phase ($t = 12$) whereas *H. ostrearia* had the lowest value ($13 \times 10^4$ cells $mL^{-1}$, Fig. 1(A)). Variation was also observed in terms of the total biovolume (μL) (ANOVA: $F_{5, 18} = 1821.1$, $p < 0.0001$). The biovolume of *P. tricornutum* was significantly higher than that of other species (Tukey's HSD: $p < 0.0001$), whereas *S. costatum* had the lowest. As to *H. ostrearia*, it showed the lowest cell concentration but a significantly higher total biovolume than the two other diatoms, *S. costatum* and *C. calcitrans* (Tukey's HSD: $p < 0.0001$ and $p = 0.0004$, respectively) (Fig. 1(B)).

Semi-continuous cultures were conducted after batch cultures by dilution of algal suspensions every 4 days to maintain cells in exponential growth phase at low cell density. Each microalga cultivated in a semi-continuous system showed a stabilization of growth, with only slight differences in cell concentration on the 4th day for each dilution (Fig. 1(C)). Variation occurred in terms of total biovolume (ANOVA: $F_{5, 54} = 132.8$, $p < 0.001$), where *P. tricornutum* has a significantly higher total biovolume than other microalgae (Tukey's HSD: $p < 0.0001$) (Fig. 1(D)). Furthermore, *T. lutea* showed the

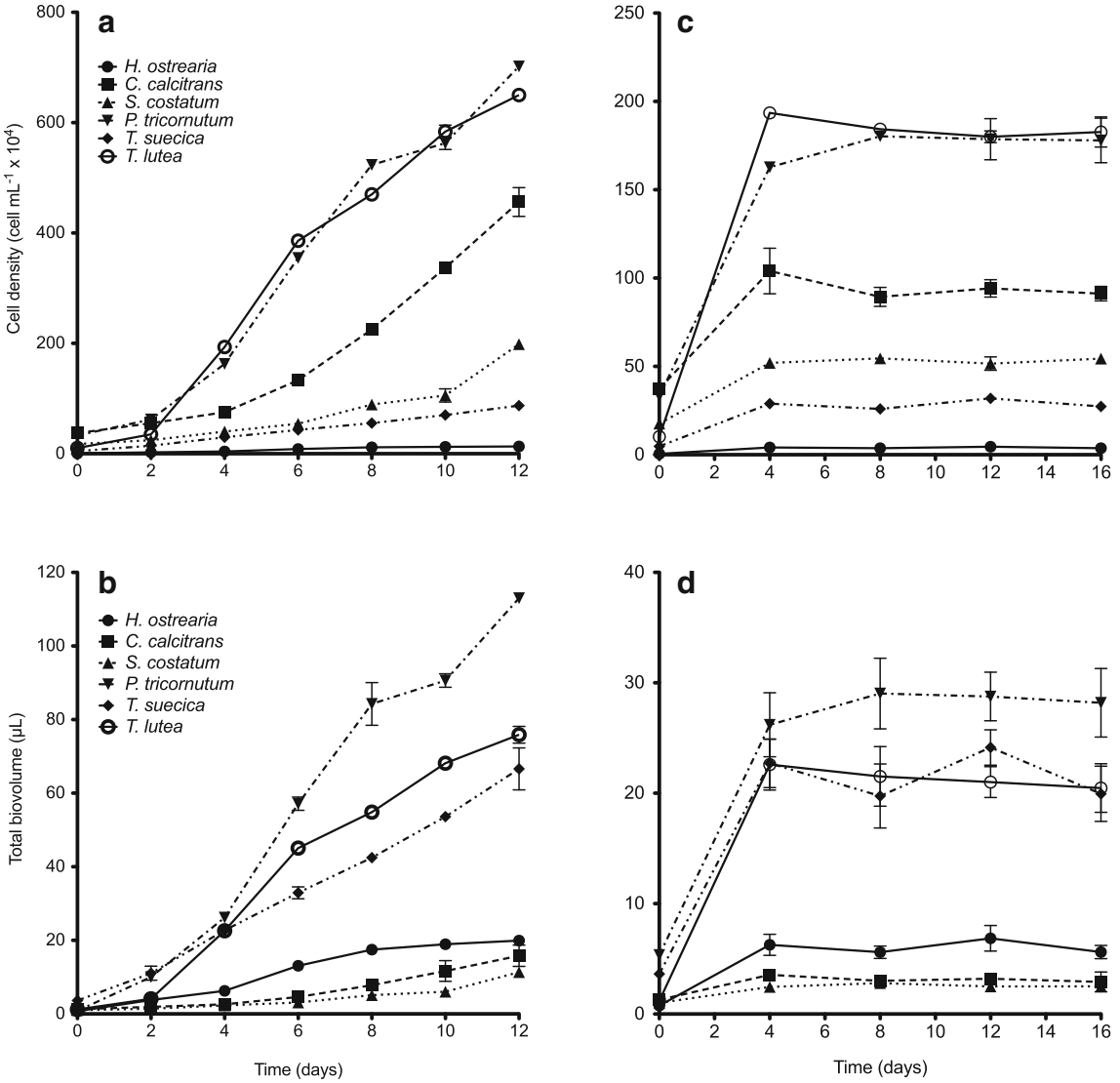


**Fig. 1** Growth kinetics of different species of microalgae cultivated in batch (*A*, *B*) and semi-continuous (*C*, *D*) modes, expressed as cell concentration (*A*, *C*) ($10^4$ cells mL$^{-1}$) and total biovolume (*B*, *D*) (µL) in 500-mL Erlenmeyer flasks, containing 250 mL of artificial seawater medium. Values are mean ± standard error ($n = 3$)

highest average growth rate whereas *C. calcitrans* exhibited the lowest value (0.75 and 0.22 day$^{-1}$, respectively) (ANOVA: $F_{5,\ 54} = 500.1$, $p < 0.001$). For each species, no significant difference was observed in terms of cell density, total biovolume, and growth rate in all cycles of dilution ($p > 0.05$), illustrating that the semi-continuous mode was stable (Fig. 1(C, D)).

### Growth inhibition in co-culture of *H. ostrearia* with different microalgae

At the beginning of allelopathic co-culture experiments, the initial cell concentration of *H. ostrearia* in each flask was 5000 cells mL$^{-1}$ while the cell number of target species was different according to cell biovolume ratios. Allelopathic test kinetics showed variations in cell concentration (not shown) and in total biovolume of the target species, in response to the presence of *H. ostrearia* when they were co-cultured (Fig. 2a, b, batch and semi-continuous mode of culture, respectively). For algae co-cultured in a semi-continuous mode, pooled mean from the three consecutive cycles of dilution showed that *H. ostrearia* significantly hampered the growth rate of all species tested as compared to the respective controls (ANOVA: $F_{15,\ 54} = 366.1$, $p < 0.001$). The decrease in growth rate, expressed as the percent inhibition (I%), was the highest for *C. calcitrans* (79.4 ± 5.5 %) and *S. costatum* (74.2 ± 2.5 %), followed by *T. lutea* (52.1 ± 1.8 %), and was the lowest for *T. suecica* (37.2 ± 3.6 %) and *P. tricornutum* (29.5 ± 1.9 %) ($p < 0.05$) (Fig. 3(A)). Pearson's correlation showed that the size of microalgae influenced the allelopathy interaction in co-culture. Percent inhibition of the target species

