Harmful Algae 39 (2014) 232–241

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Interactive effects of irradiance and temperature on growth and domoic acid production of the toxic diatom *Pseudo-nitzschia australis* (Bacillariophyceae)

Maxine Thorel [a,b], Juliette Fauchot [a,b,*], Jérôme Morelle [a,b], Virginie Raimbault [a,b], Bertrand Le Roy [a,b], Carole Miossec [c], Valérie Kientz-Bouchart [c], Pascal Claquin [a,b]

[a] Normandie Université, Université de Caen Basse-Normandie, F-14032 Caen, France
[b] UMR BOREA, CNRS-7208, IRD-207, MNHN, UPMC, UCBN, Esplanade de la paix, F-14032 Caen, France
[c] Frank Duncombe Laboratory LABEO, Saint-Contest, 14053 Caen Cedex, France

ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 17 July 2014
Accepted 17 July 2014

Keywords:
Domoic acid
Growth
Irradiance
Pseudo-nitzschia australis
Temperature

ABSTRACT

The influence of temperature and light on the growth, physiology and domoic acid (DA) production of the pennate toxic diatom *Pseudo-nitzschia australis* isolated from the English Channel was studied in semi-continuous culture. The effects of eight irradiances (35–400 μmol photons m^2 s^{-1}) and a temperature gradient (3.5–25.5 °C) were investigated. The highest growth rates (0.47–0.83 d^{-1}) were observed between 18.6 and 13.5 °C, with an optimum irradiance for growth at 100 μmol photons m^2 s^{-1}. A decrease in the optimum growth temperature was observed with an increase in irradiance. Cell chlorophyll *a* content decreased at both low and high extreme temperatures and high irradiances. Stable and high values of F_v/F_m ratios below 21 °C highlight the great acclimation capacity of *P. australis*, which may explain its wide biogeographic distribution. The strain studied produced DA without nutrient limitation and during exponential growth although at lower levels than in other studies (0.15–2.0 pg DA $cell^{-1}$). Results underline the importance of light–temperature interactions for growth and DA production. Toxin production increased with increasing temperature and light, and DA production increased exponentially with the growth rate. These results show that DA is not only produced under nutrient stress or by unhealthy cells in *P. australis*. This study underlines the need to take the ability of *P. australis* to produce DA during the exponential growth into consideration to understand the link between toxin production and bloom dynamics. These results will not only help explore the processes involved, but also help parameterize future models of growth and DA production especially for *P. australis*.

1. Introduction

In recent decades, there has been an increase in the frequency and intensity of harmful algal blooms (HABs) worldwide (Anderson et al., 2002; Hallegraeff, 2010). HABs are a serious threat to marine ecosystems, fisheries resources, and human health. Among these harmful algae, some species of the raphid pennate diatom *Pseudo-nitzschia* Peragallo are able to produce domoic acid (DA), a neurotoxic amino acid (Wright et al., 1989; Brown and Nijar, 1995). When DA bioaccumulates in trophic food webs, it causes in amnesic shellfish poisoning (ASP) events (Mos, 2001; Pulido, 2008). In coastal areas, ASP outbreaks frequently result in economic losses for fisheries and shellfish farming (reviewed by Trainer et al., 2012). Understanding the physiology of toxic *Pseudo-nitzschia* is thus crucial if we are to increase our ability to understand, predict and manage these harmful blooms.

Phytoplankton dynamics are linked to seasonal fluctuations in environmental factors (Sommer et al., 1986; Cloern, 1996), particularly light, nutrients, and temperature. These parameters can directly affect phytoplankton physiology and limit primary production. Phytoplankton spring blooms are usually triggered by changes in irradiance and water temperature (Sommer et al., 1986; Winder and Sommer, 2012). Temperature plays a crucial role in microalgae metabolic processes, affecting photosynthesis and nutrient uptake in particular, and more generally, the enzymatic activity of the whole cell (Raven and Geider, 1988; Davison, 1991; Claquin et al., 2008). Temperature is therefore an important driver of phytoplankton growth and algal bloom development (Fu et al., 2012). Acclimation of microalgae to temperature variations is

* Corresponding author at: UMR BOREA, Université de Caen Basse-Normandie, F-14032 Caen, France. Tel.: +33 2 31 56 58 37; fax: +33 2 31 56 53 46.
E-mail address: juliette.fauchot@unicaen.fr (J. Fauchot).

http://dx.doi.org/10.1016/j.hal.2014.07.010
1568-9883/

widely described (Davison, 1991; Boyd et al., 2013). The acclimation capacity of microalgae is related to the biogeographic origin of the species isolated (Davison, 1991) but also depends on strain diversity and possible deviation by clonal isolates during culture (Boyd et al., 2013). Only a few studies have characterized the acclimation capacity and metabolism regulation of *Pseudo-nitzschia* as a function of temperature (Lewis et al., 1993; Bates, 1998). Previous studies have shown that acclimation to low temperature mimics adaptation to high irradiance (Anning et al., 2001), notably in *Pseudo-nitzschia granii* and *Pseudo-nitzschia fraudulenta* (El-Sabaawi and Harrison, 2006; Claquin et al., 2008). As far as DA is concerned, the results of these studies revealed marked interspecific variability. Lewis et al. (1993) observed an increase in cell DA (cDA) content with increased temperature in *Pseudo-nitzschia multiseries*, while no effect of temperature was reported for *Pseudo-nitzschia australis* and *Pseudo-nitzschia pseudodelicatissima* (Lundholm et al., 1997; Santiago-Morales and García-Mendoza, 2011). Light also plays a key role in algal growth (Falkowski and Raven, 1997), different authors reported contrasting effects of light on DA production in *Pseudo-nitzschia* species (Lewis et al., 1993; Cusack et al., 2002; Santiago-Morales and García-Mendoza, 2011), and also underlined the need to take into account the influence of temperature and light interactions on growth and physiology (El-Sabaawi and Harrison, 2006). There are few investigations of this aspect in *Pseudo-nitzschia* species. Most studies of *Pseudo-nitzschia* spp. growth and DA production dynamics were performed under nutrient-limited conditions (reviewed in Lelong et al., 2012). It is thus essential to study *Pseudo-nitzschia* growth and DA production when nutrients are replete under different temperature and light gradients to understand their physiology and to improve models of bloom dynamics.

Currently, of the 44 known *Pseudo-nitzschia* species, 16 have been reported to produce DA (Lim et al., 2012, 2013; Orive et al., 2013; Teng et al., 2014; Fernandes et al., 2014), but not all strains are toxic (Bates et al., 1998; Thessen et al., 2009; Lelong et al., 2012). Given the observed variability in physiology and DA production among *Pseudo-nitzschia* species, species-specific studies are needed. One of the most toxic *Pseudo-nitzschia* species is *Pseudo-nitzschia australis* Frenguelli (Garrison et al., 1992; Bates, 2000; Cusack et al., 2002), which is cosmopolite (Hasle, 2002). It was identified as the source of DA in many ASP events on the west coast of the USA (Fritz et al., 1992), and in New Zealand (Rhodes, 1998), Scottish (Gallacher et al., 2001), Spanish (Mìguez et al., 1996) and French (Nezan et al., 2006) waters. *P. australis* has been reported to bloom in spring, summer and autumn in many geographic areas (Garrison et al., 1992; Villac et al., 1993; Cusack et al., 2002; Nezan et al., 2006; García-Mendoza et al., 2009; Holtermann et al., 2010; Klein et al., 2010). Different authors studied the possible role of temperature in determining the timing of *P. australis* blooms, with a coincidence between the time of blooming and warm water temperature off the coasts of California or Mexico (around 14 °C; Buck et al., 1992; García-Mendoza et al., 2009) and in the Bay of Seine in France (~17 °C; Klein et al., 2010).

The aim of the present work was to investigate the combined effects of different light and temperature conditions on the growth rate, physiological status, and DA production of *Pseudo-nitzschia australis*. To clearly understand the influence of these factors, the experiments used steady-state semi-continuous cultures in nutrient-replete conditions.

2. Materials and methods

2.1. Strain isolation

A clonal culture of *Pseudo-nitzschia australis* (UniCaen-PNaus45) was isolated on August 24th 2011 in the coastal Bay of Seine (English Channel, France). Cultures were not axenic, but the level of bacterial contamination was checked and almost no bacteria were detected during cell countings by optical microscopy observations. The species identity of this *Pseudo-nitzschia* strain was determined using morphometrics obtained by transmission electron microscopy (TEM) and molecular sequencing of the entire internal transcribed spacer region (ITS1-5.8S-ITS2) of the ribosomal RNA.

2.2. Culture conditions

The *Pseudo-nitzschia australis* strain was grown in semi-continuous cultures with a temperature gradient of 3.5–25.5 °C. Cultures (300 ml) were grown in 500 ml borosilicate Erlenmeyer flasks containing K/2-medium (Keller et al., 1987) enriched in Si (for a final concentration of 100 μM). The temperature gradient was obtained, as described in Claquin et al. (2008), using a 2-cm thick, 1.5 m × 0.6 m aluminium plate with a 1 cm diameter (0.6 m long) hole drilled 2 cm from each end, through which distilled water was pumped. Water passing through the hole at either side of the plate was circulated through a separate closed system water bath and the temperature gradient across the plate was regulated by controlling the temperature in each water bath.

