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REVIEW

Temporal offset in oceanic production and respiration processes implied by seasonal changes in atmospheric oxygen: the role of heterotrophic microbes

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ABSTRACT: Recent data on seasonal variation in the concentration of atmospheric oxygen are independent evidence for a marked annual cycle in the production/respiration (P/R) ratio of the biotic community of the ocean in both the northern and the southern hemispheres. Based on the oxygen data, the P/R ratio tends to be >1 during a 3 to 5 mo period from late winter to early spring, and <1 during the rest of the year. The amount of oxygen which accumulates in the atmosphere during spring as a result of ocean biology implies a *seasonal* unrespired production of, on average, 50 to 60 g C m^{-2}. This amount of fixed carbon is approximately one third of annual oceanic primary production, and several-fold greater than measured sinking fluxes of particulate organic matter in the open sea. Size-fractioned respiration rates in seawater imply that <5 µm sized microbes, in particular bacteria, play a major role in the establishment of the seasonal P/R cycle. Hypotheses to explain less microbial respiration compared to primary production in spring than in summer/fall in the open ocean might include: (1) temperature effect on respiration; (2) seasonal differences in bacterial growth efficiency; and (3) seasonal differences in quality of organic substrates. These processes may result in marked seasonal variation in abundance of metabolically active bacteria. Elucidating the mechanisms that contribute to the seasonal cycle in P/R ratios in the world ocean should be a goal of future research in microbial oceanography.

KEY WORDS: Respiration · Bacteria · Production/respiration ratio · Atmospheric oxygen

ANNUAL CYCLE OF PRODUCTION/RESPIRATION RATIO IN THE OCEAN

The annual cycle of primary production in the sea has been fairly well established via numerous studies and oceanographic expeditions during this century. Annual cycles of heterotrophic processes in the open ocean are less well understood. The organisms responsible for the bulk of organic carbon utilization and respiration in the sea are heterotrophic bacteria (Pomeroy 1974, Azam et al. 1983, Williams 1983, Cole et al. 1988, Ducklow & Carlson 1992). Since bacterioplankton obtain substrates for growth from phytoplankton, either directly or indirectly via the food web (Azam et al. 1983, Jumars et al. 1989), rates of bacterial production and respiration are, in general, positively related to autotrophic production (Cole et al. 1988, Ducklow & Carlson 1992). Based on this observation, the seasonal cycle of heterotrophy would be expected to more or less follow the seasonal cycle of autotrophy (Ducklow 1994).

Recent data establishing an annual cycle in the concentration of oxygen in the atmosphere suggest that seasonality in production/respiration (P/R) processes in the sea is more complex than previously thought. Innovations in gas analysis methodology have allowed detection of parts per million differences in atmos-

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pheric O_2/N_2 ratios. Keeling & Shertz (1992) were the first to report annual cycles of atmospheric oxygen concentration in the northern and southern hemispheres. Bender et al. (1996) reported similar seasonal variation in atmospheric oxygen at 2 high latitude stations in the southern hemisphere (Fig. 1).

While the well established seasonal cycle in atmospheric carbon dioxide is affected primarily by terrestrial P/R processes, the seasonal cycle in atmospheric oxygen results from biotic processes both on land and in the sea (Keeling et al. 1993). As a result, in the northern hemisphere, the annual amplitude in atmospheric oxygen concentration is approximately twice that of the seasonal cycle in carbon dioxide content (Keeling & Shertz 1992). The marine contribution to the atmospheric oxygen signal can be determined by comparing the amplitudes of seasonal atmospheric oxygen and carbon dioxide concentrations (Keeling & Shertz 1992, Keeling et al. 1993). Since there is very little seasonal signal from land in the southern hemisphere, virtually all of the annual changes in atmospheric oxygen content that result from biology can be attributed to marine organisms in this half of the globe (Keeling & Shertz 1992, Bender et al. 1996). The shape and amplitude of the annual variation in atmospheric oxygen content due to marine processes is exciting information for marine microbial ecologists, as these data can yield insights into the functional ecology of microbes in the sea integrated over longer time, and larger spatial, scales than is possible with site-specific sampling programs.

