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Heterotrophic bacterial utilization of nitrogenous and nonnitrogenous substrates, determined from ammonia and oxygen fluxes

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Abstract

We describe a simple procedure to allow the broad nature of the organic substrates used for planktonic bacterial growth to be determined. The method analyzes the coupled oxygen and ammonia fluxes in terms of the relative proportions of nitrogenous and nonnitrogenous substrates assimilated by the microheterotrophs. The model uses a stoichiometric equation that requires the knowledge of the respiratory quotient, substrate C/N ratio, cell C/N quota, and bacterial carbon growth yield and the assumption that nitrification was not occurring. We discuss the uncertainties associated with the attribution of values for these constants and illustrate the use and limitations of the approach in the interpretation of field observations on bacterioplankton metabolism in a temperate coastal ecosystem. With the exception of a single observation, we were able to interpret the data using the proposed model. Our findings are that during the spring bloom, nitrogenous substrates made up 40–80% of the total, falling to $<$20% in the postbloom period. Thus, with essentially routine methods for determining oxygen and ammonia fluxes, we have been able to determine a fundamental aspect of the cycling of organic material by the bacterioplankton.

We now have a general understanding of the role played by microorganisms in the aquatic planktonic carbon and nitrogen cycles and the magnitude of the fluxes through various parts of the microbial food web (Fasham et al. 1999; Lancelot et al. 2000; Nagata 2000). However, we have very limited knowledge of the biochemical nature of the compounds cycling through the microbial system. Early studies using ^{14}C- and ^{3}H-labeled organic substrates demonstrated and quantified the uptake and metabolism of individual organic compounds in planktonic ecosystems (e.g., Andrews and Williams 1971; Azam and Holm-Hansen 1973; Billen et al. 1980; Carlucci et al. 1986). Because of the potentially wide range of monomers that heterotrophic bacteria may be assimilating and of the restricted range of substrates that are feasible to incorporate into any study, the perspective given by these studies is inevitably limited and potentially biased by the choice of substrate. However, they do show that a wide variety of organic substrates, both nitrogenous (amino acids) and nonnitrogenous (e.g., glucose, acetate, and lactate), are assimilated. Subsequently, these studies have been extended to proteins, more strictly, to combined amino acids (Kirchman et al. 1994; Hock and Kirchman 1995; *see also* references in Kirchman 2000) and nucleic acids (Jørgensen et al. 1993, 1994; Kroer et al. 1994).

The question of the biochemical nature of the substrate for bacterial metabolism is part of the wider issue of the organic connection between autotrophic and heterotrophic processes. It has been proposed (Blight et al. 1995; Sherr and Sherr 1996) that the phasing of these two processes is governed, at least in part, by the quality and molecular size of the organic substrates for heterotrophy. The prevailing nitrogen environment may be instrumental in determining the C/N composition of the dissolved organic fraction (Williams 1995) and, thus, the balance of bacterial metabolism between nitrogenous and nonnitrogenous compounds. As long as we are ignorant of the nature of these organic substrates, these arguments remain speculations.

In the present work, we were looking for a simple procedure that would permit temporal studies of the broad nature of the substrates used for bacterial metabolism. In doing so, we were prepared to accept the compromise that we would have to forgo the detail obtained by the studies made by, for example, Jørgensen et al. (1993, 1994) and Kroer et al. (1994).

The ratio of the rate of ammonia production to that of oxygen consumed has long been used in zooplankton studies to indicate the nature of the substrate for growth (Mayzaud and Conover 1988). In principle, this approach can be extended to bacterial heterotrophs—the methods to determine oxygen and ammonia flux for natural planktonic communities are now sensitive enough for the purpose. However, as the inorganic nitrogen metabolism of bacterioplankton is more complex and diverse than metazoan metabolism, the interpretation of the observations will inevitably be more difficult and less well constrained. Bacteria, as a group, both produce and consume all major inorganic species of nitrogen. We have long known that heterotrophic bacteria produce ammonia, and in certain circumstances, chemoautotrophic nitrifying bacteria oxidize ammonia to nitrate. We are now aware that planktonic bacteria also assimilate ammonia and to a lesser extent, nitrate (*see* discussion in Kirchman 2000).

A theory exists to explain what might cause the shifts between ammonification and inorganic nitrogen assimilation by heterotrophic bacteria (e.g., Billen 1984; Lancelot and Billen 1985; Goldman et al. 1987). In many respects, the present paper is an extension of this earlier work. The theory takes as its starting point the assimilation of elemental C/N composition of the organic substrate ($S_{C/N}$). In its simplest form, the gross carbon growth efficiency (Y) and the C/N bacterial quota ($Q_{C/N}$) determine the dividing line between

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inorganic assimilation and ammonia regeneration (C/N_{crit}), such that the watershed C/N ratio is

$$C/N_{crit} = Q_{C/N}/Y$$

Organic substrates with elemental C/N ratios in the organic substrate below the C/N_{crit} ratio will give rise to nitrogen regeneration; above that ratio, inorganic nitrogen will be assimilated (ammonia in preference to nitrate). Given mean values for Y from 0.27 to 0.15 for coastal and offshore populations (del Giorgio and Cole 2000) and $Q_{C/N}$ of 4.5–6.7 (Kirchman 2000), we obtain a span for C/N_{crit} of 17 to 45. Interestingly, the C/N of few, if any, naturally occurring organic compounds fall within this range. The overwhelming majority of naturally occurring nitrogen containing-compounds have C/N ratios in the narrow range of 3 to 6, with a few (glycine, methylamine) being as low as 2. It would appear that the C/N ratio of the substrates for microbial growth in natural systems is likely to be either ≤ 6 or infinity. Thus, if microorganisms assimilate a single organic compound at a time, the exact value of C/N_{crit} (i.e., whether it is 10 or 40) is of little consequence in relation to the metabolism switching from NH_3 assimilation to NH_3 production.