Cultures were continuously illuminated using fluorescent tubes (OSRAM, DULUX L, 2G11, 55W/12-950, LUMILUX DE LUXE, daylight). Light intensity was measured in the culture using a micro-spherical quantum sensor (US-SQS/L Walz).

The *Pseudo-nitzschia australis* cultures were placed on the aluminium plate under a temperature and irradiance gradient. The light gradient was obtained by arranging the Erlenmeyers flasks at different distances from the light source. Two sets of experiments were conducted: first, we tested a high light gradient (HL; 400, 200, 150 and 100 μmol photons m^{-2} s^{-1}) and second, 6 months later, a low light gradient (LL; 90, 65, 45 and 35 μmol photons m^{-2} s^{-1}). To acclimate *Pseudo-nitzschia* cells, the cultures were daily diluted for at least five generations. Cells were considered as being acclimated (i.e., in balanced growth or steady state; MacIntyre and Cullen, 2005) when growth rates did not vary more than ±0.05 d^{-1} (Fig. 1) for at least three generations under each temperature and light condition. K/2 + Si medium (modified from Keller et al., 1987) was used for the dilutions and from 15 to 65% of the total volume of cultures was replaced daily depending on growth rates. Dilutions allowed to keep cells in exponential growth and to maintain low cell concentrations in order to avoid nutrient limitation during the experiments (Beardall and Morris, 1976; Fig. 1). Before and after daily dilution, in vivo chlorophyll *a* (chl *a*) fluorescence of all cultures was measured with a Turner TD-700 fluorometer (Turner Design, Sunnyvale, CA, USA). Daily dilution made it possible to obtain equivalent in vivo chl *a* fluorescence levels for all cultures under temperature and light condition except at extreme temperatures, when the growth rate was too low. Daily variations in biomass estimated by the in vivo chl *a* measurements made it possible to calculate the growth rates (μ, d^{-1}) of the cultures using the following equation:

$$\mu = \ln \frac{\text{chl } a_t / \text{chl } a_{t0}}{t - t_0} \quad (1)$$

where t is time in days, chl a_{t0} is initial in vivo chl *a* fluorescence (i.e. at the initial time, t_0), and chl a_t is chl *a* fluorescence at time t before dilution. Triplicate samples were taken on three consecutive days once steady state had been reached in each semi-continuous culture. The cellular concentrations were low and stable in triplicates at each condition and were comprised between 6000 and 45,000 cells ml^{-1} depending on conditions.

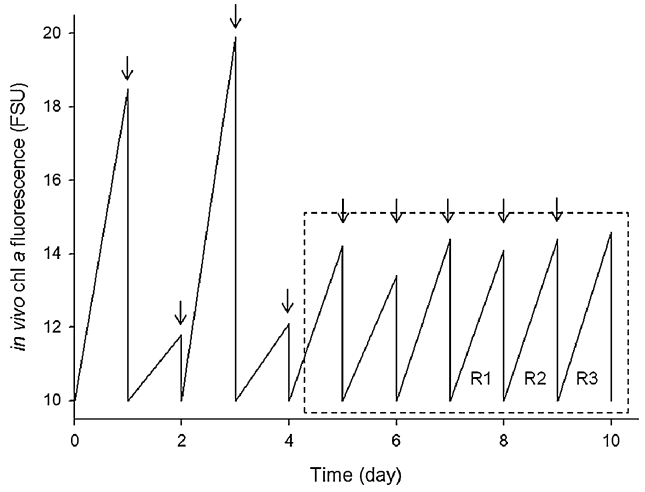

Fig. 1. Semi-continuous culture protocol applied. Example of one tested condition (100 μmol photons m^{-2} s^{-1}; 10.7 °C). Dynamics of biomass (estimated by in vivo chl *a* fluorescence – Fluorescence Standard Units (FSU)) as a function of time. Cultures were diluted daily (arrows) to maintain biomass within a restricted range. Dotted zone represents steady-state days. Steady state was reached when growth rate did not vary more than ±0.05 d-1. R1, R2 and R3 represent triplicates of culture.

2.3. Chlorophyll a and photosynthetic measurements

To measure cell chl *a* content, a 10 ml aliquot of each culture was filtrated on a GF/F filter (Whatman, 25 mm). Ten ml of 90% acetone (v/v) were then added to the filter and left for 12 h in darkness at 4 °C to extract the pigments. After centrifugation for 5 min at 1700 × *g*, the chl *a* concentration of the extracts was measured using a Turner TD-700 fluorometer (Turner Designs, Sunnyvale, USA) according to Welschmeyer (1994). The cell chl *a* content of all cultures was determined after cell counts using a Nageotte counting chamber.

Photosystem II (PSII) fluorescence kinetics were measured using a WATER/B PAM fluorometer (Walz, Effeltrich, Germany) (Schreiber et al., 1986). For each culture, one aliquot was dark adapted for 10 min at the growth temperature to fully reoxidize the electron acceptors of the PSII.

The maximum quantum yield of PSII (F_v/F_m) was calculated (Genty et al., 1989) after cell excitation by a weak blue light (1 μmol m^{-2} s^{-1}, 470 nm, frequency 0.6 Hz). F_v/F_m, considered as an indicator of the cell physiological status (Maxwell and Johnson, 2000), was calculated by:

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (2)$$

where F_v is the variable fluorescence, F_0 and F_m are the minimum and the maximum fluorescence of the dark-adapted sample during a saturating light pulse (0.6 s, 470 nm, 1700 μmol m^{-2} s^{-1}).

2.4. Domoic acid analysis

DA was estimated as described in Klein et al. (2010). At each condition, 10 ml of culture were filtered onto a GF/F filter (25-mm diameter). Filters were stored frozen at −20 °C until analysis.

The concentration of particulate DA (mg DA L^{-1}) on the filters was determined according to Wang et al. (2007), using high performance liquid chromatography (1290 Infinity, Agilent Technologies) followed by mass–mass (MS–MS) detection (6460 Agilent). DA was extracted by sonication in 5 ml of ultrapure water for 5 min, mixed for 10 min, and then filtered onto a PVDF membrane (Millex filters -HV, 0.45 μm, 13 mm, Merck Millipore). Before analysis, the extract was prepared by five-fold dilution. Twenty microliters were injected into a reversed-phase column (Zorbax SB-C18, 1.8 μm, 2.1 mm × 150 mm) with an elution gradient of acetonitrile (ACN). The initial mobile phase was 30% (v/v) 2 mM ammonium formate and 50 mM formic acid ACN with 30% acidified MilliQ ultrapure water, increased to 47.5% ACN over 3 min, then 90% over 4 min and held for 3 min at a flow rate of 0.2 ml min^{-1}. The retention time for DA was 2.2 min. The mass spectrometer was used with an electrospray interface operating in positive mode. The parameters used were capillary voltage 3.0 kV, solvent gas 12 L min^{-1}, evaporation temperature 350 °C and pressure of nebulization 40 psig.

Full scan spectra were recorded with a DA standard (LGC Promochem, UK) in order to select the most abundant *m/z*, then two MRM transitions were selected: 312 > 266 for quantification, and 312 > 161 for qualification. The detector response was linear for the concentration range from 0.1 to 20 mg DA L^{-1}.

Recovery was 86% as determined with spiked filters. The quantification limit (QL) of the method is 0.5 μg DA L^{-1} in the extract or 2.5 ng DA L^{-1} in the sample, but the presence of toxin is detected from 0.8 ng DA L^{-1}, which is the detection limit (DL) of the toxin.

2.5. Statistics

The non-linear parametric model of Blanchard et al. (1996) inspired by O'Neill (Straskraba and Gnauck, 1985), was applied to growth rates (μ, d^{-1}) and cell chl *a* content (chl*a*, μg chl *a* $cell^{-1}$) as a function of temperature (T, °C):

$$X(T) = X_{MAX}\left[\frac{T_{let} - T}{T_{let} - T_{opt}}\right]^{\beta} x\exp\left(-\beta\left\{\left[\frac{T_{let} - T}{T_{let} - T_{opt}}\right] - 1\right\}\right) \quad (3)$$

where $X(T)$ corresponds to $\mu(T)$ or chl$a(T)$. X_{MAX} represents μ_{MAX} or $chla_{MAX}$ at the optimum temperature (T_{opt}). T_{let} is the lethal temperature, and the shape parameter β is a dimensionless parameter related to Q_{10}. All curves were fitted using the least squares criterion of SigmaPlot 11 (SYSTAT Software). All the fits were tested using analysis of variance ($p < 0.001$), and the residuals were tested for normality and homogeneity of variance, and parameter significance with Student's *t*-test ($p < 0.05$).

To estimate μ_{MAX} variations and the photoacclimation process, data were plotted against irradiance (E). The Eilers and Peeters (1988) model for photosynthesis was applied to the μ_{MAX} data using SigmaPlot 11:

$$\mu_{MAX}(E) = \frac{E}{aE^2 + bE + c} \quad (4)$$

A two-way ANOVA was performed to determine the statistical significance of the effects of irradiance, temperature and interaction of both factors on growth rates ($p < 0.05$). The software SigmaPlot 11 was used.

