Chapter 10

Phaeocystis globosa: autecological data and bloom phenomena

"Es ist verwunderlich, wie gering unsere Kenntnis über die Biologie von *Phaeocystis* ist..."

P. Kornmann (1955)

Introduction

In the previous chapters, several physical, chemical and biological factors that are involved in the wax and wane of *Phaeocystis globosa* blooms have been investigated. In this chapter, several remaining questions will be examined. How do salinity and temperature influence *Phaeocystis* growth rate? Is it possible to describe growth rate as a function of the three autecological variables: salinity, temperature and daily irradiance? Autecological functions, or equations, are essential in population models because they provide first estimates of growth rates. Furthermore, they help in identifying bloom conditions in the sea. Growth rate estimates also provide vital information for mixed species experiments, in which *Phaeocystis* has to compete for nutrients with other phytoplankton. Within the boundaries (temperature, salinity, daily irradiance) set in such experiments, the choice of species may well determine the competition result. Rather surprisingly, the basic autecological equations have not been made yet for *Phaeocystis*.

In addition, some important questions remained unanswered. How can *Phaeocystis* outcompete diatoms under Si-replete conditions? *Phaeocystis* blooms are related to eutrophication, but does eutrophication ultimately determine the maximum *Phaeocystis* concentration in the sea? The wane of *Phaeocystis* blooms in the sea is notoriously associated with large amounts of foam on nearby shores; one of the major reasons to name the species a harmful alga. Is there a quantitative relation between bloom magnitude and the amount of beach-foam? These questions will be addressed in the following paragraphs. The goal is to gain a better understanding of the bloom characteristics of *P. globosa*, and to identify any remaining gaps in our knowledge that need further investigation.

Salinity

In coastal regions of the oceans, seawater salinity is subject to fluctuations due to variable freshwater input from the land. One of Europe's largest rivers, the Rhine, discharges into the North Sea leading to profound changes in salinity, nutrient concentrations, water density, water column stratification and, as a consequence, in phytoplankton development off the Dutch coast (van Bennekom *et al*. 1975, Peeters and Peperzak 1990, Simpson and Souza 1995, de Ruijter *et al*. 1997, Peperzak *et al*. 1998, chapter 6). Therefore, it would be expected that the effect of salinity variations on the growth rate of *Phaeocystis globosa*, the most abundant phytoplankton species in the Dutch coastal zone, has been well investigated. However, this is not so.

At present, only information on the salinity tolerance of *Phaeocystis* is available. In the first review of *Phaeocystis*, the genus was characterised as 'relatively stenohaline' (Kashkin 1964). This conclusion was based on a large number of field observations of *P. pouchetii* in a narrow salinity range (29.5 to 35.5‰). Furthermore, in the Gulf of Elat, *Phaeocystis* sp. was found at the maximum salinity of 40‰ (Thomsen 1978). However, the prymnesiophytes generally tolerate salinities from 10 to 30‰ (Pintner and Provasoli 1963, Hibberd 1980, Brand 1984). Nowadays, after three decades of *Phaeocystis* studies that include coastal regions as well, *P. globosa* is considered a euryhaline species (Lancelot *et al*. 1998).

In the North Sea, *P. globosa* has been found at salinities ranging from 23 to 35‰ (Wulff 1933, Rahmel *et al*. 1995, Rick and Aletsee 1989, Cadée 1991a). The maximum salinity at which *P. globosa* was found, in the Arabian Gulf, was 36-37‰ (Al-Hasan *et al*. 1990). Stefels (1997) was able to culture *P. globosa* at salinities from 25 to 50‰, which was accompanied by an increase in the osmolyte dimethylsulphoniopropionate from 6 to 38 fmol cell^{-1}. Yet, no quantitative relation between growth rate of any *Phaeocystis* species and salinity has been established.

The effect of salinity on *Phaeocystis* growth rate was tested in seawater originating from the central North Sea (36 psu) and the Oosterschelde (32 psu). Oosterschelde water was diluted with demineralised water to obtain salinities of 14.9 and 5.1 psu (in the present notation 1 psu or practical salinity unit ≅ 1‰). After sterilisation and enrichment with PEP-Si nutrients (Peperzak *et al.* 2000a), combinations of undiluted and diluted seawater were made to obtain a total of seven different salinities ranging from 10 to 36 psu. *P. globosa* clone Ph91 (Peperzak 1993) was incubated at 15°C and 110 W h m^{-2} day^{-1} (12:12 L:D cycle). Growth rate was measured from the exponential increase of in vivo fluorescence (Peperzak *et al.* 2000a). Flow cytometric measurements of exponentially growing cells, showed that there were no significant fluorescence differences between cultures of different salinities.

Growth rates were fitted in SYSTAT (Wilkinson 1990) to an adapted Eilers-Peeters (1988) equation. Under the assumption that growth rate decreases linearly towards a minimum salinity where growth rate is zero, and that growth inhibition occurs at high salinities, the growth rate - salinity equation reads:

$$\mu = (sal\text{-}sal_0) / (a\ (sal\text{-}sal_0)^2 + b\ (sal\text{-}sal_0) + c) \qquad [10.1]$$

In which: μ = growth rate (day^{-1}) and sal = salinity in psu; a, b, c and sal_0 (= sal where $\mu = 0$), are equation parameters.

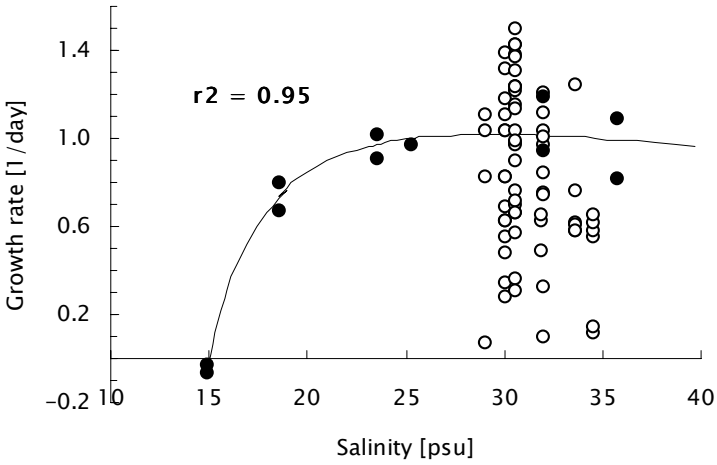

Figure 10.1. *Phaeocystis globosa* growth rate as a function of salinity. The curve is equation [10.1] fitted to the experimental data (●); o = data from the literature.

The experimental results were compared with data from Kayser (1970), Guillard and Hellebust (1971), Grimm and Weisse (1985), Jahnke and Baumann (1987), Jahnke (1989), Lubbers *et al.* (1990), Rousseau *et al.* (1990), Buma *et al.* (1991), van Boekel (1992a), Veldhuis *et al.* (1991), Stefels and van Boekel (1993), and from chapter 2 ($E > 500$ W h m^{-2} day^{-1}), 5, 7 and 8). These data pertain to *P. globosa* strains isolated in coastal areas, and cultured in the laboratory under controlled conditions. Aberrant high growth rates ($\mu > 1.5$ day^{-1} or $k > 2$ divisions day^{-1}) from Grimm and Weisse (1985) and Jahnke (1989), cannot be explained by an optimum combination of salinity, temperature and daily irradiance (this chapter) and therefore, these outliers were excluded from the database.

Under the experimental conditions, *P. globosa* grew at approximately 1 day^{-1} in the 25 to 35 salinity range (Figure 10.1). The cultures slowly died at 15 psu; at 10 psu the cells disintegrated rapidly. The results of the fit of equation [10.1] to the experimental data were good. The model explains 95% of growth rate variability ($r^2 = 0.95$). The model parameters (± standard error) are: $a = 0.012$ (± 0.014), $b = 0.65$ (± 0.30), $c = 2.36$ (± 1.23), $sal_0 = 15.0$ (± 0.2). Using equation [10.1] the calculated maximum growth rate was 1.0 day^{-1} at 29 psu.

Considering the 20 psu salinity range in which *P. globosa* is able to grow (Figure 10.1), the species is indeed euryhaline (Lancelot *et al.* 1998). However, this cannot be concluded from previous laboratory studies, because these were performed in the narrow range of 29 to 35 psu (Figure 10.1). The large range of growth rates measured in these studies (Figure 10.1), are due to the widely differing culture conditions, such as in temperature and in daily irradiance.

Brand (1984) distinguished oceanic, coastal and estuarine species on the basis of the salinity tolerance of 46 phytoplankton clones. Following Brand's classification, *P. globosa* Ph91 is a coastal species (reproduction optima at 25 to 33 psu), which is in line with the origin of this strain (Peperzak 1993). The ability of *P. globosa* to grow at widely differing salinities, may well be a factor contributing to its success in Dutch coastal waters, where salinities vary between 26 and 32 psu (chapter 4: Figure 4.2b; de Ruijter *et al.* 1997). However, the effect of fast fluctuating salinities on, for example, the speed at which *Phaeocystis* adapts its growth rate or colony buoyancy, have not been investigated yet.