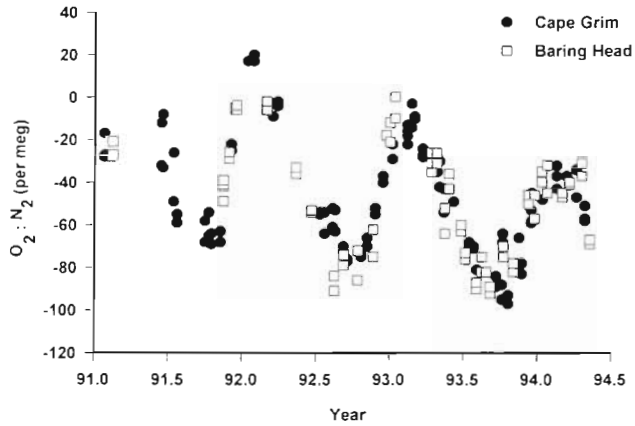

Fig. 1. Data on variation of atmospheric oxygen content over a 3 yr period at 2 sites in the southern hemisphere: Cape Grim, Tasmania (40° 41' S, 144° 41' E) and Baring Head, New Zealand (41° 20' S, 174° 50' E). Oxygen content is expressed as parts per million differences in the ratio of $O_2:N_2$ in the atmosphere (per meg) using a mass spectrometric, isotope ratio method (Bender et al. 1996). Figure based on data of Fig. 3 in Bender et al. (1996) with kind permission of Dr Michael Bender

The marine component of the annual atmospheric oxygen cycle indicates net production of oxygen in the upper water column from late winter to approximately mid-summer, and then net diffusion of oxygen into the sea, due to respiration and ventilation of subsurface oxygen-depleted water, during the rest of the year (Keeling et al. 1993). The seasonal cycle of atmospheric oxygen in the southern hemisphere, mainly due to marine processes, is asymmetrical; there is a burst of oxygen from sea to air during a 3 to 5 mo period in austral spring, and then a gradual drawdown of oxygen from air to sea during the rest of the year (Bender et al. 1996; Fig. 1). Temperature effect on oxygen saturation level in seawater is only a minor component of this cycle; most of the signal is due to biology (Keeling et al. 1993). The drawdown of atmospheric oxygen during fall and winter results from a combination of ongoing respiration in the mixed layer and ventilation of subsurface water undersaturated with oxygen due to prior organic matter decomposition. Variation in either hemispheric marine primary production or in hemispheric oceanic ventilation patterns would affect the shape and amplitude of the seasonal cycle of atmospheric oxygen concentration (Bender et al. 1996).

The atmospheric oxygen data implies a large amount of net community production during late winter/spring. By making appropriate assumptions, Keeling & Shertz (1992) estimated that the spike in atmospheric oxygen content during spring attributable to marine plant production implied an average seasonal net production of organic carbon of about 48 g C m^{-2} for the world ocean. Bender et al. (1996) calculated an average seasonal net production of 60 g C m^{-2} for the Southern Ocean. The extent to which this measure of 'seasonal net production' is related to the traditional concept of 'new production' based on exogenous nitrogen sources (Platt et al. 1992) is unclear. These values are high compared to estimates of new production based on nitrate uptake during spring blooms, suggesting production of organic matter with a C:N ratio >6 to 7. Estimates of seasonal net production based on the spring increase in atmospheric oxygen content support the contention of Sambrotto et al. (1993) that carbon fixation can become uncoupled from nitrate utilization during the bloom season.

Recent estimates of annual primary productivity in the North Atlantic and the North Pacific central gyres are in the range of 110 to 200 g C m^{-2} yr^{-1} (Table 1). Longhurst et al. (1995) estimated global oceanic productivity using a model combining satellite data on sea surface chlorophyll *a* concentrations and site-specific measures of carbon fixation in the world ocean. We calculated an average net oceanic productivity of 153 g C m^{-2} yr^{-1} from the Longhurst et al. (1995) data set. Thus the seasonal net production values estimated

Table 1. Estimates of open ocean total primary production, export production, and 'seasonal net (new) production', g C m^{-2} yr^{-1}