In this paper, we reverse this problem and lay down a theory to analyze the coupled flux of oxygen and ammonia in terms of the relative proportions of nitrogenous and nonnitrogenous substrates assimilated by bacteria. We then explore the use of this theory and discuss its limitation in the interpretation of a set of field observations on planktonic bacterial metabolism for a geographic area where we have a past record of plankton production.

Materials and methods

The present study was carried out in the temperate coastal waters of the Menai Strait, Great Britain, an environment previously described in Blight et al. (1995). Measurements were performed between early May and November. During the main spring algal bloom and its subsequent demise (May–June), sampling took place every 1–2 wk; from July onward, sampling proceeded at monthly intervals, amounting to 11 sampling occasions in total.

Seawater was collected at high tide from a depth of approximately 0.5 m by immersing and filling two 10-dm^3 opalescent polypropylene aspirators, avoiding the surface microfilm. The water was then pooled into a 25-dm^3 polypropylene aspirator, passing through a 50-μm mesh attached to its mouth to remove larger particles that could clog subsequent filters. The screened water was then filtered through 1-μm filter capsules (Whatman Polycap™ 36AS) to isolate the bacterial fraction. This processing was achieved within an hour. Aliquots of the whole (100 to 250 cm^3) and fractionated (500 to 1,000 cm^3) sample were filtered onto 47-mm Whatman GF/F filters, and the phytopigments were extracted overnight in 8 cm^3 of neutralized 90% acetone. The chlorophyll concentrations were determined using a Turner 10 Designs fluorometer in accordance with the recommendations of Tett (1987). For the determination of oxygen and ammonia dynamics in the $<$1-μm size fraction, their concentrations were determined prior to and after a 24-h incubation period in the dark. The oxygen determinations were carried out in five replicate, 100-cm^3 borosilicate bottles; three 250-cm^3 polycarbonate bottles were used for the ammonia determinations. A fourth, 250-cm^3 sample to which 1.5 μM of NH_4Cl had been added was incubated under the same conditions. Ammonia fluxes in the nonspiked sample mirrored closely those in the spiked sample (results not shown). Bottles were incubated in the dark and immersed in water at a nearly in situ temperature (12–20°C). Oxygen concentrations were measured using the Winkler technique as in Blight et al. (1995). The average standard error for the oxygen respiration estimates throughout this study was 0.39 μM O_2 d^{-1}. Ammonia concentrations were determined according to the oxidation method described by Parsons et al. (1984). The lower limit of detection, taken as blank signal plus two standard deviations of the blank (Miller and Miller 1984) was 0.030 μM.

All glassware was soaked overnight in HCl (10%) and thoroughly rinsed in deionized water, seawater, or both prior to analyses. This procedure was employed to minimize contamination of samples for NH_3 determinations.

Theoretical background

The approach we used to analyze the relative fluxes of ammonia and oxygen differs from that used for zooplankton (e.g., Mayzaud and Conover 1988). By necessity, it employs certain empirical relationships and simplifying assumptions to contend with a level of complexity not present in zooplankton physiology. Our theory has to take into account the consumption as well as the production of ammonia during growth. This will result in the flux of ammonia (ΔNH_3) being negative as well as positive. For this reason, it is more convenient to calculate a molar ratio N/O_2 (i.e., $\Delta NH_3/\Delta O_2$), rather than the reciprocal, which is conventional in zooplankton metabolism. Inverting the expression puts the quotients either side of zero rather than infinity, which greatly simplifies the presentation of the results. In the present account, all fluxes that result in a decrease in the free water concentrations are given a negative sign.

In this paper, we derive equations for the $\Delta NH_3/\Delta O_2$ ratio during heterotrophic growth for nitrogenous and nonnitrogenous substrates (NS and NNS, respectively). Using a number of assumptions, we then use the observed $\Delta NH_3/\Delta O_2$ ratios to calculate the relative proportions of these categories of substrates (as NS/[NS + NNS]) as the substrate for bacterial metabolism.

Anderson (1995) presented the stoichiometric equation for the oxidation of organic material to nitrate. Using his notation, the equation has been rewritten for the oxidation of these compounds to ammonia.

$$C_\alpha H_\delta O_\chi N_\beta P_\psi + \gamma O_2 = \alpha CO_2 + \beta NH_3 + \psi H_3PO_4 + 0.5[\delta - 3(\beta + \psi)]H_2O \quad (1)$$

Thus, γ (i.e., oxygen consumed) can be solved as

$$\gamma = \alpha + 1.25\psi + 0.25\delta - 0.75\beta - 0.5\chi \quad (2)$$

Given this equation and the elemental composition of a molecule, we can calculate the respiratory coefficient (RQ =

Table 1. Elemental protein, nucleic acid, carbohydrate, and lipid compositions and the calculated RQ. Based on Anderson (1995).

Biochemical	Stoichiometric composition	Molar ratio	
		$S_{C/N}$	RQ
Protein	$C_{3.83}H_{6.05}O_{1.25}N$	3.83	0.97
Nucleic acid	$C_{9.625}H_{12}O_{6.5}N_{3.75}P$	2.57	1.23
Carbohydrate	$C_6H_{10}O_5$	∞	1.00
Lipid	$C_{40}H_{74}O_5$	∞	0.71

$\Delta CO_2/-\Delta O_2$) as α/γ. For the complete oxidation of the organic material to carbon dioxide and ammonia, the molar $\Delta NH_3/\Delta O_2$ value will be $\beta/-\gamma$. Table 1 gives Anderson's elemental compositions for proteins, nucleic acids, polysaccharides, and lipids; their C/N ($S_{C/N}$) ratios, and RQ values.

To calculate the overall $\Delta NH_3/\Delta O_2$ ratios resulting from the metabolism of organic material ($-\Delta C_{ORG}$), we need to take account the fraction (Y) of the substrate used for growth. The full derivation of relationship in terms of the substrate C/N ratio ($S_{C/N}$), the cell C/N quota ($Q_{C/N}$), the respiratory quotient (RQ), and the growth yield (Y) is as follows.