Temperature

Seawater temperature ranges from -2°C, its freezing point, to an extreme 36°C in the Arabian Gulf (Al-Hasan *et al.* 1990). The genus *Phaeocystis* occupies a large part of this temperature range, although the tolerances of the individual species are distinct. Jahnke and Baumann (1987) measured the difference in temperature tolerance, between *Phaeocystis pouchetii* (-2 to 14°C) and *P. globosa* (4 to 22°C). The temperature tolerance of *P. antarctica* (-2 to 7°C; Baumann *et al.* 1994), resembles that of the arctic *P. pouchetii*. *Phaeocystis* sp. from the east coast of north America grows between 0 and 13°C (Guillard and Hellebust 1971, Verity *et al.* 1988a, Hegarty and Villareal 1998).

The observation that in polar regions *Phaeocystis* blooms before diatoms, in contrast to temperate seas, has been related to interspecific differences in temperature optima (Lancelot *et al.* 1998). On the other hand, Cadée and Hegeman (1986) could not find a relation between the start of the spring *Phaeocystis globosa* bloom and water temperature in the North Sea.

Colonies of *P. globosa* have been observed at widely differing temperatures, from approximately -1°C in Dutch coastal waters (Veldhuis *et al.* 1986, Cadée 1991a) to 25-26°C in the Atlantic Ocean off the South American coast (Lohmann in Kashkin (1963), Guillard and Hellebust 1971). This wide temperature tolerance makes *P. globosa* well suited for growth in the coastal regions of the North Sea, where the temperature ranges from near zero in winter (Cadée 1991a) to 22°C in summer (Cadée 1991a, Peperzak *et al.* 1996b).

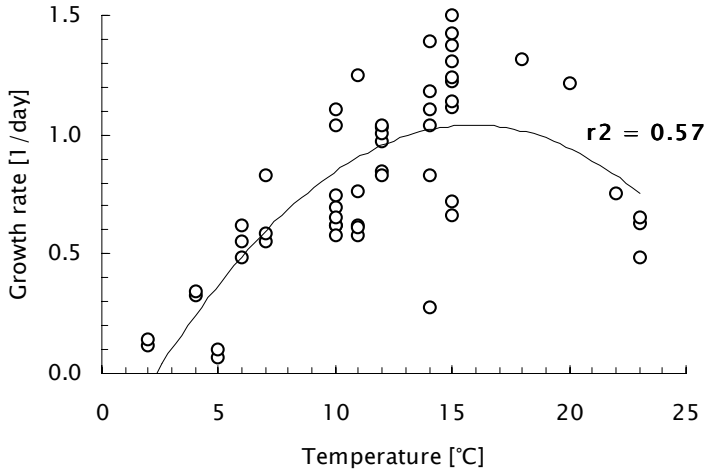

Figure 10.2. *Phaeocystis globosa* growth rate as a function of temperature. The curve is equation [10.2] fitted to data from the literature.

To evaluate the role of temperature in the development of *Phaeocystis* blooms, a quantitative relation between this variable and the algal growth rate is necessary. A first attempt was made in a review by Baumann *et al*. (1994: Figure 8), by drawing a curve by eye. However, the maximum growth rate (> 0.1 h^{-1}) they obtained would mean that, assuming continuous irradiance, the cells divided nearly four times per day, which is extremely high for a flagellate (Peperzak 1993, Furnas 1990). The temperature tolerance of a single *P. globosa* strain measured by van Boekel (1992a) was 3 to 24°C, with a maximum growth rate of 1 day^{-1} at 12°C.

Here, the relation between temperature and *P. globosa* growth rate will be re-examined. Data from the literature (see the previous paragraph on salinity), were used to fit a second degree regression equation:

$$\mu = a\,T^2 + b\,T + c \qquad [10.2]$$

In which: μ = growth rate (day^{-1}), T = temperature (°C) and a, b and c are equation parameters.

The result of applying equation [10.2] is shown in Figure 10.2. The temperature tolerance of *P. globosa*, at the prevalent experimental conditions, ranges from 2 to 23°C. Equation [10.2] explains 57% of the variance in growth rate ($r^2 = 0.57$), despite the fact that the growth rates were obtained at widely varying salinities and irradiances. The parameters (± standard errors) for equation [10.2] were: a = -0.0056 ± 0.0010, b = 0.180 ± 0.025 and c = -0.385 ± 0.154.

The choice for a second degree regression was based on the position of the data points (Figure 10.2), which form a parabola. Better known equations, that are based on the van 't Hoff rule, are the Belehrádek temperature formula ($\mu = a\ (T - b)^c$, see Eppley 1972), and the Arrhenius equation ($\mu = a\ e^{-b/RT}$, see Goldmann and Carpenter 1974). Both these equations could not be fitted properly, basically because they are unable to describe reduced growth rates beyond the optimum. Van 't Hoff based equations can, therefore, only be used to model maximum temperature dependent growth rates of a variety of algal species, not a single one.

The optimum temperature, calculated with equation [10.2] is 16°C, the same value as found by Baumann *et al.* (1994). The temperature range (2 to 23°C) is nearly the same (3 to 24°C) as found by van Boekel (1992a). From 6 to 16°C the growth rate, calculated with [10.2], increased by a factor 2.2. Goldmann and Carpenter (1974) calculated a maximum Q_{10} of 2.2 for a variety of small single-celled green algae and diatoms, and suggested that the growth rate of many algae is controlled by some master reaction.

For *P. globosa* too, temperature is an important determinant of growth rate. For example, at the start of the spring *Phaeocystis* bloom in 1992, water temperature was 7 to 8°C (chapter 4). At this temperature the cells would grow at 0.6 day^{-1}, already 60% of the maximum growth rate. Indeed, the actual growth rate calculated from the cell concentrations in Figure 4a (chapter 4), was 0.5 day^{-1}. In summer, when a relatively high temperature of 22°C is reached in the North Sea, *Phaeocystis* would still be able to grow at 80% of its maximum rate. Because of this broad temperature tolerance, it appears incorrect to call *P. globosa* less eurythermal than polar species (Lancelot *et al.* 1998). Polar *Phaeocystis* species have lower temperature optima and less broad temperature tolerances, and as such they are better adapted to polar conditions.

Daily irradiance

Sunlight drives photosynthesis and phytoplankton growth. The way phytoplankton harvest the sun's energy and convert it into new cells is, by analogy with the short-term irradiance-photosynthesis process, species-specific (Falkowski *et al.* 1985). In addition to salinity and temperature, daily irradiance, the product of irradiance (W m^{-2} or $\mu E\ m^{-2}\ s^{-1}$) and the daily light period (h day^{-1}) is the third autecological variable. An attempt is made here to model growth rate mathematically as a function of daily irradiance.

One of the first mathematical relations between growth rate and daily irradiance was described by Yoder (1979), using a hyperbolic tangent function. One drawback of his function is that, for mathematical reasons, the modelling of photoinhibition is excluded. The Yoder equation has been applied to growth

rate data of several diatoms and *Emiliania huxleyi* but, according to Nielsen (1992), with varying success. Another model, the Bannister-Law algal growth model (Bannister 1990) includes both photosynthesis and growth rate, three adaptive properties, and it treats irradiance and daily light period as separate variables. However, the complexity of the Bannister-Laws model (5 equations and 10 constants), combined with the current lack of the required data, prohibit its use for modelling *Phaeocystis* growth rate. Here, it is proposed that the daily irradiance - growth rate relation may be described using an adaptation of the Eilers-Peeters (E-P) equation (1988):

$$\mu = (E - E_c) / (a (E - E_c)^2 + b (E - E_c) + c) \qquad [10.3]$$

With μ = growth rate (day^{-1}), E = daily irradiance; a, b, c and E_c are equation parameters. E_c = E where $\mu = 0$ (E and E_c in W h m^{-2} day^{-1}).

Originally, the E-P equation describes photosynthetic rate as a function of irradiance. Unlike other equations of this kind (Kirk 1994), the E-P equation is based on phytoplankton physiology, the process of photoinhibition inclusive (Eilers and Peeters 1988). A second advantage of the of the modified E-P equation [10.3], is that it should allow the easy calculation of species-specific characteristics of the daily irradiance-growth rate curve (Table 10.1).

To test the applicability of equation [10.3], two fits were made. In both of these fits, only *P. globosa* growth rates measured at ± 5°C of the optimum temperature (11-21°C) were used. The first fit was made with the non-flagellate data from chapter 2. Subsequently, the *P. globosa* dataset used in the previous two sections was tested. The chapter 2 data were omitted, but because the dataset then contained only one measurement above 500 W h m^{-2} day^{-1}, the high daily irradiance data (E > 500 W h m^{-2} day^{-1}; n = 7) from chapter 2 were included again. Then, the second fit was made.

Figure 10.3 and Table 10.1 show that the daily irradiance growth rate equation can be fit properly to experimental data. At low daily irradiances, the chapter 2 growth rates were slightly lower than those calculated with the literature data (Figure 10.3), leading to a less steep slope (1/c) and a higher characteristic daily irradiance (E_k^{μ}, Table 10.1). This difference may be due to the short time the cells in the chapter 2 experiment had to adapt to new irradiances. The fitted compensation daily irradiance or E_c, is much lower in Figure 10.3b, compared to Figure 10.3a, due to one growth rate measured by Jahnke (1989) at 20 W h m^{-2} day^{-1}. The calculated maximum growth rate is the same for both datasets. There is no apparent inhibition at high daily irradiance (Figure 10.3), which is reflected in the high daily irradiance optima and the low inhibition constants (Table 10.1), in line with the conclusion reached in chapter 2. However, *P. globosa* mesoflagellates do show photoinhibition at high irradiances (L. Peperzak, unpublished data).

