

## Inorganic nitrogen assimilation by picoplankton and whole plankton in a coastal ecosystem

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### Abstract

$\text{NH}_4^+$  and  $\text{NO}_3^-$  seasonal uptake by the phytoplankton and  $<2\text{-}\mu\text{m}$  communities were investigated in the Menai Strait using  $^{15}\text{N}$  tracer techniques. Based on  $\text{O}_2$ -determined primary production and DIN metabolism measurements, five phases were defined: (1) prebloom (before April), (2) primary net autotrophic (April and May), (3) net heterotrophic (June), (4) secondary net autotrophic (July and August), and (5) regenerative (remainder of calendar year). The inorganic N nutrition of phytoplankton and  $<2\ \mu\text{m}$  organisms was  $\text{NO}_3^-$ -based in spring and  $\text{NH}_4^+$ -based in summer and autumn, reflecting varying ambient concentrations of these nutrients superimposed on a general preference for  $\text{NH}_4^+$  by both communities. The percent contribution of picoplankton to the total  $\text{NH}_4^+$  uptake was higher than to nitrate (seasonal averages of  $50 \pm 14\%$  and  $32 \pm 24\%$ , respectively). The contribution of the  $<2\text{-}\mu\text{m}$  size fraction was lowest during both net autotrophic phases (4–13%) and highest during the net heterotrophic, prebloom and regenerative phase (70–84%). A strong correlation between percent  $\text{NH}_4^+$  and  $\text{NO}_3^-$  assimilation by the  $<2\text{-}\mu\text{m}$  size fraction and bacteria abundance between May and July suggests significant bacterial uptake at this time. Bacterial DIN utilization was estimated by correcting uptake rates by the  $<2\text{-}\mu\text{m}$  fraction, for picophytoplankton presence. During the primary net autotrophic phase, inferred bacterial uptake constituted 13 and 34% of total  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake, respectively. In the net heterotrophic phase, it amounted to ca. 70% of the total DIN uptake, with no differences between nutrients. Competition between bacteria and phytoplankton for depleted DIN could have contributed to the phytoplankton bloom termination.

Most of the published work on the nitrogen dynamics of plankton microbial communities concentrates on short-term measurements taken over intervals of a few days to weeks. These investigations are predominantly restricted to the periods of the season of particular ecological relevance (e.g., the spring/summer, autumn, or both phytoplankton blooms). Such short term measurements are frequently directed at very specific questions (e.g., Selmer et al. 1993; Dickson and Wheeler 1995; Slawyk et al. 1997). Cost and logistical problems have almost certainly been a major impediment to this sort of work, leaving the sequential events that take place over the whole seasonal cycle (e.g., Glibert et al. 1982; Kristiansen et al. 1994; Maguer et al. 1996) and that characterize the microbial ecology of the whole system poorly documented.

If long-term studies of the phytoplankton nitrogenous nutrition are few and far between, by comparison, those regarding the seasonal variation of the uptake of inorganic N by the picoplankton or heterotrophic bacteria are scarce in the extreme. The paper by Hoch and Kirchman (1995) is the only seasonal study of this type that we are aware of. This shortage probably arises because, until the middle 1980s, the microheterotrophs were not considered to play a significant role in inorganic nitrogen assimilation in the oceans (*see*

*review by Kirchman 2000*). The acknowledgement of significant rates of bacterial uptake of inorganic N has led to the question of the quantitative participation of heterotrophs in this facet of the nitrogen metabolism, its environmental regulation, and ecological repercussions. Investigations of this aspect of the bacterial ecology have been of short duration and often bedeviled by the difficulty in isolating the activity of the microheterotrophs from the remainder of the community (Harrison and Wood 1988; Selmer et al. 1993; Kirchman and Wheeler 1998).

Sieburth et al. (1978) introduced the concept of picoplankton, defined as organisms with diameters between 0.2 and 2  $\mu\text{m}$ , comprising mainly unattached unicellular bacteria. In the present study, we followed the Sieburth et al. (1978) distinction and chose to physically separate and study the picoplanktonic fraction. A problem in appending the description “bacterial” to the picoplankton fraction is our growing awareness of small autotrophs that can pass through the 2- $\mu\text{m}$  membrane during fractionation procedures. As will be shown, the presence of phytoplankton in the small fraction precludes the straightforward quantification of the heterotrophic processes; nonetheless, valuable insights can be gained.

The primary production cycle in the Menai Strait, its timing and environmental controls, and its relation to the microheterotrophic processes (bacterial respiration and production) have been the subject of previous extensive investigations (Blight et al. 1995) and will not be addressed here in detail. The main issue we want to consider is the proportions of ammonia and nitrate taken up by the picoplankton (including the extent of bacterial inorganic N metabolism) in relation to those by the whole phytoplankton assemblage and, in particular, the seasonal changes in the relative importance of these nutrients for the two communities.

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## Methods

Seawater was taken from the Menai Strait, collected from St. George Pier, Menai Bridge, North Wales, UK (*see* Blight et al. 1995 for map and hydrography of location). Sampling was undertaken at high tide fortnightly between February and August and monthly from September until November between 0700 and 1000 h. A hand bilge pump (5 dm<sup>3</sup> min<sup>-1</sup>) was used to collect the water from approximately 1 m deep into three 25-dm<sup>3</sup> acid-washed (10% HCl), opalescent, polypropylene containers. Following collection, samples were processed immediately. Sample size fractionations were performed by means of a custom-made, gravity-driven, reverse-flow filtration system using large diameter (142 mm), 2- $\mu$ m pore size polycarbonate membrane filters (Poretics). The filtration of 10 dm<sup>3</sup> took  $\leq$ 1.5 h.

Ammonia (NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup>), nitrate, and nitrite concentrations were determined manually as described by Parsons et al. (1984). The lower limits of detection (estimated according to Miller and Miller 1984) for nitrite, nitrate, and ammonia were 0.01, 0.025, and 0.030  $\mu$ M, respectively. Chlorophyll concentrations were determined on aliquots of the whole (100 to 250 cm<sup>3</sup>) and fractionated (500 to 1,000 cm<sup>3</sup>) samples by filtering onto 47-mm-diameter Whatman GF/F filters. Phytopigments were extracted overnight in 8 cm<sup>3</sup> of neutralized 90% acetone. Chlorophyll concentrations were determined using a Turner 10 Designs fluorometer according to Tett (1987).