The O_2 and NH_3 fluxes as a consequence of respiration are

$$\Delta O_2/-\Delta C_{ORG} = -(1 + Y)RQ \quad (3)$$

$$\Delta NH_3/-\Delta C_{ORG} = (1 - Y)S_{C/N}^{-1} \quad (4)$$

The O_2 and NH_3 fluxes as a consequence of growth are

$$\Delta O_2/-\Delta C_{ORG} = 0 \quad (5)$$

$$\Delta NH_3/-\Delta C_{ORG} = Y(S_{C/N}^{-1} - Q_{C/N}^{-1}) \quad (6)$$

Combining Eqs. 3 and 5 and Eqs. 4 and 6 gives

$$\Delta O_2/-\Delta C_{ORG} = -(1 - Y)RQ \quad (7)$$

$$\Delta NH_3/-\Delta C_{ORG} = ((1 - Y)S_{C/N}^{-1}) + (Y(S_{C/N}^{-1} - Q_{C/N}^{-1})) \quad (8)$$

which simplifies to

$$\Delta NH_3/-\Delta C_{ORG} = S_{C/N}^{-1} - Y\cdot Q_{C/N}^{-1} \quad (9)$$

Equation 8 was presented by Billen (1984) and a derivation is given in Lancelot and Billen (1985). It is repeated here for the sake of completeness. The $\Delta NH_3/\Delta O_2$ ratio, in terms of $S_{C/N}$, $Q_{C/N}$, RQ, and Y, is thus

$$\frac{\Delta NH_3}{\Delta O_2} = \frac{S_{C/N}^{-1} - (Y\cdot Q_{C/N}^{-1})}{-(1 - Y)RQ} \quad (10)$$

This equation applies to both nitrogenous and nonnitrogenous substrates. For a nitrogenous substrate, the $\Delta NH_3/\Delta O_2$ can be positive or negative, depending on the relative values of Y, $S_{C/N}$, and $Q_{C/N}$. For nonnitrogenous substrates, where $S_{C/N}$ is infinity, the equation simplifies to

$$\frac{\Delta NH_3}{\Delta O_2} = \frac{-Y\cdot Q_{C/N}^{-1}}{-(1 - Y)RQ} \quad (11)$$

and will always give a positive $\Delta NH_3/\Delta O_2$ ratio because both ammonia and oxygen are consumed.

Given Eqs. 10 and 11 for nitrogenous and nonnitrogenous end members, the $\Delta NH_3/\Delta O_2$ ratio can be interpreted as a NS/(NS + NNS) quotient.

Four constants—$S_{C/N}$, RQ, Y, and $Q_{C/N}$—need to be known in order to solve Eq. 10 (two for Eq. 11) and analyze the $\Delta NH_3/\Delta O_2$ observations in terms of the relative utilization of nitrogenous versus nonnitrogenous substrates. The first two constants are a stoichiometric consequence of the elemental composition of the compound under metabolism (Table 1). If we work with the four major biochemical groupings given in Table 1, then in order to interpret the $\Delta NH_3/\Delta O_2$ observations, the main requirement is to specify the ratio of protein versus nucleic acids and the ratio of carbohydrate versus lipids. Once this is done, the $S_{C/N}$ and RQ values for the two end members of the NS/(NS + NNS) series are set. In the standard run, we used protein as the nitrogenous substrate end member and carbohydrate as the nonnitrogenous one. At first sight, the requirement to specify these ratios puts an unwelcome degree of circularity in the analysis, but there are ways of dealing with this, as will be discussed.

We now need to specify values for Y and $Q_{C/N}$. Field observations of bacterial growth yield have been reviewed by del Giorgio and Cole (2000), and their generalizations can be used as a start. They grouped the observations of growth yield for estuarine, coastal, and oceanic provinces; for the coastal waters they derived mean growth yields of 0.27 ± 0.01. The statistic they specify is the standard error, although the more appropriate statistic for the present purpose would be the standard deviation, which we estimated to be approximately 10-fold greater (i.e., ± 0.1) from the number of observations they analyzed. For the analysis, we will use a rounded-off mean plus or minus one standard deviation (i.e., $0.3 \pm 0.1 = 0.2$, 0.3, and 0.4). Kirchman has discussed values of $Q_{C/N}$ (Kirchman 2000) ranging from 4.3, based on the biochemical composition of a bacterial cell, to 6.7, based on chemical analysis. We will start with value of 4.5 and then consider the consequences of higher values.

In Fig. 1a–c, we plot the relationship between the $\Delta NH_3/\Delta O_2$ value and the proportion of nitrogenous and nonnitrogenous substrate (NS/[NS + NNS]) for a range of protein, nucleic acid, lipid, and carbohydrate mixtures and the effect of the bacterial growth yield (Y) on the relationship. These relationships are the basis for the analysis of our field observations of $\Delta NH_3/\Delta O_2$ values. In Fig. 1a and 1c, the lines are computed for a $Q_{C/N}$ value of 4.5. Higher values for $Q_{C/N}$ generally reduce the spread due to Y, especially at the nonnitrogenous end of the series. For example (*see Fig. 1a,b*), increasing $Q_{C/N}$ from 4.5 to 6.5 reduces the spread by about one third.

