Vol. 41: 155–165, 1987 | MARINE ECOLOGY – PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published December 2

Activity of heterotrophic bacteria in the euphotic zone of the Celtic Sea

I. R. Joint, A. J. Pomroy

Natural Environment Research Council, Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH, England

ABSTRACT: As part of a study to determine the importance of picophytoplankton, we have used [methyl-^{3}H] thymidine incorporation as an indicator of heterotrophic bacterial production in the Celtic Sea. Over a 4 yr period, a number of measurements were made on the same day that picophytoplankton production was measured by *in situ* incubations with ^{14}C. ^{3}H-thymidine incorporation rates are not closely coupled to primary production estimates of either total phytoplankton or picoplankton. Vertical profiles of the 2 processes are very different and there is no significant correlation between ^{3}H-thymidine uptake rate and primary production at any depth or when the rates are integrated to the base of the euphotic zone. Certain correlations were found: bacterial number with chlorophyll *a*, and ^{3}H-thymidine incorporation with bacterial number. However, the most significant correlations are between bacterial production and temperature; temperature explains 62 % of the variance in bacterial number and 35 % of the variance in ^{3}H-thymidine incorporation of samples taken at all depths throughout the euphotic zone. When the data are integrated throughout the euphotic zone, temperature explains 41 % of the variance in depth-integrated ^{3}H-thymidine incorporation rate. Using recently published factors for converting ^{3}H-thymidine incorporation rates into estimates of bacterial production and data obtained in all seasons, we estimate that the production of heterotrophic bacteria is usually less than 10 %, but occasionally up to 18 %, of the picoplankton production and is less than 6 % of the total phytoplankton production. The use of other conversion factors may result in higher estimates of bacterial production, but some published factors are shown to be physiologically unreasonable.

INTRODUCTION

The role of heterotrophic bacteria in pelagic ecosystems has been the subject of considerable research in the last decade. Bacterioplankton were previously considered not very abundant and unimportant; however, with the introduction of epifluorescence microscopy we now know that bacteria are much more abundant than had been indicated by standard microbiological techniques such as plate counts. In addition, several techniques have been introduced which enable the production of heterotrophic bacteria to be estimated. Foremost amongst these is a method based on the uptake of [methyl-^{3}H] thymidine which was introduced by Fuhrman & Azam (1980) and Fuhrman et al. (1980) and subsequently critically assessed by them (Fuhrman & Azam 1982). Other methods of estimating bacterial production have also been suggested, including determining the frequency of dividing cells (Hagström et al. 1979, Newell & Christian 1981), incorporation of ^{3}H-adenine into nucleic acid (Karl et al. 1981a, b) and changes in bacterial cell numbers following dilution to reduce grazing pressure (Ducklow & Hill 1985a). However, perhaps because of the simplicity of the procedure, the incorporation of ^{3}H-thymidine is increasingly used to determine bacterial production.

The method does not give an unequivocal estimate of bacterial production despite the fact that incorporation by natural populations appears to be specific to heterotrophic bacteria; autotrophs and protozoa do not utilise ^{3}H-thymidine at the concentrations suggested by Fuhrman & Azam (1982), largely because many of these organisms lack thymine kinase (Moriarty & Pollard 1981). The uncertainties in the method arise from the factors that are used to convert incorporation rate of ^{3}H-thymidine into production of new bacterial biomass. Specifically, there are uncertainties over whether the number of cells produced per mol of ^{3}H-thymidine incorporated is constant, what the factor is to convert ^{3}H-thymidine incorporation into cell number, and even over the carbon content of bacteria from natural oceanic and shelf populations (Ducklow & Hill 1985b).

0171-8630/87/0041/0155/$ 03.00

Our own interest in estimating bacterial production arose because we were measuring the production of photosynthetic picoplankton by the incorporation of ^{14}C bicarbonate. In the Celtic Sea in summer, we found a substantial proportion (up to 30 %) of the fixed carbon was in organisms which passed through 1 μm but were retained by 0.2 μm Nuclepore filters (Joint & Pomroy 1983). The label in this small fraction might have resulted from photosynthetic fixation by picophytoplankton (< 1 μm) or, equally, from the release of label from large phytoplankton as dissolved organic carbon which could then be assimilated by heterotrophic bacteria. This label would be trapped on 0.2 μm filters after passing through 1 μm filters but would be a consequence of high heterotrophic bacterial activity and not the result of photosynthesis by picophytoplankton. To distinguish between these 2 processes, an independent estimate of bacterial activity was required and we reported (Joint & Pomroy 1983) estimates of bacteria production based on the incorporation of ^{3}H-thymidine using the method of Fuhrman & Azam (1982). These measurements suggested that heterotrophic bacterial production was much less than the amount of ^{14}C fixed in the < 1 μm fraction and we therefore concluded that production by photosynthetic picoplankton was significant in summer in the Celtic Sea.

We have subsequently carried out a large number of determinations of picophytoplankton production (Joint & Pomroy 1986, Joint et al. 1986) and on each cruise, the activity of heterotrophic bacteria was measured using the ^{3}H-thymidine technique. The purpose of this paper is to report the results of these measurements made in all seasons in the Celtic Sea. Since measurements of photosynthetic ^{14}C fixation by size-fractionated phytoplankton were also done at the same stations, we are able to examine the relationships of ^{3}H-thymidine incorporation rate with phytoplankton and picophytoplankton production and to determine which environmental factors influence bacterial activity.

METHODS

The stations studied in the Celtic Sea were those that formed a seasonal study of picophytoplankton production (Joint et al. 1986, Joint & Pomroy 1986); additional measurements were made at Stn CS2 (Joint & Pomroy 1983) in May and June 1986. Sampling procedures and data on phytoplankton production can be found in Joint & Pomroy (1983) and Joint et al. (1986). Water samples were taken with clean NIO water bottles and transferred to clean plastic carboys; care was taken not to expose the samples to light or temperature differences and all ^{3}H-thymidine incorporation experiments were begun within 20 min of sampling. The procedures adopted were designed to minimise contamination of the water samples but no attempt was made to follow the ultra-clean procedures advocated by Fitzwater et al. (1982).