The total number of bacteria was determined using DAPI (4',6-diamino-2-phenylindole; final concentration of 1.25  $\mu$ g cm<sup>-3</sup>; Sigma) and epifluorescence UV microscopy (Leitz Ortoplan Microscope;  $\times$ 1,250 magnification) according to the method of Porter and Feig (1980).

Gross and net community production and respiration were determined from *in vitro* changes in the concentration of oxygen, determined according to classical procedures. Incubations of fractionated (<2  $\mu$ m) and unfractionated samples were made in 50 cm<sup>3</sup> borosilicate bottles (three to five replicates). The incubations took place outdoors in water-cooled incubators. One incubator was kept in the dark (dark incubations) and the other was subjected to the natural diurnal light and dark cycle (light/dark incubations) for 24 h. The incubation temperature was kept as close as possible to the *in situ* temperature ( $\leq$ 3°C). The irradiance was attenuated to approximately 60% of the total incident light by a neutral density mesh so that it was close to the *in situ* irradiance. The concentration of dissolved oxygen in the bottles was determined by Winkler titrations using a PC-based system with a photometric endpoint detector based on the original design described by Williams and Jenkins (1982).

The uptake of <sup>15</sup>N-labeled ammonium [(<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; 99.3 atom %; Europa Scientific] and nitrate (Na<sup>15</sup>NO<sub>3</sub>; 99.4 atom %; Europa Scientific) by the whole community and <2- $\mu$ m organisms was estimated according to the classical method of Dugdale and Goering (1967). The isotopic additions were generally  $\leq$ 10% of the ambient concentration of nitrate and ammonia. Incubations over the natural light/dark cycle were carried out for 24 h in the same conditions as those described for the oxygen flux determinations. Following incubation,

samples were size fractionated, and the particulate matter in the whole and <2- $\mu$ m samples was collected onto Whatman GF/F glass fiber filters for further elemental and isotopic analysis (Europa Scientific elemental analyzer "Roboprep" coupled with a mass spectrometer "Tracermass"). Reasons for performing the fractionation after rather than before incubation were twofold: (1) It minimized handling stress, and (2) because the comparison of nitrogen uptake by phytoplankton and bacteria was of interest in this experiment, by allowing these two communities to coexist during the incubation, a more realistic scenario of their interactions *in situ* was expected. The same considerations apply to the oxygen flux measurements, but there is no alternative to fractionation of samples prior to incubation. Although we experience the occasional anomaly with size-fractionated measurements with this approach, our impression (Blight et al. 1995) is that they are not pervasive and they provide us with a reliable general picture. It is without a doubt that any sample manipulation or treatment can give rise to the possibility of errors. Although there are reports claiming damage to the cells during sampling fractionation, an examination of the physics associated with shear (the major form of stress in the procedure we were using) makes this unlikely in the present circumstances. For example, the organism *Mesodinium rubrum*, an ultradelicate flagellate, swims at speeds that can approach 8,500  $\mu$ m s<sup>-1</sup> (Jonsson and Tiselius 1990) without damage, only to be ripped apart by the surface tension forces when it makes contact with the meniscus. The linear flow of sample water through the pores of the membrane was on average 300  $\mu$ m s<sup>-1</sup> in our fractionations (i.e., significantly lower). Therefore, damage from shear forces seems unlikely to us.

The experimental procedure for the determination of <sup>13</sup>C-uptake rates by the whole and picoplankton communities was identical to that applied to the <sup>15</sup>N-technique, with seawater samples labeled with both isotopes and incubated in light/dark conditions for 24 h. A 5% addition of labeled material was used (i.e., 100  $\mu$ mol <sup>13</sup>C L<sup>-1</sup> sample (as NaH<sup>13</sup>CO<sub>3</sub>; 99.0 atom %; Europa Scientific), assuming an ambient TCO<sub>2</sub> concentration of 2,000  $\mu$ mol dm<sup>-3</sup>).

Nitrate and ammonium absolute uptake rates by the whole and picoplankton communities were calculated following equations discussed by Collos (1987), with no corrections made for the recycling of nitrogen. Although N recycling (particularly that of NH<sub>4</sub><sup>+</sup>) is likely during long incubations and constitute a possible source of error, the reasonable C:N (ammonia) uptake ratios obtained (median of 17), indicate that it was not extensive in the present circumstances. Exceptions are days 143 and 219 when C:N uptake ratios between 220 and 260 suggest that N recycling might have been significant, and these data points should be treated with due care. Furthermore, on five occasions (days 158, 173, 188, 219, and 233), nitrate uptake rates were estimated assuming the ambient nitrate concentration (0.025  $\mu$ M—the lower limit of detection of the method used to determine ambient NO<sub>3</sub><sup>-</sup>) because the actual levels were below detection limits. Therefore, the calculated rates could constitute overestimations of the true values, and the results obtained for these dates should be regarded with due uncertainty. The same applies to the calculation of ammonium uptake rates on days

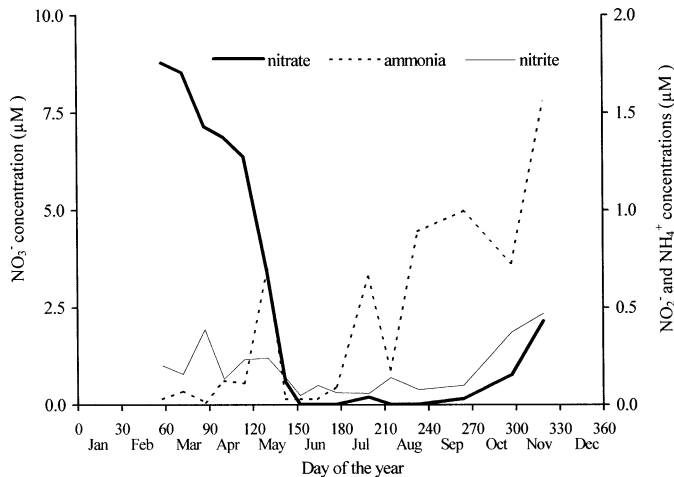


Fig. 1. Seasonal variation of dissolved inorganic nitrogen in the Menai Strait during 1995. The average standard errors for ammonia, nitrite, and nitrate measurements were  $\pm 0.005$ ,  $\pm 0.006$ , and  $\pm 0.03$   $\mu\text{M}$ , respectively.