The above calculations only take account of heterotrophic nitrogen metabolism. Autotrophic nitrification will also result in the flux of both ammonia and nitrate—the ratio depending on the product:

$$NH_3 + 1\frac{1}{2}O_2 = HNO_2 + H_2O$$

$$\text{i.e.,} \quad \Delta NH_3/\Delta O_2 = 0.67 \quad (12)$$

$$NH_3 + 2O_2 = HNO_3 + H_2O$$

$$\text{i.e.,} \quad \Delta NH_3/\Delta O_2 = 0.5 \quad (13)$$

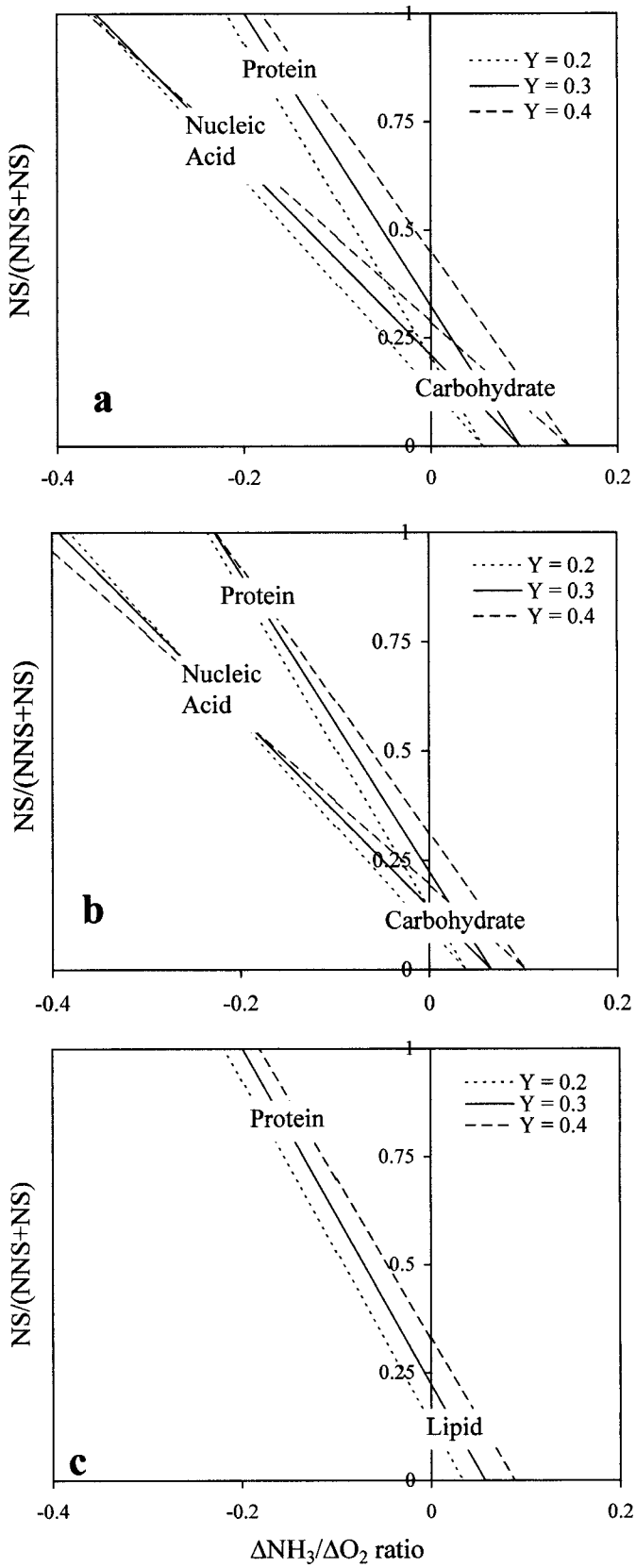

Fig. 1. The relationship between the $\Delta NH_3/\Delta O_2$ value and the proportion of nitrogenous and nonnitrogenous substrate (NS/[NS + NNS]) for combinations of the four major biochemical groupings. Shown also is the effect of the value assumed for growth yield (Y) on the relationship. (a) Lines are shown separately for proteins and nucleic acids with carbohydrate as a common nonnitrogenous end member. The elemental compositions given in Table 1 and Eqs. 10 and 11 are the basis for the calculations. The $Q_{C/N}$ ratio is set to 4.5. (b) As in (a) but with $Q_{C/N} = 6.5$. (c) As in (a) but with the lines shown for lipid as the nonnitrogenous end members and with protein as the nitrogenous end member.

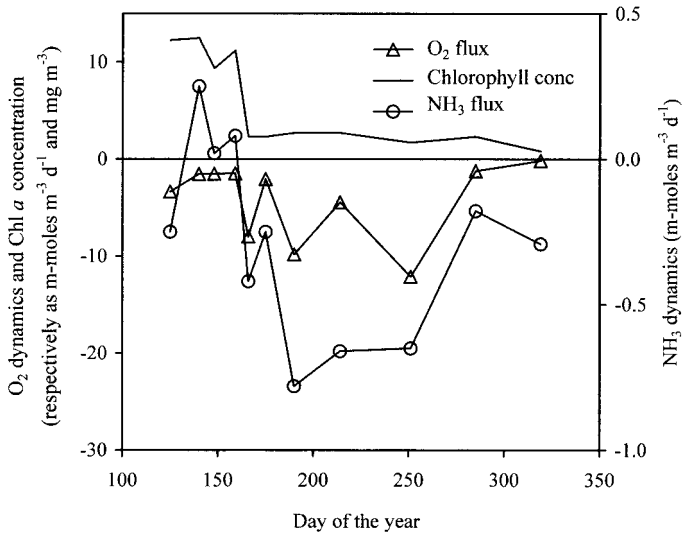

Fig. 2. Variation of NH_3 and O_2 fluxes and chlorophyll *a* (Chl *a*) concentrations between May and November. Chl *a* concentrations are given for the whole community. The NH_3 and O_2 fluxes are for the <1-μm fraction.

In the present study, we make the initial assumption that no nitrification is occurring. As will be seen, this assumption may not be sustainable.

Results and discussion

The heterotrophic fluxes of ammonia and oxygen and of chlorophyll concentrations were followed from the peak to the demise of the main spring bloom through the midsummer heterotrophic period into the autumn, when characteristically the rates of oxygen metabolism fall from low to undetectable rates. Fluctuations in the chlorophyll concentrations and oxygen fluxes (Fig. 2) are similar to those described and discussed by Blight et al. (1995).