Samples for enumeration of bacteria were fixed with 2.5 % (v/v) glutaraldehyde (electron microscope grade, obtained from BDH Chemicals Ltd, Poole, England, and filtered through 0.2 μm Nuclepore filters before use) and stored in clean glass bottles at 4 °C for up to 2 mo until counted. Samples were stained with DAPI (4'6-diamidino-2-phenylindole) as described by Porter & Feig (1980) and fluorescent bacteria were counted with an epifluorescence microscope by the method of Hobbie et al. (1977). The microscope used was a Leitz Ortholux II equipped with 50W HBO light source, Ploempak 2.2 fluorescence vertical illuminator with filter block A, and an NPL Fluorotar 100/1.32 oil objective lens. For each sample, 15 to 20 fields of view were counted, giving a minimum count of 300 cells sample^{-1}.

^{3}H-thymidine incorporation experiments followed the method of Fuhrman & Azam (1982). The [methyl-^{3}H] thymidine was obtained from Amersham International immediately before each cruise; experiments done in summer 1982 used ^{3}H-thymidine solutions which were diluted with unlabelled thymidine (Joint & Pomroy 1983) but subsequent experiments used ^{3}H-thymidine at specific activities of 85 to 90 Ci mmol^{-1}. Stock solutions were prepared before each cruise by diluting the radiochemical with filter-sterilized Milli-Q water; stocks were stored at 2 °C during the cruise and a fresh stock bottle was used for each experiment. All experiments were done within 3 wk of dispatch of the radiochemical from the manufacturer; under the storage conditions adopted, Amersham International suggest that decomposition should be less than 2 % mo^{-1} if stored as an aqueous solution at 2 °C.

The experimental procedure was as follows. Water samples were taken with clean NIO water bottles and 5 replicate 10 ml samples were immediately dispensed into sterile plastic tissue-culture tubes which were kept in the dark at ambient seawater temperature throughout the experiment; ^{3}H-thymidine was added to each tube to give a concentration of 5 nmol l^{-1}. As a control, electron microscope grade glutaraldehyde was added to 1 of the 5 replicate tubes taken from each depth immediately after the addition of the ^{3}H-thymidine. The samples were routinely incubated for 1 h but time-course assays showed that incorporation was linear for at least 2 h and frequently longer. At the end of the incubation period, the tubes were placed in an ice-water bath and ice-cold trichloroacetic acid (TCA) was added to give a final concentration of 5 % v/v. The samples were left in the ice bath for 15 min and then filtered through 25 mm 0.2 μm pore-size Nuclepore filters; each filter was rinsed 5 times with 1 ml 5 % ice-

cold TCA and the filters were then placed in scintillation vials and dried in a desiccator with active silica gel. The samples were counted in a liquid scintillation counter on return to the laboratory, usually within 3 wk of the experiment. Counting efficiency was determined with an external standard but this was checked in occasional vials of each series by the addition of internal standards.

RESULTS

Seasonal abundance of heterotrophic bacteria

There were no large seasonal variations in numbers of bacteria in the euphotic zone of the Celtic Sea. Fig. 1

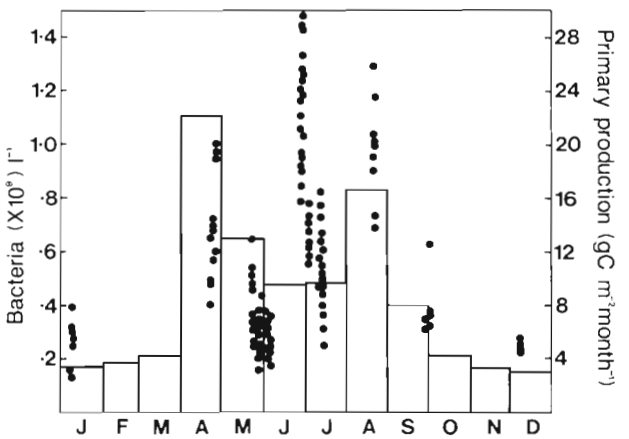

Fig. 1. Numbers of bacteria present in the surface mixed layer on 9 cruises between 1982 and 1986. Each cruise was 2 to 3 wk duration and the points show the variation in total cell number on each cruise. The histograms are estimated monthly phytoplankton production, from Joint et al. (1986)

shows numbers present on 9 cruises between 1982 and 1986, plotted as a single seasonal distribution with the monthly depth-integrated primary production estimated by Joint et al. (1986). There was less than an order of magnitude variation in bacterial number throughout the different months and the seasonal variation was of the same order of magnitude as the variation in primary production. Lowest bacterial numbers were found in January and December and there was a minimum again in May/June; highest numbers were found in late June/early July and in August. There was an increase in bacterial number at the time of maximum phytoplankton biomass and production during the spring diatom bloom in April; however, the highest numbers of bacteria occurred in July and August, when phytoplankton biomass was low but picoplankton accounted for 30 % of the primary production. Heterotrophic bacteria were 2 to 3 orders of magnitude more abundant than cyanobacteria at this time. The most conspicuous feature of heterotrophic bacterial counts was that the variation in number during a 14 to 21 d summer cruise could be of the same order as the seasonal variation. However, in winter this variability in cell numbers was greatly reduced.