59, 143, 158, and 173 when ambient ammonia was below detection concentrations (ambient  $\text{NH}_4^+$  assumed as  $0.030$   $\mu\text{M}$ , the lower limit of detection). Uptake rates of  $^{13}\text{C}$  were calculated according to the formulae in Hama et al. (1993).

## Results

**Nitrogenous nutrients**—The seasonal variation of dissolved inorganic nitrogen in the Menai Strait during 1995 is shown in Fig. 1. The main feature of the nitrate cycle was the sharp decrease in concentration observed in May, which recovered after October. The highest concentrations ( $\leq 8.8$   $\mu\text{M}$ ) were registered early in the year, prior to the spring phytoplankton bloom. Nitrite concentrations were low throughout the season ( $\leq 0.5$   $\mu\text{M}$ ), although there was a tendency to increased levels toward autumn. The annual pattern of the variation of ammonia concentration was variable, featuring short intervals of ammonia accumulation in the water (e.g., day 131;  $0.7$   $\mu\text{M}$ ) followed by periods of near exhaustion. Despite increased concentrations toward the autumn, winter concentrations tended to be low ( $\leq 0.1$   $\mu\text{M}$ ).

**Chlorophyll**—Chlorophyll concentrations (Fig. 2) peaked in May ( $8.3$   $\mu\text{g dm}^{-3}$ ), associated with the vernal blooming of phytoplankton. There was also a later peak in the autumn, but of much lower magnitude. During summer (July–September), the standing stock was low ( $\leq 1$   $\mu\text{g dm}^{-3}$ ). The observed chlorophyll seasonal pattern was comparable to that described by Blight et al. (1995), with the early phase of the phytoplankton bloom (Fig. 2A) predominantly diatomaceous, followed by a mixed population of diatoms and flagellates (Fig. 2B), the latter becoming more predominant as the former declines. The largest proportion of the flagellate population belonged to the genus *Phaeocystis*, corresponding to the highest seasonal chlorophyll concentration. The low algae standing crop present during summer and the small bloom in the autumn (Fig. 2C) was constituted mainly by diatoms. The chlorophyll content of the  $<2$ - $\mu\text{m}$  size frac-

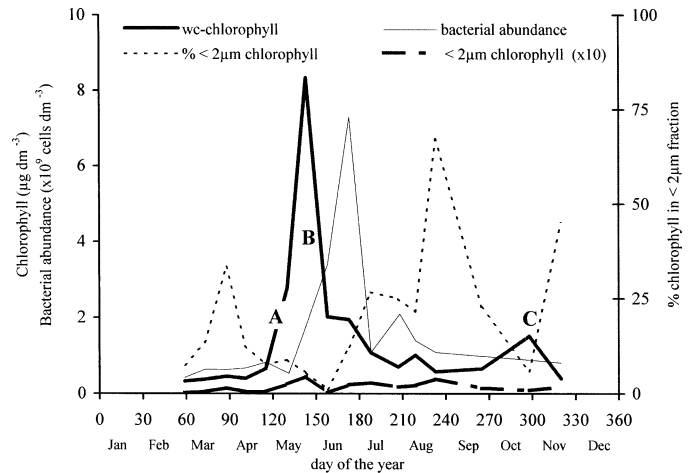


Fig. 2. Seasonal variation of bacterial abundance, whole-community (wc) chlorophyll concentration,  $<2$ - $\mu\text{m}$  absolute concentration ( $\times 10$ ) and  $<2$ - $\mu\text{m}$  percent chlorophyll (in relation to the whole community) in the Menai Strait during 1995. (A) Diatom preponderance; (B) *Phaeocystis*-dominated bloom; (C) diatom-dominated autumn bloom.

tion varied throughout the year, with the highest level registered in May ( $0.4$   $\mu\text{g dm}^{-3}$ ) and the lowest early in the year. The chlorophyll peak in the  $<2$ - $\mu\text{m}$  size fraction during spring coincided with that in the whole community, although the seasonal patterns of variation of these two size fractions were generally dissimilar (Fig. 2). The percent contribution of the picoplankton to the whole phytoplankton community was highly variable (Fig. 2). The lowest percentages of chlorophyll in the picoplanktonic fraction were registered during the spring algal bloom, varying between 1% (day 158) and 13% (day 101). This could be an artefact of the fractionation procedure, because clogging of the fractionation membranes by the high concentration of phytoplankton in the water at this time of the year, in particular by *Phaeocystis*, could have prevented small phytoplankton cells from passing through the membrane. The absence of such conditions in the summer and autumn allowed more chlorophyll-containing organisms to pass the membrane, with the proportion of picoplanktonic chlorophyll reaching 67% and 45% of the total in two sampling occasions (days 233 and 320, respectively).

**Bacterial abundance**—The seasonal variation of bacterial abundance (Fig. 2) exhibited a maximum in June ( $7 \times 10^9$  cells  $\text{dm}^{-3}$ ), followed shortly by a lesser peak a month later. The number of bacteria then slowly declined toward the autumn, reaching minimum values in winter.