	Reference	Production
Annual primary production, ^{14}C clean technique	Sargasso Sea, BATS, Lohrenz et al. (1992)	110, 144
	North Pacific Central Gyre, Station ALOHA, Karl et al. (1995)	139, 198
	Global ocean average, Longhurst et al. (1995)	153
	Southern hemisphere ocean average, Longhurst et al. (1995)	135
		Mean = 146
Export production time-series sediment trap	North Pacific, VERTEX, Knauer et al. (1990)	11.4
	Sargasso Sea, BATS, Carlson et al. (1994)	9.4
	Sargasso Sea, BATS Lohrenz et al. (1992)	9.2, 9.4
	North Pacific, Station ALOHA, Karl et al. (1995)	9–13
		Mean = 10.2
'Seasonal net (new) production'	North Atlantic subsurface oxygen deficits, Jenkins & Goldman (1985)	55
	North Atlantic seasonal DIC deficit in euphotic zone, Sambrotto et al. (1993)	48
	Seasonal atmospheric oxygen increase, global average, Keeling & Shertz (1992)	48
	Southern hemisphere seasonal atmospheric oxygen increase, Bender et al. (1996)	60
		Mean = 53

from the atmospheric oxygen data are about one third of estimates of annual oceanic primary production. Measured sinking particle fluxes in the open ocean are less: 9 to 13 g C m^{-2} yr^{-1} (Table 1). Indirect estimates of total carbon consumption based on changes in dissolved organic carbon or in subsurface oxygen concentrations in seawater are higher than sinking fluxes of particulate carbon (Table 1). The atmospheric oxygen data thus support the idea that accumulation of organic carbon due to net (or new) primary production in the sea is greater than the measured sinking flux of particulate organic matter, and may involve transport or storage of non-sinking dissolved and particulate organic matter in the upper ocean (Jenkins & Goldman 1985, Sambrotto et al. 1993, Carlson et al. 1994, Michaels et al. 1994).

One challenge for marine microbial ecologists is to explain the mechanisms that establish the marked seasonal offset in P/R processes in the open ocean implied by the annual cycle in atmospheric oxygen concentration. As an approximation, for every molecule of oxygen that accumulates in the atmosphere in the spring, 1 molecule of carbon dioxide must be fixed into organic matter that is not immediately respired. Since seasonality in primary production is damped at lower latitudes, most of the seasonal offset in marine P/R should be due to biological processes in higher latitude regions of the sea (Keeling et al. 1993, Bender et al. 1996). In the spring, production is greater than respiration, causing net diffusion of oxygen into the atmosphere. During the rest of the year, respiration and advection of oxygen-depleted water to the surface are more significant than is oxygen production in the upper ocean, causing a drawdown of atmospheric oxygen into the sea (Keeling et al. 1993). Respiration is characteristic of all aerobic organisms. However, microbes, and heterotrophic bacteria in particular, are responsible for the bulk of respiratory activity in marine systems (Pomeroy 1974, Williams 1983, Pomeroy et al. 1995).

HETEROTROPHIC BACTERIA ARE MAJOR RESPIRERS IN THE SEA

In his seminal 'changing paradigm' paper, Pomeroy (1974) proposed that the bulk of respiration in the sea was due to microorganisms rather than to metazoans. Total community dark respiration is a measure of oxygen utilization by all organisms, including phytoplankton, bacteria, and heterotrophic protists. Pomeroy (1974) focused primarily on heterotrophic bacteria, and their protistan consumers, as dominant respirers in the plankton.

Although the idea that heterotrophic bacteria are the major decomposers of organic matter in the sea is an old one, study of marine heterotrophic microbes began in earnest after publication of Pomeroy's (1974) paper. Use of standard methods such as estimates of dark respiration via rate of decrease in oxygen content of seawater, along with new approaches such as epifluorescence microscopy and radiolabel tracer methods, has since allowed quantification of bacterial abundance and activity in the sea. Empirical data on the role of bacteria in marine food webs has resulted in verification of the concept that heterotrophic microbes, bacteria and their protistan grazers, shunt a large portion of phytoplankton production into regenerative pathways via respiration (Williams 1983, Ducklow et al. 1986, Cole et al. 1988, Ducklow & Carlson 1992).

A common approach to estimating the contribution of bacteria to overall plankton respiration has been comparison of rates of oxygen utilization in unfractionated and size-fractionated seawater Williams (1983) reviewed the findings of such studies and concluded that bacterioplankton (<1 µm size fraction) contributed substantially to overall dark respiration. A number of other studies have been completed since Williams' review (Table 2). In general, the results of these respiration experiments indicate that the <1 to 5 µm size fractions account for >50% of total community dark respiration (Table 2). Most of this work has been carried out in coastal systems; there are few data sets of size-fractioned respiration rates for open ocean habitats. However, there is no reason to think that bacteria would be less important in overall respiration in the open ocean compared to coastal systems (Ducklow & Carlson 1992).