3. Results

3.1. Pseudo-nitzschia australis growth rate

Pseudo-nitzschia australis growth rate (μ) ranged from 0 to 0.83 d^{-1} and followed the Blanchard et al. (1996) model (Fig. 2A and B). The fits of the model presented a bell curve shape and were significant for all irradiances tested, indicating that growth was strongly controlled by temperature. An increase in growth rate was observed with increasing temperature up to an optimum temperature ($Topt_{(\mu)}$) for all light intensities. $Topt_{(\mu)}$ varied with the irradiance rate tested and decreased significantly with increasing irradiance (Table 1). The lowest $Topt_{(\mu)}$ (13.7 °C) was

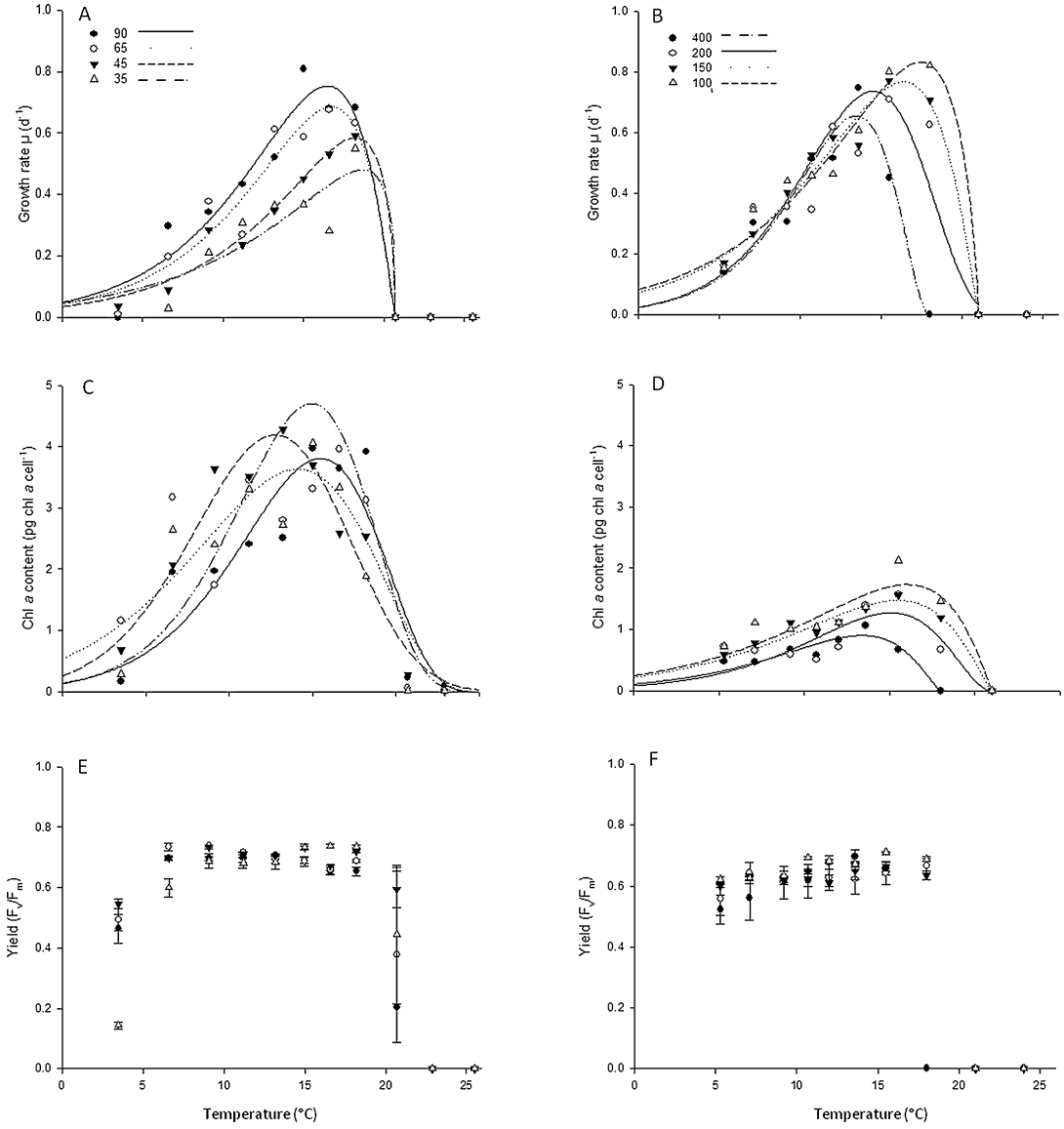

Fig. 2. *Pseudo-nitzschia australis* growth and photosynthetic responses as a function of temperature in the low light experiment (left column) and in the high light experiment (right column): (A and B) Growth rate; (C and D) cellular chl *a* content; (E and F) maximum quantum yield (F_v/F_m). Dots represent means (±SE for F_v/F_m data) of triplicate cultures; fitted lines represent the Blanchard et al. (1996) model.

recorded for the highest irradiance (400 μmol photons m^{-2} s^{-1}) and the highest $Topt_{(\mu)}$ (18.6 °C) for the lowest irradiance (35 μmol photons m^{-2} s^{-1}). $Topt_{(\mu)}$ at intermediate irradiances (200, 150, 100, 90, 65 and 45 μmol photons m^{-2} s^{-1}) ranged from 14.5 to 18.2 °C.

The lethal temperature ($Tlet_{(\mu)}$) also depended on irradiance (Table 1) and decreased significantly with increasing irradiance. A negative influence of high temperature on growth was observed at the highest irradiance: at 400 μmol photons m^{-2} s^{-1}, *Pseudo-nitzschia australis* did not grow at temperatures above 18 °C,

Table 1
Growth and chl *a* parameters (means ± SE) for the different irradiances tested (μmol photons m^{-2} s^{-1}). μ_{MAX} is the maximum growth rate (d^{-1}) for *Pseudo-nitzschia australis*; $Topt_{(\mu)}$ (°C) and $Tlet_{(\mu)}$ (°C) are, respectively, the optimum and the lethal temperature of growth; $\beta_{(\mu)}$ and $\beta_{(chla)}$ are dimensionless parameters related to Q_{10}; $Chla_{MAX}$ is maximum cellular chl *a* content (pg chl *a* $cell^{-1}$) at the optimum temperature ($Topt_{(chla)}$; °C). These parameters were determined using the Blanchard et al. (1996) model.

Irradiance	μ_{MAX}	$Topt_{(\mu)}$	$Tlet_{(\mu)}$	$\beta_{(\mu)\mu max}$	$Chla_{MAX}$	$Topt_{(chla)}$	$\beta_{(chla)}$
35	0.47 ± 0.14*	18.6 ± 3.3*	20.7*	0.3 ± 0.8	3.6 ±0.4*	13.4 ± 1.1*	6.5 ± 12.1
45	0.58 ± 0.05*	18.2 ± 0.9*	20.7*	0.5 ± 0.3	4.2 ± 0.2*	NA	NA
65	0.68 ± 0.05*	16.7 ± 0.7*	20.7*	1.1 ± 0.5*	3.6 ± 0.5*	14.1 ± 1.3*	4.4 ± 7.6
90	0.75 ± 0.05*	16.5 ± 0.6*	20.7*	1.2 ± 0.5*	3.8 ± 0.4*	15.5 ± 0.9*	5.5 ± 8.9
100	0.83 ± 0.04*	17.4 ± 0.7*	21*	0.7 ± 0.3*	1.7 ± 0.2*	16.0 ± 0.9*	1.1 ± 0.7*
150	0.77 ± 0.03*	16.4 ± 0.3*	21*	1.2 ± 0.2*	1.5 ± 0.1*	15.5 ± 0.7*	1.3 ± 0.6*
200	0.74 ± 0.06*	14.5 ± 0.6*	23*	5.0 ± 6.0	1.3 ± 0.2*	15.1 ± 1.3*	2.2 ± 6.1
400	0.66 ± 0.07*	13.5 ± 0.7*	18*	2.1 ± 4.5	0.98 ± 0.2*	13.4 ± 0.8*	1.4 ± 3.0

* Statistic significances ($p < 0.05$) of parameters tested by Student's *t*-test.

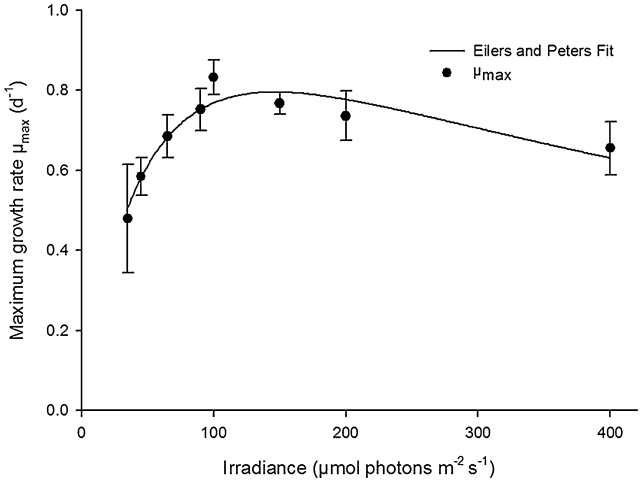

Fig. 3. Maximum growth rates (μ_{MAX}) of *Pseudo-nitzschia australis* as a function of irradiance. Means ± SD of triplicate cultures are shown; fitted line represents the Eilers and Peeters (1988) model.