**Biological oxygen fluxes**—The cycle of production in the Menai Strait has been described and analyzed in detail by Blight et al. (1995); therefore, only a brief description will be given here. The main features of the gross and net oxygen production seasonal cycles were the well-defined peaks during spring and summer (Fig. 3), with the highest rates ( $48$  and  $38$   $\mu\text{mol O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ , respectively) coinciding with the season's highest concentration of chlorophyll. Oxygen production then declined sharply, leading to low gross com-

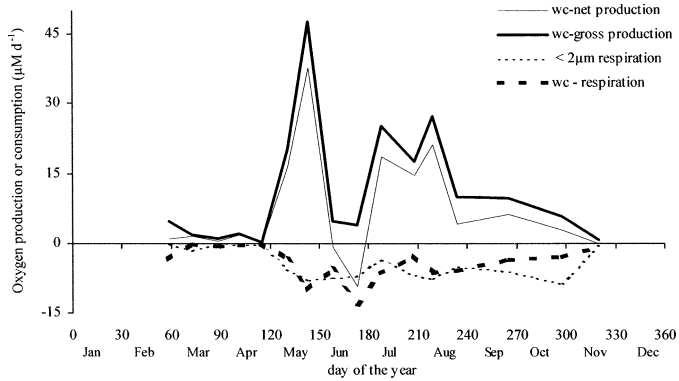


Fig. 3. Seasonal variation of gross and net whole-community (wc) oxygen production and of whole-community and  $<2\text{-}\mu\text{m}$  oxygen respiration. The average standard errors of these measurements were  $\pm 0.88$ ,  $\pm 0.77$ ,  $\pm 0.55$ , and  $\pm 0.61 \mu\text{M d}^{-1}$ , respectively.

community production and to negative net community production by the end of June (day 173). The summer period was characterized by two peaks on days 188 and 219 of smaller magnitude than the spring maxima. There were also peaks of production in September and October, but again of much lower significance than those observed before, with rates declining continuously during fall. Respiration (Fig. 3) was highest in May and June ( $10$  and  $13 \mu\text{mol O}_2 \text{ dm}^{-3} \text{ d}^{-1}$ , respectively). A smaller respiration peak was observed in August, after which rates decreased toward the autumn. The May respiration peak coincided with the maximum seasonal net oxygen production, whereas the June rise was concurrent with negative net production rates. During July and August, the respiration profile did not follow closely that of the net production, but high rates of respiration coincided with the peaks of net community production. The seasonal profile of picoplanktonic respiration (Fig. 3) did not simply track that of the whole community: rates were generally less variable throughout the summer.

**Uptake of inorganic nitrogen— $^{15}\text{N}$ -nitrate uptake:** The seasonal variation of  $\text{NO}_3^-$  uptake by the whole community and  $<2\text{-}\mu\text{m}$  size fraction is shown in Fig. 4. Early in the year (winter and early spring), the profiles for these two communities are somewhat different from each other, but from April, the seasonal variation of  $\text{NO}_3^-$  uptake by the  $<2\text{-}\mu\text{m}$  size fraction followed a pattern similar to that of the whole community. The main characteristic of those seasonal profiles is the peak in May. This uptake maximum was associated with the spring phytoplankton bloom, coincided with the highest seasonal net oxygen production rates and chlorophyll concentrations, and was accompanied by a rapid decline of ambient  $\text{NO}_3^-$ . The lowest uptake of  $\text{NO}_3^-$  by both communities was during summer, mirroring depleted  $\text{NO}_3^-$  conditions.

**$^{15}\text{N}$ -ammonium uptake:** Contrary to the seasonal profiles of  $\text{NO}_3^-$  uptake by the whole community and picoplankton, those for  $\text{NH}_4^+$  uptake were characterized by three distinct peaks in May, July, and August, with rates reaching up to  $0.62 \mu\text{M d}^{-1}$  (Fig. 5). These uptake maxima coincided with

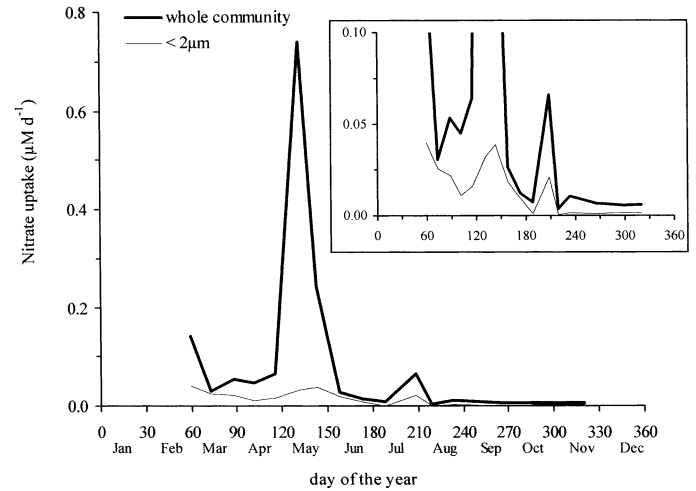


Fig. 4. Seasonal variation of nitrate uptake by the whole community and the  $<2\text{-}\mu\text{m}$  size fraction in light/dark incubations over a 24-h period. Insert shows a detail of the lower part of the graph. The average standard errors for the whole-community and  $<2\text{-}\mu\text{m}$  nitrate uptake measurements were  $\pm 0.003$  and  $\pm 0.0003 \mu\text{M d}^{-1}$ , respectively.

periods of increased concentration of ambient  $\text{NH}_4^+$  and high primary production. The May peak was registered a fortnight earlier than the net community oxygen production and chlorophyll maxima. From August, the increasing ambient availability of ammonia appeared to be sufficient to sustain the last and protracted uptake period, well into the autumn. The summer uptake maxima occurred at a time of low phytoplankton biomass (July and August) but the later peak appears to have sustained the minor autumn chlorophyll bloom. The spring and summer  $\text{NH}_4^+$  uptake peaks were separated by a period of very low uptake rates, characterized by decreased chlorophyll concentrations and depleted levels of ambient  $\text{NH}_4^+$ . For both communities, the lowest uptake rates

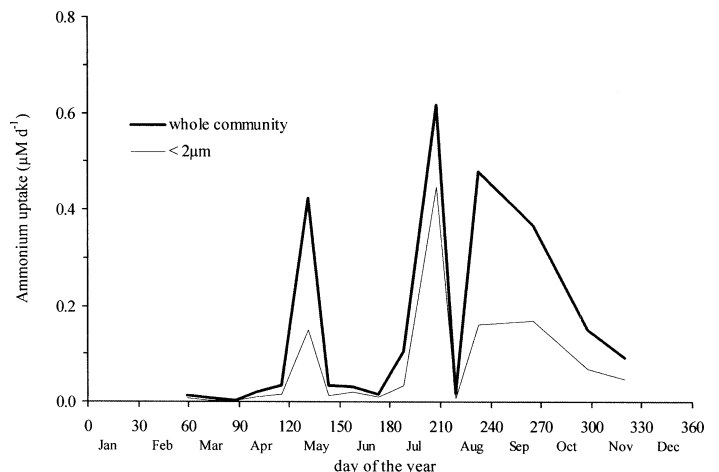


Fig. 5. Seasonal variation of ammonium uptake by the whole community and the  $<2\text{-}\mu\text{m}$  size fraction in light/dark incubations over a 24-h period. The average standard errors for the whole-community and  $<2\text{-}\mu\text{m}$  ammonium uptake measurements were  $\pm 0.004$  and  $\pm 0.001 \mu\text{M d}^{-1}$ , respectively.