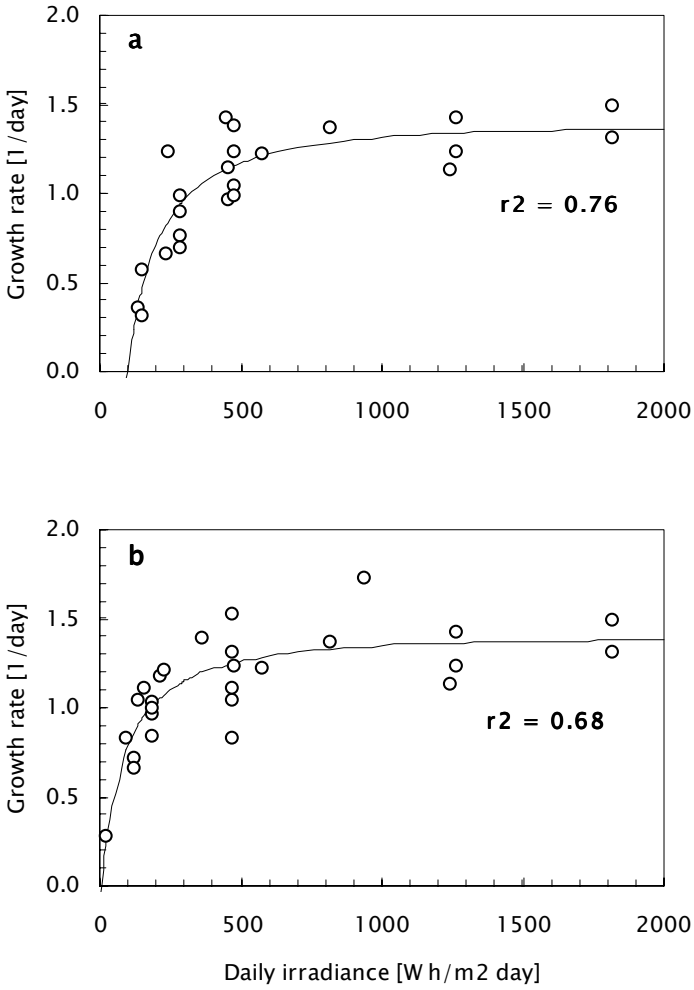

Figure 10.3. Growth rate of *Phaeocystis globosa* as a function of daily irradiance. Curves are equation [10.3] fitted to: (**a**) chapter 2 data of non-flagellate cells, (**b**) literature data, including chapter 2 growth rates measured at daily irradiances above 500 W h m^{-2} day^{-1}.

The characteristic variables of the daily irradiance-growth rate curve, can be useful in quantitative comparisons of *Phaeocystis* with other phytoplankton species. Until now, the comparison of *Phaeocystis* strains and morphotypes with each other, or with diatoms, was usually made on the basis of short-term photosynthesis data (Verity *et al.* 1991, Baumann *et al.* 1994). Only Moisan

Table 10.1. Daily irradiance-growth rate equation [10.3], fitted to data obtained from chapter 2, and from the literature (including chapter 2 data at >500 W h m^{-2} day^{-1}). All data measured at 16 ± 5°C. Characteristic variables of *P. globosa* growth were calculated with the parameters a, b and c.

			chapter2 data	literature data	unit
fit:					
explanation	r^2	-	0.76	0.68	-
number of data	n	-	23	27	-
parameters [a] :					
compensation d.i. [b]		E_c	99	4	W h m^{-2} day^{-1}
		a	0.000020	0.000011	-
		b	0.659	0.675	-
		c	75.2	56.5	-
characteristic variables:					
initial slope	s	1 / c	0.013	0.018	-
optimum d.i. [b]	$E_m\mu$	√ (c/a)	1900	2300	W h m^{-2} day^{-1}
maximum μ [c]	μ_{max}	1 / (b + 2√(ac))	1.4	1.4	day^{-1}
characteristic d.i. [b]	$E_k\mu$	c / (b + 2√ (ac))	102	78	W h m^{-2} day^{-1}
inhibition parameter	w	b / √(ac)	17	27	-

[a] parameters of equation [10.3] [b] daily irradiance [c] growth rate

and Mitchell (1999) compared daily irradiance growth rate data of *P. antarctica* with *P. globosa*. Although the shape of their curve (Figure 8b, Moisan and Mitchell 1999) is comparable to that of *P. globosa* (Figure 10.3), the maximum growth rate of *P. antarctica* (0.3 day^{-1} at 4°C) is only one third that of *P. globosa*. In contrast, Baumann *et al.* (1994) claim that the maximum growth rate of *P. antarctica* is 1.9 day^{-1}.

Because *Phaeocystis* and diatoms often co-occur it seems obvious to compare their growth rates. In early spring *Phaeocystis* outcompetes diatoms in polar regions, but not in temperate waters. In general, diatoms have much higher growth rates than other phytoplankton groups (Furnas 1990) and, under Si-replete conditions, diatoms are expected to gain dominance over *Phaeocystis* (Egge and Aksnes 1992). Jahnke (1989) compared the generation times of *P. globosa* and the diatom *Thalassiosira rotula*, and concluded that the diatom would outcompete *Phaeocystis* at daily irradiances < 500 W h m^{-2} day^{-1}. Hegarty and Villareal (1998) found that in semi-continuous cultures, the diatom *Skeletonema costatum* always dominated over *P.* cf. *pouchetii* flagellates at either low (100 W h m^{-2} day^{-1}) or high (570 W h m^{-2} day^{-1}) daily irradiance and irrespective of N- or P-limitation.

Table 10.2. Equations describing the growth rate of non-flagellate *P. globosa* cells as a function of three autecological variables: salinity, temperature and daily irradiance.

Variable	unit	growth rate (μ) =
salinity (sal)	psu	$(sal-15.0)/(0.012\ (sal-15.0)^2 + 0.65\ (sal-15.0)+ 2.36)$
temperature (T)	°C	$- 0.0056\ T^2 + 0.180\ T - 0.385$
daily irradiance (E)	$Whm^{-2}day^{-1}$	$(E-99) / (0.000020\ (E-99)^2 + 0.66\ (E-99) + 75)$

The application of equation [10.3] on daily irradiance-growth rate data of different *Phaeocystis* species, morphotypes and of relevant diatom species, will provide a better quantitative basis for explaining the competition results observed in the sea. However, in order to do so, growth rates must have been measured at a range of ecologically significant daily irradiances (cf. chapter 2).

three autecological relations

In the previous paragraphs, three autecological equations have been presented that are useful in calculating the growth rate of *Phaeocystis* as a function of salinity, temperature and daily irradiance (Table 10.2). These equations should be used carefully. In the first place, they pertain to non-flagellate cells of *P. globosa* only. A lack of data is the reason that similar sets of equations for *P. pouchetii* and *P. antarctica*, nor for flagellate cells or colonies of either three species, can be made. Second, irradiance and daylength, which are often treated as separate variables, were combined here in daily irradiance. Third, the autecological equations were derived from data collected in experiments where only one factor was varied. Interactions between the autecological variables have not been taken into account.

Several examples can be given where interactions between autecological variables have been observed. For instance, factorial experiments with the dinoflagellate *Karenia mikimotoi* have shown that its growth rate is influenced by interactions between salinity and temperature, and between irradiance and daylength (Yamaguchi and Honjo 1989, Nielsen and Tønseth 1991, Nielsen 1992). Interactions of irradiance and daylength were also found in the prymnesiophyte *Emiliania huxleyi*, but not in the diatom *Ditylum brightwellii* (Yoder 1979). Yoder (1979) also found an effect of daylength on the growth rate of *S. costatum*, when temperature was 10°C or higher. The temperature optimum of the prasinophyte *Dunalliela tertiolecta* is influenced by salinity (Eppley 1972). Such interactions have not yet been investigated in *Phaeocystis*.

Other reasons to use the autecological equations in Table 10.2 with caution are that (i) autecological data are generally derived from steady state cultures, while in the sea fast acclimatisation in response to rapid environmental changes may be an important part of a species' competitive abilities, (ii) the

autecological data were acquired from nutrient-replete *P. globosa* cultures; nutrient limitation will not only lead to lower growth rates, but it may also affect *Phaeocystis* morphology (Riegman *et al.* 1992), and (iii) autecological equations for mortality rates (see chapter 5) have not been considered at all.

Despite their limitations, the equations in Table 10.2, and comparable sets for other phytoplankton species, are valuable in making first estimates of growth rates for a given set of environmental data. For instance, the competition among phytoplankton species for a growth rate limiting nutrient has been tested in chemostats in which the species are mixed (Riegman *et al.* 1992, Hegarty and Villareal 1998). In chemostats, the growth rates are a function of the supply rate of the limiting nutrient. However, the growth rates will also depend on the prevalent set of environmental data (salinity, temperature, daily irradiance), that determines the species-specific maximum growth rates. Therefore, the success of certain species in mixed chemostat experiments, and the failure of others, not only depends on their competitive capabilities for the limiting nutrient, but also on their species-specific response to the environmental conditions.

pH, CO_2 and the diatom-*Phaeocystis* succession

ABSTRACT. Observations made in the Dutch coastal zone and in marine mesocosms did not provide evidence for the widely accepted hypothesis that the flagellate *Phaeocystis* blooms only after Si has been depleted by a preceding diatom bloom. On the contrary, at high Si concentrations *Phaeocystis* outcompeted the diatoms. The mechanism for this succession was unknown. Therefore, the mesocosm data were re-analysed in view of *Phaeocystis'* ability to grow at an intracolonial pH over 9. It was found that the abundance of diatoms in the mesocosms (mostly *Skeletonema costatum*), declined when pH reached 8.6 and the CO_2 concentration became less then 4 µM. The maximum *Phaeocystis* biomass was reached later, at pH 8.9 and 1 µM CO_2. In addition, measurements made in batch cultures revealed that the growth rate of *S. costatum* declined from 0.7 day^{-1} at pH 8.0 to 8.4, to 0.3 day^{-1} at pH 8.7 to 8.9. The growth rate of *Phaeocystis* at low pH, 0.7 day^{-1}, decreased by only 6% in the high pH range. The ability to maintain a high growth rate at high pH gives *Phaeocystis* a competitive advantage over diatoms.