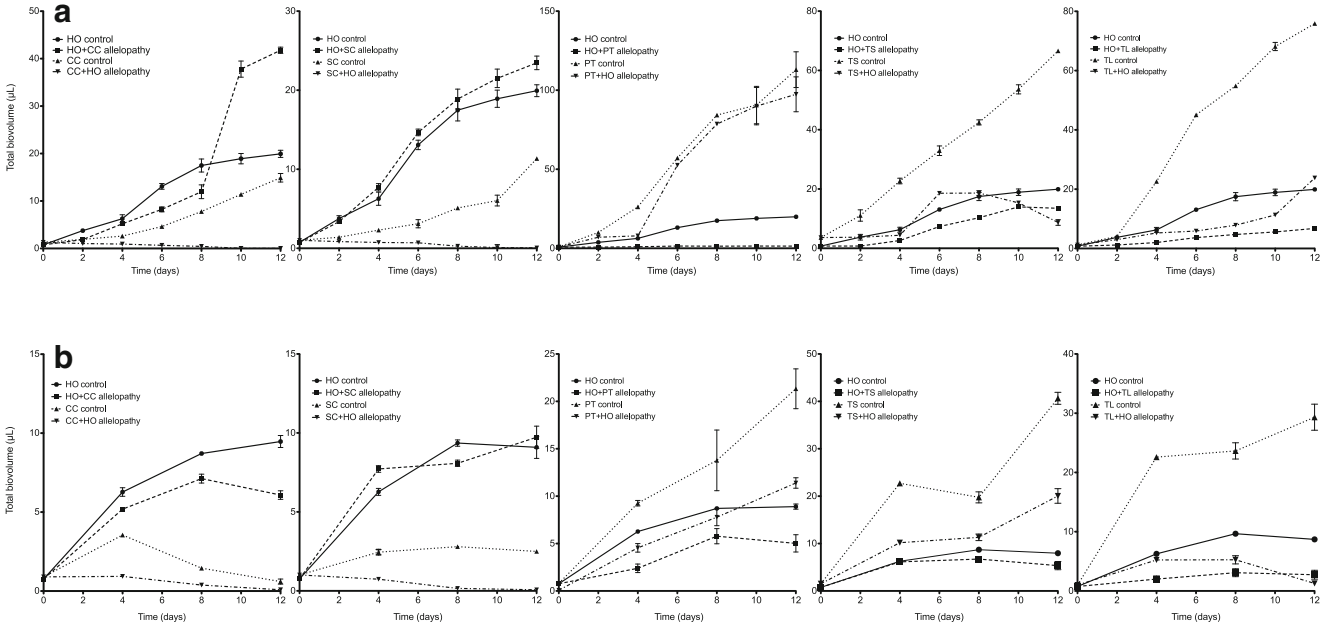


**Fig. 2** Growth kinetics of different microalgae co-cultured in allelopathic tests in batch (**a**) and semi-continuous (**b**) modes. Values are means ± standard error ($n = 3$) of total biovolumes (µL). *CC Chaetoceros calcitrans*, *SC Skeletonema costatum*, *PT Phaeodactylum tricornutum*, *TS Tetraselmis suecica*, *TL Tisochrysis lutea*, *HO Haslea ostrearia*

tested was negatively correlated with their cell biovolume and also their cell surface area ($r = -0.727$ and $-0.754$, respectively) (Fig. 4a, b). The percent inhibition was positively correlated with the cell surface area-to-volume ratio ($r = 0.776$) (Fig. 4c).

## Concentration of extracellular marennine in co-culture supernatants

During growth, the blue diatom *H. ostrearia* synthesizes and releases to the culture medium the pigment marennine, which has been shown responsible for the inhibitory effect of growth of target species when they were co-cultured (Pouvreau et al. 2007). In the present work, a posteriori quantification of extracellular marennine (EMn) concentration in the supernatants was conducted every 4 days, at the end of each dilution cycle yet during the exponential growth phase. For all co-cultures, the EMn concentration was not significantly different between all three cycles of dilution ($p > 0.05$). The highest EMn concentration was observed in the co-culture of *S. costatum*, *C. calcitrans*, or *T. suecica* + *H. ostrearia* (maximum value of $1.04 \pm 0.25$ mg $L^{-1}$, mean ± SE, $n = 3$) and the lowest in the co-culture of *P. tricornutum* or *T. lutea* + *H. ostrearia* (maximum value of $0.49 \pm 0.10$ mg $L^{-1}$, mean ± SE, $n = 3$) (Fig. 3(B)).

A significant relationship was observed between the concentration of EMn and the percent inhibition (I%) in target species tested. Pearson's correlation showed that I% was positively correlated with the amount of EMn released into the medium ($r = 0.775$) (Fig. 4d). In contrast, a negative relationship was observed between EMn concentration and the growth rate of microalgae tested in co-culture ($r = -0.750$), suggesting that a higher decrease in growth rate of the target microalgae resulted from a higher EMn released in the culture.

A complementary experiment was conducted with the sensitive species *S. costatum* to test the hypothesis that growth inhibition was not the direct consequence of the presence of *H. ostrearia* cells in the co-culture. Actively growing *S. costatum* was subcultured at low density (initial cell concentration = $37 \times 10^4$ cell $mL^{-1}$) with a mixture of fresh medium and *H. ostrearia* culture supernatant (no nutrient supplementation), in proportions (100:0, 70:30, and 30:70 *v/v*), which corresponded to 0.0, 3.6, and 8.4 mg $L^{-1}$ of marennine, respectively. In comparison with the control (no marennine, 100 % fresh medium), the growth of *S. costatum* decreased significantly when exposed to marennine-containing supernatant, percent inhibition I% increasing with time, and proportion of supernatant or marennine concentration (Fig. 5). Percent inhibition (I%) values were in accordance with I% observed in the co-culture experiment in batch mode (Fig. 2a) on days 2 and 4, $78.59 \pm 4.1$ and $87.7 \pm 1.4$ % (mean ± SE, $n = 3$), respectively.

## Effect of irradiance level on allelopathic activity of *H. ostrearia*

In this series of experiments, *S. costatum* was again chosen as the vulnerable species to determine whether the irradiance level may influence the allelopathic activity of *H. ostrearia*. For monospecific batch and semi-continuous cultures, the

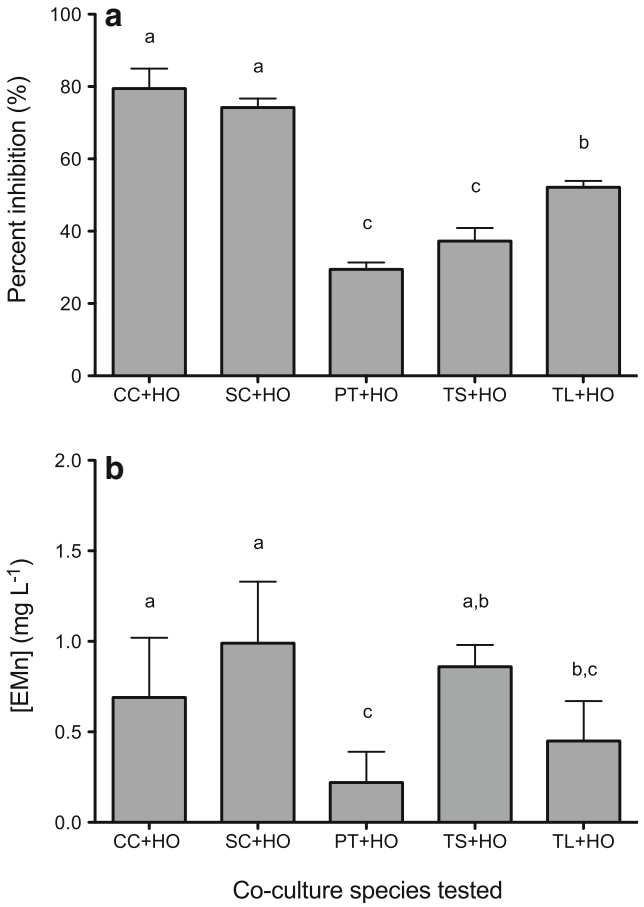


**Fig. 3** Percent inhibition (I%) of target algae (*A*) and concentration of extracellular marennine (EMn, mg $L^{-1}$) (*B*) in co-cultures with *Haslea ostrearia*. Values are means ± standard error ($n = 3$) obtained from three consecutive cycles of dilutions. *Different letters* indicate significant differences. *CC Chaetoceros calcitrans*, *SC Skeletonema costatum*, *PT Phaeodactylum tricornutum*, *TS Tetraselmis suecica*, *TL Tisochrysis lutea*, *HO Haslea ostrearia*

growth rate of *S. costatum* increased significantly with irradiance ($p < 0.05$) (Table 2). As for *H. ostrearia*, both in semi-continuous mode and in batch mode, however, the growth rate of *H. ostrearia* at low irradiance was significantly lower compared to medium and high irradiance but no significant difference was observed between the last two levels of irradiance ($p > 0.05$).

