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Factors controlling the production of domoic acid by *Pseudo-nitzschia* (Bacillariophyceae): A model study

Nathan Terseleer *, Nathalie Gypens, Christiane Lancelot

Université Libre de Bruxelles, Écologie des Systèmes Aquatiques, CP-221, Bd du Triomphe, B-1050 Brussels, Belgium

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ABSTRACT

A mechanistic model has been developed to explore the factors controlling the production of domoic acid (DA) by the pennate diatom *Pseudo-nitzschia*. The idealized model allows consideration of the uncoupling between photosynthesis and growth, while DA production has been set as a secondary metabolism sharing common precursors with growth. Under growth limitation, these precursors can accumulate, resulting in an increased DA production. The model was first evaluated based on its ability to simulate the observed DA production by either silicon (Si) or phosphorus (P) limited batch cultures of *Pseudo-nitzschia* available in the literature. Sensitivity tests were further performed to explore how the ambient nutrients and the light regime (intensity and photoperiod length) are possibly directing the *Pseudo-nitzschia* toxicity. The general pattern that emerged is that excess light, in combination with Si or P limitation, favours DA production, provided nitrogen (N) is sufficient. Model simulations with varying nutrient stocks supporting *Pseudo-nitzschia* blooms under non-limiting light suggest two potential ways for nutrients to control DA production. First, N excess in comparison to available Si and P relieves DA production from its limitation by N, an absolute requirement of the DA molecule. Second, increased nutrient stocks amplify the DA production phase of the blooms (in addition to enhancing *Pseudo-nitzschia* biomass) which leads to an even more toxigenic bloom. Simulations investigating the light regime suggest a light threshold below which an important delay in DA production could be expected in *Pseudo-nitzschia* cultures. In the natural environment, the monitoring of light conditions during *Pseudo-nitzschia* blooms might help to anticipate the magnitude of the toxic event. *Pseudo-nitzschia* toxicity is indeed linked to the excess of primary carbon that accumulates during photosynthesis under growth limitation by nutrients.

1. Introduction

Diatoms are a key component of the planktonic community, mostly because of their worldwide distribution, the food web basis they constitute, and the major role they play in global biogeochemical cycles (e.g. reviewed by Armbrust, 2009). However, some species, mostly of the genus *Pseudo-nitzschia*, are also capable of synthesizing domoic acid (DA), a powerful neurotoxin responsible for amnesic shellfish poisoning (ASP) of humans and domoic acid poisoning (DAP) of marine fauna. *Pseudo-nitzschia* is a cosmopolitan genus (Hasle, 2002) of pennate diatoms with about one-third of the 37 described species that are toxigenic (reviewed by Lelong et al., 2012; Trainer et al., 2012). Their occurrence has been reported under both natural (upwelling events) and cultural (anthropogenic inputs) nutrient enrichment (Parsons et al., 2002; Kudela et al., 2008; Trainer et al., 2012). Toxic events associated with *Pseudo-nitzschia* have been observed in many coastal areas of all inhabited continents (reviewed by Trainer et al., 2012). These cause substantial economic losses through the closure of fisheries for extended periods and ecological damage through the mortality of mammals or seabirds (reviewed by Lelong et al., 2012). Therefore *Pseudo-nitzschia* constitutes a target phytoplankton among the harmful algal bloom (HAB) species, and the understanding of environmental conditions that favour the related toxic events is prerequisite for their prediction. Together with experimental work, modelling studies can improve our understanding of *Pseudo-nitzschia* ecophysiology.

Beside the existing statistical–empirical models (Blum et al., 2006; Lane et al., 2009; Anderson et al., 2009, 2010; Palma et al., 2010) that relate *Pseudo-nitzschia* blooms and DA production to various environmental variables (e.g. nutrient concentrations, sea surface temperature, salinity, etc.), mechanistic models that integrate best knowledge on ecophysiological processes are good tools to understand the link between toxin production and environmental conditions. Attempts for developing a comprehensive mechanistic model describing DA synthesis associated to

* Corresponding author. Tel.: +32 2 6505924; fax: +32 2 6505993.
E-mail address: ntersele@ulb.ac.be (N. Terseleer).

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Pseudo-nitzschia wax and wane is seldom and to our knowledge limited to two studies (Siopsis, 2003; Davidson and Fehling, 2006). Davidson and Fehling (2006)'s model is based on the increased DA production observed under silicon (Si) or phosphorus (P) limitation (Fehling et al., 2004) and relates DA synthesis to the nutrient status of *Pseudo-nitzschia* by imposing a threshold nutrient quota at which DA production occurs. The Individual-Based Model of Siopsis (2003) includes the description of nutrient uptake, photosynthesis, synthesis of the cell components (protein, lipid, polysaccharide, frustule and DA), and catabolism of storage products. In this model, DA production occurs under conditions of excess energy and nitrogen (N) and is related to low ambient Si.

In the same line as Siopsis (2003), we propose a conceptual model of *Pseudo-nitzschia* growth and toxin production by considering DA as a secondary metabolite, i.e. not involved directly in growth. The structure of the model is based on the existing observational knowledge of conditions favouring DA production, i.e. minimal DA production during active growth while increasing when growth slows or ceases due to nutrient stress (Si, P; reviewed by Bates, 1998; Bates and Trainer, 2006). Furthermore, several studies reveal a strong link between DA production and the light regime (Bates et al., 1991; Bates and Léger, 1992; Whyte et al., 1995; Cusack et al., 2002; Fehling et al., 2005) with little DA production when light is limiting. Under conditions of sufficient light but unfavourable nutrients for growth, energy and monomeric carbon precursors produced by photosynthesis are made available for apparently non-essential cellular constituents (like DA) synthesis (Pan et al., 1998). The structure of the idealized phytoplankton model AQUAPHY (Lancelot et al., 1991) is suitable for testing and implementing this observation. Based on the explicit description of three cellular constituents (monomeric, reserve and functional) metabolic pathways, its structure explicitly takes into account the decoupling between photosynthetic and growth processes, this latter being related to an internal pool of monomers that can be directed to the production of DA when growth is nutrient-limited. The AQUAPHY model was therefore further developed to describe DA synthesis and release in the external medium. The new model was first tested for its ability to describe DA production and release observed in the same two batch culture experiments (Fehling et al., 2004) as used by Davidson and Fehling (2006) for testing their own model. Afterwards, the model was run in several nutrient and light conditions to explore and better understand the factors triggering DA production by these peculiar diatoms.