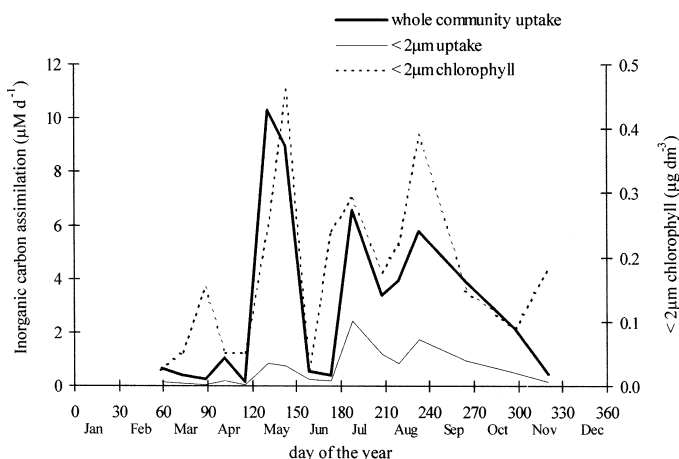


Fig. 6. Seasonal variation of  $^{13}\text{C}$  uptake by the whole community and the  $<2\text{-}\mu\text{m}$  size fraction. Comparison between the  $<2\text{-}\mu\text{m}$  chlorophyll concentrations and  $^{13}\text{C}$  uptake by this size fraction. The average standard errors for the whole-community and  $<2\text{-}\mu\text{m}$   $^{13}\text{C}$  uptake measurements were  $\pm 0.2$  and  $\pm 0.04 \mu\text{M d}^{-1}$ , respectively.

( $\leq 0.01 \mu\text{M d}^{-1}$ ) were generally measured early in February and March when ambient  $\text{NH}_4^+$  was low. Also, from early June onward, rates of  $\text{NH}_4^+$  uptake by the  $<2\text{-}\mu\text{m}$  size fraction exceeded those of nitrate uptake, whereas in the spring, nitrate uptake exceeded that of  $\text{NH}_4^+$ .

**$\text{H}^{13}\text{CO}_3^-$  uptake**—The uptake of  $\text{H}^{13}\text{CO}_3^-$  by the whole community shows a seasonal pattern characterized by a maximum in May ( $10 \mu\text{M d}^{-1}$ ) and two smaller peaks in the beginning of July and end of August (Fig. 6). The May maximum coincided with the peak of nitrate uptake, confirming the importance of this nutrient to the early stages of the primary production cycle in these waters. The timing of the summer uptake maxima agreed well with that of the phytoplankton net growth, and fluctuations in the  $\text{H}^{13}\text{CO}_3^-$  uptake exhibited a profile identical to that of ammonium uptake. This suggests a close relationship between the uptake of regenerated nitrogen and production at this time of the year.

The seasonal fluctuation of the picoplankton  $\text{H}^{13}\text{CO}_3^-$  uptake rates followed a pattern identical to that of the unfractionated sample (Fig. 6), but the main peak of uptake was in July rather than in May. The picoplankton profile was similar to that of the seasonal variation of the chlorophyll content in the  $<2\text{-}\mu\text{m}$  size fraction (Fig. 6). This suggests that the picoplanktonic  $\text{H}^{13}\text{CO}_3^-$  uptake was predominantly associated with the autotrophic component of the small size fraction and that the chlorophyll was photosynthetically active and not related to chloroplast because of damage during filtration. The mean seasonal contribution of the  $<2\text{-}\mu\text{m}$  size fraction to the total  $\text{H}^{13}\text{CO}_3^-$  uptake was  $27 \pm 12\%$ , with the lowest values (8%) during the spring bloom (days 131–143) and the highest (45%) in June.

## Discussion

As judged from historical data on the seasonal variation of nitrogenous nutrient concentrations (Ewins and Spencer 1967), bacterial abundance (Blight et al. 1995), primary pro-

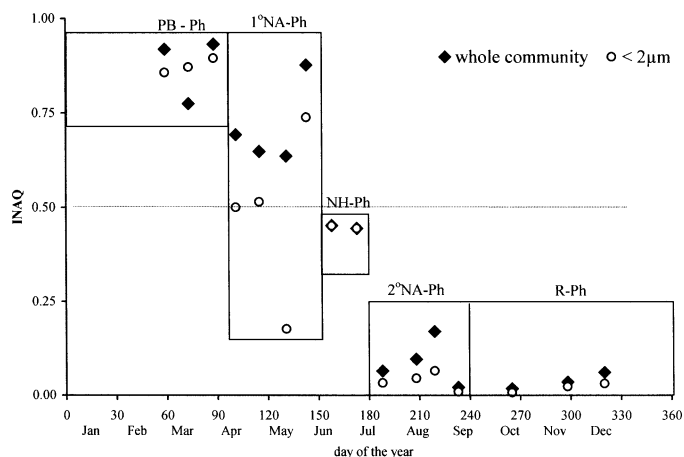


Fig. 7. Seasonal variation of the INAQ during 1995 in the Menai Strait for the whole community and the picoplankton. Comparison with the five phases established based on the oxygen production/consumption by the whole community: prebloom phase (PB-Ph); primary and secondary net autotrophic phases ( $1^\circ$  NA-Ph,  $2^\circ$  NA-Ph); regeneration phase (R-Ph), and net heterotrophic phase (NH-Ph).

duction and respiration (Blight et al. 1995), and chlorophyll concentration (Ewins and Spencer 1967; Jones and Spencer 1970; and Blight et al. 1995), 1995 was a typical year. The seasonal trends described were also found to be characteristic of temperate coastal waters (Glibert et al. 1982; Dauchez et al. 1991; Maguer et al. 1996).