Only after diatoms have depleted their essential nutrient silicon, the flagellate *Phaeocystis* is able to bloom, and take advantage of the excess nitrogen and phosphorus in eutrophied marine waters. This hypothesis was tested in 1992 by monitoring nutrient concentrations and phytoplankton development in the Dutch coastal zone. Quite unexpected, it was found that *Phaeocystis* starts blooming well before the silicon has been depleted by diatoms (chapter 4).

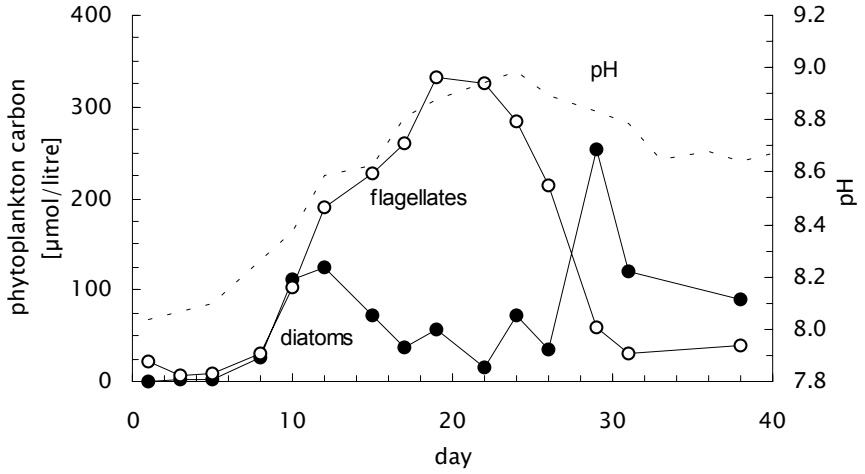

Figure 10.4. Diatom and flagellate biomass and the development of pH during the 1992 mesocosm experiment. Diatom biomass is multiplied by 10 for clarity. Shown are the means of two duplicate mesocosms. *S. costatum* was the dominant diatom; *P. globosa* was the dominant flagellate. *P. globosa* biomass was computed from cell abundance. The diatom peak on day 29 is from benthic diatoms that detached from the mesocosm walls.

In the same year, the Si-depletion hypothesis was tested in two similarly treated, nutrient-replete marine mesocosms. Again, *Phaeocystis* bloomed before Si-depletion and the flagellate outcompeted the diatom community (chapter 3). Several hypotheses were proposed to explain the diatom-*Phaeocystis* succession in the mesocosm experiment, but none could be substantiated.

In chapter 8, a model was presented in which *Phaeocystis* cells sequester inorganic carbon from the bulk seawater by employing the high intracolonial pH of 9. In contrast, the growth rates of several species of diatoms are reduced at such a high pH (Hinga 1992, Riebesell *et al.* 1993, Chen and Durbin 1994). Therefore, the rise in pH and the concomitant decline in CO_2 concentration during *Phaeocystis*-diatom blooms, could provide an explanation why *Phaeocystis* outcompetes diatoms at high Si concentrations.

This hypothesis was tested in two ways. First, the mesocosm data were re-analysed by comparing the temporal development of diatom and flagellate biomass with pH. Then, the biomass of *S. costatum* and *P. globosa* were plotted as a function of CO_2 concentration. Second, the effect of pH on growth rate of *S. costatum* and *P. globosa* was measured in batch cultures, incubated at 15°C at 100 W h m^{-2} day^{-1}. The pH in the PEP culture medium (chapter 5) was buffered at values between 8 and 9 using 10 mM Tris (Provasoli *et al.* 1957). Growth

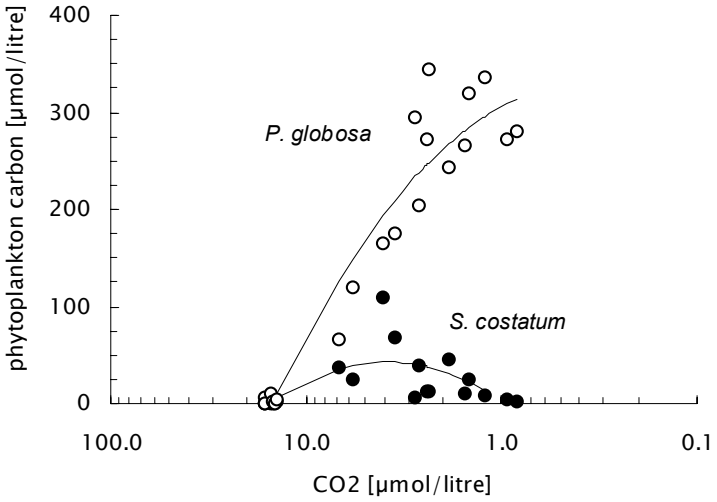

Figure 10.5. *S. costatum* and *P. globosa* biomass as function of CO_2 concentration. Data of two mesocosms (day 0 to 24) were combined. The curved lines are second degree regression equations of phytoplankton carbon over CO_2 concentration: *S. costatum* r^2 = 0.46; *P. globosa* r^2 = 0.92.

rates were calculated from the exponential increase of in vivo fluorescence (chapter 5) in the first week of growth, when only 5% of the maximum biomass in these batch cultures had been reached.

In the mesocosms, the development of the diatoms (60% *Skeletonema costatum*) stopped at day 12, when pH rose above 8.5 (Figure 10.4). The flagellates (99% *Phaeocystis globosa* cells) continued to grow until the pH rose to 8.8. The increase in *S. costatum* biomass stopped when the CO_2 concentration had declined to 4 µM (Figure 10.5). At the top of the *P. globosa* bloom, the CO_2 concentration was approximately 1 µM.

The mean growth rate of *S. costatum* in the pH experiment (Figure 10.6), declined from 0.7 day^{-1} at pH 8.0 to 8.4, to 0.3 day^{-1} (-40%) at pH 8.7 to 8.9. This agrees well with the 30% decline in *S. costatum* growth rate between the pH range of 6.5 to 8.5 and pH 9.0 found by Taraldsvik and Myklestad (2000). Hinga *et al.* (1994) also found that pH values in the range of 7.5 to 8.3 did not influence the growth rate of *S. costatum*; however, they did not culture the diatom at higher pH values. The mean growth rate of *P. globosa* at low pH values was 0.7 day^{-1}. In contrast to *S. costatum*, the prymnesiophyte's growth rate decreased by only 6% in the high pH range.

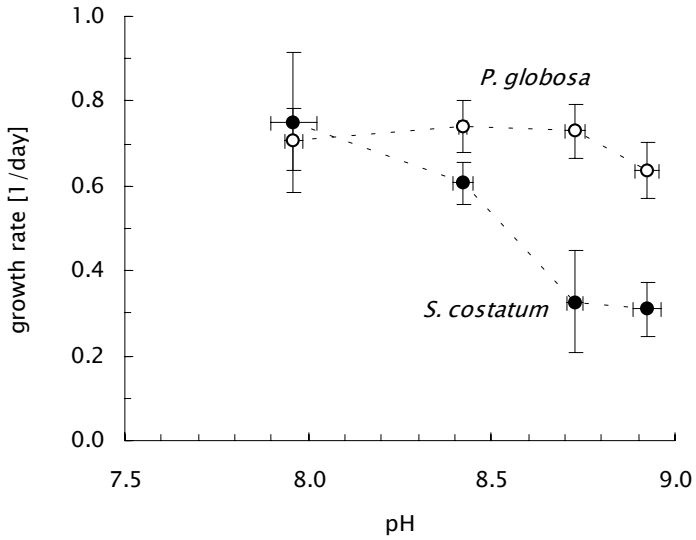

Figure 10.6. Growth rates of *S. costatum* and *P. globosa* in pH buffered batch cultures. Error bars are standard deviations (n = 3).

Reports on the negative effect of a rising or high pH on diatom growth rates are not limited to *S. costatum*. According to Hinga (1992) diatoms grow optimum at pH 8.1 with a notable growth rate decrease above pH 8.5. Riebesell *et al.* (1993) showed that the growth rates of three diatom species decreased rapidly above pH 8.6 due to CO_2-limitation. Chen and Durbin (1994) found that a pH over 8.8 reduced the growth rate of the diatoms *Thalassiosira pseudonana* and *T. oceanica*. Therefore, the rise in pH during *Phaeocystis* blooms may well be a competitive mechanism, leading to a reduction of diatom growth rates.