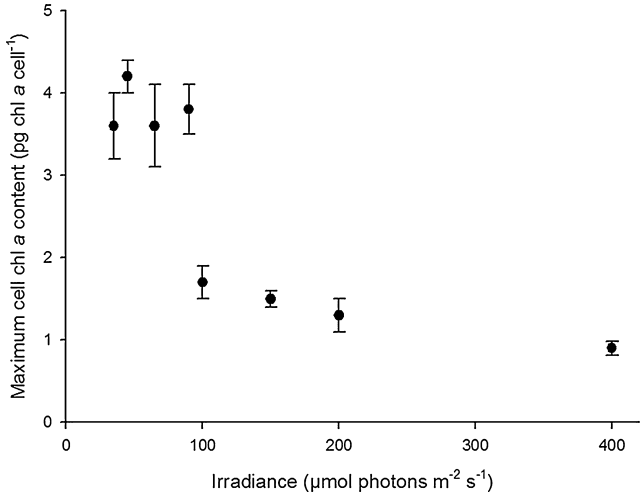

Fig. 4. Maximum cell chl *a* content (pg chl *a* cell^{-1}) in *Pseudo-nitzschia australis* as a function of irradiance. Means ± SE of triplicate cultures are shown.

whereas at lower irradiances, cells were able to grow at temperatures up to 21 °C (Table 1).

The effects of irradiance, temperature and interaction of both on growth rates were significant for the HL experiment (ANOVA two-way; $p < 0.001$). For the LL experiment, only temperature presented a significant effect on growth rates (ANOVA two-way; $p < 0.001$).

Fig. 3 shows that the maximum *Pseudo-nitzschia australis* growth rate (μ_{MAX}) as a function of irradiance μ_{MAX} increased significantly ($p < 0.05$) between 35 and 100 μmol photons m^{-2} s^{-1}. The optimum irradiance for growth was 100 μmol photons m^{-2} s^{-1} for *P. australis*. Above this irradiance, μ_{MAX} underwent a slight but significant decrease ($p < 0.05$). These results show that *P. australis* is able to grow under a wide range of temperatures (from 3.5 to 21 °C) and of irradiances (from 35 to 400 μmol photons m^{-2} s^{-1}) and emphasize the interactive effects of temperature and light on growth.

3.2. Chlorophyll a content

The variations in cell chl *a* content as a function of temperature (Fig. 2C and D) also significantly fitted Blanchard's model. Chl *a* content increased with increasing temperature until the optimum temperature ($Topt_{(chla)}$) was reached. The highest cell chl *a* contents were observed between 13 and 16 °C (Table 1; Fig. 2C and D). These results showed that part of the chl *a* variations were temperature dependent.

In Fig. 4, the maximum cell chl *a* content ($chla_{MAX}$) is expressed as a function of irradiance. No significant difference in $chla_{MAX}$ was measured between 35 and 90 μmol photons m^{-2} s^{-1} ($p > 0.05$). In contrast, a significant decrease ($p < 0.05$) in $chla_{MAX}$ was observed with increasing irradiance. There was a sharp decrease in $chla_{MAX}$ between cultures grown under low or high irradiance. The highest $chla_{MAX}$ (4.2 pg chl *a* cell^{-1}) was observed at 45 μmol photons m^{-2} s^{-1} and was 4.3 times higher than the $chla_{MAX}$ at the highest irradiance (0.98 pg chl *a* cell^{-1} at 400 μmol photons m^{-2} s^{-1}; Table 1).

3.3. Maximum quantum yield

Cultures were acclimated for at least five generations or until their growth rate stabilized. Growth rate and maximum quantum yield (F_v/F_m) were monitored to check balanced growth in the cultures. After acclimation, F_v/F_m reached a maximum and remained constant (~0.6; Fig. 2E and F), showing that the cultures were acclimated and in a healthy physiological steady state. In the low light (LL) experiment, the F_v/F_m was lower at low temperatures and underwent a significant increase between 3.5 and 6.6 °C ($p < 0.05$; from ~0.5 to 0.7, except at 35 μmol photons m^{-2} s^{-1} when F_v/F_m increased from 0.2 to 0.6). A plateau of F_v/F_m values was observed between 6.6 and 18.2 °C in the LL experiment. We observed high F_v/F_m values (between 0.6 and 0.7) and no significant difference in F_v/F_m ($p > 0.05$) between treatments. Above 18 °C, F_v/F_m decreased ($p < 0.05$; from 0.7 to 0.2–0.5). In this light range (LL experiments), the *Pseudo-nitzschia australis* maximum quantum yield of PSII was affected by extreme temperatures, i.e. at 3.5 °C and above 20.7 °C. At the highest irradiances, F_v/F_m did not significantly differ ($p > 0.05$) and remained high (between 0.5 and 0.7) at all the temperatures tested. Over 21 °C, values of the F_v/F_m were zero, in accordance with the lethal temperature we observed. Stable and high F_v/F_m ratios under 21 °C point to the high acclimation capacities of cells in the different experimental conditions and confirm that growth was balanced in all semi-continuous cultures performed.

3.4. Domoic acid production

DA was detected in all balanced growth cultures under the different conditions tested. However, at 35, 45 and 65 μmol photons m^{-2} s^{-1}, cellular DA (cDA) was very low and mainly around or below the limit of quantification (data not shown). At 90 μmol photons m^{-2} s^{-1}, cDA content was between the quantification limit and 0.09 pg DA cell^{-1} (data not shown). In the high light (HL) experiment, cDA was higher than in the low light (LL) experiment and increased exponentially ($p < 0.0001$; $R^2 = 0.4$) as a function of temperature under all irradiances tested (Fig. 5). Maximum cDA was 0.7 pg DA cell^{-1} at 400 μmol photons m^{-2} s^{-1} and 12 °C. At 200, 150 and 100 μmol photons m^{-2} s^{-1}, the maximum cDA was, respectively, 0.6, 0.7 and 0.5 pg DA cell^{-1}. These maximum values were measured at a temperature of 15.5 °C with 200 μmol photons m^{-2} s^{-1} and at 18 °C with 150 and 100 μmol photons m^{-2} s^{-1}. Maximum DA production showed a shift to lower temperatures with increasing irradiance (Fig. 5).

Fig. 6 shows cDA contents in the HL experiment as a function of the growth rate. For irradiances equal to or above 100 μmol photons m^{-2} s^{-1}, the toxin content of the cells increased significantly and exponentially with increasing growth rate ($p < 0.0001$; $R^2 = 0.5$).

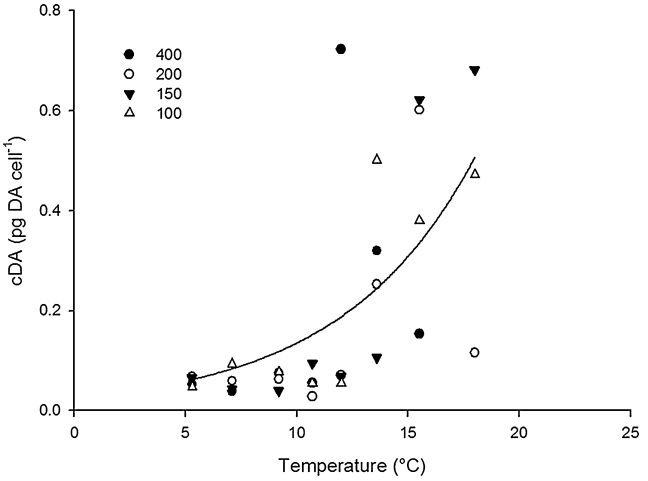

Fig. 5. *Pseudo-nitzschia australis* cell DA (cDA) content as a function of temperature for the four highest irradiances tested (high-light experiment); $f = e^{(0.1652(x-22.12))}$; $p < 0.0001$; $R^2 = 0.42$.

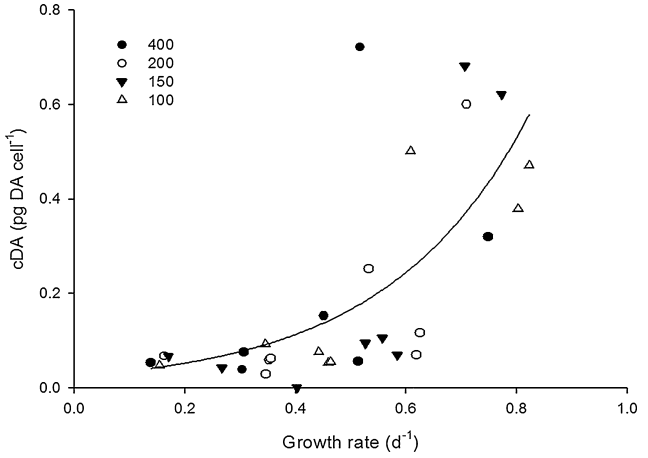

Fig. 6. *Pseudo-nitzschia australis* cell DA (cDA) content as a function of growth rate for the four highest irradiances; $f = 0.0247e^{3.8339x}$; $p < 0.0001$; $R^2 = 0.5$.

