Journal of Experimental Marine Biology and Ecology 469 (2015) 83–92

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Growth and biochemical composition of a microphytobenthic diatom (*Entomoneis paludosa*) exposed to shorebird (*Calidris alpina*) droppings

Thierry Jauffrais [a,b], Sigrid Drouet [a], Vincent Turpin [a], Vona Méléder [a], Bruno Jesus [a,c], Bruno Cognie [a], Patrick Raimbault [d], Richard P. Cosson [a], Priscilla Decottignies [a], Véronique Martin-Jézéquel [a,e,*]

[a] Université de Nantes, Mer Molécules Santé EA2160, Faculté des Sciences et des Techniques, 44322 Nantes Cedex 3, France
[b] Université d'Angers, Laboratoire des Bio-Indicateurs Actuels et Fossiles, UMR CNRS 6112 LPG-BIAF, 2 Bd Lavoisier, 49045 Angers Cedex 1, France
[c] University of Lisboa, Faculty of Sciences, BioISI — Biosystems & Integrative Sciences Institute, Campo Grande, 1749-016 Lisboa, Portugal
[d] Université d'Aix Marseille, CNRS INSU, IRD, MIO, UM 110, Marseille, France
[e] Université de La Rochelle, UMR CNRS 7266 Littoral Environnement et Sociétés, LIENSs, La Rochelle, France

ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 14 April 2015
Accepted 15 April 2015

Keywords:
Carbon
Dunlin
Guano
Microphytobenthos
Mudflat
Nitrogen

ABSTRACT

Intertidal mudflats are important feeding areas for migrating and wintering shorebird species. Partial release of organic and inorganic nutrients through bird excretion may enrich the nutrients flow of mudflat ecosystems, especially in areas with a low flushing rate. The objective of the present work was to experimentally evaluate the effect of Dunlin droppings on the growth and biochemical composition of the microphytobenthic diatom, *Entomoneis paludosa* (W. Smith) Reimer. Different nutritional conditions were tested to investigate the impact on *E. paludosa* growth rate, biomass, elemental (CHN) and biochemical composition (lipids, proteins, carbohydrates, pigments). Various culture media were used going from plain artificial seawater to F/2 culture media containing variable nitrate concentrations (0; 50 or 882 μM-NO_3) to which bird dropping extract was added or not. The faeces extracts contained inorganic nitrogen (9.1 μM-NH_4), inorganic phosphorus (8.2 μM-PO_4), traces of silicate (0.2 μM-Si), organic nitrogen in the form of urea (16 μM-N) and other dissolved organic nitrogen (120 μM-N). Faeces extract inartificial seawater was sufficient to sustain *E. paludosa* growth (up to 6.8 cell divisions in 9 days). A significant growth rate increase (+20%) and higher biomasses were observed when faeces extract was added to inorganic media enriched with 50 μM-NO_3. Bird droppings had a significant effect in *E. paludosa* final biochemical composition with the addition of faeces extract to a culture medium containing 50 μM-NO_3 increasing *E. paludosa* protein content and decreasing carbohydrate content. Pigment content per cell increased with the addition of bird dropping extract but ratios of light-harvesting and photo-protective pigments to chlorophyll *a* were unaffected. *E. paludosa* grown with faeces extract showed high cellular nitrogen and carbon contents, close to those obtained when cells were grown in F/2 medium. This study showed that shorebird droppings, through the addition of dissolved material, can significantly affect microphytobenthic diatom growth and biochemical composition.

1. Introduction

Microphytobenthic biofilms are often the main primary producers of intertidal flats. Microphytobenthos (MPB) assemblages can be composed of benthic microalgae, cyanobacteria and photosynthetic bacteria (MacIntyre et al., 1996). However, MPB mudflat assemblages are most often dominated by benthic diatoms that accumulate in the upper sediment layers (first millimetres) (Meleder et al., 2007; Ribeiro et al., 2013). Microphytobenthos is considered a major contributor to mudflats primary production providing up to 1 g of C m^{-2} day^{-1} (Underwood and Kromkamp, 1999) and is an important energy source for secondary producers (Blanchard et al., 2001), such as deposit and suspension feeders (Austen et al., 1999; Kang et al., 1999; Pinckney et al., 2003; Riera, 2010). Intertidal mudflats are also important feeding areas for migrating and wintering shorebird species strongly supported by MPB at the base of their trophic chain (Kuwae et al., 2012).

Allochthonous nutrient inputs (e.g. from fertilizer, sewage, fish, or birds) may have significant effects in food web and ecosystems (Polis et al., 1997, 2004). Bird droppings (guano) deposition adds inorganic and organic matter to coastal sediments. These natural guano depositions are recognized as important organic and inorganic nutrient sources for intertidal primary producers located nearby seabird breeding colonies off the west coast of South Africa (Bosman and Hockey, 1986; Bosman et al., 1986). Droppings of several birds (e.g. *Fulmarus glacialis*, *Eudyptes chrysolophus*, *Larus michahellis*) were shown to increase natural concentrations of nitrogen, phosphorus, carbon

* Corresponding author at: Université de Nantes, Mer Molécules Santé EA2160, Faculté des Sciences et des Techniques, 44322 Nantes Cedex 3, France. Tel.: +33 2 51 12 56 66.
E-mail address: veronique.martin-jezequel@univ-nantes.fr (V. Martin-Jézéquel).

http://dx.doi.org/10.1016/j.jembe.2015.04.014
0022-0981/

(Keatley et al., 2009; Signa et al., 2012), as well as some trace elements (i.e., As, Cd, Cr, Cu, K, Ni, Pb, Mn, Zn) (Brimble et al., 2009; Signa et al., 2013); which can be essential elements for autotrophic or heterotrophic microalgal growth. Nutrients released from bird excretion may affect local nutrient fluxes in coastal ecosystems (Bosman and Hockey, 1986; Golovkin, 1967), and increases in guano nutrient loads may impact some ecosystems, e.g. lakes, tidal pools, reservoirs or bays with low flushing rates (Gwiazda et al., 2010; Keatley et al., 2009; Loder et al., 1996; Marion et al., 1994). Namely, guano nutrient inputs can affect diatom biomass and diversity induced by changes in water quality (nutrients, pH) caused by eutrophication (Keatley et al., 2009). Guano nutrient input can also increase macroalgae and phytoplankton biomass (Bosman et al., 1986; Gwiazda et al., 2010).

Calidris alpina is present along most of the European coastlines and is amongst the shorebirds that could have an impact on mudflat primary production. In intertidal coastal habitats *C. alpina* mainly feeds on marine polychaetes, molluscs and crustaceans (Davidson, 2009; Dierschke et al., 1999a,1999b; Durell and Kelly, 1990; Mouritsen, 1994) but is also able to feed on MPB (Elner et al., 2005; Kuwae et al., 2012; Mathot et al., 2010). Thus, a direct nutrient loop can exist between shorebirds and MPB. Particularly, guano nutrients may have an effect on benthic diatoms that are capable of assimilating organic and inorganic nitrogen (Admiraal et al., 1987; Antia et al., 1991; Linares and Sundback, 2006; Sundback et al., 2011; Syrett, 1981) and organic carbon compounds (Admiraal and Peletier, 1979). Studies about the effect of guano nutrient loadings on primary producers have been mainly focused on rocky coastal areas, near nesting sites or on closed areas such as lakes, reservoirs or tidal pools (Gwiazda et al., 2010; Keatley et al., 2009; Loder et al., 1996; Marion et al., 1994). To our knowledge no studies were carried out about the effect of shorebird droppings on MPB dominated mudflat ecosystems, which are well known to be shorebird feeding areas. The objective of the current work was to evaluate the effect of Dunlin (*C. alpina*) droppings as a potential nitrogen source, on the growth and biochemical composition of the pennate diatom *Entomoneis paludosa* found in mudflat microphytobenthos (Meleder et al., 2007; Ribeiro et al., 2003, 2013; Sabbe, 1997). We thus carried out experiments in controlled systems using an *E. paludosa* strain isolated from a mudflat of the French Atlantic Coasts and grown in a laboratory with different bird droppings enrichments.

2. Material and methods

2.1. Sampling site

C. alpina droppings were collected at La Coupelasse, a mudflat located in Bourgneuf Bay (47°01 N, 2°02 W, Fig. 1). The bay is situated on the Atlantic French coast and is characterized by 100 km^2 of intertidal mudflats. It is one of the five French main wintering wader sites, harbouring each year 30,000 birds including 19,000 Dunlin (Mahéo and Le Dréan-Quénec'hdu, 2013; Wetlands International, 2012). The primary production in this bay is usually limited by major inorganic nutrients (N, P or Si) during spring and summer before nutrient replenishment in autumn and winter due to runoff (Barille-Boyer et al., 1997).