To date, there is little information on seasonal variations in heterotrophic activity in the open ocean. In one such study in the open Gulf of Mexico, Pomeroy et al. (1995) reported lower respiration rates of the total microbial community in January compared to June (Table 2). Griffith & Pomeroy (1995) also found strong seasonal changes in community respiration rate in southeastern US continental shelf waters, with summer maxima and winter minima. In outer shelf water at depths of >40 m, community respiration exceeded primary production in summer (Griffith & Pomeroy 1995). Pomeroy & Wiebe (1993) concluded that this and other site-specific studies in the sea comparing respiration and photosynthesis indicate that 'microbial activity in the upper mixed layer is seasonal, with a pronounced winter-spring minimum even at a subtropical latitude'. Additional studies demonstrating low productivity of bacteria during spring blooms at high latitudes, and increasing bacterial biomass as the spring bloom progresses (e.g. Nielsen & Richardson 1989, Ducklow et al. 1993, Kirchman et al. 1993, Lignell et al. 1993), provide further evidence for Pomeroy & Wiebe's (1993) contention. These observations support the idea that the annual variation in atmospheric oxygen content reflects seasonal changes in oceanic P/R ratios due to lack of synchrony in the cycles of activity of heterotrophic microbes and of autotrophic microbes.

Table 2. Summary of recent studies of total community and bacterial (<0.8 to 5 µm size fraction) respiration in marine waters

Site	Total µg O^2 l^{-1} h^{-1}	Bacterial µg O^2 l^{-1} h^{-1}	Bacterial/total (%)	Reference
Grand Banks (Canada)		(<1–5 µm)		Smith et al. (1986)
	3.5–12.9	–	50–100%	
Georgia (USA) nearshore shelf waters		(<1 µm)		Hopkinson et al. (1989)
1.6 km	20–45	16–40	80–89%	
10 km	10–40	12–25	62–120%	
Georgia (USA) shelf waters		(<1 µm)		Griffith et al. (1990)
0–20 km	32–128	16–93	50–80%	
20–120 km	19–98	18–93	80–99%	
North Sea		(<3 µm)		Iriarte et al. (1991)
	2.6–5.1	2.1–4	70–100%	
SE USA coast and shelf waters, Caribbean Sea	0.8–12.8	–	–	Pomeroy et al. (1994)
Louisiana coast		(<1 µm)		
Shelf	7.2	3.5	49%	Biddanda et al. (1994)
Slope	3.7	0.32	9%	
Chesapeake Bay (USA)		(<3 µm)	23–89%	Sampou & Kemp (1994)
	<1–70	<1–50	Surface 56%	
Southeastern USA continental shelf				
Inner shelf	1.6–48	–	50–94%	Griffith & Pomeroy (1995)
Outer shelf	1.6–43	–		
Gulf of Mexico				
January	0–4.8	–	50–90%	Pomeroy et al. (1995)
June	<0.5–23	–		
Menai Straight, N. Wales (UK)		(<0.8 µm)	21–101%	Blight et al. (1995)
	0.7–20	0.3–12	Average 49%	

HYPOTHESES TO EXPLAIN CONTRIBUTION OF MICROBES TO SEASONAL P/R CYCLE

If in fact heterotrophic bacterioplankton are major respirers in the sea, then determining the mechanism(s) that result in the seasonal offset between community production and respiration processes in the world ocean will require understanding seasonality in activity of heterotrophic microbes. Three hypotheses that might explain why the community of heterotrophic bacteria respire at lower rates in winter/spring compared to summer/fall are presented below.

Hypothesis 1: Low temperatures in spring disproportionately suppress respiration compared to photosynthesis, thus heterotrophic bacteria grow more slowly, and respire less, during spring than during the well stratified, higher temperature conditions of late summer/early fall. ***Rationale for Hypothesis 1:*** The positive relation between temperature and metabolic processes in living organisms is formalized in the Q_{10} approximation:

$$Q_{10} = (K_1/K_2)^{10/(t1-t2)}$$

in which K_1 and K_2 are velocity or rate constants pertaining to some quantifiable aspect of metabolic activity (growth rate, feeding rate, respiration rate, etc.) at temperatures $t1$ and $t2$ (in °C), where $t1 > t2$. In general, studies of microbial processes have shown that for a 10°C rise in temperature (i.e. the exponent of the Q_{10} equation becomes 1), metabolic activity increases by a factor of 1 to 3, and on average is about 2. Of course, $t1$ and $t2$ must be equal to or below the optimum temperature for growth of a particular organism for the Q_{10} relation to be valid.