It would be possible that a decline of diatom growth rate at high pH (Figure 10.6), is the result of a reduction in the availability of $Si(OH)_3$. However, the concentration of $Si(OH)_3$ in PEP medium, calculated using a pK_{Si} of 9.5 (Butler 1992), merely declines from 97 µM (pH 8) to 68 µM (pH 9). Therefore, it is improbable that $Si(OH)_3$ became limiting in the pH experiment. The same holds for NO_3^-. In fact, Thoresen *et al.* (1984) measured maximum NO_3^- uptake rates of *S. costatum* at pH values between 8.5 and 9. Phosphate or trace metal availability can also be influenced by pH, but it is unclear how that would effect only *S. costatum* and not *P. globosa*.

Can the pH-dependence of growth rates be used to explain the 1992 phytoplankton succession in the mesocosms? In the cultures the growth rate of *S. costatum* declined by 60% when the pH was over 8.5 (Figure 10.6). In the

mesocosm the net growth rate of the diatoms (Figure 10.4), changed from 0.6 day^{-1} (day 3-12), to -0.2 day^{-1} (day 12-22). Assuming a grazer biomass in excess of diatom biomass at day 12, growing at 0.26 day^{-1} with a growth efficiency of 20% (Table 4.2) and a 60% drop in the overall growth rate (from 1.3 to 0.5 day^{-1}) due to the rise in pH, a -0.2 day^{-1} "growth rate" is indeed feasible.

Phaeocystis cells are offered protection against grazing by the colonial matrix (e.g. Admiraal and Venekamp 1986). Despite this, the net *P. globosa* growth rate in the mesocosms was only 0.45 day^{-1} (day 5-12), and would have been insufficient to win the competition from *S. costatum* (μ = 0.6 day^{-1}) at low pH (Figure 10.4). The ability of *P. globosa* to maintain its growth rate at pH values over 8.5 (Figure 10.6) apparently was a major factor for its success over *S. costatum* in the mesocosm experiment.

Maximum achievable biomass of *Phaeocystis* blooms

ABSTRACT. The pH inside the colonies of *Phaeocystis globosa* is one unit higher then the bulk seawater pH, leading to a flux of OH^- out of, and a flux of HCO_3^- into the colonies. Assuming that (i) the final bulk seawater pH can ultimately increase by one unit with no changes in alkalinity, (ii) the rate of CO_2 diffusion from the atmosphere into the sea can be neglected, and (iii) a *Phaeocystis*-carbon model from the literature is valid, it is calculated that a maximum of 0.7 mol inorganic C m^{-3} can be converted into an equal amount of organic colonial *Phaeocystis* carbon, which is equivalent to a *Phaeocystis* concentration of 10^{11} cells m^{-3}. Such high *Phaeocystis* concentrations have indeed been observed in highly phosphate-eutrophied parts of the North Sea. It is concluded that inorganic carbon availability is the prime determinant for the height of the *Phaeocystis* bloom. The second limiting nutrient phosphate determines the length of the bloom.

CO_2 is the substrate for algal photosynthesis. Because CO_2 in water is an acid ($CO_2 + H_2O \Rightarrow H_2CO_3$), the diffusive uptake of CO_2 by algal cells leads to a rise of the bulk medium pH. As a consequence, the carbonate system ($H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2\ H^+ + CO_3^{2-}$) shifts to the right, meaning that, if there is no equilibrium with the atmosphere, the CO_2 concentration declines even further and may ultimately limit photosynthesis. Here, calculations will be made to investigate, how the rise in pH inside *Phaeocystis* colonies may influence the abundance of *Phaeocystis* in the sea.

The calculations are based on the C_i uptake model developed in chapter 8. In short, this model consists of the following steps: (i) colonial *Phaeocystis* cells split HCO_3^-, the major C_i species, into CO_2 and OH^- using the extracellular enzyme carbonic anhydrase, (ii) CO_2 diffuses into the cell, is fixed, and

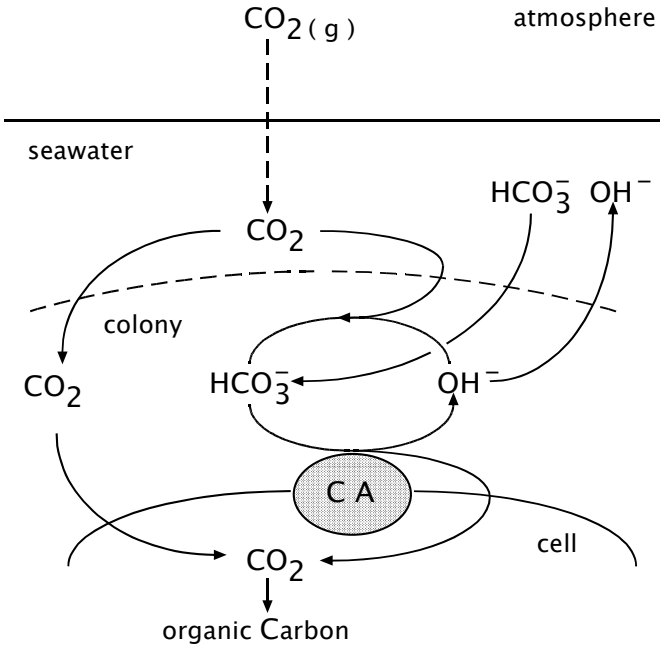

Figure 10.7. Inorganic carbon uptake by *Phaeocystis*. CO_2 in the seawater diffuses into the colony and (i) directly into the cells, or (ii) is hydrated by OH^- to yield HCO_3^- that is dehydrated at the cell surface by the enzyme carbonic anhydrase (CA). The pH inside the colony is higher than in seawater due to CO_2 uptake by the cells and by OH^- production. The diffusion of OH^- out of the colony is balanced by the diffusion of HCO_3^- into the colony (chapter 8). Diffusion of CO_2 from the atmosphere to the seawater is negligible with respect to the rate of photosynthesis.

converted to organic carbon, (iii) the colony interior does not contain sufficient HCO_3^- to sustain a prolonged C_i fixation rate, (iv) due to steep concentration gradients, OH^- will diffuse out of the colony, and HCO_3^- and CO_2 diffuse into the colony (maintaining a neutral charge balance), (v) the rate limiting OH^- diffusion is a function of colony size and the pH difference between the colony interior and the external seawater, (vi) during colony growth, the relative rate of OH^- diffusion decreases because the colonies increase in size, and because the increase in seawater pH decreases the concentration gradients. A model of C_i-uptake by *Phaeocystis* colonies is shown in Figure 10.7.

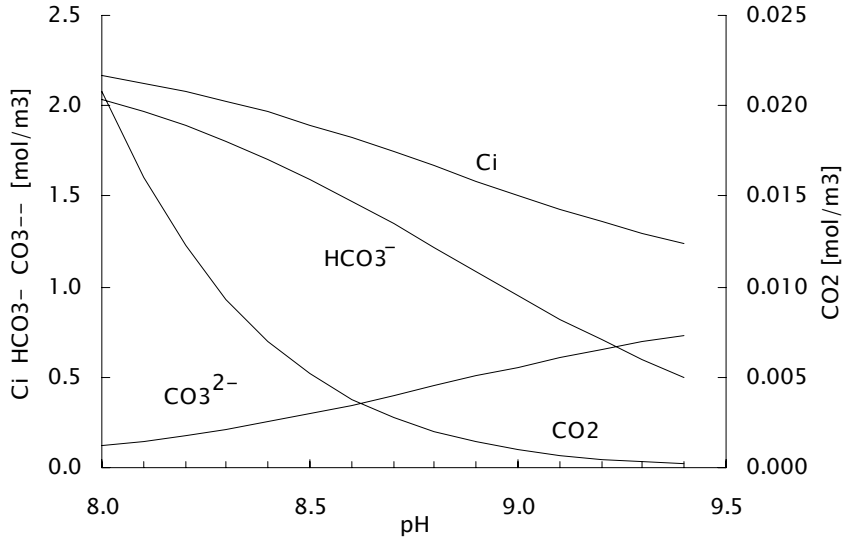

Figure 10.8. Concentrations of total inorganic carbon (C_i) and of the inorganic carbon species, as a function of pH when HCO_3^- is converted to CO_2 and OH^-, and CO_2 is taken up by the phytoplankton cells (alkalinity = constant).

The CO_2 flux into the colony will eventually stop when the pH in the bulk medium has also risen to 9.1, due to the diffusion of OH^- out of the colony (Figure 10.7). The rise in bulk medium pH is slow because the total colony volume is low, even in dense blooms, relative to the volume of the bulk medium: OH^- ions diffusing out of the colonies will be strongly diluted. This is corroborated by measurements of steep pH gradients between the outside and the inside of *Phaeocystis* colonies (Ploug *et al*. 1999a).

Theoretically, the carbonate system is completely defined by alkalinity, total inorganic (C_i) concentration and pH (Stumm and Morgan 1996). The diffusion of CO_2 from the atmosphere to the seawater is generally slower than the rate of photosynthesis (Stumm and Morgan 1996). This means that the bulk medium pH will not be influenced significantly by CO_2 diffusion. Alkalinity does not change by the reaction: $HCO_3^- \Rightarrow CO_2 + OH^-$, and the consequent uptake of CO_2 by the cells (chapter 8 and Figure 10.7). Therefore, the rise in seawater pH to a potential maximum of 9.1 can be used to calculate the maximum amount of C_i taken up by *Phaeocystis* cells, and converted into organic *Phaeocystis* carbon.