2.2. E. paludosa

E. paludosa (W. Smith) Reimer, strain NCC 18.2 (Nantes Culture Collection) was isolated from Bourgneuf Bay intertidal sediments (France,

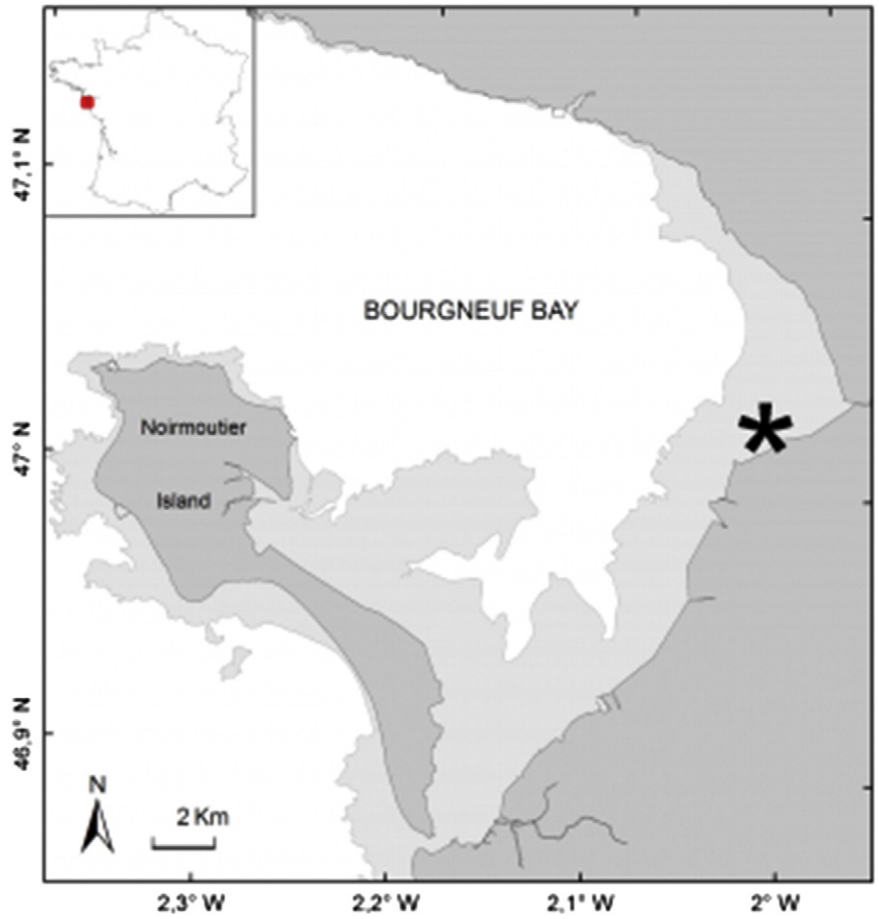

Fig. 1. Map of Bourgneuf Bay (Atlantic coast of France), the star indicates the place where the bird droppings and *E. paludosa* strain were collected; the intertidal area is represented in light grey.

Fig. 1). The diatom was maintained in 400 mL batch cultures (500 mL Erlenmeyer flask) at 17 °C, salinity of 35, photon flux density (PFD) of 90 μmol m^{-2} s^{-1} using cool light fluorescent lamp (Lumix day light, L30W/865, Osram) and a photoperiod of 14:10 h light:dark. The culture medium was made from autoclaved artificial seawater (ASW) containing inorganic nutrients (P, Si and traces metals) as described in Wolfstein and Stal (2002), vitamins as described in the F/2 vitamin solution (Guillard, 1975; Guillard and Ryther, 1962) and 882 μM-NO_3. Diatom stock-culture was kept in exponential growth phase by weekly transferring an inoculum into fresh culture medium.

2.3. Faeces extract

Fresh Dunlin droppings and sediments (without droppings) were collected in February 2013 at La Coupelasse (Fig. 1), put in Eppendorf vials (1.5 mL) previously washed with MeOH, frozen with liquid nitrogen and stored at −80 °C in the laboratory. Before use, the guano and the sediment were prepared as follow: samples were dried (70 °C, 48 h) and ground to a thin powder.

The faeces extract was made with 7.5 g of guano powder (75 droppings) solubilised in 30 mL of MilliQ water over night (12 h, 5 °C) using a magnetic stirrer. After centrifugation (3000 *g*, 30 min, 5 °C) the supernatant was filtered (0.22 μm) and frozen (−80 °C) as a stock-solution. The culture enrichments were made using 3 mL of this faeces extract stock-solution in 1 L of medium (see Table 1).

2.4. Experimental setup

To test the effect of shorebird droppings on diatom growth and biochemical composition, aliquots (1 mL) of *E. paludosa* stock culture were inoculated in triplicates in 500 mL aerated Erlenmeyer flasks (Pyrex) filled with 400 mL of culture medium and incubated in conditions similar to the stock culture. The initial cell concentration was equal to 1400 ± 380 cell mL^{-1} for all culture conditions. Besides the two control cultures, artificial seawater (ASW) and stock-culture medium containing 882 μM-NO_3 (M882), four culture conditions were used, ASW + faeces extract (FE), culture medium without nitrate but with faeces extract (MFE), culture medium plus 50 μM-NO_3 (M50), and culture medium plus 50 μM-NO_3 and faeces extract (M50FE). The exact composition of each medium is presented in Table 1.

Microalgal growth was monitored by counting cells until reaching the stationary phase of growth. Microalgae were sampled daily and fixed with one drop of lugol before counting. Samples for biochemical and nutrients analyses were collected at the end of the experiment at the beginning of the stationary phase of growth.

2.5. Microalgal cell count

Cell counts were made in triplicate, using a Nageotte haemocytometer and an optical microscope (×400), after sample sonication (Elma, S30 Elmasonic) for 5 min to avoid cellular aggregation.

Average growth rates were estimated during logarithmic growth phase following:

$$\mu = \frac{LnC_t - LnC_0}{t} \quad (1)$$

where C_0 was the initial cell concentration and C_t the cell concentration (cells mL^{-1}) after t days (Guillard, 1973).

Maximum growth rate μ_{max} (day^{-1}), maximum cell concentration α ($\alpha = \log(C_t / C_0)$) and latency time λ in day (if present) were determined after fitting a Gompertz model to the growth data set expressed in Log, using MatLab software:

$$f(t) = \alpha \times \exp\left(-\exp\left(\frac{\mu_{max} \times \exp(1)}{\alpha \times (\lambda - t) + 1}\right)\right). \quad (2)$$

2.6. Biochemical analyses

2.6.1. Particulate carbon and nitrogen

10 mL of culture was filtered through pre-combusted Whatman GF/C glass filters and dried at 70 °C for 48 h. Particulate nitrogen and carbon were determined by elemental analysis (Thermo Scientific FLASH 2000

Table 1
Microalgal media composition for the different culture conditions (μM ± SD, n = 3): ASW: artificial seawater; FE: ASW + faeces extract; MFE: medium without N + faeces extract; M50FE: medium with 50 μM N-NO_3 + faeces extract; M50: medium with 50 μM N-NO_3; M882: medium with 882 μM N-NO_3.

Elements	Molar concentration in final medium (μM)	ASW	FE	MFE	M50FE	M50	M882
NaCl	4.12×10^5	+	+	+	+	+	+
Na_2SO_4	2.25×10^4	+	+	+	+	+	+
$MgCl_2$, $6H_2O$	4.28×10^4	+	+	+	+	+	+
KCl	7.24×10^3	+	+	+	+	+	+
$CaCl_2$, $2H_2O$	1.09×10^4	+	+	+	+	+	+
$NaSiO_3$, $9H_2O$	1.15×10^2	−	−	+	+	+	+
NaH_2PO_4, H_2O	7.24×10^1	−	−	+	+	+	+
$NaHCO_3$	2.12×10^3	−	−	+	+	+	+
H_3BO_3	1.78×10^2	−	−	+	+	+	+
KBr	1.25×10^1	−	−	+	+	+	+
$SrCl_2$, $6H_2O$	3.75×10^1	−	−	+	+	+	+
Trace metal solution	Wolfstein and Stal (2002)	−	−	+	+	+	+
Vitamin solution	(F/2)[a]	−	−	+	+	+	+
$NaNO_3$	μM-N	0	0	0	50	50	882
FE composition	concentration in final medium						
NH4-N	μM-N	−	9.1 ± 1.2	9.1 ± 1.2	9.1 ± 1.2	−	−
NO2-N	μM-N	−	<LOD	<LOD	<LOD	−	−
NO3-N	μM-N	−	<LOD	<LOD	<LOD	−	−
Urea-N	μM-N	−	16 ± 2.2	16 ± 2.2	16 ± 2.2	−	−
DON[b]	μM-N	−	120 ± 0.7	120 ± 0.7	120 ± 0.7	−	−
DOC	μM-C	−	416 ± 7	416 ± 7	416 ± 7	−	−
PO4-P	μM-P	−	8.2 ± 1.1	8.2 ± 1.1	8.2 ± 1.1	−	−
$Si(OH)_4$-Si	μM-Si	−	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	−	−
Initial inorganic N/P ratio		0	1.1	0.1	1.3	0.7	12.2
Initial total N/P ratio		0	17.7	1.8	2.4	0.7	12.2

[a] Guillard and Ryther (1962), Guillard (1975).
[b] Without urea.

Series CHNS Analyzer). Cellular nitrogen (QN) and carbon (QC) quotas were calculated on the basis of the mean cell concentration. The analyses were also performed both on dry faeces (3 mg) and sediment (3 mg).

2.6.2. Carbohydrates

10 mL of culture was centrifuged and the pellet used to determine diatom carbohydrate content according to the sulphuric acid colorimetric method (Dubois et al., 1956), based on phenolphthalein absorbance at 490 nm.