4. Discussion

4.1. Effects of temperature and light on growth

Under all irradiances tested, *Pseudo-nitzschia australis* growth rate increased slightly until the optimum temperature ($Topt_{(\mu)}$; 13.5–18.6 °C depending on light intensity) was reached and then decreased sharply with increasing temperature. No growth was observed below 5 °C or above 21 °C at any of the irradiances tested. Temperature control of enzymatic kinetics is known to influence phytoplankton growth. At high temperatures, enzyme denaturation may occur and can affect the cellular concentrations of Rubisco, the Calvin cycle enzymes (Davison, 1991), and hence photosynthetic activity and efficiency (Claquin et al., 2008). At low temperatures, the overall metabolism of phytoplankton slows down, leading to a reduction in division rate (Fiala and Oriol, 1990). The diffusion properties and the composition of cellular membranes are also modified at low temperatures. This reduces the efficiency of electron transport, which, in turn, alters photosynthesis processes. As previously reported by Li and Morris (1982), the observed decrease in growth rate at low temperatures was smaller than the decrease under high temperatures over the optimum. At high temperatures, destructive processes occurred, while at low temperature there was simply a reduction in metabolic efficiency, which may have been partially offset by thermal acclimation processes. For example, photosynthetic activity can be maintained at low temperature by increasing the Rubisco concentration (Li and Morris, 1982), which compensates for the decrease in activity (Anning et al., 2001). In these conditions, light–temperature interactions play an important role in physiological responses. It is therefore not surprising that the lethal temperature measured in the present study was very close to the optimum growth temperature for *P. australis* at high temperatures and reflected the effects of light–temperature interactions. This pattern has already been reported in other diatom species (Eppley, 1972; Kaeriyama et al., 2011) and was previously observed in *Pseudo-nitzschia fraudulenta* (Claquin et al., 2008) and *P. australis* under Si-limitations (Santiago-Morales and García-Mendoza, 2011). We found that *P. australis* was able to grow under a wide range of temperatures. Few data are available on the range of growth temperatures of *Pseudo-nitzschia* spp. Santiago-Morales and García-Mendoza (2011) observed very low growth rates below 12 °C (0.32 d^{-1} at 10 °C) for *P. australis* from Mexico and no growth above 15 °C, i.e., a narrower temperature range than in the present study. Other strains of *P. australis* isolated in New Zealand might have different temperature preferences since Rhodes et al. (2004) and Holland et al. (2005) reported culture temperature of 19 °C. This can be attributed to differences between *P. australis* ecotypes since the temperature growth range is known to depend on the region from which the strains were isolated (Lelong et al., 2012; Boyd et al., 2013). Intraspecific variability in *P. australis* physiology in response to temperature thus appears to be an important factor that should be taken into account. It is worth noting that the temperature growth range and the optimum growth temperature of the *P. australis* strain studied in this work are consistent with the previously reported occurrence of toxic *P. australis* in the Bay of Seine in September, when the temperature was 17–18 °C (Klein et al., 2010). Temperature growth range is dependent on the strain isolation region and also on intraspecific variability. In Boyd et al. (2013), temperature effects on growth rate were tested on 25 strains of eukaryotic and prokaryotic phytoplankton isolated across a wide range of marine environments. Different physiological responses to temperature were described for strains isolated from a single region thus highlighting the importance of studying multiple strains from a region, when testing for region-specific responses to environmental parameters. Only one strain was studied in this work and the intraspecific variability in the physiological responses of *P. australis* isolated from the English Channel remains to be investigated.

The observed $Topt_{(\mu)}$ for *Pseudo-nitzschia australis* growth varied as a function of irradiance and was lower under high irradiance. Under high irradiance, saturation of the photosynthetic apparatus occurred at a lower temperature than under low irradiance (data not shown), which affected the growth rate. This regulation resulted in a reduction in the temperature growth range under high irradiance. In contrast to *P. australis*, no shift in $Topt_{(\mu)}$ was observed for *Pseudo-nitzschia granii* under similar temperatures and with irradiances ranging between 20 and 150 μmol photons m^{-2} s^{-1} (El-Sabaawi and Harrison, 2006). It thus appears that *Pseudo-nitzschia* species are able to grow under high irradiances but interactive effects of light and temperature on growth range depend on the studied species.

According to the Eilers and Peeters (1988) formulation, we found a significant correlation between μ_{MAX} and light intensity. The optimum irradiance for *Pseudo-nitzschia australis* growth was 100 μmol photons m^{-2} s^{-1}. This result is in agreement with that of Cochlan et al. (2008) for *P. australis*. Above 100 μmol photons m^{-2} s^{-1}, maximum growth rates decreased

and photoinhibition was observed, due to an excess of light energy, which was deleterious for cell growth. In Bill et al. (2012), a Californian *P. australis* strain presented its higher growth rate (1.44 d^{-1}) for an irradiance of 334 μmol photons m^{-2} s^{-1}. The irradiance of saturation of this *P. australis* strain was approximately 200 μmol photons m^{-2} s^{-1} but cells still presented increasing growth rate above this irradiance. This light optimum was higher than the optimum found for our strain (100 μmol photons m^{-2} s^{-1}). At the same time, these results confirm the great acclimation capacities of *P. australis*. This highlights again the need for a better understanding of intraspecific variability in *P. australis* through ecophysiological studies.

4.2. Photoacclimation: variations in chlorophyll a content

Variations in chl *a* content enable photoacclimation of phytoplankton cells. Maximum cellular chl *a* ($chla_{MAX}$) content decreased with increasing irradiance. Under higher irradiances, *Pseudo-nitzschia* cells reduced their cellular chl *a* levels to avoid damage caused by an excess of energy. Photoacclimation allows the organism to balance light energy with energy demands for carbon fixation and growth (Geider et al., 1997). Chl *a* concentrations in our *Pseudo-nitzschia australis* strain (0.98–4.2 pg chl *a* $cell^{-1}$) appeared to be in the same order of magnitude as *P. australis* strains studied in Howard et al. (2007) and Santiago-Morales and García-Mendoza (2011) (2.5 pg chl *a* $cell^{-1}$). These results should be taken with caution because cultures, in these studies, were not in the same conditions as in our study. Cellular chl *a* content varies widely, depending on the species, growth conditions and biovolume (Geider et al., 1997) and comparisons of cellular chl *a* concentrations among studies are therefore delicate. We observed an increase in chl *a* concentration with increasing temperature up to an optimum that depended on light intensity. Variations in cellular chl *a* content allowed cells to adjust their ability to harvest light. Cells grown under high temperatures need more energy to support higher enzymatic activity (Thompson et al., 1992). It has been shown that at high temperatures, feedback processes for chl *a* synthesis are affected, leading to a reduction in the photoacclimation capacities of the cells (El-Sabaawi and Harrison, 2006) and to photoinhibition of growth, as we observed in this study. The observed decrease in cellular chl *a* content at low temperatures supports the hypothesis that the physiological response of low-temperature acclimated cells mimics high-light acclimation. The decrease in cellular chl *a* content protects cells from excess energy (Maxwell et al., 1994). The slowdown of cell metabolism at low temperatures could cause an overflow of electrons in the photosystem and the formation of toxic reactive oxygen species (ROS). A decrease in pigment concentration reduces the amount of light energy received, avoiding saturation and hence damage to the photosynthetic apparatus.

4.3. F_v/F_m as a good proxy for acclimation

The maximum quantum yield of PSII, F_v/F_m estimated by PAM fluorescence, is widely used as an indicator of the physiological state (Kolber et al., 1988; Lippemeier et al., 2001), with optimum values around 0.6 for most phytoplankton species. In our study, F_v/F_m did not reflect *Pseudo-nitzschia australis* growth dynamics. F_v/F_m remained constant and high under all temperatures and irradiances tested.

Within this wide temperature range, high F_v/F_m values and the discrepancies with growth indicate, as already suggested by some studies (Parkhill et al., 2001; Kruskopf and Flynn, 2006; Claquin et al., 2010; Napoléon et al., 2013), that F_v/F_m is not a good proxy for growth capacities in balanced systems. The observed lack of sensitivity of F_v/F_m to the temperature gradient demonstrates that the cultures were in steady state, but not necessarily in the absence of thermal stress. This decoupling between growth rate and F_v/F_m has already been reported in other studies. Parkhill et al. (2001) observed a constant F_v/F_m for the diatom *Thalassiosira pseudonana* acclimated to N-limitation, whereas growth was in fact affected. No difference in F_v/F_m was detected in the polar diatom *Fragilariopsis cylindrus* after a long acclimation in steady-state conditions at −1 °C and +7 °C (Mock and Hoch, 2005). In *Pseudo-nitzschia granii*, El-Sabaawi and Harrison (2006) showed that F_v/F_m did not differ significantly ($p > 0.05$) between 10 and 17 °C under four continuous light treatments (20, 50, 100 and 150 μmol photons m^{-2} s^{-1}), whereas it decreased at 8 and 20 °C, especially under high irradiance.