Following the acclimation in batch mode, co-cultures of *H. ostrearia* and *S. costatum* were performed in semi-continuous mode at limiting or saturating irradiance to observe the manifestation of the allelopathic effect, in terms of growth rate and percent inhibition. The pooled mean from three consecutive cycles of dilution in co-culture showed that a significant difference was observed in the cell concentration (not shown) and growth rate of *S. costatum* at all irradiance levels comparative to the respective controls ($p < 0.05$) (Table 2). The decrease in *S. costatum* cell concentration and growth rate at all irradiances was concomitant with an increase in I%. At low irradiance, I% was significantly lower than at medium and high irradiance ($p = 0.012$ and 0.011, respectively). Furthermore, I% increased with EMn concentration (Table 2). Pooled data from all cycles of dilution showed that EMn concentration at medium and high irradiance levels in co-culture was significantly higher than at low irradiance ($p < 0.05$). It appeared that EMn concentration in the medium was not correlated significantly with *H. ostrearia* growth rate ($r = 0.314$, Fig. 6a) but with irradiance ($r = 0.931$), cell density ($r = 0.724$), and percent inhibition ($r = 0.876$) (Fig. 6b–d, respectively). Furthermore, a significant correlation was observed between EMn production per day and EMn production per cell in a function of the irradiance level, in both batch and semi-continuous co-cultures (Fig. 7(A, B), respectively).

## Discussion

Green oysters (known as “vertes de claires” in France) contribute to the economy and the standing local oyster production in Marennes-Oléron and Bourgneuf bays. The importance of *H. ostrearia* and marennine as a greening agent in oyster-fattening ponds has long been recognized (Gaillon 1820; Lankester 1886). It has also been demonstrated that *H. ostrearia* as a monospecific algal diet can sustain the growth of the oyster *Crassostrea gigas* for 8 weeks (Piveteau 1999; Cognie 2001) and that it is well digested with almost 90 % of digestibility (Barillé et al. 1994). Moreover, recent findings on marennine biological activities have awaken interest as a possible natural antibiotic and antiviral compound for aquaculture (Gastineau et al. 2012c, 2014) but also demonstrated that it has an allelopathic activity on some diatoms usually encountered in oyster ponds (Pouvreau et al. 2007). Thus, for potential industrial applications in aquaculture or larviculture, it has become important to determine the possible allelopathic pressure of *H. ostrearia* cultures on other microalgae in realistic conditions. For this reason, in the present work, *H. ostrearia* cultures or raw supernatants were tested, in contrast to purified pigments in Pouvreau et al. (2007). As a consequence, theoretically, any inhibitory effect observed in co-culturing the blue *Haslea* with aquaculture-relevant algae could be interpreted as a true allelopathic effect, either due to marennine or another compound released in the supernatant or to a competition between phytoplankton species or cell-to-cell interactions (effects of contact). However, a competition for light and nutrients was highly minimized, as co-cultures were grown in a semi-continuous mode and maintained in exponential phase at low cell density. Moreover, effects of contact interactions should have been rare, if not unlikely, *H. ostrearia* behaving like a benthic species, whereas most of the target microalgae are rather pelagic and *Haslea* culture supernatants (not containing cells) have been shown to exert allelopathic pressure.

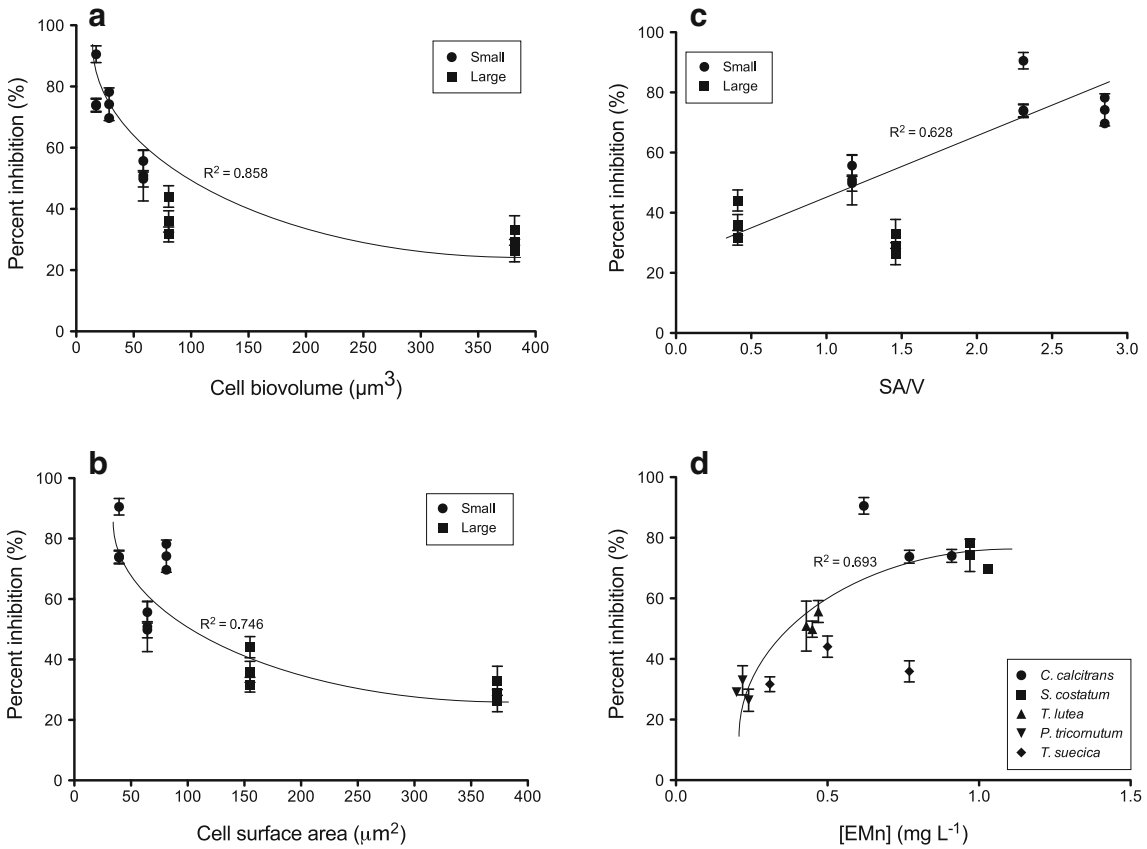


**Fig. 4** Percent inhibition (I%) of microalgal target species tested in co-culture with *Haslea ostrearia* as a function of their respective cell biovolume (**a**), cell surface area (**b**), surface area-to-volume ratio (**c**), and marennine concentration measured at the end of the cycles of dilution (**d**). Data points are means ± standard error ($n = 3$). Pearson's correlation showed a strong negative correlation between I% and cell biovolume ($r = -0.731$) and total surface area ($r = -0.778$), yet positive correlation was observed between percent inhibition and the total surface area-to-volume ratio ($r = 0.774$) and marennine concentration in a co-culture of microalgal target species tested ($r = 0.775$)