2.6.3. Proteins

The extraction was adapted from Marchetti et al. (2013). Samples (10 mL of culture) were centrifuged (2500 *g*, 5 °C, 30 min) to remove the culture medium and the algal pellet was stored at −80 °C until protein extraction. The pellet was resuspended in 0.5 mL of acetone (100%), put in an iced bath for 30 min for protein precipitation and centrifuged (15000 *g*, 5 °C, 10 min) to collect the protein pellet. Proteins were then rinsed twice with 70% acetone (2.5:1, v/v) and solubilised in 0.5 mL of ultra-pure water. Solubilised proteins were quantified using a BCA protein assay kit (Pierce) based on alkaline copper colorimetric quantification at 562 nm (Lowry et al., 1951).

2.6.4. Lipids

Samples (50 mL of culture) were centrifuged (2500 *g*, 5 °C, 30 min) and pellets were stored at −20 °C until extraction. Lipids were extracted from the pellet with 1 mL chloroform:methanol (2:1 v/v) (Folch et al., 1957) and stored at −20 °C over night. The Bligh and Dyer's (1959) method was then used, in which dichloromethane and ethanol were replaced by chloroform and methanol in the same solvent mixing proportion (1:2 v/v). Hence, 1 mL of $CHCl_3$ and 0.9 mL of water were added to 1 mL of Folch extract. After centrifugation, the organic phase was recovered in a pre-weighed haemolysis tube. The water/methanol phase was rinsed twice with 1 mL $CHCl_3$ to collect the residual organic phase, which was then added to the previous organic fraction. The whole organic phase was then dried under nitrogen flow, stored in a desiccator for 24 h and weighed.

2.6.5. Pigments

Analyses were performed by high-performance liquid chromatography (HPLC) using an Ultimate 3000 RS (Dionex) following an adaptation of Mantoura and Llewellyn (1983). Briefly, 10 mL of culture was centrifuged (2500 *g*, 5 °C, 30 min), the supernatant was discarded and the algal pellet was stored at −80 °C until analysis. For the analysis, the algal pellet was suspended in 0.5 mL of methanol at 95% (MeOH: H_2O v/v) with 2% of ammonium acetate. The sample was transferred and agitated in an Eppendorf (2 mL vials), subsequently maintained at −20 °C for 15 min, and finally centrifuged (15000 *g*, 5 °C, 10 min). The supernatant was collected and the pellet resuspended again with 0.5 mL methanol:ammonium acetate solution. This operation was repeated three times for complete pigment extraction, and the three pigment extracts were pooled. Before HPLC analysis, 0.5 mL of the pigment extracts (1.5 mL) was diluted in a HPLC vial with 0.5 mL of ammonium acetate (1 M) to optimise pigment elution and 100 μL was injected. The chromatographic analysis is described in Meleder et al. (2003). The different pigments were identified using their absorption spectra between 400 and 800 nm measured with a photodiode-array detector. The quantification was carried out at 440 nm by comparison with pigment standards (DHI, Denmark). In addition to chlorophyll *a*, the main diatom pigments were also quantified, i.e. chlorophyll *c*, fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene. For some of these pigments, concentrations were given as the sum of their different chemical forms: chlorophyll *a* = Chl *a* + allomer + epimer, chlorophyll *c* = Chl c_1 + Chl c_2, fucoxanthin = fucoxanthin + by-products.

2.7. Nutrient analysis

All dissolved nutrients were analysed after filtering the culture conditions on Nauclepore polycarbonate filters (2 μm). The concentration of nitrates, nitrites, silicates and phosphates in the culture medium was determined using a continuous-flow auto-analyser (SKALAR) and standard chemical methods (Strickland and Parsons, 1972). Ammonium and urea were quantified from freshly filtered samples of culture medium using respectively the indophenol-blue method adapted for seawater (Koroleff, 1970) and the diacetylmonoxime thiosemicarbazide method (Mulvenna and Savidge, 1992) modified for room temperature reaction (Goeyens et al., 1998). Dissolved organic nitrogen and carbon were analysed using a wet oxidation technique associated to automated colorimetry as described in Raimbault et al. (1999).

2.8. Data analysis

All data are expressed as mean ± standard deviation (SD). Statistical differences were tested using one-way analysis of variance (ANOVA) or with Kruskal–Wallis tests when homoscedasticity and normality were not observed. When necessary, a Fisher's least significant difference procedure or a box-and-whisker plot was applied. Differences were considered significant at $p < 0.05$. Linear regression analyses were used to assess relationship between data expressed in per cell or in particulate carbon basis. Statistical analyses were carried out using Statgraphics Centurion XV.I (StatPoint Technologies, Inc.).

3. Results

3.1. Bird droppings composition

The faeces dry extract used in the experiments was composed of 0.78 ± 0.24% nitrogen, 9.67 ± 3.48% carbon and 0.47 ± 0.11% hydrogen, the remaining ~89% was mineral fraction (i.e. undigested sediment), whereas dry sediment was composed of 0.04 ± 0.01% nitrogen, 7.89 ± 0.25% carbon and 0.41 ± 0.11% hydrogen.

In the FE culture condition (ASW + faeces extract), 9.1 μM-N of inorganic nitrogen was added, mainly in the form of ammonia; whereas nitrites and nitrates were not detected (Table 1). Organic nitrogen was also quantified as urea (16 μM-N) and as other unidentified DON compounds (120 ± 0.7 μM-N). Dissolved inorganic phosphate was detected in the form of ortho-phosphate (8.2 μM-P) and Si (ortho-silicic acid) was only detected at a very low level (Table 1).

3.2. Growth performances

At the beginning of the experiment, the inorganic N/P ratio in the media calculated on a molar basis was very low in each culture condition (Table 1), and only the N/P ratio of the M882 condition, with a value of 12.2, was close to the Redfield value (16) which is considered the good elemental condition for growth (Table 1). Adding organic to inorganic nitrogen to calculate the N/P ratio in the initial FE condition increased its value to 17.7, thus close to the value found in nutrient-replete condition (M882). Bird droppings extract added to the artificial sea water (FE condition) was sufficient to sustain several microalgal cell divisions (6.78 ± 0.24) and allowed the culture to reach a biomass of 1.4 10^5 cell mL^{-1}, whereas no growth was observed in pure artificial sea water (ASW condition) (Fig. 2 and Table 2).

Adding faeces extract to culture medium had a significant positive effect on *E. paludosa* growth. Microalgal growth rates (μ and μ_{max}, Table 2) increased between 0.1 and 0.2 day^{-1} (Table 2) in cultures where bird droppings extract was added to the media MFE and M50FE compared to the cultures without faeces extract but with nitrate as unique nitrogen source (M50 and M882 conditions). The highest nitrogen enrichment (M882 condition) resulted in the highest biomass and the addition of faeces extract in the media already enriched with

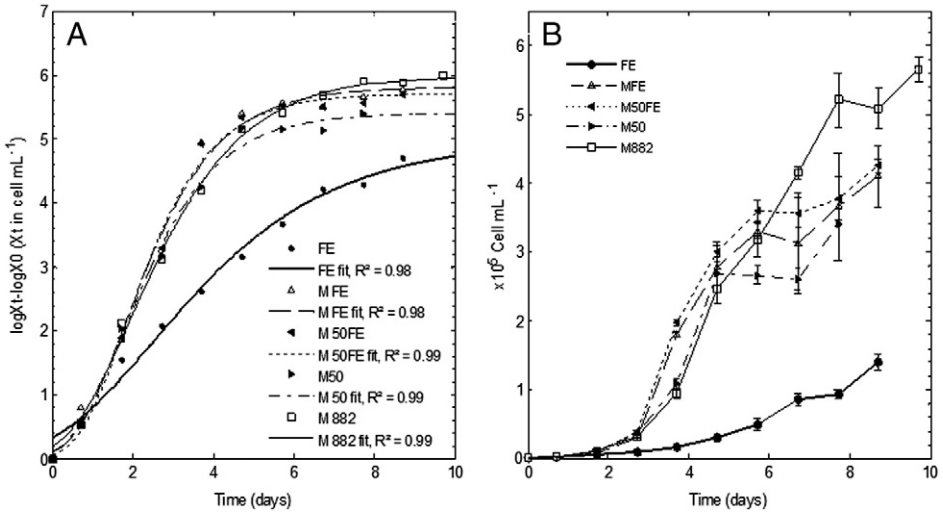

Fig. 2. Growth curves of *E paludosa*. A: Gompertz model fitted to cell concentration as a function of time with the adjusted R^2; B: *E. paludosa* concentration ($\times 10^5$ cell mL^{-1}) as a function of time. (FE: ASW + faeces extract, MFE: medium without N + faeces extract, M50FE: medium with 50 μM $N\text{-}NO_3$ + faeces extract, M50: medium with 50 μM $N\text{-}NO_3$, M882: medium with 882 μM $N\text{-}NO_3$), n = 3.

nitrogen also increased significantly the algal biomass, as observed between M50 and M50FE conditions (Table 2).