2. Materials and methods

2.1. Mathematical model

The AQUAPHY model (Lancelot et al., 1991, 2005) describes key processes associated with phytoplankton growth and mortality as a function of light, temperature and nutrients (Si, P, and N). This model represents phytoplankton as composed of different cellular pools. Adapted to *Pseudo-nitzschia*, these different cellular constituents (Fig. 1) include: the monomeric precursors for macromolecule synthesis (PNS, containing C only); the storage products (PNR, polysaccharides and lipids, C only); the structural and functional macromolecules (PNF, containing C and N, P, Si); and the internal DA pool (PDA, composed of C and N). The total C biomass of *Pseudo-nitzschia* (PN) is given by:

$$\mathrm{PN} = \mathrm{PNS} + \mathrm{PNR} + \mathrm{PNF} + \mathrm{PDA} \ [\mathrm{mmol\ C\ m^{-3}}] \quad (1)$$

A fifth state variable representing the extracellular DA pool (DDA) has been introduced.

The state equations for PNS, PNR and PNF are those described for the diatom component of the ecological model MIRO (Lancelot et al., 2005). Processes (Fig. 1) and parameterization can be found in the Web Appendix.

The state equations describing DA synthesis and extracellular release (Fig. 1) are:

$$\frac{d\mathrm{PDA}}{dt} = \mathrm{DA_{prod}} - \mathrm{excr_{PDA}} - \mathrm{lys.}\frac{\mathrm{PDA}}{\mathrm{PN}} \ [\mathrm{mmol\ C\ m^{-3}\ h^{-1}}] \quad (2)$$

$$\frac{d\mathrm{DDA}}{dt} = \mathrm{excr_{PDA}} + \mathrm{lys.}\frac{\mathrm{PDA}}{\mathrm{PN}} \ [\mathrm{mmol\ C\ m^{-3}\ h^{-1}}] \quad (3)$$

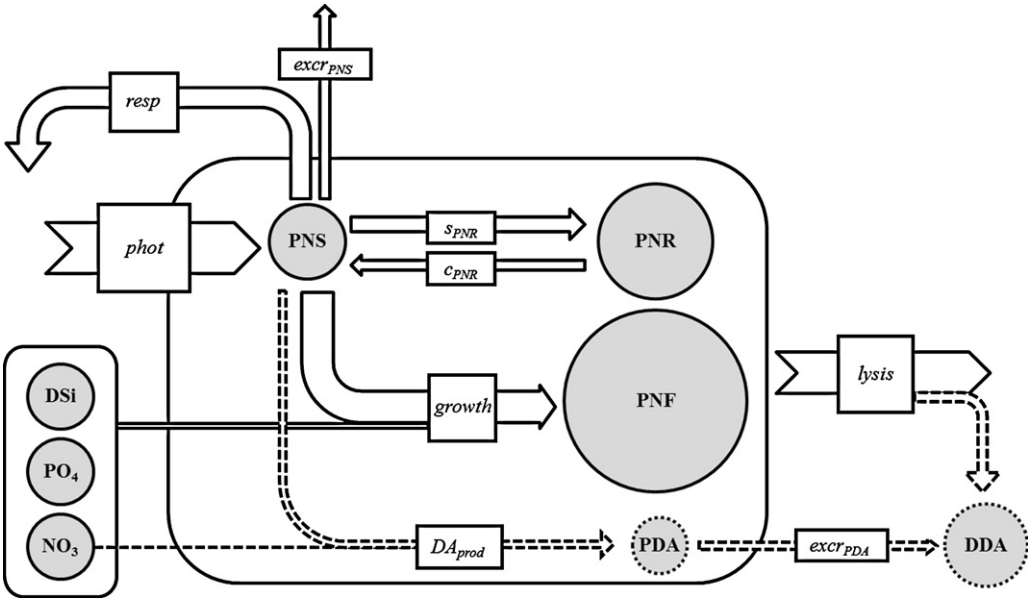

Fig. 1. Structure of the *Pseudo-nitzschia* model composed of four internal pools: monomeric precursors (PNS), reserve materials (PNR), functional pool (PNF) and intracellular DA (PDA). Extracellular DA (DDA) is the dissolved DA in the ambient medium originating from *Pseudo-nitzschia* lysis and PDA excretion ($\mathrm{excr_{PDA}}$). Dotted arrows for DA processes. All nutrients are used for growth, NO_3^- is further taken up to cover the DA production ($\mathrm{DA_{prod}}$) needs. phot, photosynthesis; $\mathrm{excr_{PNS}}$ excretion of PNS; resp, respiration; s_{PNR} and c_{PNR} respectively synthesis and catabolism of PNR (reserve materials).
Adapted from Lancelot et al. (1991).

where t is the time, DA_{prod} the DA cellular production, $excr_{PDA}$ the passive excretion of PDA in the ambient medium, and lys the cell lysis which releases PDA into the ambient medium.

DA production directly depends on the amount of monomeric precursors (PNS) also available for growth (PNF synthesis) or reserve (PNR) synthesis (Fig. 1). These different processes therefore share common precursors in the model, as suggested by observations (Pan et al., 1998). DA production is described by:

$$DA_{prod} = k_{DA} \cdot lim_N \cdot PNS \ [mmol\ C\ m^{-3}\ h^{-1}] \quad (4)$$

with k_{DA} (h^{-1}) the specific rate of DA production. We fixed k_{DA} at $2 \times 10^{-4}\ h^{-1}$ which is in the higher range computed based on the literature range of $\sim 1 \times 10^{-3}$ to 2 pg DA $cell^{-1}$ day^{-1} given for *P. multiseries* (reviewed by Bates, 1998; see also Pan et al., 1996a, b, c; Maldonado et al., 2002) and making use of the observed corresponding C:Cell (20–450 pg C $cell^{-1}$; Pan et al., 1991, 1996b, c, d). lim_N is a function describing the N limitation of DA synthesis owing to the N content of the DA molecule ($C_{15}H_{21}NO_6$) and in agreement with the observed break of DA production under severe N limitation (Bates et al., 1991). This limitation is described by a Michaelis–Menten function (details in the Web Appendix).

The passive excretion of PDA is described as for PNS in the MIRO model, and is given by:

$$excr_{PDA} = k_{excr} \cdot PDA \ [mmol\ C\ m^{-3}\ h^{-1}] \quad (5)$$

with k_{excr} the excretion constant identical to that of PNS excretion (0.001 h^{-1}).

The lysis of the *Pseudo-nitzschia* cells is a function of nutrient stress as in the original MIRO model (Lancelot et al., 2005; details in the Web Appendix). No degradation of DA is considered during the experiments, so no loss term for DDA has been introduced in the model.