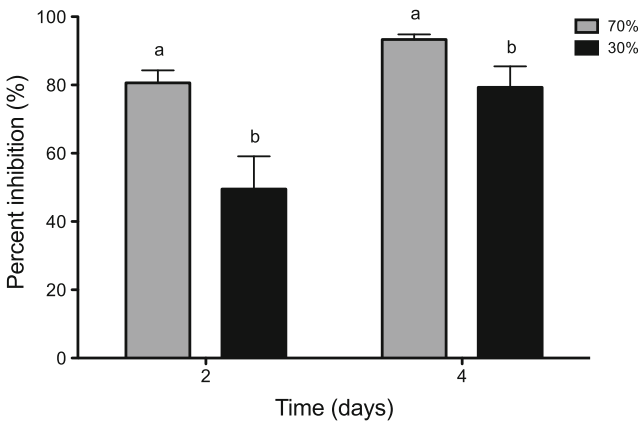


**Fig. 5** Percent inhibition of *Skeletonema costatum* grown for 4 days at low density (initial cell concentration, $37 \times 10^4$ cells $mL^{-1}$) in a mixture of fresh medium and *H. ostrearia* culture supernatant (70:30 and 30:70 *v/v*, which corresponded to 3.6 and 8.4 mg $L^{-1}$ of marennine, respectively). The results are expressed in a function of control (*S. costatum* subcultured in 100 % fresh medium, without marennine). Values are means ± standard error ($n = 3$)

## Allelopathic effect of *H. ostrearia* on the growth of co-cultured algae

The allelopathic effect of *H. ostrearia* on the growth of other microalgal species has already been demonstrated in the laboratory, both using pigmented supernatants of *H. ostrearia* cultures (crude extracts, Neuville and Daste 1978; Robert and Turpin 1993) and purified marennine solutions (Pouvreau et al. 2007). Moreover, the growth of *H. ostrearia* can itself be inhibited by marennine, at concentrations ranging between about 30 and 50 mg $L^{-1}$, depending on the form used, either purified pigment (Pouvreau et al. 2007) or crude extract (Robert and Turpin 1993), respectively. Crude extracts thus correspond to marennine produced and excreted by *H. ostrearia* and accumulated in the growth medium. In the present study, the allelopathic effect of *H. ostrearia* presumably caused by the release of marennine was investigated by co-culturing this species with other microalgae commonly used in aquaculture as bivalve feeds, thus by testing the allelopathic effect of *H. ostrearia* culture supernatants. Our in vivo experiments showed that for the same initial biovolume of *H. ostrearia* and under stable environmental

**Table 2** Mean cell growth rate ($day^{-1}$) during the exponential growth phase of mono- and co-cultured (allelopathy) microalgae (Mo. and Al., respectively), in batch and semi-continuous mode, under different irradiances

| Parameters | Type of culture | Species | Irradiance (μmol photons $m^{-2}$ $s^{-1}$) | | |
|---|---|---|---|---|---|
| | | | 20 | 100 | 500 |
| Growth rate ($day^{-1}$) | Mo. batch | Ho | 0.21 ± 0.03 | 0.53 ± 0.06 | 0.59 ± 0.05 |
| | | Sc | 0.14 ± 0.20 | 0.21 ± 0.04 | 0.40 ± 0.01 |
| | Mo. semi-continuous | Ho | 0.36 ± 0.00 | 0.52 ± 0.01 | 0.57 ± 0.03 |
| | | Sc | 0.16 ± 0.00 | 0.23 ± 0.01 | 0.41 ± 0.01 |
| | Al. batch | Ho | 0.20 ± 0.03 | 0.55 ± 0.01 | 0.58 ± 0.01 |
| | | Sc | −0.03 ± 0.00 | −0.08 ± 0.01 | −0.11 ± 0.01 |
| | Al. semi-continuous | Ho | 0.37 ± 0.01 | 0.51 ± 0.03 | 0.57 ± 0.02 |
| | | Sc | −0.07 ± 0.03 | −0.16 ± 0.06 | −0.15 ± 0.02 |
| [EMn] (mg $L^{-1}$) | Al. batch | – | 5.15 ± 0.53 | 8.43 ± 1.68 | 11.03 ± 0.33 |
| | Al. semi-continuous | – | 0.57 ± 0.03 | 0.99 ± 0.20 | 1.43 ± 0.40 |
| Percent inhibition (I%) | Al. batch | Sc | 60.87 ± 0.72 | 87.66 ± 0.41 | 89.57 ± 0.26 |
| | Al. semi-continuous | Sc | 61.99 ± 0.73 | 74.22 ± 2.48 | 82.10 ± 4.19 |

EMn concentration (mg $L^{-1}$) and percent inhibition (I%) were measured at the end of allelopathy experiment and at each cycle of dilution in batch and semi-continuous culture, respectively. Values are means ± standard error ($n = 3$) obtained from three consecutive cycles of dilutions

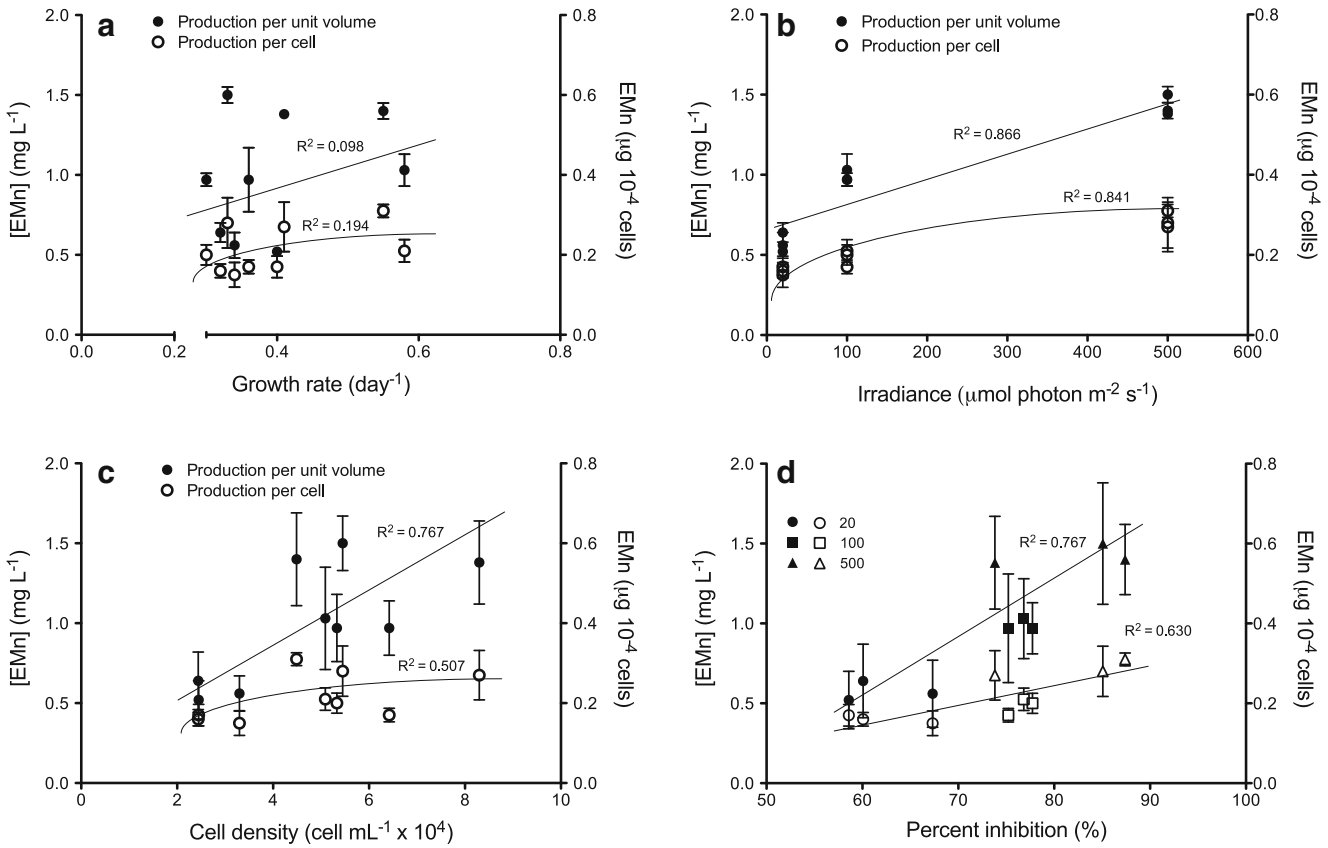


**Fig. 6** Extracellular marennine (*EMn*) concentration (mg $L^{-1}$ or μg × $10^{-4}$ cells) in co-cultures of *Skeletonema costatum* with *Haslea ostrearia* at different irradiances as a function of *H. ostrearia*'s growth rate (**a**), irradiance level (**b**), cell density (**c**), and percent inhibition I% (**d**). *Filled symbols* indicate marennine production per unit volume, and *empty symbols* indicate marennine production per cell. Data points are means ± standard error ($n = 3$). Pearson's correlation for **a**–**d** in marennine production per unit volume are $r = 0.314$, 0.931, 0.724, and 0.876, respectively. Pearson's correlation coefficient values for **a**–**d** in marennine production per cell are $r = 0.440$, 0.959, 0.507, and 0.794, respectively