Isolation of the heterotrophic component of metabolism—Autotrophic nitrogen metabolism continues after CO_2 fixation ceases (Eppley et al. 1971). Thus, dark incubations by themselves will not eliminate autotrophic processes. Therefore, we isolated heterotrophic processes by removing autotrophs through 1-μm filters prior to incubation. To establish the effectiveness of this procedure, the chlorophyll content in the filtrates was measured. The average chlorophyll content of the <1-μm fraction was 0.007 mg m^{-3}. It is highly unlikely that algal biomasses of this order would

give rise to measurable ammonia or oxygen metabolism; thus, it can be expected that the processes measured were heterotrophic. During the filtration process, bacteria will be lost (at worst, during periods of high planktonic abundance and particulate matter, these losses will be ~35%) and predators removed; thus, systematic errors inevitably will result (both over- and underestimates are possible) in the absolute rates of bacterial oxygen and nitrogen metabolisms. We are also aware that the bacterial numbers can change in the course of the incubation. This was looked at in detail by Blight et al. (1995) for the same population. What appears to happen is that, although there may be an increase in the bacteria number in the fractionated sample, metabolic rates of the microbial fraction (as judged by O_2 consumption) remain constant over a period of 36 h. Because the calculations are based on the ratio of nitrogen and oxygen flux, the errors in the absolute rates ought not to seriously bias the conclusions drawn from relative rates.

Analysis of field observations of ammonia and oxygen fluxes—Figure 2 shows that ammonia metabolism shifted from an initial negative (ammonia assimilation) to a positive flux (ammonia production) throughout the period of the bloom. This reverts to a negative flux in the postbloom period and for the remaining time of the study. We are unaware of any extensive seasonal time series of ammonia production and consumption with which to draw a comparison; indeed, Kirchman (2000) notes that there are very few modern studies of bacterial mineralization. Oxygen respiratory flux was less variable than that of ammonia, being low during the initial bloom period. As a consequence of the difference in the metabolisms of these two molecules, there was a switch from negative to positive $\Delta NH_3/\Delta O_2$ values following the bloom. The abrupt rise in $\Delta NH_3/\Delta O_2$ at the end of the study (shown in Fig. 3 as the dashed part the $\Delta NH_3/\Delta O_2$ line) is beyond that accountable by any stoichiometric theory associated with the metabolism of ammonia. We have examined the analyses for this sample carefully and can find no explanation for the high rates of ammonia production.

The analysis of the $\Delta NH_3/\Delta O_2$ values, in terms of the relative proportion of nitrogenous and nonnitrogenous substrates, is shown in Fig. 3a for the standard run (with 100% protein as the nitrogenous substrate). During the mid- to late bloom period, when there is a positive flux of ammonia, the calculations give values for NS/(NS + NNS) of 0.50 or more. The exact value is sensitive to assumptions made about the protein/nucleic acid proportion. Fig. 3b shows a run for an assumed 25% : 75% mixture of protein and nucleic acid; the pattern remains much the same as the standard run, although the peaks are suppressed by <15%. As will be discussed later, this is probably a pessimistic calculation because the nucleic acid component of bacterial nitrogen assimilation is probably no greater than 10%; thus, a figure of 5% suppression may be more likely. Out of the bloom period, the NS/(NS + NNS) value is low—generally <0.25, the exact value in this case being dependent on the value of Y. The NS/(NS + NNS) value is much less sensitive to assumptions about the composition of the nonnitrogenous substrate (cf. Fig. 3a–c). Thus, the trend during the bloom is for bacteria to use predominantly nitrogen-rich compounds for their growth and, as a consequence, excrete ammonia. In the postbloom and subsequent period, bacteria are assimilating mainly nonnitrogenous compounds and so have to assimilate ammonia. This is consistent with the observation (Williams 1995) that dissolved organic matter (DOM) that accumulates during the bloom and is removed in the latter part of the year is carbon-rich.

We know of no observations that allow direct comparison with the above calculations. Billen (1984), Goldman et al. (1987), and Goldman and Dennett (2000) used a similar theory to the one we derived, but their analysis is based on carbon rather than oxygen. Furthermore, their measurements were restricted to seawater samples supplemented with additions of individual organic substrates. The observations are valuable in the context of the present work in that they provide verification of the approach. However, as physiological studies, what they cannot reveal are the shifts in the balance of metabolism between the nitrogenous and nonnitrogenous forms of the unamended environment through the plankton seasonal cycle.

There have been a number of assessments of the utilization of various groups of nitrogenous compounds in relation to the estimated demand for bacterial production or growth. Jørgensen et al. (1994) and Kroer et al. (1994) both measured the utilization of free and combined amino acids and DNA and estimated the sum of their utilization to account for 26–43% of heterotrophic carbon metabolism. The source of the remaining carbon demand was not determined because the major groups of nitrogenous compounds were covered in the study. One can infer that the remaining uptake was of nonnitrogenous compounds, so the above values would be equivalent to the NS/(NS + NNS) value estimated in the present work. Rich et al. (1996) examined the uptake of glucose (the predominant neutral monosaccharide) and total free amino acids: glucose uptake was generally about 25% of that of the amino acids. These three studies would be similar to the state of affairs that we observed during the bloom. Billen and Fontigny (1981) made measurements of bacterial growth; growth yield; and amino acid, monosaccharide, and glycolate uptake over a season in the southern North Sea, where *Phaeocystis* was the major bloom species. The annual budget ascribed 44% of carbon assimilation to the amino acids. Unfortunately, the paper gives no indication of the variations through the season, which would have allowed comparison with the present set of observations.