Nutrient (nitrate NO_3^-, phosphate PO_4^{3-} and dissolved silicon DSi) uptake is calculated from the needs of the newly synthesized PNF biomass, making use of the upper range stoichiometry of 0.015 mol P:mol C, 0.20 mol Si:mol C and 0.20 mol N:mol C found in the literature for *Pseudo-nitzschia* (Pan et al., 1991, 1996b, c, d; Fehling et al., 2004). In the absence of internal N pool in our simplified *Pseudo-nitzschia* model, N needs associated to DA synthesis are directly estimated from Eq. (4) (Fig. 1) and making use of the N:C molar ratio of DA (1:15). Nutrient and light control the PNF synthesis rate. The former is determined based on half-saturation constants set to 0.5 mmol N m^{-3}, 0.03 mmol P m^{-3} and 0.6 mmol Si m^{-3} as in the last revision of the MIRO model for diatoms (Lancelot et al., 2011). Photosynthetic efficiency of *Pseudo-nitzschia* has been set to $1 \times 10^{-3}\ h^{-1}$ ($\mu mol\ m^{-2}\ s^{-1}$)$^{-1}$ in order to fit with a light adaptation to the irradiance of 120 $\mu mol\ m^{-2}\ s^{-1}$ used in the *Pseudo-nitzschia* culture experiments.

2.2. Simulated experiments.

The batch culture experiments of Fehling et al. (2004) were used for a first test of our model. These were performed for 21 days at 15 °C under 120 $\mu mol\ m^{-2}\ s^{-1}$ illumination and a 12:12 h light:dark cycle with either Si or P as limiting nutrient, the others (including N) being in large excess.

Available data for model comparison include Si and P concentrations, Chlorophyll *a* (Chl *a*), extracellular (DDA) and total DA (DDA + PDA) concentrations. Intracellular PDA is obtained by difference between the measured total DA and DDA. In the model, Chl *a* is estimated from the simulated PNF making use of a constant Chl *a*:C ratio of 0.24 mg Chl *a*:mmol C for this pool (i.e. in the upper range of literature values; Pan et al., 1991, 1996b, c, d; Fehling et al., 2004). This allows consideration of a variable cellular Chl *a*:C through the variations of PNS, PNR and PDA concomitant to PNF.

For the Si-limited experiment, day 1 observations (instead of day 0) have been used as initial conditions for the simulation since an unexplained increase in Si concentration from ~103 μM (day 0) to ~132 μM (day 1) accompanied by the maintenance of elevated concentrations for 3 days is observable in data (Fig. 1a in Fehling et al., 2004) and therefore considered as true initial conditions for the growth of the Si-limited culture.

3. Results

Comparison between model results (simulated *Pseudo-nitzschia* Chl *a*, nutrients, and DA production) and observations are shown in Figs. 2 and 3 for both the Si- and P-limited experiments of Fehling et al. (2004).

3.1. Chl a and nutrients

Examination of Fig. 2a–c indicates that the model describes fairly well the observed evolution – in time and magnitude – of nutrients for both experiments. The switch in nutrient limitation from Si to P consequent to the important reduction in initial PO_4^{3-} relative to DSi concentration (initial Si:P from ~8 to ~37 mol Si:mol P) is well represented by the model (Fig. 2a and b). The timing of the limiting nutrient depletion is correctly captured in both simulated experiments (Fig. 2a and b). Overall, the Si evolution is better traced by the model than PO_4^{3-}, especially the values reached at the plateau. Under P limitation, a full depletion is simulated when data show a constant value of ~1 mmol PO_4^{3-} m^{-3} (Fig. 2b) suggesting some P remineralisation in the batch culture that is not considered by the model. On the contrary the PO_4^{3-} plateau simulated under Si limitation is slightly overestimated compared to observations (Fig. 2b). In both experiments, simulated NO_3^- show a decrease in the early days after which a plateau is reached at concentrations in far excess (>700 mmol m^{-3}; Fig. 2c) as designed by the experimental study (Fehling et al., 2004).

As suggested by the time evolution of Chl *a* (Fig. 2d), the model correctly captures the exponential growth phase as well as the timing of the stationary phase and decay of *Pseudo-nitzschia* when experimenting Si- or P-limited conditions. Less represented is the simulated Chl *a* during the decay phase of the culture, though overall the Chl *a* levels simulated at the end of the decay phases agree with observations (Fig. 2d). Some of the Chl *a* fluctuations observed during the decay phase and suggesting some re-growth associated to either P or Si remineralisation occurring in the batch cultures are not shown by the model as nutrient remineralisation is not considered.

3.2. DA synthesis and extracellular release

Fig. 3 shows the evolution of observed and simulated DA forms (total, cellular and extracellular) over the 21 days of the two experiments. In agreement with observations, the model does not prevent the synthesis of DA during the growth phase (Figs. 2d and 3a), as also reported in other studies (e.g. Maldonado et al., 2002; Marchetti et al., 2008). Yet, the highest DA production is simulated when cells are limited by nutrient depletion (Figs. 2 and b and 3a), in agreement with most observations (reviewed by Bates, 1998; Bates and Trainer, 2006; Lelong et al., 2012; Trainer et al., 2012). In the model, this corresponds to the use for DA synthesis of accumulated PNS (primary carbon) resulting from the slowdown/shutdown of PNF synthesis caused by nutrient limitation.

In Si-limited experiment, total DA (Fig. 3a) reaches a maximum concentration of ~0.8 mmol DA m^{-3} at the end of the experiment,