Measurements of fluorescence in the present study showed that, except at extreme temperatures, F_v/F_m was independent of both irradiance and temperature. However, the fact that extreme temperatures (3.5 and 21 °C) have a significant effect on F_v/F_m points to a strong effect of extreme temperature on PSII efficiency (Maxwell and Johnson, 2000). At extreme temperatures, light–temperature interactions did not allow efficient photosynthesis and the cells were not able to acclimate, thus explaining the decrease in F_v/F_m. Thermal stress was stronger under these conditions and may have affected PSII integrity, especially at the antenna level and enzyme kinetics (Rubisco) involved in carbon fixation (Raven and Geider, 1988; Davison, 1991), which explains the decrease in the physiological state of *Pseudo-nitzschia australis* at 3.5 and 21 °C.

4.4. Domoic acid production

Whenever growth occurred at the temperature and light conditions tested, *Pseudo-nitzschia australis* always produced DA under balanced growth and without nutrient limitation. The values of cDA we measured are similar to previously observed DA concentrations in *P. australis*, although in the lower range (0.04–37 pg DA $cell^{-1}$; Garrison et al., 1992; Cusack et al., 2002; Rhodes et al., 2004; Álvarez et al., 2009).

Many studies on several *Pseudo-nitzschia* species reported that DA production occurs during the stationary phase of growth and generally under stress conditions, especially nutrient limitation, light excess or high temperatures (Bates, 1998; Lelong et al., 2012). However, this pattern is not true for all *Pseudo-nitzschia* species and some studies found contradictory results. For *Pseudo-nitzschia australis*, Cusack et al. (2002) and Garrison et al. (1992) showed that DA production started during the exponential growth phase and continued during the stationary phase. Our results confirm that *P. australis* produces DA during exponential growth. Pan et al. (2001) observed DA production during the exponential phase in *Pseudo-nitzschia.* sp. cf. *pseudodelicatissima*. These contradictory results suggest that the mechanism of DA production may differ among species that produce DA during the stationary phase, like *Pseudo-nitzschia multiseries* (Bates et al., 1991) or *Pseudo-nitzschia seriata* (Lundholm et al., 1994), and *Pseudo-nitzschia* species that produce DA during the exponential phase. The factors that trigger DA production in *Pseudo-nitzschia* species are still not well understood but two mechanisms are frequently cited to explain it: a slowing of division rate, and depletion of Si or P (Bates et al., 1998). In the present study, *P. australis* cells were not nutrient limited. Many studies showed that nutrient limitation can increase DA production by *Pseudo-nitzschia* species (Lelong et al., 2012; Trainer et al., 2012). This could explain why the DA concentrations measured during our experiment without nutrient limitation were in the low range of the DA production range reported for *P. australis* (0.15–2.0 pg DA $cell^{-1}$). Nutrient limitation may thus result in higher cDA concentrations in *P. australis* under similar

temperature and light conditions. This hypothesis requires further investigations.

During our experiments, *Pseudo-nitzschia australis* DA production was stimulated by increasing temperature under high light conditions. Currently, the effects of temperature on DA production by *Pseudo-nitzschia* species reported in the literature are not clear. Some authors report marked differences in toxicity as a function of temperature among different *Pseudo-nitzschia* species but also among different strains isolated from the same geographical area (Bates, 1998; Bates and Trainer, 2006). For example, the results reported by Santiago-Morales and García-Mendoza (2011) were unclear, with wide variability in DA production as a function of temperature between two different strains of *P. australis* and even among replicates. For *Pseudo-nitzschia multiseries*, however, an increase in temperature was reported to stimulate DA production (Lewis et al., 1993). In contrast, *Pseudo-nitzschia seriata* presented an increase of DA production with a decreasing temperature (Lundholm et al., 1994). Furthermore, in *Pseudo-nitzschia multistriata*, DA production was lower at 27 °C than at 18 °C (Amato et al., 2010). In the present study, thermal stress could have explained the increase in cDA with increasing temperature, but this hypothesis is not in agreement with the observed link between growth rate and DA production.

In our study, cultures grown in high-light conditions produced more DA than cultures grown in low-light conditions. Under lower irradiance (<90 μmol photons m^{-2} s^{-1}), DA was detected but was below the quantification limit. However, it is interesting to note that DA continued to be produced under low-light conditions, although to a lesser extent than in higher light. These results agree with observations on *Pseudo-nitzschia australis* (Cusack et al., 2002) and *Pseudo-nitzschia multiseries* (Bates, 1998). In these studies, DA production was considerably higher under higher irradiance. With our *P. australis* strain, maximum DA production was 18 times higher at 400 than at 35 μmol photons m^{-2} s^{-1}, which is slightly lower than the difference in DA production between high and low irradiance observed in *P. australis* by Cusack et al. (2002) (24–130 times higher at higher irradiance). In our study, the difference between LL and HL experiments may also be explained by a loss of toxicity of our *P. australis* strain, since the LL experiment began 6 months after the HL experiment. A decrease in toxicity of a culture over time has already been shown in *P. multiseries* (Bates et al., 1999) and *P. australis* (Rhodes et al., 2004). *P. multiseries* DA production decreased with the age of the culture, as did cell size (Bates et al., 1999). In any case, in our HL experiment, DA production increased with increasing irradiance. These results are in agreement with the hypothesis that DA production depends on the metabolic energy derived from photosynthesis (Bates et al., 1991; Pan et al., 1998) and that light intensity can thus affect cellular DA content (Cusack et al., 2002).

In our test conditions, toxin production appeared to be exponentially linked to growth rate as a result of the influence of temperature and light. And, like growth rate, DA production was influenced by temperature and light interactions; maximum DA production appeared to shift to lower temperatures with increasing irradiance. Consequently, higher values of cDA were observed at higher growth rates. In contrast, an inverse relationship between the cell division rate and DA production was observed in *Pseudo-nitzschia multiseries* (Bates et al., 1996; Pan et al., 1996a, 1996b) and was explained by the slowing of cell division and accumulation of DA in cells due to physiological stress. Our results show that, under balanced growth conditions and without nutrient limitation, *Pseudo-nitzschia australis* cells produce more DA when conditions are optimal for their growth. DA thus appears to be produced when photosynthesis is efficient. Similar results were obtained in *Pseudo-nitzschia* sp. cf. *pseudodelicatissima* (Pan et al., 2001). However, (Pan et al., 1996a, 1996b) suggested that DA is synthesized by *P. multiseries* when photosynthetic energy is not used for primary metabolism, i.e. during a decline in physiological activity when growth rates are low. These authors suggested that DA metabolism depends on secondary metabolic pathways. Our results do not fully support this hypothesis, since they showed that small amounts of DA can be produced by actively growing *P. australis* cells when primary metabolism is high. This confirms the results obtained by Pan et al. (2001) in *P.* sp. cf. *pseudodelicatissima* and supports their hypothesis that DA production dynamics and, hence, DA production mechanisms, may differ among *Pseudo-nitzschia* species. These findings suggest that shellfishes could begin to accumulate DA during the initial phase of a *P. australis* bloom, even at low *P. australis* cell concentrations. This may have important consequences on the dynamics and the extent of ASP events. These results stress the importance of studying DA production over the entire growth cycle of *Pseudo-nitzschia*.

5. Conclusion

This study shows that a *Pseudo-nitzschia australis* strain from the English Channel can grow at a temperature range of 5–21 °C and irradiances between 35 and 400 μmol photons m^{-2} s^{-1}. These results are in agreement with the wide biogeographic distribution of this species. Differences in optimum temperature for growth reported in the literature suggest the existence of *P. australis* ecotypes. In our study, the optimal irradiance for growth was 100 μmol photons m^{-2} s^{-1}, which is frequently described in the literature as the optimum irradiance for growth for many *Pseudo-nitzschia* species. Light–temperature interactions also had a significant influence on growth and DA production. One important finding of this study is that *P. australis* produced DA under nutrient replete-conditions and during exponential growth although at decreased levels, irrespective of the temperature and light conditions that allowed growth. DA appears to be produced by *P. australis* under optimum conditions for growth without nutrient or physiological stress. This type of regulation, i.e. DA production during exponential growth, has major consequences for toxin production dynamics during *P. australis* blooms. DA production may occur throughout the duration of the bloom and not only during its decline, leading to toxic events when environmental conditions are favourable. Besides exploring processes, these results will help parameterize future models of growth and DA production, especially for *P. australis*.

Acknowledgements

The authors are most grateful to Dr. Didier Goux for his assistance with species identification by TEM. We thank the BOREA laboratory staff for help with the experiments, Dr. Francis Orvain for his help with statistical analyses and Nicolas Schiffrine for his valuable help during the study. We also thank two anonymous reviewers for their constructive comments. This work was supported by the ANR-2010-STRA-010 COMANCHE.

Maxine Thorel received a PhD fellowship from the Ministère de la Recherche et de l'Enseignement Supérieur and this paper is part of her Ph.D. thesis.[SS]

References

Álvarez, G., Uribe, E., Quijano-Scheggia, S., López-Rivera, A., Blanco, J., Marin, C., 2009. Domoic acid production by *Pseudo-nitzschia australis* and *Pseudo-nitzschia calliantha* isolated from North Chile. Harmful Algae 8, 938–945.