In the present study, in the period following the bloom, it is only possible to account for observations if a high value (0.4) is used for Y and a lower value (4.5) for $Q_{C/N}$ (cf. Fig. 3a,b). Values for Y = 0.4 and bacterial $Q_{C/N}$ = 4.5 are both at the extremes of their ranges and might call to question some of the assumptions built into our analysis. The value of Y we obtained is notably higher than the mean of 0.27 reported in the review by del Giorgio and Cole (2000) for coastal waters. There are, however, studies pointing toward Y values in the region of 0.4. Ducklow et al. (2000), from an analysis of rates of carbon flux and bacterial growth in the Ross Sea, were able to constrain Y to the range 0.35–0.45; mass balance considerations would not allow values as low as 0.27. The study of Billen (1984) in this context is helpful. He analyzed observations of carbon and ammonia

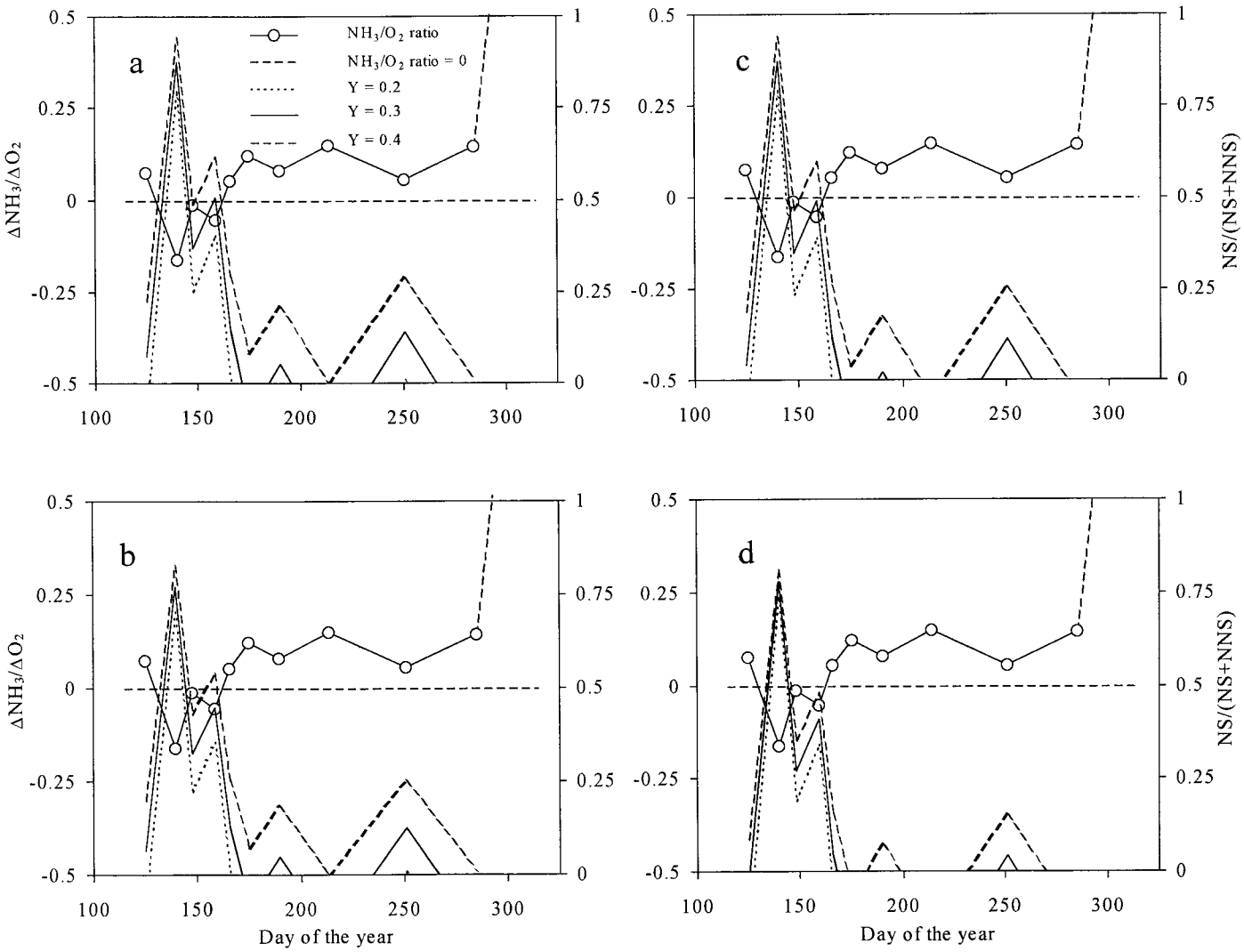

Fig. 3. The observed $\Delta NH_3/\Delta O_2$ value and the calculated proportion of nitrogenous and nonnitrogenous substrate (NS/[NS + NNS]) used by the heterotrophic bacteria for various assumptions. (a) The nitrogenous substrate is 100% protein, the nonnitrogenous substrate is 100% carbohydrate, and the bacterial C/N quota is 4.5. (b) The nitrogenous substrate is 75% protein and 25% nucleic acid, the nonnitrogenous substrate is 100% carbohydrate, and the bacterial C/N quota is 4.5. (c) The nitrogenous substrate is 100% protein, the nonnitrogenous substrate is 75% carbohydrate and 25% lipid, and the bacterial C/N quota is 4.5. (d) The nitrogenous substrate is 100% protein, the nonnitrogenous substrate is 100% carbohydrate, and the bacterial C/N quota is 6.5.

flux from added organic substrates and derived a mass ratio for C/N_{crit} of 10. This would require the molar $Q_{C/N}/Y$ to be equal to 11.7, which could only be achieved with a low value for $Q_{C/N}$ and a high value for Y (e.g., 4.5 and 0.38, respectively); our required values (4.5 and 0.4, respectively) would give a C/N_{crit} value of 11, close to his field observations. Support for our findings also come from the studies with seawater cultures by Goldman et al. (1987). They concluded that the bacterial $Q_{C/N}$ values were ''low and invariant,'' reporting a mean of 5.1. Their analysis suggests values for Y in the range 0.4–0.5. In a subsequent paper, Goldman and Dennett (2000) observe that in carbon-limited continuous and batch seawater cultures, bacterial growth yields were generally high (0.498 ± 0.19, mean ± two standard deviations, $n = 72$). Thus, there is a fair measure of support for our bacterial Y and $Q_{C/N}$ observations, even though at first they seem to be at the extremes of accepted values. If more conventional values of 0.2–0.3 are used for Y, a value >6 is used for $Q_{C/N}$, or both, the majority of the NS/(NS + NNS) values fall below zero (*see Fig. 3d*). In these cases, we would need to invoke concomitant nitrification for which the $\Delta NH_3/\Delta O_2$ values are 0.5–0.67 (*see Eqs. 12, 13*).