Based on the results obtained, the seasonal cycle of production in these waters was divided into five phases.

- I. Prebloom phase (up to the end of March)
- II. Primary net autotrophic phase (April and May)
- III. Net heterotrophic phase (June)
- IV. Secondary net autotrophic phase (July and August)
- V. Regenerative phase (remainder of the calendar year)

These phases are shown in Fig. 7 and will be used as a basis for discussing the results.

**Contribution of nitrate and ammonium to the nitrogenous nutrition of Menai Strait's phytoplankton**—To facilitate the description of the balance between nitrate- and ammonium-supported production during the seasonal cycle in the Menai Strait, we calculated a quotient (inorganic nitrogen assimilation quotient, INAQ) from the uptake rates of these nutrients by the whole phytoplankton community (Fig. 7).

$$\text{INAQ} = \frac{\text{uptake of nitrate } (\mu\text{M d}^{-1})}{\text{uptake of ammonia } (\mu\text{M d}^{-1}) + \text{uptake of nitrate } (\mu\text{M d}^{-1})}$$

Although mathematically the same as the  $f$ -ratio (Dugdale and Goering 1967; Eppley and Peterson 1979), we were disinclined to use the same term because the calculated quotient does not bear the legitimate meaning associated with the terms of "new" and "regenerated" production used in Dugdale and Goering (1967) and Eppley and Peterson (1979). This is because in the Menai Strait, the production zone is in close contact with the sediments where nitrate regenera-

tion can occur because of the shallow water column. Notwithstanding this, the value and seasonal variations of our INAQ are similar to those of  $f$ -ratios reported by several investigators for coastal waters (e.g., Glibert et al. 1982; Dauchez et al. 1991). In particular, the seasonal cycle of events in the Menai Strait regarding the phytoplankton nitrogenous assimilation resembled closely those of Kristiansen et al. (1994) for the Barents Sea marginal ice zone, despite differences between these two environments. The high estimates of nitrate-based production during the spring bloom are also in accordance with Platt and Harrison's (1985) suggested value of 80% in coastal areas.

There was a temporal progression from the predominant use of  $\text{NO}_3^-$  (INAQ > 0.5) to that of  $\text{NH}_4^+$  (INAQ < 0.5). The seasonal pattern (Fig. 7) can be divided into the five phases described above. High INAQ are characteristic of the prebloom phase and, most important, of the primary net autotrophic phase, with the spring algal bloom being 64 to 88% nitrate based. The postbloom period can be considered transitional, corresponding to the net heterotrophic phase with INAQ = 0.45. This indicates that although nitrate was still being used, ammonium was becoming more important as a nutrient than previously. From July onward, INAQ were < 0.15, indicating that at least 85% of the primary production peaks that define the secondary net autotrophic phase was supported by ammonium. After September, and equivalent to the regenerative phase, the low net production was initially  $\text{NH}_4^+$  based, the contribution of this nutrient to primary production decreasing from 80% in September to only 40% in November. This could be because of the increased nitrate concentration, which surpassed that of ammonium on days 297 and 319.

The balance between nitrate and ammonium assimilation throughout the year was almost certainly influenced by the preference for a particular form of inorganic N by the predominant phytoplanktonic community and the nature of the algal assemblage itself at any point in time during the seasonal cycle. Furthermore, the proportionately greater utilization of nitrate early in the year, in comparison to that in summer despite the observed overall preference for  $\text{NH}_4^+$  (given by ammonium and nitrate relative preference indices, McCarthy et al. 1977; data not shown), could be a function of the composition of the predominant algal assemblage during those periods. The early blooms of phytoplankton in the Menai Strait are dominated by diatoms (Blight et al. 1995). This group of algae appears to be able to exploit high nitrate concentrations at the low temperatures typical of early spring better than the nanoplankton, which predominate in the summer. There are many examples demonstrating that nitrate-based production is associated with larger rather than smaller phytoplankters (Glibert et al. 1982; Sambroto et al. 1993). This could explain the high contribution of nitrate to the Menai Strait phytoplankton nutrition early in the year, despite preference for  $\text{NH}_4^+$ .

*The role of picoplankton in DIN uptake*—The contribution of the <2- $\mu\text{m}$  size fraction to the total uptake of nitrate and ammonium varied widely. The highest percent uptake was observed at the least productive periods of the seasonal cycle (i.e., during the prebloom phase—84 and 67% for nitrate and

ammonium, respectively) and the net heterotrophic phase. In the latter, the average percent contribution was approximate for both nutrients ( $73 \pm 5\%$ ) and coincided with the season's highest bacterial abundance, high <2- $\mu\text{m}$  size fraction oxygen respiration, low chlorophyll, negative net oxygen production, and ambient depletion of ammonium and nitrate. The lowest percent uptake by the small size fraction was during the autotrophic phases of the seasonal cycle.

The percent  $\text{NH}_4^+$  uptake by the picoplankton fraction is in the range reported by Selmer et al. (1993) for the NW Mediterranean where, at the time of the chlorophyll maximum, 40–50% of the total ammonium uptake was by the <1- $\mu\text{m}$  size fraction. In the Delaware estuary, Hoch and Kirchman (1995) and Glibert (1982) estimated that <0.8- $\mu\text{m}$  micro-organisms were responsible for 10–20% and 30%, respectively, of total  $\text{NH}_4^+$  uptake during summer. The estimates of Hoch and Kirchman are lower than those obtained in the Menai Strait for the same time of the year. This could be because their <0.8- $\mu\text{m}$  size fraction excluded a larger proportion of autotrophs than our <2- $\mu\text{m}$  size fraction. The values of Glibert (1982) are, however, of the same magnitude as those in the Menai Strait during the secondary net autotrophic phase.

In the Menai Strait, the average contribution of the small fraction to the uptake of ammonium ( $50 \pm 14\%$ ) was higher than that of nitrate ( $32 \pm 24\%$ ) and did not vary as much, indicating a higher affinity for the former, by these organisms. INAQ estimates for the small fraction confirm this (Fig. 7). The INAQ seasonal trend for the <2- $\mu\text{m}$  size fraction is similar to that for the whole community, except during the primary net autotrophic phase. This agrees closely with the pattern of  $\text{NH}_4^+$  uptake by a <0.8- $\mu\text{m}$  size fraction throughout the year reported by Hoch and Kirchman (1995).