3.3. Biochemical composition

A significant correlation was found between values calculated as pg $cell^{-1}$ and g g^{-1} C for proteins ($p < 0.001$, adjusted $R^2 = 0.94$, $n = 15$); lipids ($p < 0.05$, adjusted $R^2 = 0.43$, $n = 15$); and carbohydrates ($p < 0.001$, adjusted $R^2 = 0.83$, $n = 15$). The addition of bird droppings to the culture medium did not have a significant effect on cellular carbon quota (QC) ($p = 0.086$, 30 pgC $cell^{-1} < Qc < 46$ pgC $cell^{-1}$; Table 3). Thus, no effects due to cell cycle or cell size were observed in this experiment and we focused the results and discussion in the per-cell unit data. Cellular nitrogen quota showed significant differences between the treatments ($p = 0.01$, Table 3). Three culture conditions showed significant lower nitrogen concentrations (pgN $cell^{-1}$: 2.9 ± 0.8 (MFE), 3.9 ± 0.6 (M50FE) and 1.8 ± 0.9 (M50)) compared to culture enriched solely with faeces extract (6.2 ± 0.6 pgN $cell^{-1}$ (FE condition)) or with a high nitrogen concentration (7.7 ± 0.7 pgN $cell^{-1}$ (M882 condition)). The same pattern was observed for the ratio of total cell nitrogen by total cell carbon (g g, see Table 3).

Microalgae protein concentrations varied according to culture conditions, from 7.7 to 46.2 pg $cell^{-1}$ ($p = 0.015$, Table 3). Protein content was affected by the amount of nitrogen added at the beginning of the experiment. The highest protein concentration was obtained in diatoms grown in the cultures with the highest nitrogen concentration (882 μM-N, Table 3). Lipid contents were significantly different amongst the experimental conditions ($p = 0.038$); however, the observed differences were mainly due to the FE condition that contained a higher lipid content (89.9 pg $cell^{-1}$) compared to the other four conditions that did not showed major differences (43 to 48 pg $cell^{-1}$, Table 3). Carbohydrate contents also showed significant differences, from 39.6 to 116.3 pg $cell^{-1}$ ($p < 0.001$) in cells grown in different culture conditions (Table 3). In particular, the highest values were obtained for cells grown in MFE, M50FE and M50 conditions with significant differences ($p < 0.05$) between the three cultures; whereas very close values were obtained for cells grown in FE and M882 conditions (Table 3).

3.4. Pigment composition

Significant differences were observed for chlorophyll *a*, fucoxanthin, chlorophyll *c*, diadinoxanthin, diatoxanthin and total pigment content, but not for β-carotene concentration on a per-cell basis (Table 4). The initial nitrogen concentration was the main factor that affected cell pigment concentrations, similar to what was observed for proteins, with cells growing in M882 condition showing the highest pigment concentration (Table 4). Total pigment content also showed significant differences between M50 and M50FE conditions (0.84 and 2.04 pg $cell^{-1}$, respectively). Light harvesting and photo-protective pigment ratios ((fuco + Chl *c*)/Chl *a* and (Dd + Dt)/Chl *a*, respectively) showed no significant differences ($p = 0.20$ and $p = 0.11$, respectively, Table 4).

4. Discussion

In the present study *E. paludosa*, a benthic diatom representative of the main life form inhabiting muddy sediments was grown under

Table 2
General growth characteristics of *E. paludosa* grown under the different culture conditions (symbols as in Table 1). Values with the same letter (a, b or c) are not significantly different (Fisher, $p > 0.05$).

	Maximum growth rate μ_{max} (day^{-1})[a]	Mean growth rate μ (day^{-1})[b]	Number of divisions[b]	Maximal biomass (10^5 cell mL^{-1})[b]
ASW	–	–	–	–
FE	0.74 (0.58, 0.89)	0.64 ± 0.04 c	6.78 ± 0.24 c	1.40 ± 0.21 d
MFE	1.67 (1.28, 2.06)	1.34 ± 0.01 a	8.31 ± 0.27 a	4.20 ± 0.93 bc
M50FE	1.78 (1.46, 2.10)	1.33 ± 0.04 a	8.22 ± 0.35 a	4.32 ± 0.13 b
M50	1.55 (1.25, 1.85)	1.15 ± 0.06 b	7.72 ± 0.20 b	3.40 ± 0.68 c
M882	1.41 (1.24, 1.58)	1.13 ± 0.04 b	8.64 ± 0.08 a	5.67 ± 0.32 a
p-Value***	–	0.021	0.001	<0.001

[a] μ_{max} is calculated using the Gompertz model and is presented with its range of confidence at 95%.
[b] Mean ± SD.
*** The ANOVA p-value is considered significant when $p < 0.05$, $n = 3$.

Table 3
Cellular quota in carbon, nitrogen, protein, lipid, and carbohydrate of *E. paludosa* expressed in pg cell^{-1} and in per carbon (g g^{-1}) as a function of the different culture conditions (symbols as in Table 1) at the end of the experiment (±SD). Values with the same letter (a, b, c) are not significantly different (Fischer, $p > 0.05$).

	FE	MFE	M50FE	M50	M882	p-Value*
pg cell^{-1}						
Carbon	45.5 ± 1.5	32. 4 ± 3.6	39.2 ± 9.4	30.0 ± 6.7	45.7 ± 3.4	0.086
Nitrogen	6.2 ± 0.6 b	2.9 ± 0.8 c	3.9 ± 0.6 c	1.8 ± 0.9 cd	7.7 ± 0.7 a	0.010
Proteins	22.9 ± 6.1 b	14.9 ± 2.6 c	21.1 ± 2.4 b	7.70 ± 0.97 d	46.2 ± 1.9 a	0.015
Lipids	89.9 ± 6.8 a	43.8 ± 2.8 b	48.2 ± 5.2 b	44.8 ± 5.9 b	48.8 ± 2.9 b	0.038
Carbohydrates	39.6 ± 18.5 d	66.9 ± 3.3 c	87.3 ± 9.3 b	116.3 ± 5.4 a	40.9 ± 5.6 d	<0.001
g g^{-1}						
Nitrogen	0.14 ± 0.01 b	0.09 ± 0.02 c	0.10 ± 0.01 c	0.06 ± 0.03 cd	0.17 ± 0.01 a	0.012
Proteins	0.50 ± 0.13 b	0.46 ± 0.03 b	0.55 ± 0.08 b	0.27 ± 0.12 c	1.05 ± 0.09 a	0.020
Lipids	1.98 ± 0.15	1.36 ± 0.21	1.27 ± 0.34	1.60 ± 0.49	1.07 ± 0.02	0.063
Carbohydrates	0.87 ± 0.40 c	2.09 ± 0.30 b	2.28 ± 0.36 b	4.04 ± 1.07 a	0.89 ± 0.05 c	<0.001

* The ANOVA p-value is considered significant when $p < 0.05$, $n = 3$.

various conditions, either in condition enriched with different amounts of nitrate or none, and either with faecal extract from bird droppings or none. The objective was to determine if Dunlin droppings have a fertilizing effect on microphytobenthos growth and biomass and if they induce change in diatom biochemical composition, and to evaluate the potential impact of wintering shorebirds on Bourgneuf Bay's pennate diatoms. Results show that adding Dunlin faecal extract to *E. paludosa* growth media significantly affects growth, biomass and biochemical composition of this diatom.

4.1. Growth

The Dunlin dropping extract used for our experimental enrichments was prepared as in Loder et al. (1996), a study designed to follow the dynamics of ammonia in rockpools near gull rookeries. Similar to Loder et al. (1996), almost all inorganic nitrogen in the faeces extract was found in the form of ammonia, but other N-forms, such as urea and undetermined organic species (DON), were also detected. In our experiment, *E. paludosa* grew under all nutritional conditions tested, in a similar range of growth rates than those measured in a previous study (Barnett et al., 2014), except in the ASW control condition (artificial sea water), that contained no micro- or macronutrients (Table 2, Fig. 2). It was noticeable that the addition of faeces extract to artificial seawater (FE condition) was sufficient to sustain a growth of up to 6.8 cell divisions which is close to the maximum of 8.6 cellular divisions obtained with the NO_3-enriched culture condition M882 (Table 2). In addition the highest growth rates have been obtained when faeces extract was added to the growth media (MFE and M50FE conditions, Table 2). Maximum and mean growth rates in these two conditions represent respectively an increase of 26% and 18% compared to the growth rates measured in M882 condition. Furthermore, the addition of bird droppings to culture medium enriched with 50 μM-NO_3 (M50FE condition) increased both the growth rate and to a smaller extent the maximal biomass, in comparison with growth obtained with NO_3 as sole nitrogen source (M50 condition, Table 2, Fig. 2). These results support the hypothesis that shorebird droppings could have a fertilizing effect on microphytobenthic habitats and could boost diatom growth even under high nutrient conditions.

The observed increased growth rates can be explained by the presence of multiple nitrogen sources when faeces extract was added, whereas the other culture media (M50 and M882 conditions) contained only nitrate as a nitrogen source. Diatoms can use ammonia, urea and DON as nitrogen sources (Admiraal et al., 1987; Antia et al., 1991; Linares, 2006; Linares and Sundback, 2006; Sundback et al., 2011). Moreover, there is a strong interaction between ammonium and nitrate when both are present in the medium, and it is generally assumed that ammonia is often used first (Dortch, 1990; Vincent, 1992). It is thus very likely that *E. paludosa* has found nitrogen sources in the faeces extract that favoured its growth. Furthermore, guano can also be a source of trace elements (Brimble et al., 2009; Signa et al., 2012, 2013)

Table 4
Pigment quota of *E. paludosa*, expressed in pg cell^{-1} and in per carbon ($\times 10^3$ g g^{-1}), ratio in g g^{-1} for the different culture conditions (±SD, symbols as in Table 1). Values with the same letter (a, b or c) are not significantly different (Fisher, $p > 0.05$).