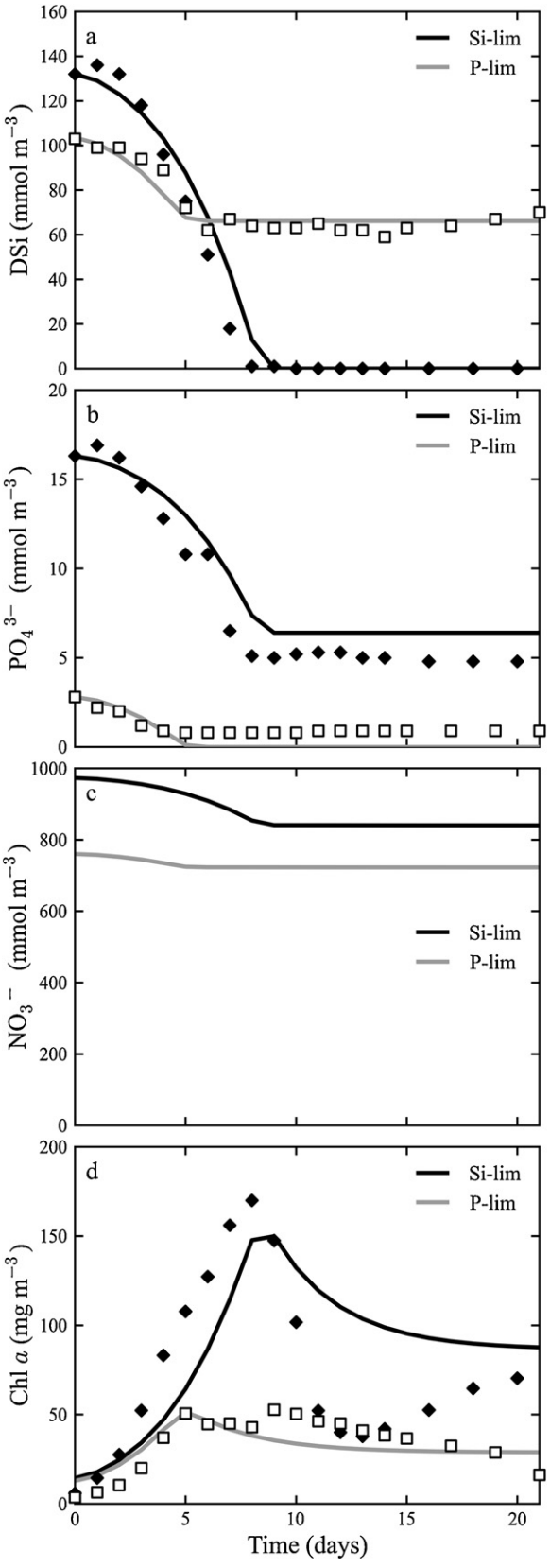

Fig. 2. Model simulation (continuous lines) and observations of nutrients (a–c) and *Pseudo-nitzschia*-Chl *a* (d) for Si-limited (black, diamonds) and P-limited (grey, open squares) limited batch experiments.
Data from Fehling et al. (2004).

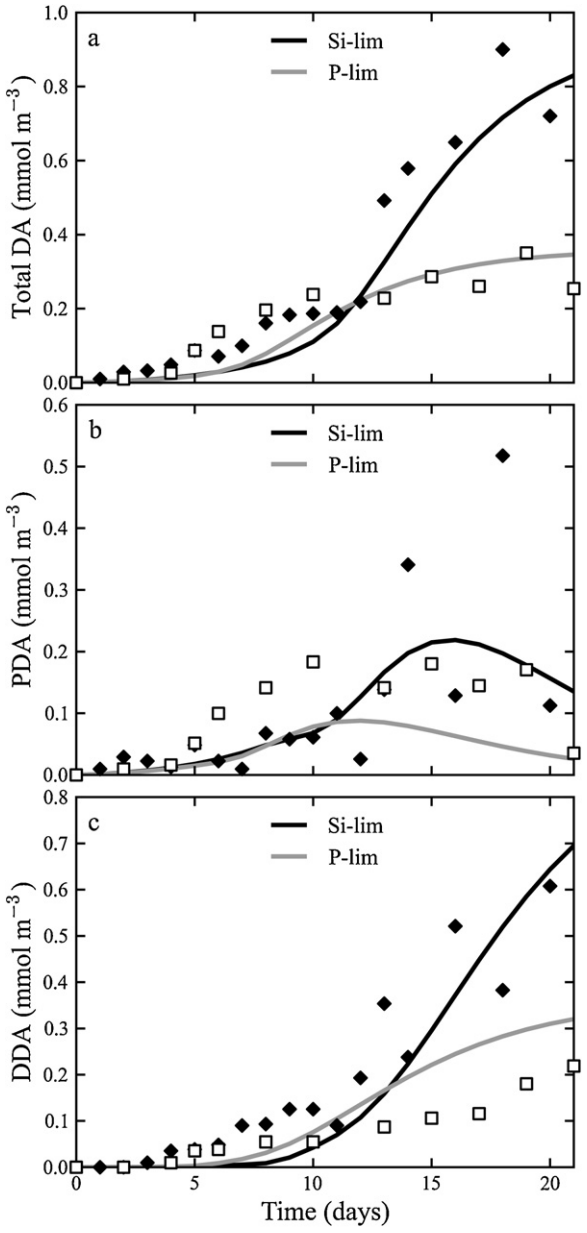

Fig. 3. Model simulation (continuous lines) and observations of total (a), intracellular (PDA, b) and extracellular (DDA, c) DA concentrations obtained for Si-limited (black, diamonds) and P-limited (grey, open squares) limited batch experiments.
Data from Fehling et al. (2004).

in agreement with observations. Overall, the model correctly simulates the observed partitioning between intracellular (PDA, Fig. 3b) and extracellular DA (DDA, Fig. 3c), this latter constituting the main form of DA at the end of the simulation. Fig. 4 compares the simulated evolution of intracellular PNS with that of DA synthesis and extracellular release by either direct excretion or lysis. The DA production is directly linked to the accumulation of PNS and increases more markedly with the accumulation of PNS coinciding with DSi depletion (day 9, Figs. 2a and 4). With the decline of *Pseudo-nitzschia* biomass (and, hence, of PNS), the DA production is reduced at the end of the experiment. The simulated accumulation of DA in the ambient medium (DDA; Fig. 3c) results mainly from enhanced lysis under Si limitation (Figs. 2a and 4), while the contribution of excretion to the external DA pool is minor (Fig. 4). Despite the decreased apparent PDA in the end of the experiment (Fig. 3b; responding to the decrease in *Pseudo-nitzschia*

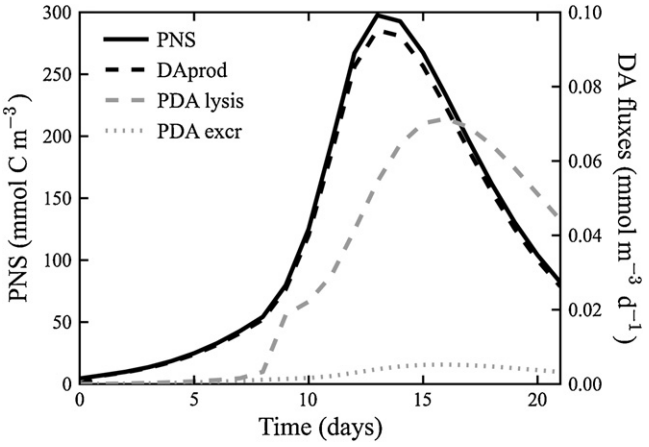

Fig. 4. Model simulation of PNS concentrations (continuous line, left axis) and DA processes (dotted lines, right axis; cf. Eqs. (2) and (3)) in the Si-limited experiment: DA production (DA_{prod}), PDA lysis and PDA excretion (PDAexcr).

biomass), the specific cellular content of DA is higher, in agreement with observations (Fehling et al., 2004).