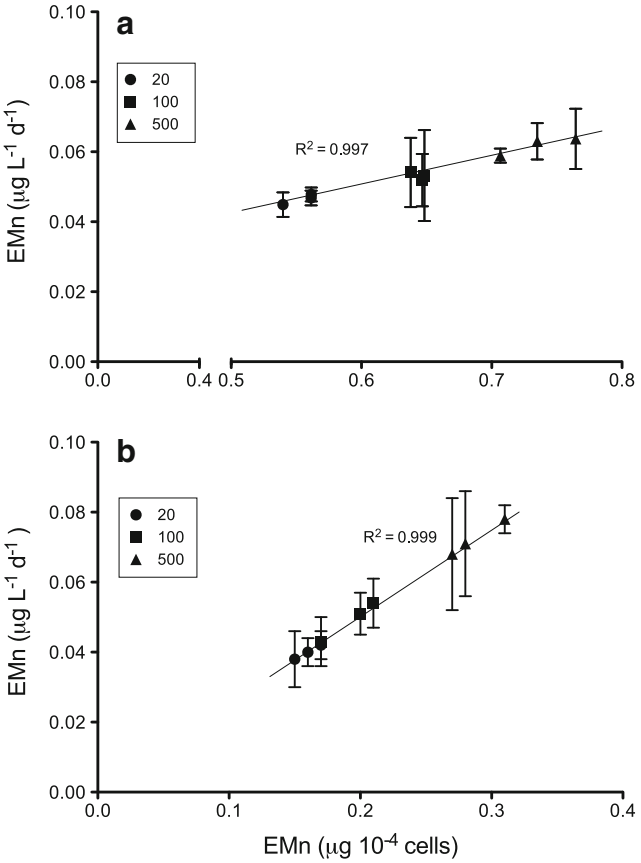


**Fig. 7** Relationship between the production of marennine per day and per cell for monospecific cultures of *Haslea ostrearia* grown at different irradiances in batch (*A*) and semi-continuous (*B*) modes. Data points are means ± standard error ($n = 3$). Pearson's correlation coefficient values between the production of marennine per day or per cell and irradiance are $r = 0.915$ and 0.875 in batch culture and $r = 0.959$ and 0.956 in semi-continuous culture, respectively

conditions, without light or nutrient limitation (cells maintained in exponential growth at low cell concentration, using a semi-continuous mode of culture), the diatom *H. ostrearia* triggered a reduction in cell concentration of several target species and that the magnitude of this impact was species dependent. For instance, *C. calcitrans* and *S. costatum* were significantly inhibited by *H. ostrearia* and marennine. Their growth rate was negatively affected when cultured in the presence of *H. ostrearia* cells and marennine concentration ranging from 0.7 to 1 mg $L^{-1}$, with percent inhibition (I%) of more than 70 %. Thus, these two microalgae can be considered as highly susceptible species, a result in accordance with Pouvreau et al. (2007), who observed that *S. costatum* was inhibited by marennine concentration less than 2 mg $L^{-1}$ (purified pigment). A complementary experiment run without the presence of *H. ostrearia* cells (mixture of fresh medium and *H. ostrearia* culture supernatant) also showed *S. costatum* growth inhibition, I% increasing with the proportion of marennine-containing supernatant. Although no nutrient was added to the supernatant, this result is in agreement with the I% of *S. costatum* growth observed in the co-culture experiment (batch mode) on days 2 and 4 (Fig. 2a), when cell density was low enough to minimize competition for nutrients and light. This allows hypothesizing that the inhibition was likely caused by the presence of marennine in the supernatant. Although less susceptible, the haptophyte *T. lutea* is also a species in which growth was slowed in the presence of *H. ostrearia*. Interestingly, a lower relative growth was also observed for *H. ostrearia* when co-cultured with *T. lutea*, suggesting that there is an inhibition caused by an allelopathic substance released by *T. lutea*, as previously observed, for instance using *Chaetoceros gracilis*, *Chaetoceros muelleri*, and *Nitzschia closterium* (Sun et al. 2012).

In contrast, *P. tricornutum* and *T. suecica* were less affected, as confirmed by an increase in cell concentrations and total biovolume despite the presence of *H. ostrearia* and marennine. Among the target species tested, *P. tricornutum* and *T. suecica* exhibited a certain tolerance, displaying an increase of cell concentrations and total biovolume despite allelochemical exposure, although their cell numbers in the treatment were still lower than those in controls. Moreover, these two microalgae outcompete *H. ostrearia*, not only in terms of cell concentration but also of the total biovolume. The present work, however, cannot allow conclusions about the intrinsic insensitivity of *P. tricornutum* and *T. suecica*, as experiments were not designed to study specifically their response to change in marennine concentration. Indeed, previous studies have demonstrated that biotic factors such as changes in cell concentration or dilution rates can modulate allelopathic effects between species (Sharp et al. 1979; Tillmann et al. 2007, 2008; Lyczkowski and Karp-Boss 2014).

Susceptibility or insensitiveness of the target species could be related to their cell biovolume, as the largest species (*P. tricornutum* and *T. suecica*) were less sensitive to marennine than the smallest ones (Fig. 4a). Additionally, our study revealed that the susceptibility of target species to allelopathy was also influenced by the cell surface area as it is negatively correlated to percent inhibition (I%) (Fig. 4b). Marennine allelopathic effect is linearly correlated to the surface area-to-volume (SA/V) ratio as I% in target species increased with the SA/V ratio (Fig. 4c), which could reflect interactions at the cell membrane level. Indeed, our results are in coherence with the previous work by Lyczkowski and Karp-Boss (2014) who observed the negative relationship between cell size and allelochemical impact of *Alexandrium fundyense* on *Thalassiosira* cf. *gravida*. These authors suggested that the toxicity of allelochemicals was biovolume or biosurface dependent, meaning that large cells would need to absorb a greater amount of the allelochemical compound to be as inhibited as smaller species, which could present a higher flux rate of allelochemicals inside the cells. In other words, small cells would tend to accumulate more allelochemicals or toxins per unit volume due to a higher flux rate (Ribalet et al.

2007). Thus, these mechanisms could explain why, in our study, microalgae that have a smaller cell biovolume were significantly more vulnerable to marennine although the initial total biovolume for both microalgae species at the beginning of their growth kinetics in co-culture was the same.

The allelopathic effect could also be influenced by the biomass of target species, which is also related to their growth rate. In our study, we observed that the percent inhibition I% due to allelochemical released was negatively correlated with the growth rate of target species ($r = -0.890$). This result is in agreement with previous studies, where the growth rate of target species had a significant role in the susceptibility to allelochemicals. For instance, Tillmann (2003) observed that the lytic activity of *Prymnesium parvum* decreased when increasing the cell concentration and growth rate of target organisms.

If a lower susceptibility to allelopathic compounds in large cells could be related to a lower surface-to-volume ratio, in some species, resistance against allelochemical compounds could also be related to the structure and properties of cell wall, which could represent an impermeable barrier. For instance, the resistance of *P. tricornutum* to allelochemicals has been reported where this microalga can outcompete *Thalassiosira pseudonana* (Sharp et al. 1979). Additionally, Vasconcelos and Leal (2008) also demonstrated that the allelopathic exudates could promote the growth of *P. tricornutum*. Ribalet et al. (2007) hypothesize that membrane characteristics and cell wall properties may have a role in cell vulnerability to allelochemical compounds. This would be a possible explanation why among large cells, *P. tricornutum* was less sensitive to marennine than *T. lutea* since the diatom has a more highly structured cell wall compared to the haptophyte *T. lutea* (Johansen 1991; Bartual et al. 2008; Tesson et al. 2009).