	FE	MFE	M50FE	M50	M882	p-Value*
pg cell^{-1}						
Chl *a*	1.36 ± 0.60 b	0.89 ± 0.18 bc	1.19 ± 0.12 bc	0.49 ± 0.24 c	3.33 ± 0.63 a	0.039
Fuco	0.66 ± 0.11 b	0.39 ± 0.08 b	0.61 ± 0.22 b	0.25 ± 0.01 b	2.11 ± 0.2 a	0.030
Chl c_2	0.16 ± 0.03 b	0.05 ± 0.02 b	0.12 ± 0.02 b	0.03 ± 0.01 b	0.57 ± 0.2 a	0.023
Diadino	0.16 ± 0.02 b	0.07 ± 0.01 cd	0.11 ± 0.02 bc	0.05 ± 0.02 d	0.35 ± 0.06 a	0.024
β-Caro	0.06 ± 0.06	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.18 ± 0.02	0.131
Diato	0.008 ± 0.001 b	0.004 ± 0.001 b	0.003 ± 0.002 b	<LOD	0.06 ± 0.03 a	0.039
Total	2.41 ± 0.81 b	1.42 ± 0.29 bc	2.04 ± 0.29 b	0.84 ± 0.36 c	6.59 ± 1.58 a	0.031
$\times 10^3$ g g^{-1}						
Chl *a*	29.72 ± 12.46	27.34 ± 4.76	31.05 ± 3.9	18.33 ± 13.79	75.47 ± 10.75	0.174
Fuco	14.57 ± 2.25	12.14 ± 2.54	15.64 ± 0.99	9.15 ± 5.32	47.82 ± 12.30	0.090
Chl c_2	3.62 ± 0.63 b	1.69 ± 0.53 b	3.09 ± 0.18 b	1.19 ± 0.66 b	12.92 ± 3.96 a	0.031
Diadino	3.42 ± 0.38 b	2.28 ± 0.33 c	2.82 ± 0.20 cb	1.77 ± 1.13 c	7.93 ± 0.95 a	0.026
β-Caro	1.29 ± 1.24	0.27 ± 0.47	0.18 ± 0.31	0.94 ± 0.78	4.07 ± 0.21	0.132
Diato	0.17 ± 0.02 b	0.11 ± 0.04 b	0.07 ± 0.06 b	<LOD	1.02 ± 0.52 a	0.030
Total	52.79 ± 16.40	43.84 ± 8.25	52.84 ± 4.85	31.39 ± 21.67	149.24 ± 28.70	0.129
g g^{-1}						
(Fuco + Chl *c*)/Chl *a*	0.66 ± 0.17	0.50 ± 0.03	0.61 ± 0.04	0.62 ± 0.14	0.80 ± 0.10	0.200
(Dd + Dt)/Chl *a*	0.13 ± 0.03	0.09 ± 0.002	0.09 ± 0.01	0.10 ± 0.02	0.12 ± 0.002	0.111

* The ANOVA p-value is considered significant when $p < 0.05$, $n = 3$.

and some of them, such as Zn, Cu, Cd, Ni, and Mn are required for algal growth (Ho et al., 2003; Twining and Baines, 2013). In our experiments, these elements could have enhanced growth rate during the exponential growth phase. However, adding faeces extract to unenriched artificial sea water (FE condition) did not allow *E. paludosa* to reach biomass levels equivalent to that measured in the four other conditions enriched with major and minor nutrients. The final biomass produced with only faeces extract as nutrients (FE condition) represents 24% of the biomass obtained in M882 condition. This suggests that faeces extract alone lacks some nutritional elements needed to sustain high productivity. Because diatoms are known to have silica requirements (Martin-Jezequel et al., 2000), it is very likely that *E. paludosa* growing solely with faeces extract was silica limited. Indeed, the growth medium with faeces extract (FE) contained low silica concentrations (0.2 μM-Si, Table 1) and silicates were exhausted in this medium at the end of the growth (Table 5). Contrary to silica, phosphorus was not found to be exhausted in any culture (Table 5), even in the FE medium which had the lowest initial (8.9 μM-P, Table 1) and residual (1.8 μM-P, Table 5) phosphate concentrations.

4.2. Biochemical composition

Biochemical composition is an indicator of the diatom physiological status since some nutritional deficiencies can modify this composition in a predictable way (Beardall et al., 2001; Finkel et al., 2010). The standard elemental stoichiometry is generally assumed to be 106 C:16 N:1 P, referred to as the Redfield ratio (Redfield, 1958). As a consequence C/N ratio for cells in good nutritional status is assumed to be close to 6.6. In diatoms, this ratio is closer to 8 for species growing in nutrient replete conditions (Brzezinski, 1985; Sarthou et al., 2005). This ratio can vary at the most ten-fold when cells are nutrient-limited, the maximum being measured in *Thalassiosira fluviatilis* from 6.3 to 43.5 in N-non limited and N-limited conditions respectively (Darley, 1977). In our study, *E. paludosa* cellular C/N ratio was in the range of what was measured in other diatom species (Sarthou et al., 2005). According to previous works on diatoms (Claquin et al., 2002; Harrison et al., 1977; Sarthou et al., 2005), this ratio increased between N-non limited and N-limited conditions, respectively from 6 and 7.4 for M882 and FE conditions, to 10–17 for M50, M50FE, and MFE conditions. The C/N variation amongst our culture conditions was mainly related to the decrease of cellular nitrogen which was significantly lower in M50, M50FE and MFE conditions (Table 3). In accordance with the literature (Beardall et al., 2001; Claquin et al., 2002) nitrogen deficiency seemed thus to affect the nitrogen cellular quota but had no major effect on the carbon cellular quota of *E. paludosa* (Table 3).

Algal cells are mainly constituted of proteins, lipids and carbohydrates (Brown et al., 1997; Morris, 1981) and when diatoms are not limited by major nutrient availability, proteins should be the major cellular component (±50% ash free dry weight (AFDW)) followed either by lipids (±20–30% AFDW) or by carbohydrates (±20–30% AFDW) (Brown et al., 1997; Parsons et al., 1961; Scholz and Liebezeit, 2013). Conversely, diatoms grown in nitrogen-limited media show a decrease in protein content (Dortch, 1982; Guerrini et al., 2000; Harrison et al., 1990; Jiang et al., 2012) and an increase in carbohydrate or lipid contents (up to 50–60% AFDW for both) (Guerrini et al., 2000; Jiang et al., 2012). In the present study, the culture enriched with 882 μM of nitrate (M882 condition) can be considered similar to a N- and P-non-limited condition. The good nutritional N/P ratio of the M882 condition equal to 17 (Table 5), explains the high cellular protein content (46.2 pg/cell, Table 3). However proteins in cells grown in M882 condition were not the major macromolecular component, as it is generally found in many studies where proteins account for around 40% in nutrient replete cells (Darley, 1977; Geider and La Roche, 2002; Harrison et al., 1990; Taguchi et al., 1987). Indeed, fairly equivalent percentages of proteins (34%), lipids (36%) and carbohydrates (30%) were measured in *E. paludosa* (Fig. 3), a proportion already measured in another diatom, *Stephanodiscus minutulus* (Lynn et al., 2000). In the same way, even if the lipid content is generally lower than 30% in diatoms, the lipid percentage in M882 condition was close to those obtained in some other nutrient replete diatoms (Lynn et al., 2000; Shifrin and Chisholm, 1981; Taguchi et al., 1987). However, because the silicate concentration was around 0.9 μM-Si in M882 condition at the end of the experiment (Table 5) the high proportion of lipids (36%) may be the result of a slight Si-limitation in this condition.

The highest cellular lipid content (Table 3) and percentage (59%, Fig. 3) found in cells grown in FE condition were the result of the silicate starvation of the medium at the end of the experiment (see Table 5). It is well known that Si limitation in diatoms results in the production of lipids (Roessler, 1988; Shifrin and Chisholm, 1981; Yu et al., 2009). In the case of Si limitation at the beginning of the stationary growth phase, lipid synthesis rates can for example increase by a factor of 1.7 to 3.1 (e.g., *Chaetoceros gracilis*, *Hantzschia* sp., *Cyclotella* sp.) compared to the exponential growth phase (Taguchi et al., 1987). On the other hand, the cellular carbohydrate content under the FE condition did not vary in comparison to the one measured in the M882 condition, whereas proteins decreased by half (Table 3, Fig. 3), confirming that carbon assimilation has been directed to lipids at the expense of proteins.

In all the other tested nutritional conditions, MFE, M50FE and M50 the protein content largely decreased (at least by half) in comparison to cells grown in the M882 condition; whereas cellular carbohydrates increased, in particular in the M50 condition (Table 3). The low protein (7–13%) and high carbohydrate percentages (53–67%) were in accordance with the high C/N ratio. This relates to the low concentrations of available nitrogen measured at the end of the growth in these conditions (1 to 4 μM-N, Table 5) with a N/P ratio close to 0 indicating a nitrogen limitation. The increase of carbohydrates and the decrease of protein content is a well known regulation in N-limited microalgae, and was already observed in other diatom species (Cook et al., 2007; Corzo et al., 2000; Guerrini et al., 2000; Lynn et al., 2000; Smith and Geider, 1985). On the other hand, lipid contents and percentages were very close to the values obtained in the M882 condition (Table 3), indicating that no major regulation of this biochemical pathways had been induced in these culture conditions. Nevertheless, low silicate concentrations were measured at the end of the experiment, below the

Table 5
Concentration in macronutrients (±SD) for all conditions at the end of the experiment (n = 3, symbols as in Table 1).