Amato, A., Lüdeking, A., Kooistra, W.H.C.F., 2010. Intracellular domoic acid production in *Pseudo-nitzschia multistriata* isolated from the Gulf of Naples (Tyrrhenian Sea, Italy). Toxicon 55, 157–161.

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication nutrient sources, composition, and consequences. Estuaries 25, 704–726.

Anning, T., Harris, G., Geider, R.J., 2001. Thermal acclimation in the marine diatom *Chaetoceros calcitrans* (Baxillariophyceae). Eur. J. Phycol. 36, 233–241.

Bates, S.S., 1998. Ecophysiology and metabolism of ASP toxin production. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Phycological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 405–426.

Bates, S.S., 2000. Domoic-acid-producing diatoms: another genus added! J. Phycol. 985, 978–983.

Bates, S.S., Trainer, V.L., 2006. The ecology of harmful diatoms. In: Ecology of Harmful Algae. Springer-Verlag, Heidelberg, pp. 81–93.

Bates, S.S., de Freitas, A.S.W., Milley, J.E., Pocklington, R., Quilliam, M.A., Smith, J.C., Worms, J., 1991. Controls on domoic acid production by the diatom *Nitzschia pungens* f. *multiseries* in culture: nutrients and irradiance. Can. J. Fish. Aquat. Sci. 48, 1136–1144.

Bates, S.S., Garrison, D.L., Horner, R.A., 1998. Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Heidelberg, pp. 267–292.

Bates, S.S., Hiltz, M.F., Léger, C., 1999. Domoic acid toxicity of large new cells of *Pseudo-nitzschia multiseries* resulting from sexual reproduction. In: Martin, J.L., Haya, K. (Eds.), Sixth Canadian Workshop on Harmful Marine Algae. Can. Tech. Rep. Fish. Aquat. Sci., 2261. St. Andrews, Canada, pp. 21–26.

Bates, S.S., Léger, C., Smith, K.M., 1996. Domoic acid production by the diatom *Pseudo-nitzschia multiseries* as a function of division rate in silicate limited chemostat culture. In: Yasumoto, T., Oshima, Y., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission, UNESCO, Paris, pp. 163–166.

Beardall, J., Morris, I., 1976. The concept of light intensity adaptation in marine phytoplankton: some experiments with *Phaeodactylum tricomutum*. Mar. Biol. 37, 377–387.

Bill, B.D., Cochlan, W.P., Trainer, V.L., 2012. The effect of light on growth rate and primary productivity in *Pseudo-nitzschia australis* and *Pseudo-nitzschia turgidula*. In: Pagou, P., Hallegraeff, G. (Eds.), Proceedings of the 14th International Conference on Harmful Algae. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, pp. 78–80.

Blanchard, G.F., Guarini, J.M., Richard, P., Gros, P., Mornet, F., 1996. Quantifying the short-term temperature effect on light-saturated photosynthesis of intertidal microphytobenthos. Mar. Ecol. Prog. Ser. 134, 309–313.

Boyd, P.W., Rynearson, T.A., Armstrong, E.A., Fu, F., Hayashi, K., Hu, Z., Hutchins, D.A., Kudela, R.M., Litchman, E., Mulholland, M.R., Passow, U., Strzepek, R.F., Whittaker, K.A., Yu, E., Thomas, M.K., 2013. Marine phytoplankton temperature versus growth responses from polar to tropical waters – outcome of a scientific community-wide study. PLOS ONE 8, e63091.

Brown, J.L., Nijar, M.S., 1995. The release of glutamate and aspartate from rat-brain synaptosomes in response to domoic acid (Amnesic Shellfish Poisonning) and kinetic acid. Mol. Cell. Biochem. 151, 49–54.

Buck, K.R., Uttal-Cooke, L., Pilskaln, C.H., Roelke, D.L., Villac, M.C., Fryxell, G.A., Cifuentes, L., Chavez, F.P., 1992. Autecology of the diatom, a domoic acid producer, from Monterey Bay, California. Mar. Ecol. Prog. Ser. 84, 293–302.

Claquin, P., Longphuirt, S., Fouillaron, P., Huonnic, P., Ragueneau, O., Klein, C., Leynaert, A., 2010. Effects of simulated benthic fluxes on phytoplankton dynamic and photosynthetic parameters in a mesocosm experiment (Bay of Brest France). Estuar. Coast. Shelf Sci. 86, 93–101.

Claquin, P., Probert, I., Lefebvre, S., Veron, B., 2008. Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. Aquat. Microb. Ecol. 51, 1–11.

Cloern, J.E., 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay. Calif. Rev. Geophys. 32, 127–168.

Cochlan, W.P., Herndon, J., Kudela, R.M., 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). Harmful Algae 8, 111–118.

Cusack, C.K., Bates, S.S., Quilliam, M.A., Patching, J.W., Raine, R., 2002. Confirmation of domoic acid production by *Pseudo-nitzschia australis* (Bacillariophyceae) isolated from Irish waters. J. Phycol. 38, 1106–1112.

Davison, I.R., 1991. Environmental effects on algal photosynthesis: temperature. J. Phycol. 27, 2–8.

Eilers, P.H.C., Peeters, J.C.H., 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecol. Model. 42, 199–215.

El-Sabaawi, R., Harrison, P.J., 2006. Interactive effects of irradiance and temperature on the photosynthetic physiology of the pennate diatom *Pseudo-nitzschia granii* (Bacillariophyceae) from the northeast subarctic Pacific. J. Phycol. 42, 778–785.

Eppley, R.W., 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70, 1063–1085.

Falkowski, P.G., Raven, J., 1997. Aquatic Photosynthesis. Blackwell, Malden, MA, USA.

Fernandes, L.F., Hubbard, K.A., Richlen, M.L., Smith, J., Bates, S.S., Ehrman, J., Léger, C., Mafra, L.L., Kulis, D., Quilliam, M., Libera, K., McCauley, L., Anderson, D.M., 2014. Diversity and toxicity of the diatom *Pseudo-nitzschia* Peragallo in the Gulf of Maine, Northwestern Atlantic Ocean. Deep Sea Res. II 103, 139–162.

Fiala, M., Oriol, L., 1990. Light–temperature interactions on the growth of Antarctic diatoms. Polar Biol. 10, 629–636.

Fritz, L., Quilliam, M.A., Wright, J.L.C., Beale, A.M., Work, T.M., 1992. An outbreak of domoic acid poisoning attributed to the pennate diatom *Pseudonitzschia australis*. J. Phycol. 28, 439–442.

Fu, F., Tatters, A., Hutchins, D., 2012. Global change and the future of harmful algal blooms in the ocean. Mar. Ecol. Prog. Ser. 470, 207–233.

Gallacher, S., Howard, G., Hess, P., MacDonald, E., Kelly, M.C., Bates, L.A., Brown, N., MacKenzie, M., Gillibrand, P., Turrell, W.R., 2001. The occurrence of amnesic shellfish poisons in shellfish from Scottish waters. In: Hallegraef, G.M., Blackburn, S.I., Bolch, C.J., Lewis, D. (Eds.), Harmful Algal Blooms 2000: Proceedings of the Ninth International Conference on Harmful Algal Blooms. UNESCO, Hobart, Australia, pp. 30–33.

García-Mendoza, E., Rivas, D., Olivos-Ortiz, A., Almazán-Becerril, A., Castañeda-Vega, C., Peña-Manjarrez, J.L., 2009. A toxic *Pseudo-nitzschia* bloom in Todos Santos Bay, northwestern Baja California, México. Harmful Algae 8, 493–503.

Garrison, D.L., Conrad, S.M., Eilers, P.H.C., Waldron, E.M., 1992. Confirmation of domoic acid production by *Pseudo-nitzschia australis* (Bacillariophyceae) cultures. J. Phycol. 28, 604–607.

Geider, R.J., Macintyre, H.L., Kana, T.M., 1997. Dynamic model of phytoplankton growth and acclimation: responses of the balanced growth rate and the chlorophyll *a*: carbon ratio to light, nutrient-limitation and temperature. Mar. Biol. 148, 187–200.

Genty, B., Briantais, J., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and of quenching chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.

Hallegraeff, G.M., 2010. Ocean climate, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J. Phycol. 46, 220–235.

Hasle, G.R., 2002. Are most of the domoic acid-producing species of the diatom genus *Pseudo-nitzschia cosmopolites*? Harmful Algae 1, 137–146.

Holland, P.T., Selwood, A.I., Mountfort, D.O., Wilkins, A.L., McNabb, P., Rhodes, L.L., Doucette, G.J., 2005. Isodomoic acid C, an unusual amnesic shellfish poisonning toxin from *Pseudo-nitzschia australis*. Chem. Res. Toxicol. 18, 814–816.

Holtermann, K.E., Bates, S.S., Trainer, V.L., Odell, A., Armbrust, E.V., 2010. Mass sexual reproduction in the toxigenic diatoms *Pseudo-nitzschia australis* and *P. pungens* (Bacillariophyceae) on the Washington Coast, USA. J. Phycol. 46, 41–52.

Howard, M.D.A., Cochlan, W.P., Ladizinsky, N., Kudela, R.M., 2007. Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. Harmful Algae 6, 206–217.