## Marennine allelopathic effect on vulnerable species is a function of irradiance

This series of experiments was based on the hypothesis that *H. ostrearia* cultured at different irradiances could generate different allelopathic effects, depending on marennine production and accumulation at cell apices (intracellular marennine, IMn) and excretion in the growth medium (EMn). Our study showed that *H. ostrearia* growth rate increased with irradiance, a result in agreement with a previous study by Mouget et al. (1999), where *H. ostrearia*-specific growth rate was high and almost constant from 100 to more than 750 μmol photons $m^{-2}$ $s^{-1}$. These authors also observed that at high irradiance, *H. ostrearia* cells displayed a reduction in size of the chloroplasts and an increase of marennine accumulation at the apices (IMn), a result confirmed by Rech et al. (2008), who showed that IMn increased with irradiance. In the present study, we observed that *H. ostrearia* produces and releases more marennine (EMn) at high irradiances (100 and 500 μmol photons $m^{-2}$ $s^{-1}$), as compared to low irradiance (20 μmol photons $m^{-2}$ $s^{-1}$). Furthermore, a significant correlation was observed between growth irradiance and EMn production per day and EMn per cell, which might influence the allelopathic susceptibility of the target species. Indeed, this demonstrated that change in irradiance level can modulate marennine concentration in the medium and significantly influence *H. ostrearia* allelopathic pressure on sensitive co-cultured microalgae.

Marennine allelopathic effect varied with irradiance and thus with concentration, *S. costatum* co-cultured with *H. ostrearia* being significantly more inhibited at high irradiance, as compared to co-culture at low irradiance. Thus, light plays a significant role in the allelochemical production by *H. ostrearia*, which consequently inhibited the growth of *S. costatum* despite non-limiting nutrient and light conditions. This result illustrates that light is possibly an important abiotic factor on allelopathy, not only because of possible photo-oxidation and degradation of allelochemicals, as hypothesized by Granéli and Hansen (2006), but also because of its direct influence on photosynthesis (e.g., Figueredo et al. 2007), growth and relative cell concentrations of emitter and target species, and the production of allelochemical compound (this study).

## Chemical warfare between algae and ecological importance of marennine

When greening occurs in oyster ponds, *H. ostrearia* blooms and produces a huge amount of marennine, a phenomenon that is accompanied by the decrease in abundance of other microalgal species (Robert 1983; Turpin et al. 1999). Turpin et al. (1999) observed that the concentration of marennine in oyster ponds during a bloom of *H. ostrearia* might reach 5 mg $L^{-1}$. This concentration is sufficiently high to inhibit the growth of *S. costatum* and other sensitive species (Pouvreau et al. 2007; this study), thus participating in the decline of their abundance (Robert 1983; Turpin et al. 1999). Consequently, marennine released in oyster ponds is likely able to participate in the dominance of *H. ostrearia*, thus changing the community structure of microalgae through allelochemical mediation in such closed ecosystems.

Greening of marine invertebrates possibly due to blooms of *H. ostrearia* does not occur in oyster ponds in the west coast of France only. It has been observed spontaneously elsewhere such as in Great Britain (Sprat 1669), Denmark (Petersen 1916), the USA (Mitchell and Barney 1918), Canada (Medcof 1945), and Australia (Gastineau et al. 2014). Moreover, recent findings revealed other species of pennate diatoms from the genus *Haslea* that produce a blue pigment such as *Haslea karadagensis* in the Black Sea (Gastineau et al. 2012a), *Haslea provincialis* from the Mediterranean Sea

(Gastineau et al. 2015), *Haslea silbo* sp. *inedit* from the Canary Islands (Gastineau et al. 2014). Thus, different species of blue *Haslea* produce "marennine-like" pigments, which can interact with bivalves (Gastineau et al. 2012b) in the same way as purified marennine or *Haslea* culture supernatants, and the resulting greening of marine invertebrates elsewhere in the world could probably be linked to as many allelopathic phenomena and competitions between phytoplankton.

### Co-culture of *H. ostrearia* with aquaculture-relevant microalgae: why and how?

In aquaculture, the massive use of conventional antimicrobials for disease control and growth promotion in animals increases the selective pressure exerted on the microbial communities and favors the natural emergence of bacterial resistance (Alderman and Hastings 1998; Van den Bogaard and Stobberingh 2000; Cabello 2006). For biohazard control and health management consideration, the utilization of *H. ostrearia* as oyster feed could be advantageous since marennine produced during algal growth could act as natural antibiotics or bacteriostatic agent. Indeed, confirming previous studies on the biological properties observed using *H. ostrearia* supernatants (e.g., Carbonnelle et al. 1999; Bergé et al. 1999), Gastineau et al. (2012b, c, 2014) showed that purified marennine inhibited the growth of bacterial pathogens such as *Vibrio aestuarianus* and *Vibrio splendidus*. For larviculture, considering that the cell size of *H. ostrearia* is overly large for larvae, supernatant containing extracellular marennine could be added to a diet consisting of resilient species of microalgae, in order to benefit from marennine's protecting properties. In contrast, for adult stages, *H. ostrearia* could be provided, either as the sole source of feed or alongside robust species for an optimization of the feeding. Hence, aside being a "greening agent," *H. ostrearia* could act as a nutraceutical, a feed with several benefits regarding animal health.

As a feed source, *H. ostrearia* has been used as a monospecific diet to sustain the growth of *C. gigas* for weeks (Piveteau 1999; Cognie 2001), with almost 90 % of digestibility (Barillé et al. 1994). Although the specific nutritional value of *H. ostrearia* for aquaculture is not yet documented like other diatoms, it has been shown that this diatom is a good source of eicosapentaenoic acid (EPA, 20:5n-3), with a relative proportion of total fatty acids of 12.2 % (Dunstan et al. 1994) and 14.5 % (Groth-Nard 1994). These authors showed that other main fatty acids in *H. ostrearia* are 16:0 (20.4 and 21.3 %, respectively), 16:1 (28.6 and 29.9), and 16:3 (10.6 and 15.1 %). The diatom *H. ostrearia* could thus be used as a unique source of feeding for animals, depending on their growth phase. However, several studies conducted over the last decades on the alternative feeding of oysters, especially on their larval stage in which they are particularly vulnerable to pathogens, emphasized the idea that a mixed diet composed of several microalgal species gave better results than a monospecific diet (Guedes and Malcata 2012; Becker 2004). An explanation is that microalgae vary significantly in their nutritional value (Enright et al. 1986; Brown et al. 1997). For instance, the haptophyte *T. lutea* is a rich source of docosahexaenoic acid (DHA, 22:6n-3), which represents 8–10 % of total fatty acids, while diatoms are a good source of EPA (20:5n-3) and arachidonic acid (AA, 20:4n-6) (Volkman et al. 1989; Dunstan et al. 1994). However, the utilization of *T. lutea* as monospecific diet seems not sufficient to optimize growth and survival, particularly for bivalve larvae (Da Costa et al. 2015; Marshall et al. 2010; Pernet and Tremblay 2004; Pernet et al. 2005). In our study, the allelopathic tests showed that *T. suecica* was one of the most tolerant species to marennine. However, we demonstrated also that diatoms rich in EPA and AA are susceptible to marennine and other sources of these essential fatty acids will need to be identified. One possibility could be a mixed diet composed of *H. ostrearia* and *T. suecica*, but such suggestion needs to be validated by a nutritional study.