Final concentration (μM)	FE	MFE	M50FE	M50	M882
NH_4-N	1.7 ± 0.4	<LOD	0.1 ± 0.1	<LOD	6.5 ± 2.5
NO_2-N	<LOD	<LOD	<LOD	<LOD	4.0 ± 1.1
NO_3-N	<LOD	<LOD	<LOD	1.2 ± 1.6	631.5 ± 115.5
Urea-N	4 ± 0.4	1.1 ± 0.3	1.7 ± 0.1	0.3 ± 0.4	19.5 ± 29.3
DON[a]	74 ± 8	79 ± 13	94 ± 13	41 ± 2.3	184 ± 49.5
DOC	1198 ± 88	1362 ± 480	1491 ± 360	369 ± 42	859 ± 93
PO4-P	1.8 ± 0.5	45.8 ± 7.6	46.9 ± 3.6	42.8 ± 0.4	37.2 ± 5.5
Si	<LOD	<LOD	0.8 ± 0.7	0.4 ± 0.3	0.9 ± 0.6
Inorganic N/P ratio	0.9	0	0	0	17.4
Total N/P ratio	44.3	1.7	2	1	22.7

[a] Without urea.

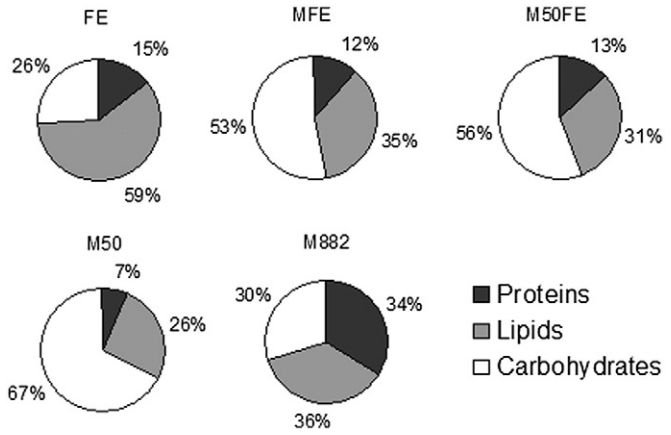

Fig. 3. Relative biochemical expressed composition (%) of *E. paludosa* as a function of the different culture conditions, *black*: proteins, *blue grey*: lipids, *white*: carbohydrates (FE: ASW + faeces extract, MFE: medium without N + faeces extract, M50FE: medium with 50 μM N-NO_3 + faeces extract, M50: medium with 50 μM N-NO_3, M882: medium with 882 μM N-NO_3).

detection limit in cultures grown with only faeces extract as nitrogen source (MFE condition, Table 5). Thus, the interaction between silicon and nitrogen limitations could have amplified the biochemical shift between the three main cell components and increased the production of lipids by up to 30%.

It is noteworthy that adding bird dropping extract in the medium M50 (M50FE condition) significantly increased the cellular protein quantity and decreased the cellular carbohydrate quantity (Table 3). Under the M50 condition, nitrates were the only available nitrogen source, whereas ammonia, urea and DON were also available as nitrogen sources in the MFE50 condition, increasing the availability of this element for growing diatoms (Table 1). Furthermore, this nitrogen source diversity could have favoured the nitrogen metabolism because of the intimate interaction between N-sources and energy for nitrogen assimilation (Bender et al., 2012). Trace elements were also present in faeces extract that could affect *Entomoneis*' biochemical composition, as already observed for other diatoms, such as *Ditylum brightwellii* (Darley, 1977). As observed with its growth rate, *E. paludosa* clearly benefitted from all these elements to assimilate nitrogen and produce proteins, as well as delay the production of stock compounds such as carbohydrates.

Adding faeces extract to the culture medium also resulted in another metabolic regulation during the *Entomoneis* growth: a four-fold increase in production of dissolved organic carbon (DOC) in the culture conditions MFE, FE, M50FE compared to M50 (Table 5). This result cannot be explained by nitrogen limitation (e.g. Corzo et al., 2000) or other limitations (Si, P) nor by the amount of DOC added by the faeces extract at the beginning of the experiment (Table 1). Because the cellular carbon was not significantly different amongst the growth conditions (Table 3), it is likely that the excess of DOC excreted into the three media enriched with faeces extract was not the result of stress, but results from an additional assimilation of carbon by the cells in these conditions. In accordance to the higher growth rates measured in these cultures, the hypothesis of an enhancement of the carbon assimilation and of the overall metabolism of the cells can be suggested. Although this effect cannot be clearly understood, it might have a role in biofilm formation in mudflat ecosystems and in microalgal cell protection against environmental stress (review in Decho (2000) and Underwood and Paterson (2003)) as DOC is partially composed of EPS (Exopolysaccharides) (Biddanda and Benner, 1997).

The six common diatom lipophilic pigments detected in *E. paludosa* were similar to those found by Meleder et al. (2013) and Barnett et al. (2014) for the same species, two chlorophylls (chlorophyll *a*, chlorophyll $c_1 + c_2$) and four carotenoids (fucoxanthin, diadinoxanthin, diatoxanthin and β-carotene). The pigment's qualitative composition did not vary but total pigment content per cell showed significant differences between growing conditions according to initial nitrogen availability (Table 4), and cells growing in the M882 condition had the highest pigment concentration. This is consistent with previous studies where pigment content and pigment ratio were used as indicators of the diatom physiological status (Geider et al., 1993; Kulk et al., 2013; Van Leeuwe et al., 2008) and where nitrogen availability affected total chlorophyll *a* cellular content. However, in our study, the light-harvesting pigment ratio Chl *c* + fucoxanthin/Chl *a* was unaffected by nitrogen limitation (Table 4). Unlike Geider et al. (1993), we did not observe any increase of the photo-protective pigment ratio diatoxanthin + diadinoxanthin/Chl *a* resulting from nutrient limitation. This could be explained by the light intensity used in our experiment which was much lower (90 μmol m^{-2} s^{-1}) than the 250 μmol m^{-2} s^{-1} used by Geider et al. (1993).

4.3. Implication for the Bourgneuf Bay nutrient fluxes and ecology

The current work showed that Dunlin droppings have the potential to enhance diatom growth either by diversifying available nitrogen sources for growth or by adding minor trace elements. Therefore, given the high wader bird densities observed annually in Bourgneuf Bay, 30,000 birds including 19,000 Dunlins (Wetlands International, 2012), it is possible that Dunlin populations have an effect on microphytobenthic biofilms in this bay. The bay is divided in two distinct parts by a rocky barrier oriented NE–SW. The northern part has a high hydrodynamism, whereas the southern is less exposed to currents and waves (Barille-Boyer et al., 1997; Haure and Baud, 1995). The southern part is also characterized by a low seawater turnover rate of two month (Haure and Baud, 1995) which enhances the residence time and the availability of nutrients from wader bird droppings. Furthermore, during the low tide and at a micro-scale (cm^2) the bird derived nutrients are directly available to the microphytobenthos. After the estimation of the annual amount of Dunlin droppings in Bourgneuf bay, these faeces could bring a minimum of 500 kg of nitrogen per year. This amount of nitrogen only represents what is brought by wintering Dunlin during their feeding time on the mudflat. It does not take into account the nitrogen produced during their resting time and the nitrogen input by other wader birds present in the bay (e.g. *Branta bernicla*, *Haematopus ostralegus*) which are generally bigger species.

Interestingly, it was also shown recently in the same mudflats that Dunlins seem to feed preferentially in zones exhibiting high microphytobenthic biofilm concentrations (Drouet et al., 2015). This suggests that in this bay there is a strong interaction between Dunlins and microphytobenthic biofilms. This also opens the possibility that Dunlin droppings contribute to a top down control of microphytobenthos biomass by directly supplying nutrients required for growth. However, with current data this remains speculative and further *in situ* research is still required.

5. Conclusion

Bird dropping extract induced a substantial growth of *E. paludosa* in artificial sea water (up to 6.8 cell divisions) producing 24% of the biomass obtained in nutrients replete conditions. Furthermore, adding faeces extract to growth media containing all major and traces nutrients lead to a significant increase of growth rates (around 20%) and biomass ($+92\ 10^5$ cell mL^{-1}). Nutrients or trace elements present in the faeces also had an effect on *E. paludosa* biochemical composition, regulating both nitrogen and carbon contents and their allocation between the three macromolecular components protein, lipid and carbohydrate. However, this did not seem to affect the photosynthetic apparatus and the light-harvesting pigments to chlorophyll *a* ratios or the photo-protective pigments to chlorophyll *a* ratios, which were both unchanged by the addition of bird droppings extract.