Kaeriyama, H., Katsuki, E., Otsubo, M., Yamada, M., Ichimi, K., Tada, K., Harrison, P.J., 2011. Effects of temperature and irradiance on growth of strains belonging to seven *Skeletonema* species isolated from Dokai Bay, Southern Japan. Eur. J. Phycol. 46, 113–124.

Keller, M.D., Selvin, R.C., Claus, W., Guillard, R.R.L., 1987. Media for the culture of oceanic ultraphytoplankton. J. Phycol. 23, 633–638.

Klein, C., Claquin, P., Bouchart, V., Le Roy, B., Véron, B., 2010. Dynamics of *Pseudo-nitzschia* spp. and domoic acid production in a macrotidal ecosystem of the Eastern English Channel (Normandy, France). Harmful Algae 9, 218–226.

Kolber, Z., Zehr, J., Falkowski, P., 1988. Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. Plant Physiol. 88, 923–929.

Kruskopf, M., Flynn, K.J., 2006. Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. New Phytol. 169, 525–536.

Lelong, A., Hégaret, H., Soudant, P., Bates, S.S., 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia 51, 168–216.

Lewis, N.I., Bates, S.S., McLachlan, J.L., Smith, J.C., 1993. Temperature effects on growth, domoic acid production and morphology of the diatom *Nitzschia pungens* f. *multiseries*. In: Smayda, T.J., Shimuzu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Sci Publ. B.V., Amsterdam, pp. 601–606.

Li, W.K.W., Morris, I., 1982. Temperature adaptation in *Phaeodactylum tricornutum* Bohlin: photosynthetic rate compensation and capacity. J. Exp. Mar. Biol. Ecol. 58, 135–150.

Lim, H.-C., Leaw, C.-P., Su, S.N.-P., Teng, S.-T., Usup, G., Noor, N.M., Lundholm, N., Kotaki, Y., Lim, P.-T., 2012. Morphology and molecular characterization of *Pseudo-nitzschia* (Bacillariophyceae) from Malaysian Borneo, including the new species *Pseudo-nitzschia circumpora* sp. nov. J. Phycol. 48, 1232–1247.

Lim, H.C., Teng, S.T., Leaw, C.P., Lim, P.T., 2013. Three novel species in the *Pseudo-nitzschia pseudodelicatissima* complex: *P. batesiana* sp. nov., *P. lundholmiae* sp. nov., and *P. fukuyoi* sp. nov. (Bacillariophyceae) from the Strait of Malacca, Malays. J. Phycol. 49, 902–916.

Lippemeier, S., Hintze, R., Vanselow, K., Hartig, P., Colijn, F., 2001. In-line recording of PAM fluorescence of phytoplankton cultures as a new tool for studying effects of fluctuating nutrient supply on photosynthesis. Eur. J. Phycol. 36, 89–100.

Lundholm, N., Skov, J., Pocklington, R., Moestrup, Ø., 1994. Domoic acid, the toxic amino acid responsible for amnesic shellfish poisonning, now in *Pseudonitzschia seriata* (Bacillariophyceae) in Europe. Phycologia 33, 475–478.

Lundholm, N., Skov, J., Pocklington, R., Moestrup, Ø., 1997. Studies on the marine planktonic diatom *Pseudo-nitzschia*. 2. Autecology of *P. pseudodelicatissima* based on isolates from Danish coastal waters. Phycologia 36, 381–388.

MacIntyre, H.L., Cullen, J.J., 2005. Using cultures to investigate the physiological ecology of microalgae. In: Andersen, R.A. (Ed.), Algal Culturing Techniques. Elsevier Academic Press, Amsterdam, pp. 287–326.

Maxwell, D.P., Falk, S., Huner, N.P.A., 1994. Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. Plant Physiol. 105, 535–543.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence – a practical guide. J. Exp. Bot. 51, 659–668.

Mìguez, A., Fernandez, M.L., Fraga, S., 1996. First detection of domoic acid in Galicia (NW of Spain). In: Yasumoto, T., Fukuyo, Y. (Eds.), Harmful and Toxic Algal Blooms. Intergovernmental Oceanographic Commission, UNESCO, Paris, pp. 143–145.

Mock, T., Hoch, N., 2005. Long-term temperature acclimation of photosynthesis in steady-state cultures of the polar diatom *Fragilariopsis cylindrus*. Photosynth. Res. 85, 307–317.

Mos, L., 2001. Domoic acid: a fascinating marine toxin. Environ. Toxicol. Pharmacol. 9, 79–85.

Napoléon, C., Raimbault, V., Claquin, P., 2013. Influence of nutrient stress on the relationships between PAM Measurements and carbon incorporation in four phytoplankton species. PLOS ONE 8 (6) .

Nezan, E., Antoine, E., Fiant, L., Amzil, Z., Billard, C., 2006. Identification of *Pseudo-nitzschia australis* and *P. multiseries* in the Bay of Seine. Was there a relation to presence of domoic acid in king scallops in autumn 2004? Harmful Algae News 31, 1–3.

Orive, E., Perez-Aicua, L., David, H., Garcia-Etxebarria, K., Laza-Martinez, A., Seoane, S., Miguel, I., 2013. the genus *Pseudo-nitzschia* (Bacillariophyceae) in a temperature estuary with description of two species: *Pseudo-nitzschia plurisecta* sp. nov. and *Pseudo-nitzschia abrensis* sp. nov. J. Phycol. 49, 1192–1206.

Pan, Y., Subba Rao, D.V., Mann, K.H., Li, W.K.W., Harrison, W.G., 1996a. Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. II Continuous culture studies. Mar. Ecol. Prog. Ser. 131, 235–243.

Pan, Y., Subba Rao, D.V., Mann, K.H., 1996b. Changes in domoic acid production and cellular chemical composition of the toxigenic *Pseudo-nitzschia multiseries* under phosphate limitation. J. Phycol. 32, 371–381.

Pan, Y., Bates, S.S., Cembella, A.D., 1998. Environmental stress and domoic acid production by *Pseudo-nitzschia*: a physiological perspective. Nat. Toxins 6, 127–135.

Pan, Y., Parsons, M.L., Busman, M., Moeller, P.D.R., Dortch, Q., Powell, C.L., Doucette, G.J., 2001. *Pseudo-nitzschia* sp. cf. pseudodelicatissima – a confirmed producer of domoic acid from the northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 220, 83–92.

Parkhill, J., Maillet, G., Cullen, J.J., 2001. Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J. Phycol. 529, 517–529.

Pulido, O.M., 2008. Domoic acid toxicologic pathology: a review. Mar. Drugs 6, 180–219.

Raven, J., Geider, R.J., 1988. Temperature and algal growth. New Phycol. 110, 441–461.

Rhodes, L., Holland, P., Adamson, J., Selwood, A., Mcnabb, P., 2004. Mass culture of New Zealand isolates of *Pseudo-nitzschia australis* for production of a new isomer of domoic acid. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, Intergovernmental Oceanographic Commission, UNESCO, pp. 125–127.

Rhodes, L.L., 1998. Identification of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) in New-Zealand coastal waters, using lectins. Nouv. J. Mar. Freshw. Res. 32, 537–544.

Santiago-Morales, I.S., García-Mendoza, E., 2011. Growth and domoic acid content of *Pseudo-nitzschia australis* isolated from northwestern Baja California, Mexico, cultured under batch conditions at different temperatures and two $Si:NO_3$ ratios. Harmful Algae 12, 82–94.

Schreiber, U., Schliwa, U., Bilger, W., 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. 10, 51–62.

Sommer, U., Gliwicz, Z.M., Lampert, W., Duncan, A., 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. 106, 433–471.

Straskraba, M., Gnauck, A., 1985. Freshwater Ecosystems: Modelling and Simulation. Elsevier, Amsterdam.

Teng, S.T., Lim, H.C., Lim, P.T., Dao, V.H., Bates, S.S., Leaw, C.P., 2014. *Pseudo-nitzschia kodamae* sp. nov. (Bacillariophyceae), a toxigenic species from the Strait of Malacca, Malaysia. Harmful Algae 34, 17–28.

Thessen, A.E., Bowers, H.A., Stoecker, D.K., 2009. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. Harmful Algae 8, 792–810.

Thompson, P., Guo, M., Harrison, P.J., 1992. Effects of variation in temperature on the biochemical composition of eight species of marine phytoplankton. J. Phycol. 28, 481–488.

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., Trick, C.G., 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14, 271–300.

Villac, M.C., Roelke, D.L., Villareal, T.A., Fryxell, G.A., 1993. Comparison of two domoic acid-producing diatoms: a review. Hydrobiologia 269, 213–224.

Wang, Z., King, K.L., Ramsdell, J.S., Doucette, G.J., 2007. Determination of domoic acid in seawater and phytoplankton by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1163, 169–176.

Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. 39, 1985–1992.

Winder, M., Sommer, U., 2012. Phytoplankton response to a changing climate. Hydrobiologia 698, 5–16.

Wright, J.L.C., Boyd, R.K., de Freitas, A.S.W., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCulloch, A.W., McInnes, A.G., Odense, P., Walter, J.A., Gilgan, M., Richard, D.J.A., Dewar, D., 1989. Identification of domoic acid, a neuroexcitatory amino-acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem. 67, 481–490.