Similar trends are obtained for total DA simulation under P-limited conditions although the agreement between simulated and observed DA intra- and extracellular forms is less good (Fig. 3). This discrepancy might be due to a severe P control of *Pseudo-nitzschia* growth in our model lacking consideration of P regeneration. The low positive PO_4^{3-} concentration observed at the culture stationary phase possibly reflects P regeneration that maintains little growth (Fig. 2b and c). In contrast, the early slowdown of *Pseudo-nitzschia* growth in our model corresponds to PO_4^{3-} exhaustion (Fig. 2b) which remains to zero and prevents a sufficient PDA accumulation (Fig. 3b). Further, the increased lysis under P limitation causes an important release of intracellular DA in the ambient medium which is overestimated in the simulated stationary phase (Fig. 3c).

A consequence of the formulation used for DA production in the model (Eq. (4)) is that a fixed value for kDA does not preclude the effective specific toxin production rate to vary since it depends on PNS levels (cf. Fig. 4) whose proportion in the total *Pseudo-nitzschia* biomass is influenced by other physiological pathways. Accordingly, the specific DA production rate in the model varies with more than one order of magnitude depending on the conditions of the simulated cultures. A sensitivity analysis (with kDA set to 2×10^{-3} h^{-1} and 2×10^{-5} h^{-1} compared to the reference value of 2×10^{-4} h^{-1}; not shown) showed that the value used for kDA had no effect on *Pseudo-nitzschia* growth while DA production was modified proportionally to the change in kDA. This emphasizes the importance of this parameter for DA production, but indicates that this latter does not compete with growth since it consumes very small amounts of monomers (PNS). In the model, the accumulation of these monomers is directly influenced by light and indirectly by nutrients through their control on growth (PNF synthesis) and reserve (PNR) storage (Fig. 1). The reported controls of these environmental factors on DA production by *Pseudo-nitzschia* are discussed in the next section.

4. Discussion

4.1. Pseudo-nitzschia *mechanistic modelling*

This study aimed to develop a conceptual model of *Pseudo-nitzschia* growth and toxin synthesis in order to better understand mechanisms behind DA production. Compared to the model of Davidson and Fehling (2006) that relies the *P. seriata* DA production to their internal nutrient status (P:C and Si:C), our model links explicitly a secondary metabolism (DA synthesis) to essential processes like photosynthesis, growth, and reserve material synthesis. In our model, DA synthesis is seen as an alternative path using primary carbon and energy originating from photosynthesis when growth is limited by Si or P while N is sufficient. Distinction can thus be made between primary metabolism (growth) and a secondary metabolism through the channelling of excess carbon monomers (PNS) to the DA synthesis (Fig. 1). Davidson and Fehling (2006)'s model chooses a fixed cellular Si:C or P:C of ~0.15 and ~0.0028 mol:mol as tipping points for triggering DA production. In comparison, our model, while conceptually different, simulates the increase of DA production for Si:C or P:C of respectively ~0.14 and ~0.01 mol:mol (not shown), i.e. comparable to Si but nearly four times higher than the P thresholds set by Davidson and Fehling (2006). In an optimized version of their model for P-limited experiment, they found a better DA production with a critical P:C of ~0.0039 mol:mol. This last value corresponds to the lowest P:C (0.0041 mol:mol) observed for *P. multiseries* under PO_4^{3-} limitation in chemostat continuous culture (Pan et al., 1996c), while the maximum value observed was ~0.01 mol:mol (i.e. the Redfield ratio). Considering this uncertainty, we suggest that our approach of DA production seen as a consequence of the misbalance between pathways involved in the primary metabolism is more flexible than using fixed thresholds. Conceptually, this approach is closer to the Individual-Based Model of Siopsis (2003), who also considers DA as a secondary metabolite. In her model, Siopsis (2003) describes the nutrient uptake, the photosynthesis, the synthesis of protein, lipid, polysaccharide, frustule and DA, and the catabolism of storage products of *P. multiseries*. The excess energy and N left after formation of the cell structures and storage components permits DA synthesis which is further related to low external Si concentration. Though the aggregation level of cellular components is higher in our model, it also relates the DA production to shared precursors with major metabolic pathways (i.e. growth and reserve products synthesis). Moreover, our model includes all nutrient forms (N, P, Si) allowing consideration of P or Si limitation. Such a model structure enables to study the effect on DA production of factors affecting major metabolic pathways. The present study focuses on macronutrients and light; other physico-chemical factors affecting DA production (not implemented in the model yet) have been reported and include trace metal stress, salinity, temperature or pH (reviewed by Lelong et al., 2012).

4.2. Factors controlling the DA synthesis and release in the ambient medium

The chosen structure of the model allows exploring how different nutrient (Section 4.2.1) and light (Section 4.2.2) conditions might affect the cellular DA synthesis and its release in the ambient medium. In the following simulations, initial *Pseudo-nitzschia* biomass and nutrient concentrations of the reference simulation are modified and set to a tenth of those defined for the reference Si-limited simulation (DSi = 13.2 mmol m^{-3}, PO_4^{3-} = 1.63 mmol m^{-3}, NO_3^- = 97.3 mmol m^{-3}) in order to simulate DSi and PO_4^{3-} concentrations closer to in situ levels when *Pseudo-nitzschia* is blooming (reviewed by Trainer et al., 2012) while N is in excess. Further differences in the nutrient ratio or in the initial stock are however used and reported when the control of nutrient on DA production is investigated (Section 4.2.1). Similar results would be obtained considering P limitation instead of Si limitation.

4.2.1. Nutrients

In the model, nutrient (N, P or Si) limitation indirectly affects DA synthesis by controlling the availability of primary carbon (PNS)

Table 1
Effect of nutrient initial conditions on total DA produced during the simulated *Pseudo-nitzschia* blooms and growth stage (either exponential growth phase or decay phase of the bloom) of the maximum DA production rate (max DAprod rate) reached. Limiting nutrient in bold, others are twice in excess compared to *Pseudo-nitzschia* needs (with N:Si:P = 13.3:13.3:1 mol N:mol Si:mol P following the N:C, Si:C and P:C ratios, cf. Section 2).