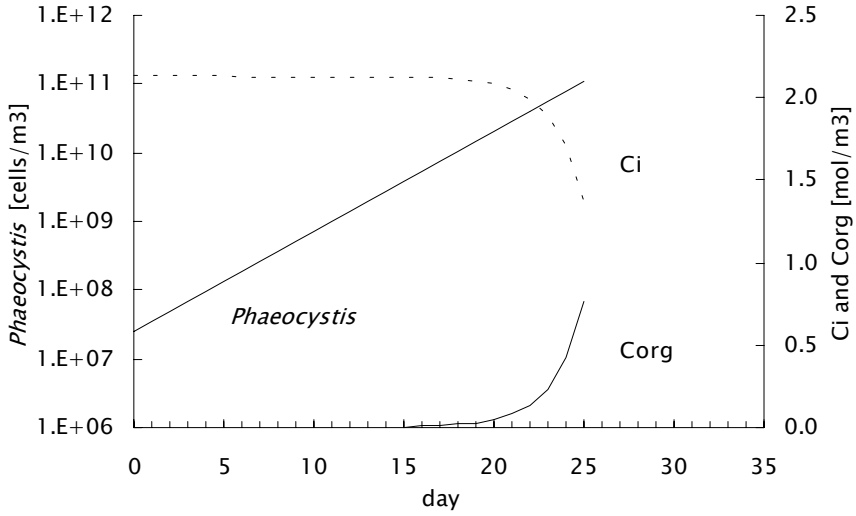

Figure 10.9. Calculated development of a *Phaeocystis* bloom. *Phaeocystis* biomass is expressed as cells m^{-3} and as organic carbon (C_{org}). The decrease of inorganic carbon (C_i) was calculated from the increase in C_{org}. At day 25, pH has reached a value larger than 9.1 and the growth of *Phaeocystis* stops.

Concentrations of C_i and the C_i-species (CO_2, HCO_3^- and CO_3^{2-}) were calculated as a function of alkalinity and pH using the equations in Stumm and Morgan (1996). The necessary dissociation constants (except $pK_{B(OH)3}$ = 8.80) were computed as a function of salinity (30 ‰) and temperature (10°C) (Stumm and Morgan 1996). The initial pCO_2 was set at $10^{-3.45}$ atmosphere, resulting in a calculated total alkalinity of 2.33 eq. m^{-3} at pH 8.1. By raising the pH to 9.1, and keeping total alkalinity constant, the C_i concentration dropped by 0.70 mol m^{-3}. Near pH 9.2, HCO_3^- and CO_3^{2-} reached equimolar concentrations (Figure 10.8).

The 0.7 mol C_i m^{-3} resulting from a 1 unit pH increase is converted to organic *Phaeocystis* carbon (Figure 10.7). The concentration of *Phaeocystis* cells, in colonies with a diameter of 10^{-3} m, calculated from 0.7 mol carbon by using the equations of Rousseau *et al.* (1990), is 1.3 x 10^{11} cells m^{-3}. This calculated concentration is nearly equal to peak numbers observed in the Menai Street in 1970 (Morris 1971), in the Marsdiep over the years 1978 to 1995 (Cadée and Hegeman 1986, Cadée 1990, Cadée 1991b, Cadée and Hegeman 1991b and 1993, Brussaard *et al.* 1996, Cadée 1996), and between 1985 and 1988 in German coastal waters (Lancelot *et al.* 1991). Apparently, the model calculation does not produce an extraordinary result in terms of cell numbers.

The development of inorganic and organic carbon concentrations during a bloom can also be calculated. The mean *Phaeocystis* growth rate in spring is 0.4 divisions day^{-1} (Cadée and Hegeman 1986). The final colony concentration is calculated from 10^{11} cells m^{-3} and 2^{12} cells $colony^{-1}$ (diameter 1.1×10^{-3} m), as 24×10^6 colonies m^{-3}. If the colony concentration is constant during the bloom, the initial *Phaeocystis* cell concentration is known, because the bloom started with 2^0 cells $colony^{-1}$. As shown in Figure 10.9, a concentration of 10^{11} cells m^{-3} is reached on day 25. Any further exponential increase in *Phaeocystis* biomass would be halted by a rapid exhaustion of inorganic carbon. However, in the present model, the biomass increase already stopped when the seawater pH became equal to the intracolonial pH.

By using straightforward carbonate system calculations, simple assumptions on the diffusion of CO_2, and an organic carbon model for *Phaeocystis* from the literature, the prediction of the maximum achievable *Phaeocystis* biomass in the sea was quite similar to actually measured values in the North Sea coastal zone. The calculations and assumptions will be discussed.

First, the calculations were performed for a pH rise in the bulk medium of exactly 1 unit, with no change in alkalinity. Comparable high rises in pH have been measured in the chapter 3 mesocosm experiments (ΔpH = 1.0; Figure 10.4), and in the Marsdiep in 1993, during a *Phaeocystis* bloom (ΔpH = 0.8; Brussaard *et al.* 1996). In batch cultures of *Phaeocystis* the pH may well increase by 0.8 pH units (Table 8.1) and even reach values up to pH 9.2 (Elzenga *et al.* 2000). As Figure 10.8 shows, any change between start and end pH will lead to a change in the achievable C_i difference. Figure 10.8 also makes clear, that the pH in *Phaeocystis* colonies will not reach values much higher than those measured by Lubbers *et al.* (1990). The convergence of HCO_3^- and CO_3^{--} concentrations at pH > 9 means an increase in buffer capacity, which reaches a maximum at pH 9.1 to 9.2, the pK'_2 of the carbonate system in seawater of 31 psu at 10 to 15°C (Stumm and Morgan 1996, L. Peperzak, unpublished data). Because the carbonic anhydrase catalyzed conversion of HCO_3^- is not coupled to an exergonic reaction, the enzyme does not change the equilibrium of the C_i species (Falkowski and Raven 1997). Indeed, at pH 9.16, the intracolonial concentration of CO_2 is close to the CO_2 compensation concentration for Rubisco, giving only a minimal net C-fixation in *Phaeocystis* cells (Elzenga *et al.* 2000). In other words, *Phaeocystis* stops growing when the intracolonial pH is between 9.1 and 9.2.

Changes in alkalinity that would impair the calculations, could arise from HCO_3^- uptake by the cells, the formation of $CaCO_3$ and by calcium binding by the colonial mucus. In chapter 8 it was reported that alkalinity does not change appreciably during photosynthesis, implying that *Phaeocystis* cells do not take up HCO_3^-. Furthermore, the formation of $CaCO_3$ in seawater at high pH is negligible (Johnson *et al.* 1979). Binding of Ca^{2+} by the *Phaeocystis* mucus

polymers (van Boekel 1992b) is, in an alternative way of defining alkalinity (Stumm and Morgan 1996), equivalent to the release of $2H^+$ in the colony. This would decrease both alkalinity and pH and increase the CO_2 concentration. The significance of this process can only be estimated when the Ca^{2+} to mucus ratio is known. However, this is not the case. A preliminary calculation, using an estimate of the number of negative charges in the mucus in a 10^{-3} m diameter colony, showed that Ca^{2+}-binding would provide only 3% of the CO_2 necessary for one division (L. Peperzak, unpublished). Therefore, it is probable that the effect of Ca^{2+}-binding on the colonial carbonate system may be neglected.

Second, it was assumed that diffusion of CO_2 from the atmosphere into the seawater was negligible. With a diffusion film model (Stumm and Morgan 1996) the diffusion rate of CO_2 through the water-air interface layer (z_w = 50 x 10^{-6} m) into the bulk seawater can be calculated with $pCO_2 = 10^{-3.45}$ atmosphere and a diffusion coefficient $D_{CO2} = 2 \times 10^{-9}$ m^{-2} s^{-1}. The CO_2 diffusion coefficient is independent of temperature and wind speed (Peng *et al.* 1987). In the interface layer, where CO_2 is in equilibrium with the atmosphere, the concentration is 16 mmol CO_2 m^{-3} and independent of pH. The bulk medium CO_2 concentration is set at 1 mmol CO_2 m^{-3} (pH 9, Figure 10.8) which yields a flux of 0.05 mol CO_2 m^{-2} day^{-1}. Combined with a water depth of 20 m for the North Sea coast (chapter 2), the flux of CO_2 from the atmosphere leads to an increase of 2.5 mmol C_i m^{-3} day^{-1}. This amount has to be multiplied by a factor 3, because at pH 9 the hydration of CO_2 by OH^- leads to an enhanced CO_2 transfer (Stumm and Morgan 1996). Even so, the daily total CO_2 influx is still only 0.5 % relative to total C_i. Therefore, the flux of CO_2 is indeed negligible compared to the C_i demand at the end of the bloom (Figure 10.9).

The *Phaeocystis* maxima measured in the Marsdiep were approximately 10^{11} cells m^{-3}. However, in 1985, 1989 and in 1993 peaks of 2 x 10^{11} cells m^{-3} were measured (Cadée and Hegeman 1986, Cadée 1991b, Cadée 1996), similar to the *Phaeocystis* concentration reached in well-mixed mesocosms in 1992 (Figure 3.7). In later years, however, when mixing conditions in the mesocosms were reduced, *Phaeocystis* did not reach such high concentrations (V. Escaravage, personal communication). Therefore, it is feasible that due to waves and vigorous turbulent mixing, as during storms, more CO_2 is transferred to the water, leading to potentially higher *Phaeocystis* concentrations.