This study underlines the fertilizing effect of Dunlin droppings on MPB growth, biomass and biochemical composition, and raises the question of shorebird impact on mudflat primary producers

Acknowledgements

This study is part of the COSELMAR project carried out with the support of the "Region Pays de la Loire", the "MSH Ange Guépin", the Research Federation CNRS 3473 "Institut Universitaire Mer et Littoral — IUML", and the University of Nantes and the CNRS. The authors thank Philippe Rosa and Véronique Lagadec for their technical assistance in field sampling and DOC/DON analyses, respectively, and Denis Loquet from the CEISAM for CHNS analyses. **[SS]**

References

Admiraal, W., Peletier, H., 1979. Influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. Br. Phycol. J. 14, 197–206.
Admiraal, W., Riaux-Gobin, C., Laane, R.W.P.M., 1987. Interactions of ammonium, nitrate, and D- and L-amino acids in the nitrogen assimilation of two species of estuarine benthic diatoms. Mar. Ecol. Prog. Ser. 40, 267–273.
Antia, N.J., Harrison, P.J., Oliveira, L., 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30, 1–89.
Austen, I., Andersen, T.J., Edelvang, K., 1999. The influence of benthic diatoms and invertebrates on the erodibility of an intertidal mudflat, the Danish Wadden Sea. Estuar. Coast. Shelf Sci. 49, 99–111.
Barille-Boyer, A.L., Haure, J., Baud, J.P., 1997. L'ostréiculture en baie de Bourgneuf. Relation entre la croissance des huîtres *Crassostrea gigas* et le milieu naturel: synthèse de 1986 a 1995. Ifremer.
Barnett, A., Meleder, V., Blommaert, L., Lepetit, B., Gaudin, P., Vyverman, W., Sabbe, K., Dupuy, C., Lavaud, J., 2014. Growth form defines physiological photoprotective capacity in intertidal benthic diatoms. ISME J. 9, 32–45.
Beardall, J., Young, E., Roberts, S., 2001. Approaches for determining phytoplankton nutrient limitation. Aquat. Sci. 63, 44–69.
Bender, S.J., Parker, M.S., Armbrust, E.V., 2012. Coupled effects of light and nitrogen source on the urea cycle and nitrogen metabolism over a diel cycle in the marine diatom *Thalassiosira pseudonana*. Protist 163, 232–251.
Biddanda, B., Benner, R., 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. Limnol. Oceanogr. 42, 506–518.
Blanchard, G.F., Guarini, J.M., Orvain, F., Sauriau, P.G., 2001. Dynamic behaviour of benthic microalgal biomass in intertidal mudflats. J. Exp. Mar. Biol. Ecol. 264, 85–100.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
Bosman, A.L., Hockey, P.A.R., 1986. Seabird guano as a determinant of rocky intertidal community structure. Mar. Ecol. Prog. Ser. 32, 247–257.
Bosman, A.L., Toit, Du., Hockey, J.T., Branch, P.A.R., G.M., 1986. A field experiment demonstrating the influence of seabird guano on intertidal primary production. Estuar. Coast. Shelf Sci. 23, 283–294.
Brimble, S.K., Blais, J.M., Kimpe, L.E., Mallory, M.L., Keatley, B.E., Douglas, M.S.V., Smol, J.P., 2009. Bioenrichment of trace elements in a series of ponds near a northern fulmar (*Fulmarus glacialis*) colony at Cape Vera, Devon Island. Can. J. Fish. Aquat. Sci. 66, 949–958.
Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151, 315–331.
Brzezinski, M.A., 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. J. Phycol. 21, 347–357.
Claquin, P., Martin-Jezequel, V., Kromkamp, J.C., Veldhuis, M.J.W., Kraay, G.W., 2002. Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen, and phosphorus control. J. Phycol. 38, 922–930.
Cook, P.L.M., Veuger, B., Boer, S., Middelburg, J.J., 2007. Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. Aquat. Microb. Ecol. 49, 165–180.
Corzo, A., Morillo, J.A., Rodriguez, S., 2000. Production of transparent exopolymer particles (TEP) in cultures of *Chaetoceros calcitrans* under nitrogen limitation. Aquat. Microb. Ecol. 23, 63–72.
Darley, W.M., 1977. Biochemical composition. In: Werner, D. (Ed.), The Biology of Diatoms. University of California Press, Berkeley and Los Angeles, pp. 198–223.
Davidson, P.E., 2009. Some foods taken by waders in Morecambe Bay, Lancashire. Bird Stud. 18, 177–186.
Decho, A.W., 2000. Microbial biofilms in intertidal systems: an overview. Cont. Shelf Res. 20, 1257–1273.
Dierschke, V., Kube, J., Rippe, H., 1999a. Feeding ecology of dunlins *Calidris alpina* staging in the southern Baltic Sea, 2. Spatial and temporal variations in the harvestable fraction of their favourite prey *Hediste diversicolor*. J. Sea Res. 42, 65–82.
Dierschke, V., Kube, J., Probst, S., Brenning, U., 1999b. Feeding ecology of dunlins *Calidris alpina* staging in the southern Baltic Sea, 1. Habitat use and food selection. J. Sea Res. 42, 49–64.
Dortch, Q., 1982. Effect of growth conditions on accumulation of internal nitrate, ammonium, amino acids and protein in three marine diatoms. J. Exp. Mar. Biol. Ecol. 61, 243–264.
Dortch, Q., 1990. The interaction between ammonium and nitrate uptake in phytoplankton. Mar. Ecol. Prog. Ser. 61, 183–201.
Drouet, S., Turpin, V., Godet, L., Cognie, B., Cosson, R., Decottignies, P., 2015. Utilisation of intertidal mudflats by the Dunlin Calidris alpina in relation to microphytobenthic biofilms. J. Ornithol. 156, 75–83.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28, 350–356.
Durell, S., Kelly, C.P., 1990. Diets of Dunlin — *Calidris alpina* and grey plover — *Pluvialis squatarola* on the wash as determined by droppings analysis. Bird Stud. 37, 44–47.
Elner, R.W., Beninger, P.G., Jackson, D.L., Potter, T.M., 2005. Evidence of a new feeding mode in western sandpiper (*Calidris mauri*) and dunlin (*Calidris alpina*) based on bill and tongue morphology and ultrastructure. Mar. Biol. 146, 1223–1234.
Finkel, Z.V., Beardall, J., Flynn, K.J., Quigg, A., Rees, T.A.V., Raven, J.A., 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. J. Plankton Res. 32, 119–137.
Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
Geider, R.J., La Roche, J., 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur. J. Phycol. 37, 1–17.
Geider, R.J., La Roche, J., Greene, R.M., Olaizola, M., 1993. Response of the photosynthetic apparatus of *Phaeodactylum tricornutum* (Bacillariophyceae) to nitrate, phosphate, or iron starvation. J. Phycol. 29, 755–766.
Goeyens, L., Kindermans, N., Abu Yusuf, M., Elskens, M., 1998. A room temperature procedure for the manual determination of urea in seawater. Estuar. Coast. Shelf Sci. 47, 415–418.
Golovkin, A.N., 1967. The effect of colonial sea birds on development of the phytoplankton. Oceanology 7, 521–529.
Guerrini, F., Cangini, M., Boni, L., Trost, P., Pistocchi, R., 2000. Metabolic responses of the diatom *Achnanthes brevipes* (Bacillariophyceae) to nutrient limitation. J. Phycol. 36, 882–890.
Guillard, R.R.L., 1973. Division rates. In: Stein, J.R. (Ed.), Handbook of Phycological Methods. Cambridge University Press, Cambridge, pp. 289–312.
Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, USA, pp. 26–60.
Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea Cleve*. Can. J. Microbiol. 8, 229–239.
Gwiazda, R., Jarocha, K., Szarek-Gwiazda, E., 2010. Impact of a small cormorant (*Phalacrocorax carbo sinensis*) roost on nutrients and phytoplankton assemblages in the littoral regions of a submontane reservoir. Biologia 65, 742–748.
Harrison, P.J., Conway, H.L., Holmes, R.W., Davis, C.O., 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation III cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida*. Mar. Biol. 43, 19–31.
Harrison, P.J., Thompson, P.A., Calderwood, G.S., 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. J. Appl. Phycol. 2, 45–56.
Haure, J., Baud, J.P., 1995. Approche de la capacité trophique dans le bassin ostréicole (Baie de Bourgneuf). Ifremer.
Ho, T.Y., Quigg, A., Finkel, Z.V., Milligan, A.J., Wyman, K., Falkowski, P.G., Morel, F.M.M., 2003. The elemental composition of some marine phytoplankton. J. Phycol. 39, 1145–1159.
Jiang, Y.L., Yoshida, T., Quigg, A., 2012. Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiol. Biochem. 54, 70–77.
Kang, C.K., Sauriau, P.G., Richard, P., Blanchard, G.F., 1999. Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oleron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Mar. Ecol. Prog. Ser. 187, 147–158.
Keatley, B.E., Douglas, M.S.V., Blais, J.M., Mallory, M.L., Smol, J.P., 2009. Impacts of seabird-derived nutrients on water quality and diatom assemblages from Cape Vera, Devon Island, Canadian High Arctic. Hydrobiologia 621, 191–205.
Koroleff, F., 1970. Direct determination of ammonia in natural waters as indophenol blue. Information on Techniques and Methods for Seawater Analysis. International Council for the Exploration of the Sea, pp. 19–22.
Kulk, G., van de Poll, W.H., Visser, R.J.W., Buma, A.G.J., 2013. Low nutrient availability reduces high-irradiance-induced viability loss in oceanic phytoplankton. Limnol. Oceanogr. 58, 1747–1760.
Kuwae, T., Miyoshi, E., Hosokawa, S., Ichimi, K., Hosoya, J., Amano, T., Moriya, T., Kondoh, M., Ydenberg, R.C., Elner, R.W., 2012. Variable and complex food web structures revealed by exploring missing trophic links between birds and biofilm. Ecol. Lett. 15 (4), 347–356.
Linares, F., 2006. Effect of dissolved free amino acids (DFAA) on the biomass and production of microphytobenthic communities. J. Exp. Mar. Biol. Ecol. 330, 469–481.
Linares, F., Sundback, K., 2006. Uptake of dissolved free amino acids (DFAA) by microphytobenthic communities. Aquat. Microb. Ecol. 42, 175–186.
Loder, T.C., Ganning, B., Love, J.A., 1996. Ammonia nitrogen dynamics in coastal rockpools affected by gull guano. J. Exp. Mar. Biol. Ecol. 196, 113–129.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Lynn, S.G., Kilham, S.S., Kreeger, D.A., Interlandi, S.J., 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). J. Phycol. 36, 510–522.
MacIntyre, H.L., Geider, R.J., Miller, D.C., 1996. Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. 1. Distribution, abundance and primary production. Estuaries 19, 186–201.