*Bacterial DIN uptake*—The lack of a significant correlation between the <2- $\mu\text{m}$  size fraction chlorophyll concentration and absolute ammonia and nitrate uptake likewise suggests that a major part of the DIN assimilated by the picoplankton might not be associated with its autotrophic component. Given the importance of heterotrophic DIN uptake (reviewed in Kirchman 2000), we inferred this uptake to be predominantly bacterial.

We measured dark uptake of ammonium and nitrate (results not shown) in order to have an insight into the heterotrophic processes involved in nutrient assimilation. We are aware that it is not possible to simply equate dark uptake with heterotrophic activity. The data support the view that a large proportion of the DIN assimilated by the picoplankton indeed might be bacterial: the percent contribution of the <2- $\mu\text{m}$  size fraction to the unfractionated uptake in the dark was on average higher (57%) than that in light/dark conditions (44%). If the picoplanktonic fraction was dominated by autotrophs, it would be expected that uptake in the light/dark conditions would greatly exceed that in the dark.

Several approaches were explored to quantify bacterial DIN uptake in the <2- $\mu\text{m}$  size fraction. None yielded the complete answer, but collectively they provide evidence that, at least during part of the seasonal cycle, DIN uptake by the <2- $\mu\text{m}$  size fraction was dominated by the bacteria.

1. We examined the relationship between the picoplankton uptake of  $\text{H}^{13}\text{CO}_3^-$  and chlorophyll concentration as a way of correcting for the presence of phytoplankton in the  $<2\text{-}\mu\text{m}$  size fraction, since assimilation of  $\text{H}^{13}\text{CO}_3^-$  is essentially autotrophic. No significant correlation was found. However, the high uptake rates of  $^{13}\text{C}$  in the small fraction in relation to what would be expected given its low chlorophyll content, which could suggest bacterial  $\text{CO}_2$  assimilation (Romanenko 1964), led us to the conclusion that this approach would not resolve the matter.
2. The correlation between the absolute DIN uptake rates and bacterial abundance and between the percentage of picoplanktonic DIN uptake in relation to that by the whole community and bacterial abundance was examined. No significant correlations were found when the whole set of data was used. However, for the period between mid-May and early July (days 131 and 188, i.e., during later stages and immediately after the spring phytoplankton bloom), there was a strong correlation between percentage of DIN uptake and bacterial abundance ( $R^2 = 0.80$ ;  $P < 0.05$  for nitrate;  $R^2 = 0.83$ ;  $P < 0.05$  for ammonium;  $n = 5$  in each case). Thus, at this time of the year, the bacteria appear to have a major influence on DIN uptake in the  $<2\text{-}\mu\text{m}$  size fraction.
3. Data on dark DIN uptake (not shown) are consistent with the previous view. The proportion of dark uptake rose to 94–100% of that in light/dark conditions. Although algae take up inorganic N in the light and in the dark (Eppley et al. 1971), it would be expected that in the light, uptake would far exceed that in the dark. This was observed during the net autotrophic periods of the seasonal cycle (dark uptake was 16.3–24.8% of that in the light/dark incubations in May and between 1.4–25.3% in July and August). In addition to the above observations, the percent chlorophyll for the  $<2\text{-}\mu\text{m}$  size fraction was very low (1–6%) and bacterial abundance peaked during the net heterotrophic phase. During this period, total chlorophyll and primary production fell sharply. Collectively, this information constitutes qualitative evidence that the N uptake by the  $<2\text{-}\mu\text{m}$  size fraction was dominated by the bacteria, at least for this part of the season.
4. We explored the approach used by Kirchman et al. (1994), which assumes that the percentage of autotrophic uptake in the  $<2\text{-}\mu\text{m}$  size fraction is equivalent to the percentage of chlorophyll in this size fraction, the rest being bacterial. This tactic was applied to the whole set of data, despite a lack of correlation between DIN uptake and chlorophyll concentrations in the  $<2\text{-}\mu\text{m}$  size fraction. There also was no allowance for any allometric effect (Joint 1991), so the results obtained can only be considered as first approximations. We made similar calculations using the  $^{13}\text{C}$  uptake rates (i.e., the percentage of autotrophic DIN uptake by the  $<2\text{-}\mu\text{m}$  size fraction was considered to be equal to its percent  $\text{H}^{13}\text{CO}_3^-$  assimilation in relation to that by the whole community). The results obtained (not shown), including the seasonal profiles of the percent contribution of bacteria to total ammonium and nitrate uptake, in general were the same as those obtained with the chlorophyll approach.

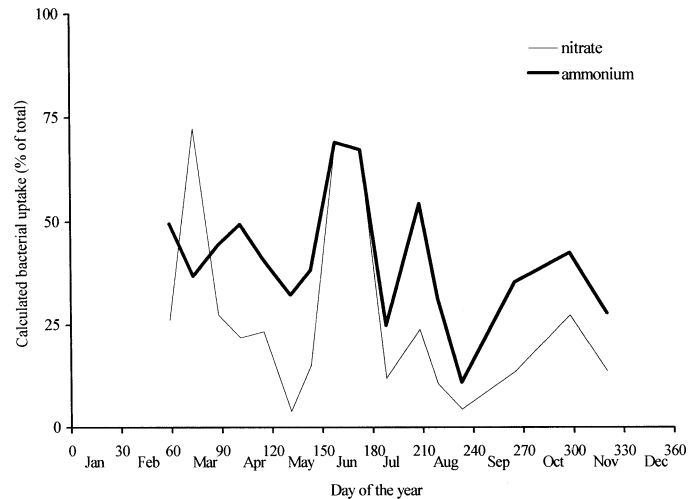


Fig. 8. Estimated percent contribution of bacteria to the unfractionated ammonium and nitrate uptake.