Limitations of the analysis—We are able in a broad way to analyze the $\Delta NH_3/\Delta O_2$ ratio in terms of the balance between nitrogenous and nonnitrogenous substrates for growth. Because the approach has a number of new aspects, it is appropriate at this juncture to review the assumptions and uncertainties associated with the analysis and to see their consequences. In order to make the analysis, we need to (1) assume that O_2 and NH_3 production and utilization are tightly coupled, (2) restrict our consideration of substrates to four

major biochemical categories, (3) specify the protein/nucleic acid and the carbohydrate/lipid ratios to calculate the RQ and $S_{C/N}$ values, (4) ascribe values, ideally single values, for Y and $Q_{C/N}$ ($S_{C/N}$ and RQ are determined as a consequence of assumptions 1 and 2), (5) assume no other forms of O_2 and NH_3 production or utilization occur (i.e., there is no nitrification).

Tight coupling of O_2 and NH_3 production and utilization—If there is loose or no coupling between the metabolisms of oxygen and ammonia, then the stoichiometric analysis would have no basis. It would be difficult to argue against some decoupling between the two at some phase during growth, but this would have to be made up at some other time during the cell cycle. The measurements span a 24-h period, which can be taken to be comparable to the cell generation time for coastal populations (e.g., Newell and Christian 1981), so the assumption that nitrogen and oxygen metabolism is coupled would not seen unreasonable over the time scale we have used.

Restriction of our consideration to four major biochemical categories—We make an implicit assumption that the classical biochemical groupings (proteins, nucleic acids, polysaccharides, and lipids) can be taken as the substrates for metabolism. We are aware that this is strictly incorrect because it is their monomers and hydrolysis products that are transported into the bacterial cell and are, in fact, the immediate substrates for metabolism. The monomers are less conservative in their elemental composition than their polymers. Thus, if the calculations of $\Delta NH_3/\Delta O_2$ were, for example, extended to individual amino acids, the range of possibilities for the $\Delta NH_3/\Delta O_2$ value for nitrogenous compounds would be broadened from -0.25 to -0.48 to -0.10 (phenylalanine) to -0.67 (glycine). This would materially increase the uncertainty of the calculations. These individual organic compounds are characteristically at low nanomolar concentrations in seawater and could not sustain the micromolar metabolic rates observed over the measurement time scale. If there were prolonged selective utilization of particular amino acids or nucleotides, then this would result in massive accumulations of the forms discriminated against. This is simply not seen on the necessary (high nanomolar) scale. It is therefore reasonable to suppose that these individual components can be regarded to be merely intermediates between a large polymeric reservoir in the dissolved and particulate organic pool and the substrates used for bacterial metabolism. Thus, the assumption that the elemental composition of the biopolymers can be used in the calculations (Table 1) would seem to be justified.

The need to specify the protein/nucleic acid and the carbohydrate/lipid ratios—We need to know the protein/nucleic acid and the carbohydrate/lipid ratios in order to specify the values for $S_{C/N}$ and the RQ. It can be argued that this requirement means that we are in danger of putting as much, if not more, into the calculation than we get out of it. Comparison of Fig. 3a with 3c shows that the analysis is rather insensitive to the carbohydrate/lipid ratio, the uncertainty being less that 10%. It means also that organic and fatty acids, if they were being metabolized, would not seriously bias the results. If we are prepared to accept a 10% uncertainty in the interpretation of the observations, then we can discount this as a problem. If not, then the problem could be resolved by direct determinations of the RQ using high-performance CO_2 titrations (e.g., Robinson et al. 1999) because the $S_{C/N}$ value is not needed in the case of the nonnitrogenous compounds.

The effect of the assumed protein/nucleic acid ratio on the calculations is greater. The overall uncertainty is close to 35% (i.e., the variation between an assumption of the nucleic acids being either 0 or 100% of the nitrogenous substrate; *see Fig. 1a*). This is large enough to give concern and so needs fuller consideration than the nonnitrogenous substrates. The problem can be examined in two ways. First, we can make an estimate of the nucleic acid fraction of DON. Values for the DON and dissolved organic phosphorus (DOP) concentration of seawater can be taken as 4.6 and 0.12 μM, respectively (Banoub and Williams 1973). Using these values and an elemental N/P ratio for nucleic acids of 3.75 (Table 1), we can calculate a maximum nucleic content for DON of 10%. This would be a maximum (because it ignores organic phosphate forms such as sugar phosphates), and as such, it would give rise to a 12% uncertainty in the calculation of the NS/(NS + NNS) value, which can be considered an acceptable error. However, one could argue that the nucleic acid fraction might be cycling around much faster than the remainder of the DOM and that the estimate of stock does not represent the flux. Jørgensen et al. (1993, 1994) and Kroer et al. (1994) measured the relative utilization of nucleic acid (DNA) and dissolved free and combined amino acids and found the nucleic acid uptake to be 5–11% of the sum of the uptake of dissolved nitrogenous material. If we can take these as representative values, then the problem of ascribing a nitrogenous end member would not appear to be a serious one. Assuming the nitrogenous substrate to be 100% protein would only give rise to a 5% uncertainty in the analysis. To resolve the matter, one could opt for the following solution. If concomitant measurements of $\Delta PO_4/\Delta O_2$ were made—and this should be technically possible with modern phosphate methods (e.g., Karl and Tien 1992)—then the data would enable the nucleic acid flux to be resolved using an equation for the phosphorous release from nucleic acids derived from Eq. 10; that is,

$$\frac{\Delta PO_4}{\Delta O_2} = \frac{S_{C/P}^{-1} - (Y \cdot Q_{C/P}^{-1})}{-(1 - Y)RQ} = \frac{0.1 - (Y \cdot Q_{C/P}^{-1})}{-(1 - Y)1.23} \quad (14)$$