Simulation	Initial concentrations (mmol m^{-3})			max Chl *a* (mg m^{-3})	DA production (10^{-3} mmol m^{-3} period^{-1})	Timing of the max DAprod rate
	NO_3^-	PO_4^{3-}	DSi			
Si-limited	26.4	1.98	**13.2**	14.5	94.9	Decay phase
P-limited	26.4	**0.99**	26.4	14.7	96.4	Decay phase
N-limited	**13.2**	1.98	26.4	14.6	5.44	Growth phase

while N availability exerts a direct control on DA production (Eq. (4)). The effect of nutrient limitation on DA production was investigated by running simulations of *Pseudo-nitzschia* batch cultures mimicking either Si, P or N limitation, the other nutrients being in excess (and chosen in order to obtain similar Chl *a* levels; Table 1). As expected, results clearly indicate a lower DA production when *Pseudo-nitzschia* is exposed to N limitation because of the N need for DA synthesis. Hence, for similar biomass reached, DA production significantly differs whether the cells are Si- (or P-) or N-limited. The ecological relevance of such difference in DA production may be estimated by comparison between the simulated concentrations and observed levels during in situ blooms. The simulated total DA after the bloom (3 weeks) when N is limiting reaches 5.44×10^{-3} mmol DA m^{-3}, which is close to the maximum of 3.3×10^{-3} mmol DA m^{-3} reported in the Chesapeake Bay between 2002 and 2007 and considered as low enough to explain the lack of documented toxic events in the area; it also compares to the 2.8×10^{-3} mmol DA m^{-3} measured during a *Pseudo-nitzschia* bloom in Mexico in 2007 where no adverse biological effects were detected (reviewed by Trainer et al., 2012). This value is by far lower than the $>30 \times 10^{-3}$ mmol DA m^{-3} reached during the toxic event in Monterey Bay, 1991 (Walz et al., 1994, cited by Bates et al., 1998), a level exceeded by the $\sim 95 \times 10^{-3}$ mmol DA m^{-3} simulated when N is not limiting (Table 1). Hence the limiting nutrient could make the difference between a problem (DSi or P limitation) or non-problem (N limitation) *Pseudo-nitzschia* bloom. Furthermore, results indicate that the simulated maximal DA production rate under N limitation is reached during the exponential growth phase, while it takes place in the decay phase of the Si- or P-limited experiments (Table 1). This particular pattern has indeed been observed in a batch culture where N was the nutrient inducing the onset of the stationary phase (Auro, 2007, cited by Trainer et al., 2012). It happens because actively growing *Pseudo-nitzschia* produce small amounts of DA in the model while this DA production is inhibited under N limitation.

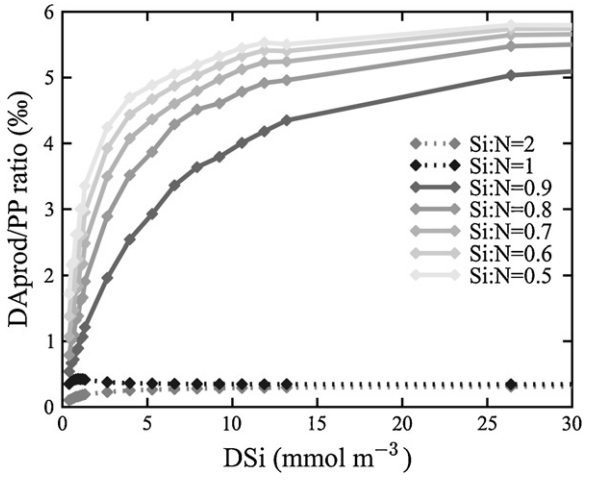

Fig. 5. Model sensitivity of DA production to nutrients: ratio between bloom-integrated DA production (DA_{prod}, mmol C m^{-3}) and primary production (PP; mmol C m^{-3}), obtained for different initial Si concentrations and Si:N stoichiometry. Si:P ratio is always set to 8 (P is in excess). Dotted (continued) lines when N (Si) is the primary limiting nutrient.

Nutrient control of DA production is further studied by running model simulations along a gradient of initial nutrient concentrations with different Si:N stoichiometry (Fig. 5). In these simulations, Si:P is fixed (with P always in excess) while either N (Si:N $\geq$ 1 mol:mol) or Si (Si:N $<$ 1 mol:mol) is the limiting nutrient. Results are expressed as DA production normalized to the primary production both integrated over the bloom period (DA_{prod}/PP ratio). This ratio (expressed in ‰) may be seen as an estimation of the harmfulness of a bloom by indicating how proportionally toxic it might be. Results show a clear switch from conditions where N is the primary limiting nutrient (here, Si:N $\geq$ 1 mol:mol) and DA production is extremely low, to those where Si becomes the primary limiting nutrient (Si:N $<$ 1) and DA production is significantly increased (Fig. 5). In the field, Anderson et al. (2006) linked low particulate DA concentrations in the Santa Barbara Channel (California) to areas where the DSi:NO_3^- ratio was $\geq$1.1, and measured the maximal levels when this ratio was $<$1 (at 0.6 mol:mol). Combining these observations with those collected in the Monterey Bay, they suggest that DA production by *P. australis* might occur when the DSi:NO_3^- ratio is $<$2. This ratio is higher than our threshold (1 mol:mol), which is related to the Si:N stoichiometry used in the model for *Pseudo-nitzschia* (cf. Section 2.1). Variability in this stoichiometry (values from $\sim$0.7 to nearly 3 mol Si:mol N are observed for *P. multiseries*; Pan et al., 1996b, c) might indeed imply different (and more particularly $>$1) threshold values for increased DA production. In Fig. 5, the DA_{prod}/PP envelope obtained for further decreasing Si:N ratios (from 0.9 to 0.5 mol:mol) shows the added control of N on DA production: although in slight excess for growth compared to Si, N still imposes a limitation on DA production. At this range of values, any N supply might be responsible for an increase in DA production. This mechanism suggests that any excess of N relative to Si (or P) *Pseudo-nitzschia* needs may be expected to stimulate DA production. An additional effect of the inorganic/organic N source on DA production has been observed (Howard et al., 2007; Thessen et al., 2009) but is not considered by the model yet.