Vertical profiles of ^{3}H-thymidine incorporation

The organic matter required by heterotrophic bacteria might be supplied principally by phytoplankton exudates and, indeed, in the literature it is common for bacterial production to be scaled with reference to phytoplankton production. If there was a close temporal and spatial link involving utilization by heterotrophic bacteria of the dissolved organic matter produced by phytoplankton, then the vertical profiles of the 2 processes should be similar. However, the uptake of ^{3}H-thymidine was essentially homogeneous within the surface mixed layer. Fig. 2 shows 2 typical situations. In the summer (Fig. 2a), the surface mixed layer was about 30 m deep and there was no variation in ^{3}H-thymidine uptake rate in the water column above the thermocline. Fig. 2b shows ^{3}H-thymidine uptake rates in April at the beginning of the diatom spring bloom. Surface water was warming to form a weak thermocline; chlorophyll *a* concentrations were 1.7 $\mu g\ l^{-1}$ in the surface 20 m and 0.8 $\mu g\ l^{-1}$ at 30 m. Again there is no indication of variation in ^{3}H-thymidine uptake rate with depth and incorporation rates were low. However, these vertical profiles of ^{3}H-thymidine uptake are very different to the vertical profiles of primary production. The results of a typical *in situ* determination of primary production (Fig. 3b) show a decrease in production rate

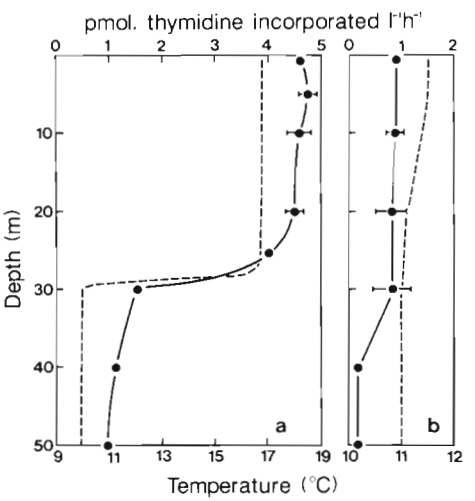

Fig. 2. Vertical profiles of temperature (---) and ^{3}H-thymidine incorporation rate (•) on (a) 2 Aug 1982 and (b) 24 Apr 1984; error bars (some of which are smaller than the symbols) represent approximate 95 % confidence intervals

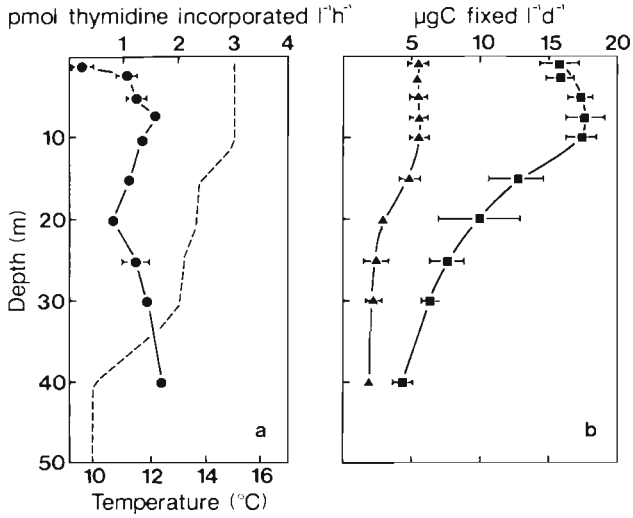

Fig. 3. (a) Vertical profiles of temperature (---) and ^{3}H-thymidine incorporation rate (●) on 5 Jul 1985. (b) Production profiles of total phytoplankton (■) and picoplankton, <1 µm (▲), determined by *in situ* incubations on 5 Jul 1985: error bars represent approximate 95 % confidence intervals

with depth, which is controlled by decreasing irradiance (Joint & Pomroy 1983).

These vertical profiles of primary production contrast with the homogeneous ^{3}H-thymidine uptake rates measured in the surface mixed layer and do not support the idea of close coupling between bacterial and phytoplankton production. However, one explanation may lie in the way in which we determine primary production. By doing *in vitro* incubations at fixed depths, we have not simulated the dispersion of phytoplankton cells that occurs due to vertical mixing. The method gives reasonable estimates of depth-integrated primary production but does not indicate how that production is mixed through the water column and, over the period of a day, the phytoplankton biomass and photosynthetic exudates may be homogeneously distributed. If the time-scale of bacterial utilization of photosynthate is similar to that of vertical mixing, then the supply of dissolved organic carbon to the water column may indeed be homogenous within the surface mixed layer.

However, there are occasions when the vertical water column structure does allow a direct comparison to be made. During periods of calm weather in the Celtic Sea, secondary thermal structure develops at intermediate depths within the euphotic zone, as a result of warming of the surface waters. Such a vertical structure offers the opportunity to determine a relationship between bacterial and phytoplankton populations since there is a separation of microbial populations by the pycnoclines. Phytoplankton production is greatest in the waters nearest to the surface and, if we assume that maximum DOC production occurs with maximum photosynthetic rate, higher rates of bacterial activity might be expected at these depths. Fig. 3a shows data from July 1985, when the rate of ^{3}H-thymidine incorporation in the 2 layers was very similar. However, the highest rates of primary production were measured in the surface water above the first pycnocline and were significantly greater than those between 15 and 30 m (Fig. 3b). At this time of greatly reduced wind-driven mixing, there was clear evidence that ^{3}H-thymidine uptake was inhibited in the surface 2 m. More significantly, the rate of ^{3}H-thymidine incorporation by the surface bacterial population was no different from that by bacteria taken from 25 m, where primary production was half that in the surface 10 m. Fig. 3b also shows the carbon fixation by organisms which pass through a 1 µm pore-size Nuclepore filter; this is the fraction which we have ascribed to picophytoplankton (Joint & Pomroy 1983, 1986, Joint et al. 1986). The vertical production profile of this <1 µm fraction is similar to the total phytoplankton fraction and is very different from the profile of ^{3}H-thymidine incorporation. If the ^{14}C label incorporated into the <1 µm fraction was largely due to assimilation by heterotrophic bacteria of labelled organic matter exuded by larger phytoplankton, then the vertical profile of ^{3}H-thymidine incorporation should be the same as the 'picoplankton' profile of ^{14}C incorporation. There does not appear to be a close relationship between the rate of ^{3}H-thymidine incorporation and the rate of ^{14}C incorporation into phytoplankton cells. Although we have assumed that DOC production was proportional to carbon fixation into phytoplankton cells, in July 1985, DO^{14}C was not measured. However, we have measured DO^{14}C production on other occasions (Joint & Pomroy 1983) and found very close correspondance. Therefore, on the basis of vertical profiles of ^{3}H-thymidine and ^{14}C uptake rates, there is no evidence of a close coupling of the 2 processes since high rates of ^{3}H-thymidine uptake do not occur in water which has the highest primary production rates.