Third, the Rousseau *et al.* (1990) model used to convert organic carbon into cell concentrations, has a cell-carbon : colonial-carbon ratio that is a function of colony diameter. In other words, large colonies have relatively fewer cell-carbon and more mucus-carbon than small colonies. Therefore, choosing a colony diameter influences the *Phaeocystis* cell concentration that is equivalent to 0.7 mol C_i m^{-3}. These calculated cell concentrations range from 1.8 x 10^{11} (2^{11} cells colony^{-1} ≡ 0.72 x 10^{-3} m), 1.3 x 10^{11} (10^{-3} m) to 1.1 x 10^{11} (2^{12} cells colony^{-1} ≡ 1.14 x 10^{-3} m) cells m^{-3}. A diameter of 10^{-3} m is representative for the North Sea

(Rousseau *et al*. 1990), and has been used for the conversion calculation. The examples also show that blooms composed of small *Phaeocystis* colonies contain more cells than blooms of large colonies. However, the calculated range of maximum cell concentrations, 1.1 to 1.8 x 10^{11} cells m^{-3}, is still within the range observed in the sea: 1 to 2 x 10^{11} cells m^{-3} (see references on page 185).

Alternatively, the van Rijssel *et al*. (1997) field model with a constant cell : carbon ratio (1 cell = 4.75 x 10^{-12} mol total, = cellular and colonial carbon) could have been used. Applying this constant ratio on the calculated cell number, 1.3 x 10^{11} cells m^{-3} in 10^{-3} m colonies (obtained from 0.7 mol C_i m^{-3}), leads to a total organic carbon biomass of 0.6 mol C m^{-3}. In other words, here the Rousseau *et al*. (1990) and the van Rijssel *et al*. (1997) conversion models give comparable organic carbon estimates.

The Rousseau model makes use of a constant cell content : colony diameter relation (Rousseau *et al*. 1990), which may not be applicable when *Phaeocystis* colonies grow under C_i stress (chapter 8). However, for the relatively large colonies of 10^{-3} m diameter used here in the calculation of maximum *Phaeocystis* biomass, C_i stress would only lead to a decline in organic carbon in the order of 10 to 20% relative to unstressed colonies (chapter 8).

A simple check of the carbon calculations in the sea would be the direct comparison of Marsdiep *Phaeocystis* concentrations, measured from 1973 onwards (Cadée and Hegeman 1986), with measurements of (in)-organic carbon concentrations. Unfortunately there are insufficient data. Although the Dutch Rijkswaterstaat-database contains pH measurements and chlorophyll *a* concentrations in the Marsdiep for 1978 to 1998, the first particulate and dissolved organic carbon (POC and DOC) data are from 1988. Only two rough estimates of the correlation between pH and organic carbon production can be made.

For the first estimation POC and DOC were combined to total organic carbon (TOC), and the linear regression between the spring rise in pH (δpH) and TOC concentration (mol m^{-3}) in April-May 1988 - 1998, was calculated. For 6 years with both TOC and pH data available this yielded: TOC = 0.84 x δpH - 0.12 (r^2 = 0.26, P = 0.30). In other words: a pH rise of 1.0 unit equals 0.72 mol TOC m^{-3}.

In the second estimation, of the TOC concentrations before 1988, the chlorophyll *a* (Chl*a*) data and an appropriate C:Chl*a* ratio were used. The frequency of Chl*a* measurements in 1978 to 1985 was once per month. Not surprisingly, the doubling of the measurement frequency in some years during the 1990s lead to 40% higher spring chlorophyll a concentrations (L. Peperzak, unpublished; see also chapter 4). Therefore, the chlorophyll data need to be corrected by at least a factor 1.4. The Marsdiep April-May C:Chl*a* ratio was 118 ± 49 (1988 to 1998, L. Peperzak unpublished data), and falls in the *P. globosa* ranges 54 to 113 (Table 8.4), 106 ± 49 (chapter 4, unpublished data) and 50 to 250 (Belgian coastal water, Lancelot *et al*. 1991). Using C:Chl*a* = 118,

and a 1.4 frequency correction, the calculated spring TOC maxima in 1978 to 1985 ranged from 0.4 to 0.7 mol m^{-3}. Therefore, it can be concluded that both methods of calculating organic carbon concentrations in the Marsdiep apparently lead to estimates near or at the value of 0.7 mol m^{-3}, which was derived theoretically from a one unit pH rise.

In conclusion, the calculations and assumptions made to estimate the maximum achievable *Phaeocystis* biomass in the sea: (i) the rise in pH by one unit, (ii) the neglect of CO_2 diffusion from the atmosphere, and (iii) the *Phaeocystis*-carbon model, seem to be valid.

Finally, the estimated maximum *Phaeocystis* biomass can be used to evaluate the effect of other potentially limiting nutrients. In the coastal North Sea, the first limiting mineral nutrient in spring is phosphorus (P) (Peeters and Peperzak 1990). Assuming a standard Redfield ratio of C:P = 106, P is in unlimited supply, relative to 0.70 mol C_i m^{-3}, at concentrations over 6.6 mmol P m^{-3}. However, under P-deficient conditions the *Phaeocystis* C:P ratio varies from 128 to 568 (Jahnke 1989). Therefore, P-stress in the coastal North Sea begins at concentrations less than 6.6 mmol P m^{-3}. Taking C:P is 128 to 568, P becomes limiting at 1.2 to 5.5 mmol P m^{-3}. This means that, under the assumption of a constant cell number : colony diameter ratio and C:P is 568, the maximum of 10^{11} *Phaeocystis* cells m^{-3} can still be attained with 1.2 mmol P m^{-3}. Maximum P concentrations during the 1970s and 1980s in the Dutch coastal zone were 3 to 5 mmol m^{-3} (de Vries *et al.* 1998) which means that both C_i and P may have been nutrients limiting the *Phaeocystis* spring bloom.

In other words, the carbonate system determines maximum biomass at P concentrations > 1.2 mmol P m^{-3}. In turn, the P surplus determines the length of the *Phaeocystis* bloom. This mechanism provides an explanation for the relation between increased eutrophication in the Dutch coastal zone and the increased length of the *Phaeocystis* spring bloom in the Marsdiep during the 1970s and 1980s (Cadée and Hegeman 1986).

Foam observations on the coast of Holland are correlated with blooms of *Phaeocystis globosa* (Prymnesiophyceae) in the North Sea

L. Peperzak, M. Rademaker and L.P.M.J. Wetsteyn, submitted to the *Journal of Plankton Research*.

ABSTRACT. A conspicuous feature of *Phaeocystis globosa* blooms is the production of large amounts of foam. However, so far quantitative relations between *Phaeocystis* concentrations and the observations of foam have never been established. Here the 1993 to 1997 data from two independent monitoring programs are reported. In the first program *P. globosa* cell numbers in the North Sea were counted; in the second foam was monitored on the beaches of Holland. Taking into account a two-week delay between

Phaeocystis blooms and foam observations a significant ($r = 0.85$, $P < 0.001$) correlation between the two was found. Above 1 million *Phaeocystis* cells per litre the chance of observing foam in the following two-week period was 90%, with a mean frequency of 0.5 (i.e. at 5 out of 10 shore locations). This is the first report that quantifies the link between *Phaeocystis* blooms and foam production.

The increase in eutrophication in the North Sea has lead to an increase in blooms of *Phaeocystis globosa* (*e.g.* Cadée and Hegeman 1986). It is widely accepted that this in turn led to a concomitant increase in the amount of foam on nearby coasts (Bätje and Michaelis 1986, Lancelot *et al.* 1987). However,a quantitative relation between the abundance of *Phaeocystis* in the sea and the amount of foam observed on shores has never been established. In fact, it is uncertain which component of the *Phaeocystis* bloom is the precursor of the foam. Eberlein *et al.* (1985) related foam production in the German Bight to *Phaeocystis*-derived dissolved organic matter. Lancelot and Rousseau (1994) assumed that the foam is derived from remnants of the *Phaeocystis* colony with a low biodegradability. Peperzak *et al.* (1998) hypothesized that when such colony remnants settle towards the seabed in the Dutch coastal zone, a shoreward bottom current would transport them to the coast, where they could be beaten to foam in the turbulent surf zone. Surface foam may be derived from buoyant 'jelly', after gas bubbles have been produced by the decaying cells (Boalch 1984). An increase in wind-induced turbulence is generally considered conducive to the formation of coastal and seasurface foam.

Sometimes even non-degraded colonies are washed ashore as jelly (GrØntved 1960, Al-Hasan *et al.* 1990). Both jelly and foam are considered a nuisance to humans (Grøntved 1960, Lancelot *et al.* 1987). On coastal meiofauna the effect of the foam varies, depending on the species, from beneficial to lethal (Armonies 1989).

In order to investigate the relation between *Phaeocystis* and foam, results of two independent monitoring programs of the Dutch Rijkswaterstaat were compared. These programs were run simultaneously from 1993 to 1997. In the first program phytoplankton cell numbers were counted in Lugol-preserved North Sea surface samples, and the *Phaeocystis* data from three stations near the coast of Holland: Noordwijk 2, Noordwijk 10 and Marsdiep (Figure 4.1) were used. These stations were sampled two to four times per month from April to September, and once per month in the rest of the year. The second monitoring program was established to evaluate beach and swimming water quality. It extended from the southern tip of Holland nearby the Rhine outflow into the North Sea, to the northern tip near the Marsdiep (Figure 4.1). At ten locations on the shore, sampled at two week intervals from week 15 (April) to week 43 (October), foam was monitored as: 0 (not present), 1/2 (some foam) and 1 (foam present). Weeks 17 and 18 were never monitored.