Mahéo, R., Le Dréan-Quénec'hdu, S., 2013. Limicoles séjournant en France (littoral). Janvier 2013. Wetlands International/ONCFS, France.
Mantoura, R.F.C., Llewellyn, C.A., 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse phase high-performance liquid chromatography. Anal. Chim. Acta 151, 297–314.
Marchetti, J., Bougaran, G., Jauffrais, T., Lefebvre, S., Rouxel, C., Saint-Jean, B., Lukomska, E., Robert, R., Cadoret, J.P., 2013. Effects of blue light on the biochemical composition and photosynthetic activity of *Isochrysis* sp. (T-iso). J. Appl. Phycol. 25, 109–119.
Marion, L., Clergeau, P., Brient, L., Bertru, G., 1994. The importance of avian-contributed nitrogen (N) and phosphorus (P) to lake Grand-Lieu, France. Hydrobiologia 279, 133–147.
Martin-Jezequel, V., Hildebrand, M., Brzezinski, M.A., 2000. Silicon metabolism in diatoms: implications for growth. J. Phycol. 36, 821–840.
Mathot, K.J., Lund, D.R., Elner, R.W., 2010. Sediment in stomach contents of western sandpipers and Dunlin provide evidence of biofilm feeding. Waterbirds 33, 300–306.
Meleder, V., Barille, L., Launeau, P., Carrere, V., Rince, Y., 2003. Spectrometric constraint in analysis of benthic diatom biomass using monospecific cultures. Remote Sens. Environ. 88 (4), 386–400.
Meleder, V., Rince, Y., Barille, L., Gaudin, P., Rosa, P., 2007. Spatiotemporal changes in microphytobenthos assemblages in a macrotidal flat (Bourgneuf bay, France). J. Phycol. 43, 1177–1190.
Meleder, V., Laviale, M., Jesus, B., Mouget, J.L., Lavaud, J., Kazemipour, F., Launeau, P., Barille, L., 2013. In vivo estimation of pigment composition and optical absorption cross-section by spectroradiometry in four aquatic photosynthetic micro-organisms. J. Photochem. Photobiol. B Biol. 129, 115–124.
Morris, I., 1981. Photosynthetic products, physiological state, and phytoplankton growth. In: Platt, T. (Ed.), Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci. 210, pp. 83–102.
Mouritsen, K.N., 1994. Day and night feeding in Dunlins *Calidris alpina*: choice of habitat, foraging technique and prey. J. Avian Biol. 25, 55–62.
Mulvenna, P.F., Savidge, G., 1992. A modified manual method for the determination of urea in sea water using diacetylmonoxime reagent. Estuar. Coast. Shelf Sci. 34, 429–438.
Parsons, T.R., Stephens, K., Strickland, J.D.H., 1961. On the chemical composition of 11 species of marine phytoplankters. J. Fish. Res. Board Can. 18, 1001–1016.
Pinckney, J.L., Carman, K.R., Lumsden, S.E., Hymel, S.N., 2003. Microalgal–meiofaunal trophic relationships in muddy intertidal estuarine sediments. Aquat. Microb. Ecol. 31, 99–108.
Polis, G.A., Anderson, W.B., Holt, R.D., 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. 28, 289–316.
Polis, G.A., Power, M.E., Huxel, G.R., 2004. Food Webs at the Landscape Level. University of Chicago Press, Chicago.
Raimbault, P., Pouvesle, W., Diaz, F., Garcia, N., Sempere, R., 1999. Wet-oxidation and automated colorimetry for simultaneous determination of organic carbon, nitrogen and phosphorus dissolved in seawater. Mar. Chem. 66, 161–169.
Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46, 205–221.
Ribeiro, L., Brotas, V., Mascarell, G., Coute, A., 2003. Taxonomic survey of the microphytobenthic communities of two Tagus estuary mudflats. Acta Oecol. Int. J. Ecol. 24, S117–S123.
Ribeiro, L., Brotas, V., Rince, Y., Jesus, B., 2013. Structure and diversity of intertidal benthic diatom assemblages in contrasting shores: a case study from the Tagus estuary. J. Phycol. 49, 258–270.
Riera, P., 2010. Trophic plasticity of gastropod *Hydrobia ulvae* within an intertidal bay (Roscoff, France): a stable isotope evidence. J. Sea Res. 63, 78–83.
Roessler, P.G., 1988. Effects of silicon deficiency on lipid composition and metabolism in the diatom *Cyclotelle cryptica*. J. Phycol. 24, 394–400.
Sabbe, K., 1997. Systematics and Ecology of Intertidal Benthic Diatoms of the Westerschelde Estuary (The Netherlands). Universiteit Gent, Gent.
Sarthou, G., Timmermans, K.R., Blain, S., Tréguer, P., 2005. Growth physiology and fate of diatoms in the ocean: a review. J. Sea Res. 53, 25–42.
Scholz, B., Liebezeit, G., 2013. Biochemical characterisation and fatty acid profiles of 25 benthic marine diatoms isolated from the Solthorn tidal flat (Southern North Sea). J. Appl. Phycol. 25, 453–465.
Shifrin, N.S., Chisholm, S.W., 1981. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light–dark cycles. J. Phycol. 17, 374–384.
Signa, G., Mazzola, A., Vizzini, S., 2012. Effects of a small seagull colony on trophic status and primary production in a Mediterranean coastal system (Marinello ponds, Italy). Estuar. Coast. Shelf Sci. 111, 27–34.
Signa, G., Mazzola, A., Tramati, C.D., Vizzini, S., 2013. Gull-derived trace elements trigger small-scale contamination in a remote Mediterranean nature reserve. Mar. Pollut. Bull. 74, 237–243.
Smith, R.E.H., Geider, R.J., 1985. Kinetics of intracellular carbon allocation in a marine diatom. J. Exp. Mar. Biol. Ecol. 93, 191–210.
Strickland, J.D.H., Parsons, T.R., 1972. A practical handbook of sea water analysis. Bull. Fish. Res. Board Can. 167, 1–311.
Sundback, K., Lindehoff, E., Graneli, E., 2011. Dissolved organic nitrogen: an important source of nitrogen for the microphytobenthos in sandy sediment. Aquat. Microb. Ecol. 63, 89–100.
Syrett, P.J., 1981. Nitrogen metabolism in microalgae. In: Platt, T. (Ed.), Physiological Bases of Phytoplankton Ecology. Can. Bull. Fish. Aquat. Sci. 210, pp. 182–210.
Taguchi, S., Hirata, J.A., Laws, E.A., 1987. Silicate deficiency and lipid synthesis of marine diatoms. J. Phycol. 23, 260–267.
Twining, B.S., Baines, S.B., 2013. The trace metal composition of marine phytoplankton. Annu. Rev. Mar. Sci. 5, 191–215.
Underwood, G.J.C., Kromkamp, J., 1999. Primary production by phytoplankton and microphytobenthos in estuaries. In: Nedwell, D.B., Raffaelli, D.G. (Eds.), Adv. Ecol. Res.Academic Press, pp. 93–153
Underwood, G.J.C., Paterson, D.M., 2003. The importance of extracellular carbohydrate production by marine epipelic diatoms. In: Callow, J.A. (Ed.), Adv. Bot. Res.Academic Press, pp. 183–240
Van Leeuwe, M.A., Brotas, V., Consalvey, M., Forster, R.M., Gillespie, D., Jesus, B., Roggeveld, J., Gieskes, W.W.C., 2008. Photoacclimation in microphytobenthos and the role of xanthophyll pigments. Eur. J. Phycol. 43, 123–132.
Vincent, W.F., 1992. The daily pattern of nitrogen uptake by phytoplankton in dynamic mixed layer environments. In: Berman, T., Gons, H.J., Mur, L.R. (Eds.), The Daily Growth Cycle of Phytoplankton. Hydrobiol. 238, pp. 37–52.
Wetlands International, 2012. Waterbird population estimates. Summary Report fifth edition. Wetlands International, Wageningen, The Netherlands.
Wolfstein, K., Stal, L.J., 2002. Production of extracellular polymeric substances (EPS) by benthic diatoms: effect of irradiance and temperature. Mar. Ecol. Prog. Ser. 236, 13–22.
Yu, E.T., Zendejas, F.J., Lane, P.D., Gaucher, S., Simmons, B.A., Lane, T.W., 2009. Triacylglycerol accumulation and profiling in the model diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (Baccilariophyceae) during starvation. J. Appl. Phycol. 21, 669–681.