Pomeroy & Deibel (1986) suggested that the activity of bacteria in very low temperature seawater (< about 1°C) is strongly suppressed compared to that of phytoplankton growing at the same temperature. They found apparent Q_{10} values of up to 147 for a bacterial isolate from Newfoundland (Canada) coastal waters, grown in laboratory culture over the temperature range −1 to +8°C. Subsequently, Pomeroy et al. (1991) and Wiebe et al. (1992) reported that strains of bacteria isolated from Newfoundland waters grew much faster at low temperature when provided with high concentrations of organic substrate.

Other workers have not found unexpectedly high Q_{10} values for microorganisms in polar waters. Robinson & Williams (1993) reported Q_{10} values of 1 to 3 for Antarctic plankton community respiration rates measured over the temperature range −2 to +14°C. A review of available literature by Robinson & Williams (1993) indicated that several other studies of temperature response of autotrophic and heterotrophic process of Antarctic microorganisms have found Q_{10} values in the general range of 1 to 4. However, results of temperature response experiments, such as those of Robinson & Williams (1993), using *in situ* microbial communities not preadapted to the temperature range being tested should be viewed with caution. Karl (1993) concluded that, while specific bacterial isolates might show temperature responses dependent on substrate concentration, 'There is no theoretical basis for an extrapolation to mixed populations of microorganisms.' Clearly, the question of whether the *in situ* activity of heterotrophic bacteria is supressed to a greater extent by very cold water temperature, compared to the activity of phytoplankton, remains to be resolved.

Even if the metabolism of heterotrophic microbes, compared to that of phytoplankton, is not, in fact, disproportionately affected by low water temperature, temperature remains a prime suspect in the list of potential factors causing a seasonal offset in oceanic P/R processes. Pomeroy & Wiebe (1993) stated that available evidence indicates that: 'During the period of minimal temperature, microbial heterotrophic metabolism is very low, and systems tend to be autotrophic, while during the warmer periods, microbial metabolism is high and systems tend to be heterotrophic.' These authors reported that bacterial isolates from Newfoundland and the Arctic Ocean had optimum growth at 20 to 25°C, and that a variety of isolates from polar and temperate waters showed high Q_{10} near minimum temperature-substrate growth conditions.

Other studies have also demonstrated temperature control of rate of bacterial growth in marine systems. Autio (1992) found a positive relation between specific growth rate of brackish-water bacteria and temperature, with highest specific growth rate at 20°C. Shiah & Ducklow (1994) reported that Q_{10} values for bacterial production ranged from 5.0 to 8.2, and for bacterial specific growth rate from 2.2 to 4.5, when water temperature was <20°C in the Chesapeake Bay (USA). During summer, however, when water temperatures in the bay were >20°C, no significant relation between bacterial activity and temperature was found. Hoch & Kirchman (1993) made a similar observation in the Delaware Estuary (USA): bacterial specific growth rates were strongly correlated with temperature only when water temperature was less than 12°C.

Growth rates of organisms other than bacteria in the sea, e.g. heterotrophic protists, phytoplankton, and metazooplankton, are also affected by temperature. Caron et al. (1986) showed that for the marine omnivorous microflagellate *Paraphysomonas imperforata* grown in culture over the temperature range of 14 to 26°C, Q_{10} values were 2.0 to 3.2 for growth rate, and 1.4 to 4.8 for weight-specific respiration rate. Choi & Peters (1992) found Q_{10} values of 2.0 to 2.7 over a lower temperature range (−1.8 to 15°C) for ingestion and

growth rates of 2 strains of *P. imperforata* isolated from Arctic Ocean and Newfoundland coastal waters. Similar temperature responses have been demonstrated for marine ciliates (Stoecker et al. 1983, Verity 1985) Rates of carbon fixation and of dark metabolic processes by phytoplankton are also positively related to temperature (reviewed in Robinson & Williams 1993). Huntley & Lopez (1992) summarized literature reports showing a strong dependence of copepod growth rates on water temperature. Thus lower temperatures in spring compared to summer would be expected to suppress total planktonic respiration, not only respiration of heterotrophic bacteria.