Fig. 5 shows in addition that under conditions of Si depletion, the proportion of DA production to primary production increases with increasing nutrient stocks. Both DA and primary productions are calculated over the bloom period, which extends with increased nutrient stocks. Concomitantly, the decay phase of the bloom, i.e. the period when the DA production is maximal, is prolonged while the primary production increases to a lesser extent. Hence, DA production increases faster than primary production with nutrient levels and the proportion of DA production over primary production rises (Fig. 5). This phenomenon occurs concomitantly with the added control of N on DA production: at lower nutrient stocks, the remaining N after Si depletion is lower and imposes a greater limitation of DA

production than with higher initial nutrient concentrations. Altogether, this suggests that *Pseudo-nitzschia* blooms are susceptible to be even more harmful with increased nutrient concentrations. Any factor lengthening the blooms of *Pseudo-nitzschia* can be expected, in addition to supporting higher abundances, to intensify the potential toxic event. *Pseudo-nitzschia* mixotrophy for N (Hillebrand and Sommer, 1996; Howard et al., 2007; Cochlan et al., 2008; Loureiro et al., 2009) when inorganic N is depleted after a phytoplankton bloom as well as natural or anthropogenic eutrophication might be among these factors.

4.2.2. Light control

Both light intensity and the photoperiod determine the phytoplankton light climate. The control of DA production by light was investigated by running batch culture simulations for photosynthetically active radiation (PAR) ranging from 20 to 500 μmol m^{-2} s^{-1} and photoperiods from 6 to 18 h. The reference simulation has initial *Pseudo-nitzschia* biomass and nutrient concentrations set to a tenth of those from the reference Si-limited simulation (cf. Section 3).

The simulated time evolution of the specific DA production rate, i.e. the amount of DA produced each day per unit of *Pseudo-nitzschia* biomass, is shown for different PAR (and a 12-h photoperiod) in Fig. 6a. Results obtained show an abrupt increase in specific DA production rate that coincides with Si limitation of growth and consequent primary carbon (PNS) accumulation. At low light, *Pseudo-nitzschia* growth is slow, nutrient limitation is significantly delayed and so is the DA production. At high light (i.e. higher than light adaptation), additional light energy has little effect on the timing at which nutrient exhaustion and enhanced specific DA production rate occur. The critical day (i.e. the day beyond which DA production is accelerated) depends on both incident PAR and the *Pseudo-nitzschia* light adaptation (expressed by the light adaptation coefficient Ik) as shown by Fig. 6b that relies the critical day to the PAR/Ik ratio. The simulations suggest an important delay at limiting PAR (PAR/Ik < 1) while DA production occurs rapidly (here within one week) at higher PAR. Besides the timing of increased DA production, the simulated maximum specific DA production rate reached does not vary much between the different light intensities (Fig. 6a). It indeed depends on the specific accumulation of PNS (originating from photosynthesis while growth is limited), which is comparable between different light regimes. The actual DA production rate on the contrary depends on the *Pseudo-nitzschia* biomass effectively reached and decreases more importantly with decreasing light conditions. This can explain why, in laboratory cultures, the timing at which increased DA production occurs and its rate may vary significantly between some light regimes, and be more conservative after a certain threshold. The latter case may be illustrated by Lewis et al. (1993) who observed DA production in cultures at 80 μmol m^{-2} s^{-1} comparable (or slightly delayed/slower) to cultures at 180 μmol m^{-2} s^{-1}. On the other hand, Bates and Léger (1992, cited by Bates, 1998) observed comparable DA production rate at 200, 130 and 90 μmol m^{-2} s^{-1} but significantly reduced at 35 μmol m^{-2} s^{-1}. Furthermore, *P. multiseries* grown at 145 μmol m^{-2} s^{-1} and 45 μmol m^{-2} s^{-1} reached similar densities but with a slowed down growth under low illumination resulting in a considerably delayed DA production (Bates et al., 1991). More markedly, Cusack et al. (2002) observed a greatly delayed and reduced DA production by *P. australis* under 12 μmol m^{-2} s^{-1} compared to 115 μmol m^{-2} s^{-1}, only trace amounts of DA being detected during the very late stationary phase of the low-light regime. Whyte et al. (1995) also explained large differences in toxin content of *P. multiseries* at different scales of culture by the distinct photon flux density per cell, the most illuminated culture being the most toxigenic. A minimum irradiance of 100 μmol m^{-2} s^{-1} was

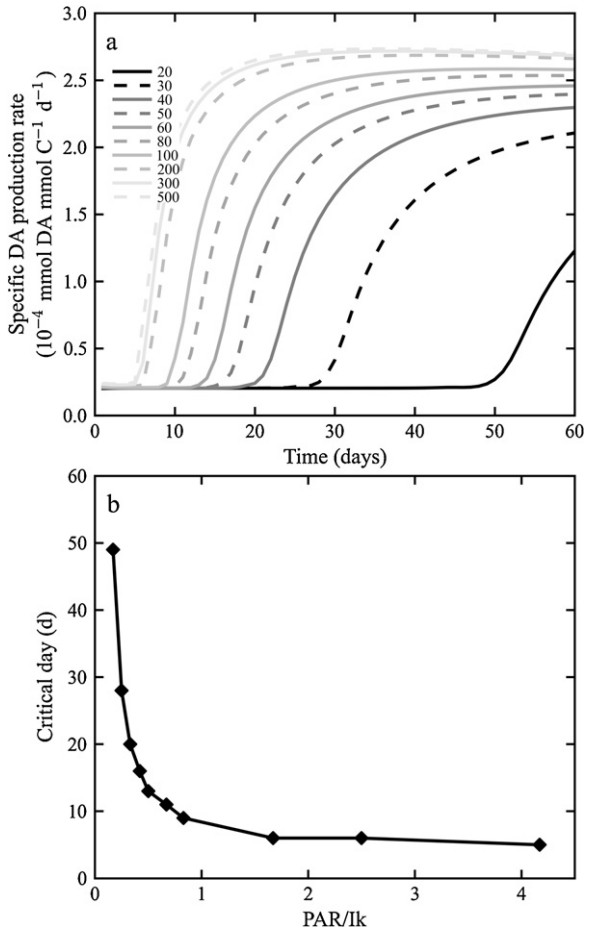

Fig. 6. Model sensitivity of DA production to incident light: (a) time evolution of the specific DA production rate for different PAR (20–500 μmol m^{-2} s^{-1}) and a 12 h:12 h L:D cycle. (b) Relationship between the critical day for enhanced DA production and the PAR/Ik ratio, with $Ik = k_{max}/\alpha$ the light adaptation of *Pseudo-nitzschia* set to 120 μmol m^{-2} s^{-1}. Each dot in (b) represents one curve from (a).

proposed in order to avoid light limitation of growth or toxin production in cultures (Bates, 1998). This value is comparable to the 120 μmol m^{-2} s^{-1} used in the present simulations (i.e. where PAR/Ik = 1) below which light limitation occurs. Such threshold may however be influenced by the light adaptation in regard to the experienced irradiance during *Pseudo-nitzschia* cultures, as well as by the photoperiod of their light regime. Hence, results presented here suggest a mechanism rather than provide a threshold value of minimum irradiance for DA production.