In conclusion, the present study shows that *H. ostrearia* can influence the growth of microalgae species relevant for aquaculture possibly through allelopathic interactions in a co-culture system. The magnitude of this allelopathic-like effect is species dependent; *S. costatum*, *C. calcitrans*, and *T. lutea* were revealed as vulnerable species, whereas *P. tricornutum* and *T. suecica* were more resistant. The study also distinctly showed that the supernatant produced in conditions of co-cultures of *H. ostrearia* with a vulnerable species such as *S. costatum* acted as an allelochemical mixture with marennine concentrations lower than ca. 1 mg $L^{-1}$. These values are consistent with those observed in oyster ponds where oysters are fattened and greened, which could explain the domination of *H. ostrearia* in these ponds. Regarding a putative exploitation in the aquaculture of marennine antibacterial properties, a mixture of *H. ostrearia* and insensitive species such as *P. tricornutum* and *T. suecica* is recommended to circumvent allelopathic interactions between phytoplankton used as feed for animals.

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## References


Adolph S, Poulet SA, Pohnert G (2003) Synthesis and biological activity of α, β, γ, δ–unsaturated aldehydes from diatoms. Tetrahedron 59: 3003–3008

Alderman DJ, Hastings TS (1998) Antibiotic use in aquaculture: development of antibiotic resistance–potential for consumer health risks. Int J Food Sci Technol 33:139–155

Arzul G, Seguel M, Guzman L, Erard-Le Denn E (1999) Comparison of allelopathic properties in three toxic *Alexandrium* species. J Exp Mar Bio Ecol 232:285–295

Barillé L, Bougrier S, Geairon P, Robert J-M (1994) Alimentation expérimentale de l'huître *Crassostrea gigas* à l'aide de navicules bleus *Haslea ostrearia* (Simonsen) de différentes tailles. Oceanol Acta 17:201–210

Bartual A, Gálvez JA, Ojeda F (2008) Phenotypic response of the diatom *Phaeodactylum tricornutum* Bohlin to experimental changes in the inorganic carbon system. Bot Mar 51:350–359

Becker W (2004) Handbook of microalgal culture. Blackwell, Oxford

Bergé JP, Bourgougnon N, Alban S et al (1999) Antiviral and anticoagulant activities of a water-soluble fraction of the marine diatom *Haslea ostrearia*. Planta Med 65:604–609

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315–331

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8:1137–1144

Carbonnelle D, Pondaven P, Morancais M, Masse G, Bosch S, Jacquot C, Briand G, Robert J, Roussakis C (1999) Antitumor and antiproliferative effects of an aqueous extract from the marine diatom *Haslea ostrearia* (Simonsen) against solid tumors: lung carcinoma (NSCLC-N6), kidney carcinoma (E39) and melanoma (M96) cell lines. Anticancer Res 19:621–624

Casotti R, Mazza S, Brunet C, Vantrepotte V, Ianora A, Miralto A (2005) Growth inhibition and toxicity of the diatom aldehyde 2-trans, 4-trans-decadienal on *Thalassiosira weissflogii* (Bacillariophyceae). J Phycol 41:7–20

Cognie B (2001) Alimentation de l'huitre *Crassostrea gigas* (Thunberg): Etude des mecanismes de selection des particules et des processus retroactifs entre le bivalve et les microalgues. PhD thesis, University of Nantes

Da Costa F, Petton B, Mingant C, Bougaran G, Rouxel C, Quéré C, Wikfors GH, Soudant P, Robert R (2015) Influence of one selected *Tisochrysis lutea* strain rich in lipids on *Crassostrea gigas* larval development and biochemical composition. Aquac Nutr. doi:10.1111/anu.12301

De BC, Meena DK, Behera BK, Das P, Das Mohapatra PK, Sharma AP (2014) Probiotics in fish and shellfish culture: immunomodulatory and ecophysiological responses. Fish Physiol Biochem 40:921–971

Desbois AP, Lebl T, Yan L, Smith VJ (2008) Isolation and structural characterisation of two antibacterial free fatty acids from the marine diatom, *Phaeodactylum tricornutum*. Appl Microbiol Biotechnol 81:755–764

Desbois AP, Mearns-Spragg A, Smith VJ (2009) A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). Mar Biotechnol 11:45–52

Dunstan GA, Volkman JK, Barrett SM, Leroi JM, Jeffrey SW (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35:155–161

Enright CT, Newkirk GF, Craigie JS, Castell JD (1986) Evaluation of phytoplankton as diets for juvenile *Ostrea edulis* L. J Exp Mar Biol Ecol 96:1–13

Figueredo CC, Giani A, Bird DF (2007) Does allelopathy contribute to *Cylindrospermopsis raciborskii* (Cyanobacteria) bloom occurrence and geographic expansion? J Phycol 43:256–265

Gaillon B (1820) Des huîtres vertes, et des causes de cette coloration. J Phys Chim d'Histoire Nat des Arts 91:222–225

Gastineau R, Davidovich NA, Bardeau JF, Caruso A, Leignel V, Hardivillier Y, Jacquette B, Davidovich OI, Rince Y, Gaudin P, Cox EJ, Mouget J-L (2012a) *Haslea karadagensis* (Bacillariophyta): a second blue diatom, recorded from the Black Sea and producing a novel blue pigment. Eur J Phycol 47:469–479

Gastineau R, Hardivillier Y, Leignel V, Tekaya N, Morançais M, Fleurence J, Davidovich N, Jacquette B, Gaudin P, Hellio C, Bourgougnon N, Mouget J-L (2012b) Greening effect on oysters and biological activities of the blue pigments produced by the diatom *Haslea karadagensis* (Naviculaceae). Aquaculture 368–369: 61–67

Gastineau R, Pouvreau JB, Hellio C, Morançais M, Fleurence J, Gaudin P, Bourgougnon N, Mouget JL (2012c) Biological activities of purified marennine, the blue pigment responsible for the greening of oysters. J Agric Food Chem 60:3599–3605

Gastineau R, Turcotte F, Pouvreau JB et al (2014) Marennine, promising blue pigments from a widespread *Haslea* diatom species complex. Mar Drugs 12:3161–3189

Gastineau R, Hansen G, Davidovich NA, Davidovich O, Bardeau J-F, Kaczmarska I, Ehrman JM, Leignel V, Hardivillier Y, Jacquette B, Poulin M, Morançais M, Fleurence J, Mouget J-L (2015) A new blue-pigmented hasleoid diatom, *Haslea provincialis*, from the Mediterranean Sea. Eur J Phycol (accepted)

Granéli E, Hansen PJ (2006) Allelopathy in harmful algae: a mechanism to compete for resources? In: Granéli E, Turner J (eds) Ecology of harmful algae. Springer, Berlin, pp 189–201

Groth-Nard C (1994) Les lipides des Diatomées: exemple d'*Haslea ostrearia* (Simonsen), responsable du verdissement des huîtres. PhD Thesis, University of Nantes

Guedes AC, Malcata FX (2012) Nutritional value and uses of microalgae in aquaculture. In: Muchlisin Z (ed) Aquaculture. InTech, Riejeka. pp 59–78

Hillebrand H, Dürselen C-D, Kirschtel D, Pollinger U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:402–424

Ianora A, Miralto A (2010) Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. Ecotoxicology 19: 493–511

Inderjit, Duke SO (2003) Ecophysiological aspects of allelopathy. Planta 217:529–539

Johansen JR (1991) Morphological variability and cell wall composition of *Phaeodactylum tricornutum* (Bacillariophyceae). West North Am Nat 51:310–315

Jüttner F (2005) Evidence that polyunsaturated aldehydes of diatoms are repellents for pelagic crustacean grazers. Aquat Ecol 39:271–282

Jüttner F, Todorova AK, Walch N, von Philipsborn W (2001) Nostocyclamide M: a cyanobacterial cyclic peptide with allelopathic activity from *Nostoc* 31. Phytochemistry 57:613–619

Keating KI (1977) Allelopathic influence on blue-green bloom sequence in a eutrophic lake. Science 196:885–887

Lankester ER (1886) On green oysters. Q J Microsc Sci 26:71–94

Leflaive J, Ten-Hage L (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshw Biol 52:199–214