Hypothesis 2: Bacterial gross growth efficiencies (GGEs) are higher in spring than in summer. Higher GGEs would imply less respiration per unit of organic carbon metabolised by bacteria in spring compared to summer. ***Rationale for Hypothesis 2:*** Respiration rates of bacterioplankton communities are determined both by overall rates of utilization of organic carbon and by the GGEs of the bacteria (Pomeroy & Wiebe 1993, Jahnke & Craven 1995). There is growing evidence that marine bacteria exhibit lower efficiencies of conversion of substrate to bacterial biomass when growing on high C:N ratio organic compounds with no external source of nitrogen. Working with bacterial isolates grown on artificially defined medium, Goldman et al. (1987) reported an inverse relationship between GGE and substrate C:N ratio. Kroer (1993) examined the relationship between these parameters using natural dissolved organic matter in an estuary near Pensacola, Florida (USA). Using mixed species assemblages of bacterioplankton from the estuary, Kroer (1993) reported that bacterial GGE varied from 26 to 61%, that bacteria growing on higher C:N ratio dissolved organic matter had lower GGEs, and that addition of ammonium increased the GGEs of the bacteria. A number of studies have demonstrated that in nutrient-limited systems, or in systems with a dominance of high C:N ratio dissolved organic matter, marine bacteria can compete successfully with phytoplankton for ammonium and, to some extent, nitrate (reviewed in Kirchman 1994).

Bacteria may also be limited by availability of phosphorus. Zweifel et al. (1993) found that bacterial growth yields were more limited by availability of inorganic phosphorus than by nitrogen in the Baltic Sea and in the northeast Mediterranean Sea, and that heterotrophic bacteria preferentially utilized inorganic nitrogen and phosphorus nutrients to support growth on the time scale of days. That bacteria can be limited by phosphorus in at least some regions of the sea has been confirmed in recent studies in the Mediterranean (Thingstad & Rassoulzadegan 1995) and in the Gulf of Mexico (Pomeroy et al. 1995).

For those regions of the sea, e.g. the temperate North Atlantic, in which nitrate and phosphate concentrations are relatively high in the upper water column at the beginning of the growing season, and low during the summer and fall, availability of inorganic nutrients and of low C:N ratio organic substrates would be expected to be greater for heterotrophic bacteria in spring compared to summer A consequently higher GGE for the bacterial community in spring would therefore contribute to overall lower respiration rates of bacteria during this period compared to later in the year when N and P were limiting. Pomeroy et al. (1995) reported lower than expected bacterial gross growth efficiencies (1 to 28%) during June in the Gulf of Mexico, at a time when bacterioplankton were subject to apparent phosphorus deficiency

For some regions of the sea, the upper water column is characterized by either continuously low nutrient concentrations, e.g. open ocean gyres, or continuously measurable nutrient concentrations, e.g. the subarctic North Pacific. In regions with little seasonal variation in surface nutrient content, bacterial GGEs would not be expected to vary due to availability of inorganic N or P. However, other factors could cause seasonal variation in GGE in bacteria. Carlson & Ducklow (1996) reported that in oligotrophic Sargasso Sea water, the average bacterioplankton GGE of 14 ± 6% was increased by addition of glucose and algal lysate, but not by addition of ammonium or phosphate, to experimental cultures. Thus quality of organic matter as well as nutrient availability could result in seasonal differences in the gross growth efficiency of marine bacteria (see Hypothesis 3).

Hypothesis 3: There are seasonal differences in quality of dissolved and particulate organic substrates available for bacterial utilization. In particular, the phytoplankton, such as diatoms, that dominate spring phytoplankton blooms may produce high molecular weight organic compounds or colloidal-sized particles that may not be immediately used by bacteria. ***Rationale for Hypothesis 3:*** New information on size classes and composition of high molecular weight dissolved and colloidal organic matter in the sea has altered previous concepts of sources of substrate for marine bacteria (reviewed in Azam et al. 1994). High molecular weight dissolved organic matter in surface seawater appears to be largely composed of polysaccharides, which may be released in large amounts during phytoplankton blooms (Benner et al. 1992). Non-sinking, or slowly sinking, organic particles can be formed from dissolved organic matter via various aggregation processes (Wells & Goldberg 1993, Kepkay 1994). Colloidal- and larger-sized organic particles must be acted upon by exoenzymes in order to be utilized by bacteria (Azam et al. 1994). Kepkay (1994)

suggested that colloidal-sized organic particles (0.2 to 2.0 µm) escape bacterial degradation due to lower bacteria-particle interception frequencies compared to larger (>2 µm) particles.