Besides the specific DA production rate, which weights the toxin production by the existing biomass of *Pseudo-nitzschia*, the absolute DA production will depend on the biomass effectively reached during the bloom. Fig. 7 shows the simulated total DA production integrated over the bloom for conditions as in Fig. 6 and supplemented with different photoperiods. The total DA production during the bloom follows a saturation curve as a function of ambient PAR (Fig. 7). Little DA production is simulated at lower intensities, supporting early observation that light is necessary (although not sufficient) for DA production (Bates et al., 1991). A slope of increasing DA production with increasing PAR (where small changes in PAR can lead to sensibly different DA production) is followed at high PAR by a plateau corresponding to the saturation of biomass and PNS accumulation. As expected, higher L:D at similar PAR stimulate higher DA production. In agreement, Fehling et al. (2005) measured higher total DA concentration in cultures under long (18:6 h) than short (9:15 h) photoperiod. Concomitantly, higher PAR in combination with lower L:D are

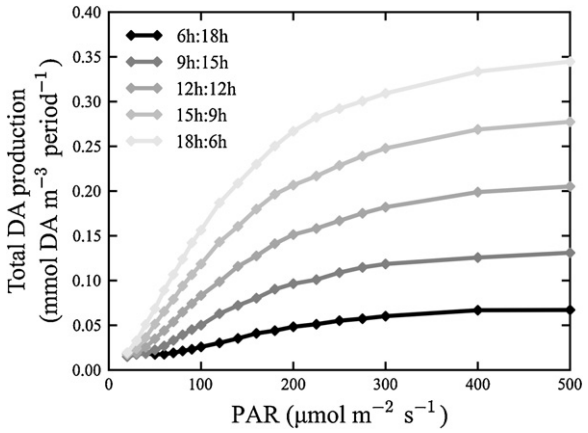

Fig. 7. Model sensitivity of DA production to incident light and photoperiod: bloom-integrated total DA production simulated for PAR ranging from 20 to 500 μmol m^{-2} s^{-1} and L:D cycles from 6 h:18 h to 18 h:6 h.

necessary to reach equivalent DA production (Fig. 7). Light is not often reported as a critical factor in natural DA toxic events, and light conditions during *Pseudo-nitzschia* blooms might actually be most of the time sufficient for toxin production. However, *Pseudo-nitzschia* toxicity in the northern Gulf of Mexico was recently found to be correlated to high irradiance (MacIntyre et al., 2011) supporting our model results linking the importance of the DA production to the excess in photosynthesis-derived energy. This pleads for adding ambient light (including incident PAR, mixed layer depth, vertical light attenuation) to other monitored environmental variables in order to forecast the extent of DA toxic events in marine waters.

Beyond the control of environmental conditions and more particularly the underwater light climate *Pseudo-nitzschia* are experiencing, their own physiology determines the amount of compounds and energy that could be directed to toxin synthesis. It should be noted that the above model sensitivity analysis to light conditions was performed assuming identical light adaptation parameters. To our knowledge, few studies focused directly on photosynthesis by *Pseudo-nitzschia*, except Pan et al. (1991, 1996d) who emphasized photoadaptation. In particular, Pan et al. (1996d) reported photosynthesis and growth acclimation to low light intensity by *P. multiseries*, which could in fact extend the window of light regime where toxicity can occur. Physiological adaptation may also, at least partly, explain differences in toxicity in different *Pseudo-nitzschia* species. For example, a comparison between non DA producing oceanic *Pseudo-nitzschia* species and one toxigenic coastal *Pseudo-nitzschia* species showed that this latter had maintained photosynthetic competences in stationary phase while the oceanic species faced a rapid degradation of the photosynthetic apparatus under nutrient (Si) starvation (Marchetti et al., 2008). The continued (coastal species) or interrupted (oceanic species) input of primary carbon (usable for DA production) while growth is nutrient-limited may explain differences in toxin production by these different species. As suggested by a simulation where the interruption of photosynthesis is imposed when growth is nutrient-limited (leading to a cessation of DA production; not shown), a better balance between photosynthesis and growth (e.g. the open-ocean species mentioned here above) would imply less toxin production.

5. Conclusion

In this paper we developed and used a conceptual model for *Pseudo-nitzschia* to better understand mechanisms of toxin (DA) production and the control by light and nutrient availability. In this model, DA production is considered as a secondary metabolite, sharing common precursors with growth and reserve products synthesis processes, while photosynthesis provides a resource (the shared primary carbon) for all these physiological pathways. Simulations with varying light and nutrient conditions conclude that Si or P limitation when N is sufficient will promote DA production (provided that light is not limiting), while light shortage will delay or hamper it. In the end, *Pseudo-nitzschia* bloom dynamics and toxicity are likely to be a complex function of the energy status and growth limitation, which together will determine the timing and the extent of DA production.

Model results suggest that increased N should enhance DA production by inducing P or more probably Si limitations which are more conducive to DA production and by relieving this latter from its control by N. Moreover, it suggests that increased nutrient stocks enhance the toxicity of *Pseudo-nitzschia* blooms by extending the phase of DA production in addition to supporting higher biomass. Besides, this modelling study particularly stresses the added effect of photosynthesis on DA synthesis by *Pseudo-nitzschia* and directly links this toxin production to the input of light energy. Here a threshold around 100–120 μmol m^{-2} s^{-1} was suggested below which light limitation is preponderant and delay the DA production expected in *Pseudo-nitzschia* cultures. This threshold as well as the critical day beyond which DA production is accelerated would however depend on both the light regime (photoperiod and intensity) and the *Pseudo-nitzschia* photoadaptation. The role played by light suggests that the underwater light conditions should be also monitored when the magnitude of toxic events is to be anticipated. Such control of the 'photon pressure' on DA production was proposed recently (MacIntyre et al., 2011), suggesting the need for further studies on photosynthetic capacities and adaptation between different light regimes of *Pseudo-nitzschia*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.hal.2013.01.004.

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