Thymidine incorporation rate and relationship with temperature, phytoplankton biomass and production, and bacterial numbers

The data from ^{3}H-thymidine incorporation determinations done at all depths within the euphotic zone were compared with a number of other parameters in a regression analysis; the parameters used were bacterial number, temperature and chlorophyll *a* (as an indicator of phytoplankton biomass, which is presumed to be one source of organic matter for the bacteria). Since phytoplankton production was strongly influenced by depth and ^{3}H-thymidine incorporation was independent of depth, it was inappropriate to attempt to relate these 2 rates. Table 1 shows the results of a correlation analysis. The most significant correlation was between tempera-

Table 1. Results of correlation analysis of ^{3}H-thymidine incorporation rate, bacterial number, temperature and chlorophyll *a*; units are $\log_e$ pmol ^{3}H-thymidine incorporated l^{-1} h^{-1}, $\log_e$ number of bacteria l^{-1}, °C, and µg chlorophyll *a* l^{-1} p: significance level of the F ratio with 1,186 degrees of freedom

Correlation	r^2 (%)	F ratio	p
$\log_e$ ^{3}H-thymidine · temperature	35.10	100.75	≦0.001
$\log_e$ ^{3}H-thymidine : chlorophyll	7.46	14.99	≦0.001
$\log_e$ ^{3}H-thymidine : $\log_e$ number	35.79	103.67	≦0.001
Temperature chlorophyll *a*	3.47	6.69	≦0.01
$\log_e$ number : temperature	61.93	302.51	≦0.001
$\log_e$ number : chlorophyll *a*	18.81	43.09	≦0.001

ture and number of bacteria; 62 % of the variance in number was explained by the variance in temperature. ^{3}H-thymidine incorporation was also significantly correlated ($p \leq 0.001$) with temperature (r^2 = 35 %) and with numbers of bacteria in the sample (r^2 = 36 %). Bacterial number and ^{3}H-thymidine incorporation were both correlated with chlorophyll *a* ($p \leqq 0.001$). Fig. 4 shows the regression of $\log_e$ bacterial number and $\log_e$ ^{3}H-thymidine incorporation rate against temperature.

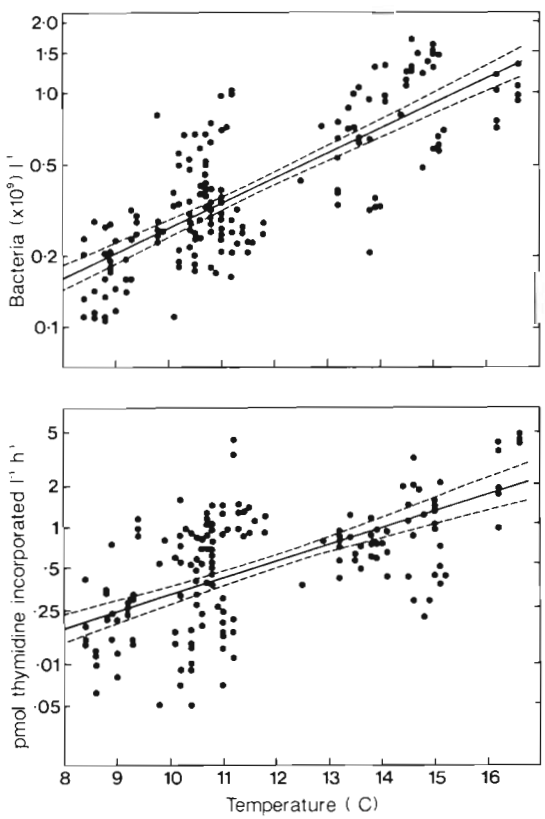

Fig. 4. Relation between $\log_e$ number of bacteria and temperature (upper) and between $\log_e$ ^{3}H-thymidine incorporation rate and temperature (lower) for all occasions when bacterial production was measured (n = 189). Approximate 95 % confidence funnels (- - -) are indicated

Although temperature explains 35 % of the variance in ^{3}H-thymidine incorporation, other factors must influence bacterial activity. In a multiple regression analysis, there was only one case when a second variable effected a significant reduction in variance; bacterial numbers resulted in an additional 1.4 % reduction in variance in a multiple regression of ^{3}H-thymidine incorporation rate against temperature and bacterial number.

Relationship between depth-integrated ^{3}H-thymidine incorporation rate, temperature and primary production

The different vertical profiles of phytoplankton production and ^{3}H-thymidine incorporation rate suggest that there is no close temporal coupling of the 2 processes and they are not significantly correlated. However, phytoplankton production might still be the major source of organic matter for bacteria if the time scale of bacterial utilization was long compared with the vertical mixing time. In this case, the products of phytoplankton production would be homogeneously dispersed within the euphotic zone and would result in homogeneous bacterial production within the surface mixed layer. There should then be a correlation between the depth-integrated primary production rate and the depth-integrated ^{3}H-thymidine incorporation rate. Table 2 shows the correlation analysis for every date between 1982 and 1986 when primary production and ^{3}H-thymidine incorporation were determined on the same day. This is a subset of the whole data set

Table 2. Results of correlation analysis of ^{3}H-thymidine incorporation rate, phytoplankton production and picoplankton production, all depth-integrated to base of euphotic zone; the temperature of the euphotic zone was homogeneous on each occasion. Units are: $\log_e$ ^{3}H-thymidine incorporation rate (µmol thymidine incorporated m^{-2} d^{-1}), $\log_e$ phytoplankton and $\log_e$ picoplankton production (mgC m^{-2} d^{-1}). p: significance level of the F ratio with 1,24 degrees of freedom; ns: not significant