The calculated $\Delta PO_4/\Delta O_2$ value would allow an estimate of the fraction of oxygen metabolism attributable to nucleic acid utilization. $S_{C/P}$ and the RQ values of 0.1 and 1.23, respectively, are set by the elemental composition of nucleic acid (Table 1); thus in this case, only Y and $Q_{N/P}$ remain unknown. Although $Q_{C/P}$ is highly variable (Kirchman 2000), because it is generally >50 and because of the structure of the equation, the $\Delta PO_4/\Delta O_2$ is insensitive to the exact value of $Q_{C/P}$. A 10-fold variation in $Q_{C/P}$ gives a 5% shift in the calculated value of $\Delta PO_4/\Delta O_2$. Given the $\Delta PO_4/\Delta O_2$ value, the protein/nucleic acid ratio can be calculated.

Ascribing unique values for bacterial $Q_{C/N}$ and Y—It is unlikely that either $Q_{C/N}$ or Y is constant in space and time. Kirchman (2000) reviewed the reported determinations of $Q_{C/N}$ of marine bacteria and found an overall variation from 3.8 to 15. The majority of the determinations fall in the range 4.5–6.5. Goldman and Dennett (2000) examined the variation of $Q_{C/N}$ in batch and continuous seawater cultures and concluded that it was constant at 4.5 during active growth but rose to ≥ 7 in the stationary phase of growth. Comparing Fig. 1a with 1b shows the effect of a variation from 4.5 to 6.5 on the outcome of the present analysis. High values of $Q_{C/N}$ narrow the uncertainty in the interpretation due to variations in Y. It also reduces the $\Delta NH_3/\Delta O_2$ value for the nonnitrogenous compounds but has a proportionately lesser effect on the value for the nitrogenous compounds. Comparison of Fig. 3a and 3d shows the consequence of these effects on the interpretation of the $\Delta NH_3/\Delta O_2$ values. Increasing $Q_{C/N}$ from 4.5 to 6.5 depresses the NS/(NS + NNS) value by $<15\%$. It also gives rise to problematic negative values.

Uncertainties over the value of Y bedevil much of the current effort to calculate bacterial carbon demand. The present study is no exception. There are differing views about whether or not Y can be assumed to be constant. Goldman and Dennett (2000) found bacterial carbon growth yield of seawater cultures to be constant, whereas del Giorgio and Cole (2000) and Jørgensen et al. (1994) found it to be dependent on the productivity of the system. In the present analysis it is more of a problem in the case of the nonnitrogenous end member and, as a consequence, when NS/(NS + NNS) is low (*see Figs. 1, 3*). We see no alternative at the present but to live with the problem. With the present approach, we may be able to invert the problem. For the sake of illustration, if we make the assumption that there were no complicating factors in our measurement of a heterotrophic $\Delta NH_3/\Delta O_2$ value (i.e., no nitrification), then we can use the sensitivity of the calculations to Y and $Q_{C/N}$ to set limits on these values.

No other forms of O_2 and NH_3 production/utilization occur (i.e., there is no nitrification)—Finally, the analysis needs to make the assumption that there is no chemoautotrophic nitrogen metabolism. Our previous observations of the study area showed no elevated levels of nitrite during the part of the year over which the observations were made (Rodrigues 1998). Accordingly, it was felt reasonable to assume we would be making our measurements in a period of the year when nitrification in the plankton would be a complicating factor. However, that we need to adopt apparently extreme values for Y and $Q_{C/N}$ in order to analyze our data leaves us more cautious about this assumption. In future work, it would be prudent to inhibit nitrification with, for example, N-serve (2-chloro-6-trichloromethyl pyridine; Ward 2000) and so avoid the need to make this assumption. This would make constraining Y and $Q_{C/N}$ values (*see above*) much more powerful.

Conclusions—We have been able to measure the associated bacterial fluxes of ammonia and oxygen through the key part of the productive season in an inshore planktonic ecosystem. We find production of ammonia by the microheterotrophs during the spring bloom and consumption during the postbloom period. With the exception of a single observation, we are able to interpret the data using a stoichiometric equation as the relative utilization of nitrogenous and nonnitrogenous substrates. Our findings are that during the bloom, nitrogenous substrates make up 40–80% of the total, falling to $<20\%$ in the postbloom period. This gives rise to a switch in the bacteria acting as producers to consumers of inorganic nitrogen (ammonia).

The analysis requires knowledge of the values for the respiratory quotient, the substrate C/N ratio, the cell C/N quota, and the bacterial carbon growth yield. The first two are determined by the elemental composition of the substrate. In order to make the analysis, we have assumed that the starting material for bacterial growth can be categorized as the four major biochemical groupings (proteins, nucleic acids, carbohydrates, and lipids), for which we can ascribe mean elemental compositions. We then have to prescribe the ratio of the two nitrogenous and nonnitrogenous groups for the two end members. Although this would seem to be a major constraint to the analysis, when considered in detail, it has little material effect on the conclusions. The greatest uncertainty in the analysis surrounds the value we assume for the bacterial carbon growth yield. Our analysis requires that it has a value of 0.4 or greater—significantly higher than the mean value derived by del Giorgio and Cole (2000). Finally, in order to make the analysis, it was necessary to assume that nitrification was not occurring in the samples. Having completed the study, we are less confident that this assumption can be made, and in future studies, it would be prudent to block the process with an inhibitor such as N-serve.

The great virtue of the approach is that with essentially routine methods for oxygen and ammonia, one can determine a fundamental aspect of the microbial cycling of organic material. There are uncertainties, but we think that they are offset by the simplicity of the technique.

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