Legrand C, Rengefors K, Fistarol GO, Granéli E (2003) Allelopathy in phytoplankton—biochemical, ecological and evolutionary aspects. Phycologia 42:406–419

Lyczkowski ER, Karp-Boss L (2014) Allelopathic effects of *Alexandrium fundyense* (Dinophyceae) on *Thalassiosira* cf. *gravida* (Bacillariophyceae): a matter of size. J Phycol 50:376–387

Marshall R, Mckinley S, Pearce CM (2010) Effects of nutrition on larval growth and survival in bivalves. Rev Aquac 2:33–55

Medcof J (1945) Green oysters from New Brunswick. Acadian Nat 2:40

Mitchell PH, Barney RL (1918) The occurrence in Virginia of green-gilled oysters similar to those of Marennes. Bull U S Bur Fish 35: 135–149

Moreau J (1970) Contribution aux recherches écologiques sur les claires à huîtres du bassin de Marennes-Oléron. Rev Trav l'Institut pêches Marit 34:381–462

Mouget JL, Tremblin G, Morant-Marceau A, Morancais M, Robert JM (1999) Long-term photoacclimation of *Haslea ostrearia* (Bacillariophyta): effect of irradiance on growth rates, pigment content and photosynthesis. Eur J Phycol 34:109–115

Mouget JL, Rosa P, Tremblin G (2004) Acclimation of *Haslea ostrearia* to light of different spectral qualities—confirmation of "chromatic adaptation" in diatoms. J Photochem Photobiol B 75:1–11

Mouget JL, Rosa P, Vachoux C, Tremblin G (2005) Enhancement of marennine production by blue light in the diatom *Haslea ostrearia*. J Appl Phycol 17:437–445

Mouget J-L, Gastineau R, Davidovich O, Gaudin P, Davidovich NA (2009) Light is a key factor in triggering sexual reproduction in the pennate diatom *Haslea ostrearia*. FEMS Microbiol Ecol 69: 194–201

Neuville D, Daste P (1978) Recherches sur le de!terminisme de la production de marennine par la diatomée marine *Navicula ostrearia* (Gaillon) Bory en culture in vitro. Bot Rev 85:255–303

Paillard C, Le Roux F, Borrego JJ (2004) Bacterial disease in marine bivalves, a review of recent studies: trends and evolution. Aquat Living Resour 17:477–498

Pernet F, Tremblay R (2004) Effect of varying levels of dietary essential fatty acid during early ontogeny of the sea scallop *Placopecten magellanicus*. J Exp Mar Bio Ecol 310:73–86

Pernet F, Bricelj VM, Parrish CC (2005) Effect of varying dietary levels of ω6 polyunsaturated fatty acids during the early ontogeny of the sea scallop, *Placopecten magellanicus*. J Exp Mar Biol Ecol 327: 115–133

Petersen CG (1916) "Grüne Austern" in Dänemark in 1911–1912. Int Rev Hydrobiol Hydrogr 7:39–41

Piveteau F (1999) Étude des arômes de l'huître creuse *Crassostrea gigas*: conséquences d'un affinage à l'aide des microalgues *Skeletonema costatum* et *Haslea ostrearia* (Aroma of oyster *Crassostrea gigas*: effect of supplementation with the microalgae *Skeletonema costatum*). Doctoral dissertation, Nantes

Pohnert G, Lumineau O, Cueff A, Adolph S, Cordevant C, Lange M, Poulet S (2002) Are volatile unsaturated aldehydes from diatoms the main line of chemical defence against copepods? Mar Ecol Prog Ser 245:33–45

Pouvreau JB, Housson E, Le Tallec L, Morançais M, Rincé Y, Fleurence J, Pondaven P (2007) Growth inhibition of several marine diatom species induced by the shading effect and allelopathic activity of marennine, a blue-green polyphenolic pigment of the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen. J Exp Mar Bio Ecol 352:212–225

Pouvreau JB, Morançais M, Taran F, Rosa P, Dufossé L, Guérard F, Pin S, Fleurence J, Pondaven P (2008) Antioxidant and free radical scavenging properties of marennine, a blue-green polyphenols pigment from the diatom *Haslea ostrearia* (Gaillon/Bory) Simonsen responsible for the natural greening of cultured oysters. J Agric Food Chem 56:6278–6286

Rech M, Morant-Manceau A, Tremblin G (2008) Carbon fixation and carbonic anhydrase activity in *Haslea ostrearia* (Bacillariophyceae) in relation to growth irradiance. Photosynthetica 46:56–62

Ribalet F, Berges JA, Ianora A, Casotti R (2007) Growth inhibition of cultured marine phytoplankton by toxic algal-derived polyunsaturated aldehydes. Aquat Toxicol 85:219–227

Ribalet F, Vidoudez C, Cassin D et al (2009) High plasticity in the production of diatom-derived polyunsaturated aldehydes under nutrient limitation: physiological and ecological implications. Protist 160: 444–451

Robert JM (1983) Fertilite des claires ostréicoles et verdissement: utilisation de l'azote par les diatomees dominantes. PhD Thesis. University of Nantes (Brittany, France)

Robert JM, Turpin V (1993) La marennine est-elle toxique pour l'algue. Cryptogam Algol 14:89

Robert JM, Morançais M, Pradier E, Mouget J-L, Trembin G (2002) Extraction and quantitative analysis of the blue-green pigment "marennine" synthesized by the diatom *Haslea ostrearia*. J Appl Phycol 14:299–305

Sharp JH, Underhill PA, Hughes DJ (1979) Interaction (allelopathy) between marine diatoms: *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. J Phycol 15:353–362

Sprat T (1669) The history of the generation and ordering of green oysters, commonly called Colchester oysters. In: The history of the Royal-Society of London, for the Improving of natural knowledge. The Royal Society, London, 307–319

Sun Y, Xu S, Li W, Zhang J, Wang C (2012) Antialgal substances from *Isochrysis galbana* and its effects on the growth of *Isochrysis galbana* and six species of feed microalgae. In: Zhu E, Sambath S (eds) Information technology and agricultural engineering. Springer, Berlin, pp 211–223

Takano K, Igarashi S, Mikami H, Hino S (2003) Causation of reversal simultaneity for diatom biomass and density of *Phormidium tenue* during the warm season in eutrophic Lake Barato, Japan. Limnology 4:73–78

Tang YZ, Gobler CJ (2011) The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. Harmful Algae 10:480–488

Tesson B, Gaillard C, Martin-Jézéquel V (2009) Insights into the polymorphism of the diatom *Phaeodactylum tricornutum* Bohlin. Bot Mar 52:104–116

Tillmann U (2003) Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat Microb Ecol 32: 73–84

Tillmann U, John U, Cembella A (2007) On the allelochemical potency of the marine dinoflagellate *Alexandrium ostenfeldii* against heterotrophic and autotrophic protists. J Plankton Res 29:527–543

Tillmann U, Alpermann T, John U, Cembella A (2008) Allelochemical interactions and short-term effects of the dinoflagellate Alexandrium on selected photoautotrophic and heterotrophic protists. Harmful Algae 7:52–64

Turpin V, Robert JM, Goulletquer P (1999) Limiting nutrients of oyster pond seawaters in the Marennes-Oleron region for *Haslea ostrearia*: applications to the mass production of the diatom in mesocosm experiments. Aquat Living Resour 12:335–342

Van den Bogaard A, Stobberingh EE (2000) Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents 14:327–335

Vasconcelos MTSD, Leal MFC (2008) Exudates of different marine algae promote growth and mediate trace metal binding in *Phaeodactylum tricornutum*. Mar Environ Res 66:499–507

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219–240

Yamasaki Y, Nagasoe S, Matsubara T, Shikata T, Shimasaki Y, Oshima Y, Honjo T (2007) Allelopathic interactions between the bacillariophyte *Skeletonema costatum* and the raphidophyte *Heterosigma akashiwo*. Mar Ecol Prog Ser 339:83–92