Correlation	r^2 (%)	F ratio	p
$\log_e$ ^{3}H-thymidine: temperature	41.19	16.81	≦0.001
$\log_e$ ^{3}H-thymidine: $\log_e$ phytoplankton	6.66	1.71	ns
$\log_e$ ^{3}H-thymidine: $\log_e$ picoplankton	4.247	1.068	ns
$\log_e$ phytoplankton: temperature	0.21	0.05	ns
$\log_e$ picoplankton: temperature	5.00	1.27	ns
$\log_e$ picoplankton: $\log_e$ phytoplankton	45.33	19.90	≦0.001

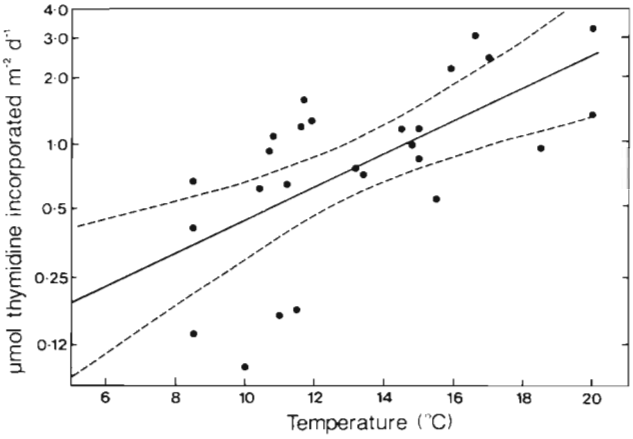

Fig. 5. Relation between $\log_e$ ^{3}H-thymidine incorporation rate, integrated to base of the euphotic zone, and temperature (n = 26). Approximate 95 % confidence funnels (- - -) are indicated

because data could only be used when the surface mixed layer was the same as the euphotic depth and temperature was uniform within the euphotic zone. The only significant correlation found was again between depth-integrated ^{3}H-thymidine incorporation and temperature (Fig. 5); temperature accounted for 41 % of the variance in depth-integrated ^{3}H-thymidine incorporation. There was no correlation between either depth-integrated phytoplankton or picophytoplankton production and depth-integrated ^{3}H-thymidine incorporation rate.

DISCUSSION

The seasonal amplitude in numbers of bacteria present in the euphotic zone of the Celtic Sea is small. Similar observations have been made by many others (e.g. Admiraal et al. 1985, Davis et al. 1985, Ducklow 1986), in a variety of environments from estuaries to the oceans which have widely varying rates of bacterial production. Since standing stock is a balance between growth of the bacteria and grazing losses, this uniformity in bacterial numbers from so many aquatic environments is increasingly taken as evidence for significant protozoan grazing (e.g. Ducklow & Hill 1985a).

A significant relation (F-test; $p \leqq 0.001$) was found between bacterial number and chlorophyll and this has also been previously reported for both fresh and marine waters (Fuhrman et al. 1980, Linley et al. 1983, Bird & Kalff 1984). A regression analysis of $\log_{10}$ transformed data of bacterial number and chlorophyll *a* concentration gave:

$$\log_{10} \text{bacterial number} = 5.683 + 0.416 \log_{10} \text{chl } a$$

where bacterial number = no of bacteria ml^{-1}; chl *a* = µg chlorophyll $a\,l^{-1}$. This equation is very similar to that reported by Malone et al. (1986) for Chesapeake Bay in July and August but the slope is approximately half that reported by Bird & Kalff (1984). However, chlorophyll *a* explains only 30 % of the variance in our bacterial counts, compared with 88 % for the analysis of Bird & Kalff (1984) and 83 % for Malone et al. (1986). Nevertheless, there is a clear indication that the numbers of bacteria present in freshwater and marine systems is controlled by unknown trophic relationships of which chlorophyll *a* concentration is a good indicator.

Fuhrman et al. (1980) suggested that bacterial production, as measured by the incorporation of ^{3}H-thymidine, was also correlated with phytoplankton biomass. However, in this study, we have found no evidence of a direct relationship between ^{3}H-thymidine incorporation and phytoplankton biomass (Table 1) or production (Table 2). Indeed, there is clearly a marked difference in the vertical profiles of phytoplankton and bacterial production, with most primary production occurring in the surface 10 m where irradiance is high. The data presented in Fig. 3 clearly fail to demonstrate a tight coupling between phytoplankton and bacterial production; the temporary pycnocline, which developed at 10 to 15 m within the seasonal surface mixed layer, resulted in a physical separation of the water masses between 15 to 30 m and that above 10 m. Primary production in the upper 10 m was approximately double that in the lower zone of the surface mixed layer, yet incorporation rates of ^{3}H-thymidine were very similar. One explanation for the observations made at this time of greatly reduced depth of vertical mixing might be that ^{3}H-thymidine incorporation was inhibited by longer exposure to sunlight than a population might receive with a 30 m deep mixed layer. Sieracki & Sieburth (1986) reported that sunlight increased the lag-phase of natural bacterial population by 2 to 4 h in summer. There was a clear indication that the ^{3}H-thymidine incorporation in the near surface (< 2 m) was inhibited (Fig. 3) but it seems unlikely that sunlight-inhibition might influence the whole population in the surface 10 m.

However, such effects should not apply to the analysis involving depth-integrated phytoplankton and bacterial production rates (Table 2). By examining the total production within the euphotic zone, we have eliminated any differential effects of vertical gradients. Yet, again there is clearly no correlation between total bacterial production and total phytoplankton production. This is at variance with many statements in the literature which have suggested a close link between the 2 processes (Cole et al. 1982, Larsson & Hagström 1982, Chrost & Faust 1983, Lancelot & Billen 1984, Ducklow & Hill 1985a, Ducklow 1986, Malone et al. 1986).