The results of the calculations using the chlorophyll approach showed bacterial contribution to the total uptake of ammonium to be higher (seasonal average of 41%) than to that of nitrate (27%) and less variable throughout the season (Fig. 8). The net heterotrophic phase was the only time of the year when bacteria appeared to be contributing equally to the total uptake of ammonium and nitrate, with absolute uptake rates of ammonium only slightly exceeding those of nitrate. This was concurrent with ambient depletion of these nutrients ( $\leq 0.025 \mu\text{M}$  for nitrate and  $\leq 0.030 \mu\text{M}$  for ammonium). When the rates were combined, the highest estimated bacterial contribution to total DIN uptake was observed during the net heterotrophic phase ( $68 \pm 1\%$ ). The lowest calculated bacterial contributions occurred at times of high primary production (average of 28 and 22% in the primary and secondary net autotrophic phase, respectively) and in the regeneration phase ( $27 \pm 11\%$ ). In the prebloom phase, bacteria accounted for an average of  $43 \pm 17\%$  of the total DIN uptake.

The estimated bacterial contribution to  $\text{NH}_4^+$  uptake in the Menai Strait in June and July compared well with that measured in the coastal region by Hoch and Kirchman (1995) for the same time of the year. Our calculated average percent bacterial contribution to total nitrate and ammonium uptake between May and September (of 24 and 40%, respectively) was also within the range of values reported by Kirchman and Wheeler (1998) for the sub-Arctic Pacific at the same period of the year.

In accordance with other published work, the analysis of our data leads us to the conclusion that bacteria play an important role in DIN assimilation throughout the year in the environment studied. Evidence suggests that during the senescent period following the spring bloom, the majority of DIN available was used by the microheterotrophs.

*Contribution of bacterial DIN uptake to the demise of the algal spring bloom*—We examined the implications of the previous conclusion on the demise of the spring phytoplankton bloom (from mid-May until the beginning of July; days

131–188). Estimates of DIN uptake by bacteria showed how dramatically the role of the microheterotrophs changed in the space of a few weeks at this time of the season. In May and July, the majority of the DIN uptake was associated with the autotrophs (62–96%), whereas in June, putative bacterial uptake predominated (60%). This variation in the attribution of the DIN uptake agrees very well with the net autotrophic character of May and July and the net heterotrophic character of June attributed earlier on the basis of oxygen production/consumption dynamics.

In the period between days 133 and 188, the major peaks of algal nitrate and ammonium uptake (calculated bacterial uptake subtracted from the whole-community uptake) observed in May were concurrent with maximum chlorophyll and whole-community production. Although bacterial nitrate and ammonium assimilation also peaked throughout this month, on days 143 and 131, respectively, the contribution of these organisms to the total DIN uptake was small (maximum of 32% for ammonium and 15% for nitrate).

The decrease in algal DIN uptake between days 131 and 143, which presumably contributed to the sharp decline in chlorophyll concentrations and gross oxygen production (of 4- and 10-fold, respectively), can be a consequence of ambient nitrate exhaustion. Nitrate was the main nutrient sustaining the spring phytoplankton bloom, amounting to 71–90% of the algal nitrogenous nutrition in May. It appears that ammonium was not of importance for the autotrophs nutrition at this stage but, by contrast, it contributed to ca. 80% to the bacterial DIN utilization on day 131 when ambient ammonium was relatively high ( $0.7 \mu\text{M}$ ). However, toward the end of May, when the concentration of this nutrient was undetectable, bacteria appeared to resort to the less favored N source, nitrate, which accounted for 74% of the total bacterial DIN consumption on day 143. Therefore, on day 143, when ambient nitrate was already low, bacterial nitrate consumption peaked at a time when the algal uptake of this nutrient was in serious decline.

In June, it appears that bacteria were able to exploit the low concentrations of both nitrate and ammonium better than the phytoplankton because the contribution of heterotrophs to total DIN uptake increased substantially (to approximately 68%) compared to a month earlier (4–38%). This is in accordance with studies showing that, by comparison with phytoplankton, bacteria appear to be more effective at taking up nutrients at low concentrations but not at high (Suttle et al. 1990), having lower half-saturation constants ( $K_s$ ) than the algae (Cotner and Wetzel 1992). Bacteria have been shown to outcompete algae for nutrients in certain situations (Rhee 1972; Parker et al. 1975; Currie and Kalff 1984). However, any negative effect on the primary producers by the heterotrophs can only be transient because bacteria ultimately depend on phytoplankton as a source of carbon and energy substrates (Kirchman et al. 1994).

It is then possible that the demise of the phytoplankton bloom, initially caused by nitrate depletion, was accelerated by the competition with bacteria for nutrients. By competing for a progressively depleted pool of DIN with the heterotrophs, the autotrophs were unable to sustain their growth. The consequence was that by the beginning of July (day 188), chlorophyll concentrations had fallen 7.6 times in re-

lation to its maximum in May, whereas at the same time, bacterial abundance had risen 13.5 times.

Other factors, such as grazing by the zooplankton, could be involved in this decrease of the autotrophic biomass, but it would be unwise to dismiss the bacterial effect on the decline of the algal spring bloom in these circumstances.

On the basis of fractionated nitrogen and oxygen metabolism, we have analyzed the dynamics of ammonium and nitrate assimilation of the autotrophs and microheterotrophs and drawn the following conclusions. The seasonal cycle of primary production in the Menai Strait can be divided into five distinct phases: the year starting with a prebloom phase, followed by a swing from net autotrophy to heterotrophy and back, finally leading to a protracted regenerative stage. There was a progression from nitrate- to ammonium-dominated production from March to November, with an intermediate stage in June when uptake of these nutrients was equal. This trend was also presented by the picoplankton. The balance between the assimilation of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was interpreted as being the result of the interplay between the ambient availability of the nutrients, the predominant algal assemblage, and its inorganic N preference. The picoplankton contributed to a significant proportion of the total  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake, with percent uptake highest during the net heterotrophic phase and lowest during both net autotrophic phases. Inferred bacterial uptake constituted up to 68% of the total DIN assimilated during the net heterotrophic phase, but only 21–28% during the net autotrophic phases, with a higher contribution to ammonium than nitrate uptake. Analyses of algal and estimated bacterial DIN uptake, algal and bacterial biomass, and oxygen dynamics in the spring suggest that bacteria could have had a role in the demise of the main phytoplankton bloom. By commanding the depleted pool of inorganic N, they might have given the final coup de grâce to the already stressed spring phytoplankton bloom by outcompeting the algae for nitrogen.

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