An example of non-sinking, polysaccharide-rich organic particles is the transparent exopolymer particles (TEP), which Alldredge et al. (1993) reported are abundant in seawater and to which a substantial portion of the 'free bacteria' in seawater appear to be attached. This polysaccharide material was first observed, using the same staining approach as that of Alldredge et al. (1993), by Wiebe & Pomeroy (1972). TEP appear to be mucilage particles originating from dissolved or colloidal acidic polysaccharide exudates (Passow et al. 1994). TEP can be produced in copious quantities by diatoms in culture; the average size and total area of TEP *in situ* were greater at sampling stations where diatoms dominated the phytoplankton community (Passow & Alldredge 1994).

Since diatoms often are a major component of spring phytoplankton blooms, especially at higher latitudes, it is possible that the formation of TEP and other non-sinking organic material from such blooms represents a pool of organic carbon that is slow to degrade. Passow & Alldredge (1994) observed that a large quantity of TEP formed during a flocculating diatom bloom was not accompanied by a similar increase in bacterial number. They concluded that 'TEP is not always a preferred energy source promoting growth of bacteria and no simple relationship exists between TEP and bacterial abundance.' That spring phytoplankton blooms result in storage of non-sinking organic matter in the upper water column is supported by the data of Carlson et al. (1994) on seasonal concentrations of dissolved organic carbon (DOC) in the euphotic zone of the Sargasso Sea. They found a large increase in total DOC m^{-2} from early spring to mid-summer, and then a decrease in DOC content of the upper water column from mid-summer to winter

In the post-bloom phase of the growing season, a multiple-trophic step pelagic food web typically develops which is based on production by <5 to 10 µm sized phytoplankton. Within such a food web, production of lower molecular weight, labile organic substrates, e.g. sugars and amino acids, for bacterial growth is likely, due to sloppy feeding and excretions of metazoan and protistan grazers (Jumars et al. 1989). Stimulation of bacterial activity by increased flux of low molecular weight organic compounds, combined with warmer water temperatures, might, in turn, enhance the rate of degradation of high molecular weight organic matter produced during the spring. An increase in bacterial respiration could result from a combination of increased bacterial biomass from spring to summer, as observed by Kirchman et al. (1993) in the subarctic North Pacific, and decreased bacterial gross growth efficiency during summer (see above).

The recent finding of Amon & Benner (1994, 1995) that marine microbes utilize >1000 MW (molecular weight) dissolved organic compounds more rapidly than <1000 MW compounds does not necessarily contradict the above scenario. Amon & Benner were concerned with the standing stock of dissolved organic matter, which includes a large amount of condensed, humic compounds of low molecular weight. Higher molecular weight material, presumably polysaccharides, had estimated turnover times of 5 to 20 d in warm seawater (Amon & Benner 1995), which is significantly slower than estimated turnover times (hours) of labile low molecular weight substrates such as sugars and amino acids.

Seasonality in abundance of active bacteria as one test of these hypotheses. The above hypotheses could be tested by seasonal studies in oceanic systems, that would include determination of the ratio of primary production to community respiration, of *in situ* bacterial gross growth efficiencies, and of the composition of particulate and dissolved organic matter and its rate of utilization by bacterioplankton. One prediction, based on the above hypotheses, is that there would be lower abundances of actively growing and respiring bacteria in the late winter/early spring compared to the rest of the year. As explained below, newly available methods should now allow estimation of percent active cells in natural bacterioplankton assemblages on a fairly routine basis.

A persistent problem in aquatic microbial ecology has been explaining the lack of marked seasonal variability in abundance of suspended bacteria. The percentage of metabolically active bacteria, estimated by autoradiography to detect uptake of radiolabeled substrates (Meyer-Reil 1978, Pedros-Alio & Newell 1989), or by indication of an active electron transport system (Tabor & Neihof 1982), is generally lower than total cell counts as determined by epifluorescence microscopy after staining cells with the fluorochromes acridine orange or DAPI. Because determination of activity on a cell-specific basis has been difficult to carry out, there are few data sets on seasonal cycles in abundance of metabolically active bacteria.