The most significant correlations found in this study have been with temperature. Both bacterial abundance (r^2 = 62 %) and ^{3}H-thymidine incorporation rate were highly correlated (r^2 = 35 %, Table 1) as were depth-integrated ^{3}H-thymidine incorporation rate (r^2 = 41 %, Table 2). Other workers have reported a significant effect of temperature; Wright & Coffin (1983), working in a salt-marsh estuary, and Väätänen (1980), working in the Baltic, found significant relations between bacteria and temperature. Wright & Coffin suggested that the effect was indirect and related to the influence of temperature on *Spartina* exudate production, which could then be utilized by the bacteria. Stn CS2 in the Celtic Sea is far removed from any allochthonous organic sources from rivers and land. An indication of the magnitude of this supply can be obtained from values of surface salinity; the mean annual surface salinity at Stn CS2 is 0.3 % less than the surface salinity at the shelf break (Bowden 1955) and, even if organic matter derived from rivers shows conservative behaviour, the potential terrestrial supply is clearly insignificant. Therefore, the relationship between bacterial production and temperature must reflect processes occurring *in situ* and is unlikely to be a temperature effect on allochthonous organic sources.

Do these correlations with temperature indicate a direct effect of temperature on ^{3}H-thymidine incorporation? In their study of bacteria in the Hudson River plume, Ducklow & Kirchman (1983) showed a 4-fold increase in ^{3}H-thymidine incorporation when water temperature was raised from 3.5 to 18°C, but full details of the temperature response of the population were not given. We measured the effect of temperature on ^{3}H-thymidine incorporation by a natural assemblage from International Oceanographic Stn E1, in the English Channel (about 150 km from the Celtic Sea), when the ambient temperature was 10.6°C (Fig. 6). This temperature response is typical of many organisms, with an optimum temperature between 15 and 20°C and rapidly decreasing activities at temperatures greater than 22°C and less than 8°C. The optimum temperature response of this assemblage is very similar to the sea surface temperature range in this region (9 to 18°C; Pingree 1980). The Q_{10} value of this curve about ambient temperature, (9 to 12°C) was 2.7 but the data for the range 6 to 22°C clearly did not fit a typical exponential temperature response curve; below 8°C and above 12°C, the rate of ^{3}H-thymidine incorporation decreased more than would be expected from a typical Q_{10} response, suggesting that marine bacteria might have a restricted temperature range.

Support for this comes from data obtained with a culture of a marine psychrophilic *Pseudomonas* (Harder et al. 1984). This bacterium showed optimum growth at 14°C and maximal growth at 20°C and

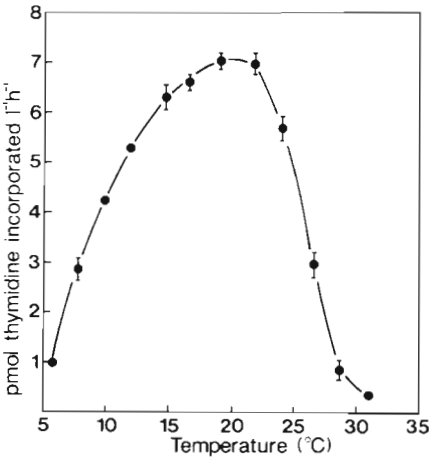

Fig. 6. Effect of temperature on ^{3}H-thymidine uptake rate of a natural assemblage from Stn E1 in the English Channel on 4 Jun 1987: error bars (some of which are smaller than the symbols) represent approximate 95 % confidence intervals

appeared to exibit a very similar temperature response to the assemblage shown in Fig. 6. These authors showed that there were 2 different physiological responses of the bacterium to temperatures lower and higher than the optimum. At lower temperatures, there was an increase in RNA content which resulted from an increase in ribosome content; it was postulated to result from a need for the bacterium to sustain the same rate of protein synthesis at the lower temperature as it did at the optimum. Above the optimum temperature, there was also a significant increase in RNA content but this was considered to be a compensation for impairment of the protein synthesis mechanism. At temperatures above the maximum, thermal damage prevented a further increase in cellular RNA and growth rate decreased. Significantly, there was no variation in cellular DNA content with temperature; if there had been, it is doubtful if the thymidine incorporation technique could be used to determine the production of natural assemblages which experience seasonal variations in temperature.

These results are of relevance to natural populations of marine bacteria and to methods of measuring bacterial production that rely on assumptions about the RNA content of bacteria (Karl et al. 1981a). If the population present in the summer, at temperatures of 18 to 20°C, is composed of the same bacterial species as occur in the winter and spring, then the cells will be growing under sub-optimal conditions and the efficiency with which organic substrates are utilized will be greatly reduced. On the basis of the data of Harder et al. (1984), it does not appear likely that a bacterial species will be able to adapt to different temperature because protein synthesis appears to be incapable of adapting. Therefore, in a nutrient-limited environment,

bacteria not growing at their optimum temperature would be at a disadvantage compared with bacteria at their optimum. It may be that the summer assemblage is composed of different species or strains of bacteria which have higher temperature optima. If this is the case, there would have to be a succession of marine bacterial strains or species throughout the season and a mechanism by which bacteria could survive when temperatures were not optimal.

Finally, how do these rates of ^{3}H-thymidine translate into bacterial production? We suggested that bacterial production was low in August 1982 (Joint & Pomroy 1983) and that heterotrophic bacterial production was generally less than 10 % of the production of photosynthetic picoplankton (< 1 µm). These estimates were based on the conversion factors suggested by Fuhrman & Azam (1982) of 1.7×10^{18} cells produced per mol thymidine incorporated and a carbon content of 1.7×10^{-15} g carbon cell^{-1}. Ducklow & Hill (1985b) rightly pointed out that our interpretation depended on these conversion factors and they suggested that our conclusion about the importance of picophytoplankton might not be valid if larger conversion factors were applied to the ^{3}H-thymidine incorporation data. The factor determined by Ducklow & Hill (1985b) from parallel changes in cell number and thymidine incorporation was 4×10^{18} cells produced per mol ^{3}H-thymidine, i.e. 2.4 times greater than the factor used by Joint & Pomroy (1983). The second conversion factor – the carbon content of bacterial cells – is more problematic since it is impossible to make carbon content measurements on bacteria from natural populations. Estimates of carbon content must, therefore, depend on extrapolation from laboratory cultures of large cells to the small cells that are commonly found in the sea. There are 2 problems; firstly, how to precisely measure bacterial volume and, secondly, whether it is appropriate to extrapolate from laboratory cultures.