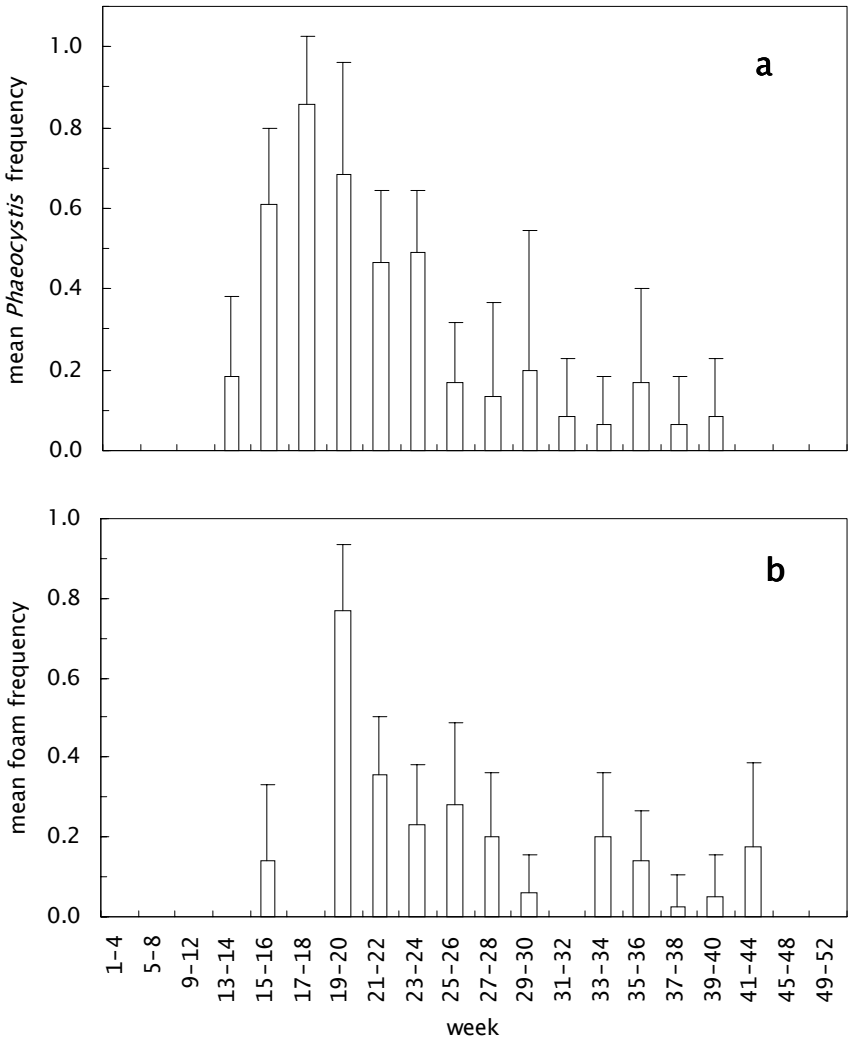

Figure 10.10. Frequencies of *Phaeocystis* blooms in the North Sea coastal zone of Holland (**a**), and of foam on the coast of Holland (**b**) in the period 1993-1997. Foam was not monitored prior to week 13, in weeks 17-18, and after week 44. Bars are standard deviations from the mean.

First, the temporal relation between *Phaeocystis* blooms, and the occurrence of foam was investigated by comparing bloom and foam frequencies. Blooms of *Phaeocystis* were defined as cell concentrations over 1 million per litre (Cadée

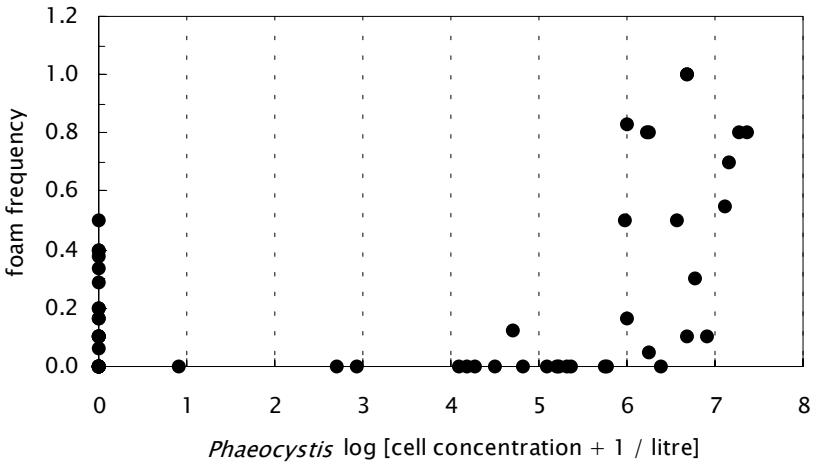

Figure 10.11. Foam frequency and *Phaeocystis* concentration in 1993-1997. A two-week delay between *Phaeocystis* concentration and foam was taken into account.

and Hegeman, 1986); during such blooms up to 85% of the cells are present in colonies (Weisse and Scheffel-Möser 1990a). For each of the three North Sea stations, the occurrence of blooms in two-week intervals was summed, and divided by the total number of samples taken. Then, the two-weekly mean *Phaeocystis* bloom frequency and its standard deviation were calculated from the three stations.

For each of the ten beach locations, the two-weekly foam data of 1993 to 1997 were summed, and divided by the total number of observations made. The two-weekly mean foam frequency and its standard deviation were calculated from the ten locations.

The occurrence of *Phaeocystis* blooms and foam vary over the year (Figure 10.10a and b). The first blooms took place in weeks 13-14, followed two weeks later by the first foam. The spring bloom attains its top in weeks 17-18 (Figure 10a), while the maximum foam frequency was observed in weeks 19-20 (Figure 10b). *Phaeocystis* blooms were not restricted to spring. In July and August (weeks 27-36) blooms took place, but with a lower frequency (Figure 10.10a), and they stopped in the weeks 39-40. This *Phaeocystis* bloom sequence is in accordance with previous reports for the Marsdiep by Cadée and Hegeman (1986).

The Pearson correlation between the frequencies of *Phaeocystis* blooms and foam was significant: $r = 0.67$ ($P < 0.05$). This correlation even improved when a two-week delay between bloom and foam frequencies was taken into account: $r = 0.85$ ($P < 0.001$).

Second, the mean foam frequency was plotted as function of the *Phaeocystis* cell concentration at the best sampled station, Noordwijk 10, in the preceding two-week interval (Figure 10.11). It appears that the bloom criterion of 1 million cells per litre (Cadée and Hegeman 1986) was aptly chosen. Above 1 million cells per litre, the chance of observing foam in the following two-week period was 90%, with a mean frequency of 0.5 (i.e. at 5 out of 10 beach locations). Above 10 million cells per litre foam was always observed, with a mean frequency of 0.7.

Foam was also observed without a preceding *Phaeocystis* bloom (Figure 10.11, vertical axis) which may be due to *Phaeocystis* blooms in the Dutch coastal zone that were not observed at Noordwijk 10. Alternatively, phytoplankton species other than *Phaeocystis* give rise to foam production. In the summer of 1995 for instance, a period of continuous foam observations in the absence of *Phaeocystis* blooms coincided with a high abundance of diatoms and flagellates. However, it was not possible to identify any particular phytoplankton species in relation to this summer foam.

On the whole it can be concluded that the observed synchrony of *Phaeocystis* blooms with the foam observations on nearby shores, both temporal and quantitative, makes it plausible that *Phaeocystis* is indeed the origin of that foam. This is the first time that this relation is quantified.

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Concluding remarks

In this chapter autecological equations were derived that relate the growth rate of non-flagellate *P. globosa* to salinity, temperature and daily irradiance. The growth rates were obtained from various batch culture studies, performed under steady-state conditions. However, the effects of fluctuating, non steady-state conditions as they appear in the sea, on growth and mortality rates of *Phaeocystis* are not known. Nor have the interactions between the autecological variables been measured. Furthermore, *Phaeocystis* flagellates have not been examined and, in general, *Phaeocystis* species other than *P. globosa* have hardly been investigated. The equations presented here, should therefore be considered a first step in a more comprehensive study of the genus *Phaeocystis*.

The role of the nutrients nitrogen, phosphorus, vitamin B1 and inorganic carbon in *Phaeocystis* growth has been described in the previous chapters. Here in chapter 10, the hypotheses was discussed that the inorganic carbon acquisition of *Phaeocystis* colonies is related to the maximum bloom biomass, and that the supply of phosphate determines bloom duration. This hypothesis has not been tested yet. To do so, the use of more sophisticated mathematical models that incorporate the intricate relations between the concentrations and fluxes of phosphate, carbon and *Phaeocystis* biomass, is necessary.

Furthermore, diatoms are outcompeted by, a *Phaeocystis*-induced, increased pH and decreased CO_2 availability in the bulk sea water. Laboratory or mesocosm experiments are needed to test this hypothesis. However, the ultimate complexity of the interacting effects of salinity, temperature, interference (CO_2), and resource (nutrient and light) competition and several loss factors that determine the structure of the phytoplankton community, will only be found under natural conditions: in the sea. Field measurements, therefore, remain essential in the study of *Phaeocystis* blooms.