A further complication has been raised by Zweifel & Hagström (1995), who suggested that counts of bacterioplankton using the standard DAPI-staining direct count method might overestimate the number of live, intact bacteria. These authors showed that at salinities greater than 12‰, the fluorochrome DAPI binds poorly with DNA, but does stain bacterial cell walls in a non-selective fashion. By diluting seawater samples with freshwater, and rinsing the sample with 2-propanol to remove DAPI not bound to DNA, Zweifel & Hagström

(1995) were able to visualize DAPI-stained nucleoids (DNA-containing regions of the cell) of living bacteria. Bacteria with visible nucleoids were found to be 2 to 32% of the number of total DAPI-stained cells prepared using the standard method. Zweifel & Hagström (1995) concluded that DAPI direct counts may include a variable, often large, number of bacterial 'ghosts' which do not contain DNA.

Work in our laboratory with Oregon coastal seawater (Choi et al. 1996) substantiated the observation made by Zweifel & Hagström (1995) that only a fraction of marine bacteria contain visible nucleoids. However, we found that cells without visible nucleoids could develop nucleoids when supplied with substrate, suggesting that they are not dead, but rather dormant cells. We also compared abundances of bacteria with 'healthy' or uncompromised membranes, and with damaged membranes, in seawater using LIVE/DEAD BacLight stain (Molecular Probes, Eugene, OR, USA); and abundances of metabolically active bacteria using the fluorescent formazan compound CTC (Polysciences, Inc., Warrington, PA, USA) (Rodriguez et al. 1992). Both 'healthy' and metabolically active bacteria were a smaller component (<10% of total cell counts) than were nucleoid-containing bacteria.

Our observations support other data showing that estimates of bacterial abundance using acridine orange and DAPI direct count methods include a variable, often large, number of inactive bacterial cells. Cells with uncompromised membranes, and cells with active electron transport systems, are an even lower proportion of total counts than are cells with visible nucleoids. There may well be strong seasonal cycles in abundance of metabolically active bacteria hidden within a large and less varying population of inactive/dormant bacterial cells. The newly available fluorescent stains for detection of metabolically active bacteria make investigation of such seasonal cycles less technically difficult compared to previous methods. Determination of the number of metabolically active cells would be a useful adjunct to process measurements carried out in order to evaluate the role of heterotrophic bacteria with respect to seasonal variations in the P/R ratio of the ocean.

CONCLUSIONS

The above hypotheses are based in large part on work done with bacterial isolates that grow well in culture, and with bacterioplankton communities present in estuarine and coastal waters. Our understanding of activity of heterotrophic microbes in the open ocean in general remains limited, and there is as yet little information on such processes with respect to seasonal cycles. Recent studies carried out in the subarctic North Pacific (Kirchman et al. 1993) and in the North Atlantic and Sargasso Sea (Ducklow et al. 1993, Carlson & Ducklow 1996) have begun to address this deficiency. More detailed research on annual variations in heterotrophic processes in the ocean, particularly in higher latitude systems with strong seasonal production cycles, is needed. In particular, measurement of rates of respiration should be routinely included in studies of ocean biology, as suggested by Kepkay (1994).

On a global basis, the amount of seasonal net community production in the sea that results from the seasonal P/R cycle is calculated to be about 20 Gt C yr^{-1} (Keeling & Shertz 1992). This is a large number, equivalent to approximately 35 to 45% of the estimated 45 to 50 Gt C yr^{-1} of total annual primary productivity in the world ocean (Longhurst et al. 1995), and 10 times the annual net sequestration of atmospheric carbon dioxide by the sea. Thus, understanding the dynamics of the seasonal offset in P/R processes in the sea should be essential to understanding both how oceanic systems function and how biology interacts with chemistry in the net storage of about 2 Gt of atmospheric carbon dioxide per year in the ocean (Siegenthaler & Sarmiento 1993).

It is clear that elucidating the mechanisms that contribute to seasonality in heterotrophic processes in the upper water column is fundamental to explaining the seasonal change in oceanic P/R ratios implied by the marine component of the annual cycle in oxygen concentration in the atmosphere. In this regard, it will be necessary to evaluate seasonal changes in (1) the abundance, biomass production, and gross growth efficiencies of metabolically active bacteria; (2) the quality of organic substrates available for bacteria; and (3) rates of microbial carbon utilization and respiration in relation to rates of carbon fixation and oxygen production by phytoplankton (Pomeroy & Wiebe 1993, Jahnke & Craven 1995).

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