We have estimated cell volume in 3 different ways, none of which is entirely satisfactory. Measurements from photographs of epifluorescence images gave a mean cell diameter of 0.26 µm for bacteria from the surface mixed layer (Joint & Pomroy 1983). However, we have subsequently found that the apparent size of bacteria is a function of intensity of fluorescence. We measured the dimensions of individual cells from a culture of *Synechococcus*, strain DC2, under 2 epifluorescent conditions; the diameter of cells stained with DAPI was approximately 60 % of the diameter of the same cells when viewed under optimum conditions for phycoerythrin fluorescence, a much brighter fluorescence than DAPI. Therefore, intensity of fluorescence influences the measured size of bacteria. In all natural populations examined, some cells appear much fainter than others but this could reflect a number of physiological causes and may not be related to cell size; measurements of cell size based on fluorescence must be treated with caution.

The second method we used was scanning electron microscope. The median diameter of a Celtic Sea bacterial assemblage was 0.335 µm. Sample preparation for scanning electron microscopy involves dehydration of the specimen and some shrinkage is possible (Fuhrman 1981), although Norland et al. (1987) have argued that small bacteria would shrink less than large bacteria because their small dimensions would make them more rigid. Estimates from scanning electron micrographs inevitably include some cyanobacteria, which are larger than heterotrophic bacteria, and may bias the estimate.

The final method we have used attempted to overcome the uncertainties over identification by using transmission electron microscopy, since cyanobacteria can be readily distinguished from heterotrophic bacteria on the basis of cell morphology (Warwick & Joint 1987). The median diameter of heterotrophic bacteria determined by this method was 0.22 µm. However, this method is also subject to a number of potential errors (Warwick & Joint 1987).

The 3 independent methods estimate the median diameter of heterotrophic bacteria in the Celtic Sea to be between 0.22 and 0.335 µm. Using the largest estimate gives a median volume of 0.0197 µm^{-3}, assuming that all cells are spherical. A wide range of carbon-to-volume conversion factors have been applied in estimations of bacterial activity. One of the highest is due to Bratbak (1985) who derived a factor of 0.56 pgC µm^{-3}; this is significantly greater than other published estimates which are usually in the range 0.05 to 0.1 pgC µm^{-3} (Norland et al. 1987). It has been supported by recent work of Lee & Fuhrman (1987) which suggested a value of 0.38 pgC µm^{-3} Taking these factors, the median carbon content of the Celtic Sea bacteria would be 11×10^{-15} gC with Bratbak's factor, 7.5×10^{-15} with the factor of Lee & Fuhrman; however, the estimate would be 1 to 2×10^{-15} gC cell^{-1} with the factors summarised by Norland et al. (1987), and very similar to the factor suggested by Fuhrman & Azam (1982) and that used by Joint & Pomroy (1983).

Table 3 compares bacterial production estimates derived from conversion factors of 4×10^{18} cells produced per mol ^{3}H-thymidine (Ducklow & Hill 1985b) and 2×10^{-15} gC cell^{-1} (Norland et al. 1987) with total euphotic zone phytoplankton and picophytoplankton production estimated on the same day; the data were obtained between 1982 and 1986, but are listed by calendar month for convenience. Using these factors, bacterial production was never more than 6 % of total phytoplankton production and never more than 18 % of the production in the picoplankton fraction (< 1 µm).

Table 3. Total euphotic zone production estimates for heterotrophic bacteria, total phytoplankton and picophytoplankton (< 1 μm), expressed as mgC m^{-2} d^{-1}, for data obtained from 1982 to 1986. Bacterial production is shown as a percentage of total phytoplankton production (A:B) and of picophytoplankton production (A:C), using the carbon content per cell of 2 fgC $cell^{-1}$ Columns D:C and E:C show the percentage of the picoplankton production that would be due to heterotrophic bacteria using conversion factors of 7.5 fg C $cell^{-1}$ (Lee & Fuhrman 1987) and 11 fg C $cell^{-1}$ (Bratbak 1985)

Date	(A) Bacterial production (mgC m^{-2} d^{-1})	(B) Phytoplankton production (mgC m^{-2} d^{-1})	A:B (%)	(C) Picoplankton production (mgC m^{-2} d^{-1})	A:C (%)	D:C (%)	E:C (%)
April							
9 Apr 1984	3.36	349	1.0	71	4.7	17.7	26.0
14 Apr 1983	1.12	544	0.2	101	1.1	4.2	6.1
16 Apr 1983	5.44	606	0.9	89	6.1	22.9	33.6
22 Apr 1984	7.44	796	0.9	77	9.7	36.2	53.1
24 Apr 1984	5.25	943	0.6	92	5.7	21.4	31.4
26 Apr 1984	12.72	717	1.8	79	16.1	60.4	88.6
May							
23 May 1986	5.04	704	0.7	80	6.3	23.6	34.7
28 May 1984	1.36	601	0.2	206	0.7	2.5	3.7
29 May 1984	1.44	382	0.4	148	1.0	3.6	5.3
31 May 1986	8.72	313	2.8	68	12.8	48.1	70.5
June							
2 Jun 1986	9.60	308	3.1	72	13.3	50.0	73.3
6 Jun 1986	10.16	264	3.9	112	9.1	34.0	49.9
28 Jun 1985	9.36	805	1.2	125	7.5	28.1	41.2
29 Jun 1985	7.92	685	1.2	107	7.4	27.8	40.7
July							
1 Jul 1985	6.88	828	0.8	189	3.6	13.7	20.0
4 Jul 1985	9.44	561	1.7	219	4.3	16.2	23.7
6 Jul 1985	4.56	441	1.0	156	2.9	10.9	16.1
August							
21 Aug 1982	24.48	542	4.5	193	12.7	47.6	69.7
22 Aug 1982	17.44	528	3.3	199	8.8	32.9	48.2
September							
29 Sep 1982	5.84	98	6.0	33	17.7	66.4	97.4
October							
2 Oct 1982	6.24	139	4.5	47	13.3	49.8	73.0
December							
10 Dec 1983	<0.8	99	<0.8	13	<6.2	<23.1	<33.8

Table 3 also lists the percentage of picoplankton production that would be due to heterotrophic bacteria using the carbon:volume ratios of Lee & Fuhrman (1987) and Bratbak (1985). These larger conversion factors result in estimates of heterotrophic bacterial production which account for a large proportion of the ^{14}C fixed by picoplankton. This would mean that the large cyanobacterial population was very unproductive but such a result would be consistent with the hypothesis of close coupling of bacterial utilization of algal exudates.

However, our data do not support this hypothesis. If there was closely coupling, there should be a strong correlation between ^{3}H-thymidine uptake rates and the incorporation of ^{14}C into the picoplankton (< 1 μm) fraction. No such relationship was observed (Table 2). We have other data which suggest that the ^{14}C label in the < 1 μm fraction of the production experiments is due to photosynthetic fixation by picophytoplankton. We have demonstrated the presence of photosynthetic picoplankton and both cyanobacteria and small (< 1 μm) eukaryotic phytoplankton are abundant (Joint & Pipe 1984). We have demonstrated that the photosynthetic characteristics of natural populations are consistent with photosynthetic ^{14}C fixation by picophytoplankton and have used the maximum rate of photosynthesis (P_m^B) to estimate a realistic doubling time of the cyanobacteria component of the picoplankton of ca 8.5 h under saturating irradiance (Joint & Pomroy 1986); this compares with a recent measurement of ca 9.7 h for the doubling time of a culture of marine *Synechococcus* by Kana & Glibert (1987). All these observations are consistent with our original conclusion (Joint & Pomroy 1983) that picophytoplankton production in the Celtic Sea is significant but are inconsistent with the hypothesis that a major fraction of the ^{14}C

found in organisms <1 µm arises from heterotrophic uptake of exudates from larger phytoplankton.

The carbon : volume ratios of Bratbak (1985) and Lee & Fuhrman (1987) appear too high. The smallest cell measured by Lee & Fuhrman (1987) had a mean volume of 0.036 μm^3, a carbon content of about 20 fg C per cell and a carbon : nitrogen ratio of about 4. Therefore, each cell must contain 20 fg C, 5 fg N and 36 fg water, giving a density of 1.69 g cm^{-3}. This is a minimum estimate, since we have not include the mass of oxygen, phosphorus etc, which is present in every cell and the density must be greater than 1.8 g cm^{-3}. This is similar to the density of a diatom but is impossible for a cell which contains no mineral phases; it is also inconceivable that carbon and nitrogen can make up 40 % of the *wet* weight of a bacterial cell. Therefore, on the basis of cellular physiology, it does not seem appropriate to use factors as high as those proposed by Bratbak (1985) and Lee & Fuhrman (1987) in any estimates of bacteria production. We urgently need direct quantification of the carbon content and biovolume of natural populations of heterotrophic marine bacteria; until those data are available, studies which attempt to use the incorporation of ^{3}H-thymidine to obtain unequivocal estimates of the proportion of primary production utilized by bacteria, including the present one (Table 3), must be interpreted with caution.

Acknowledgements. This work forms part of the Shelf-Sea Ecology Programme of the Institute for Marine Environmental Research, a component of the U.K. Natural Environment Research Council.

LITERATURE CITED

Admiraal, W., Beukema, J., Es, F. B. van (1985). Seasonal fluctuations in the biomass and metabolic activity of bacterioplankton and phytoplankton in a well-mixed estuary: the Ems-Dollard (Wadden Sea). J. Plankton Res. 7: 877–890

Bird, D. F., Kalff, J. (1984). Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. aquat. Sci. 41. 1015–1023

Bowden, K. F. (1955). Physical oceanography of the Irish Sea. Fishery Invest. Lond. Ser. II, 18 (8): 1–67

Bratbak, G. (1985). Bacterial biovolume and biomass estimations. Appl. environ. Microbiol. 49: 1488–1493

Chrost, R. H., Faust, M. A. (1983). Organic carbon release by phytoplankton: its composition and utilization by bacterioplankton. J. Plankton Res. 5: 477–493

Cole, J. J., Likens, G. E., Strayer, D. L. (1982). Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnol. Oceanogr 27: 1080–1090

Davis, P. G., Caron, D. A., Johnson, P. W., Sieburth, J. McN. (1985). Phototrophic and apochlorotic components of picoplankton and nanoplankton in the North Atlantic: geographic, vertical, seasonal and diel distributions. Mar. Ecol. Prog. Ser. 21 15–26

Ducklow, H. (1986). Bacterial biomass in warm-core Gulf Stream ring 82-B: mesoscale distributions, temporal changes and production. Deep Sea Res. 11/12: 1789–1812

Ducklow, H. W., Hill, S. M. (1985a). The growth of heterotrophic bacteria in surface waters of warm core rings. Limnol. Oceanogr. 30: 239–259

Ducklow, H. W., Hill, S. M. (1985b). Tritiated thymidine incorporation and the growth of heterotrophic bacteria in warm core rings. Limnol. Oceanogr. 30: 260–272

Ducklow, H. W., Kirchman, D. L. (1983). Bacterial dynamics and distribution during a spring diatom bloom in the Hudson River plume, USA. J. Plankton Res. 5: 333–355

Fitzwater, S. E., Knauer, G. A., Martin, J. H. (1982). Metal contamination and its effect on primary production measurements. Limnol. Oceanogr. 27: 544–551

Fuhrman, J. A. (1981). Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescence microscopy compared to scanning electron microscopy. Mar. Ecol. Prog. Ser. 5: 103–106

Fuhrman, J. A., Azam, F. (1980). Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. environ. Microbiol. 39: 1085–1095

Fuhrman, J. A., Azam, F. (1982). Thymidine incorporation as a measure of heterotrophic bacterioplankton production in surface waters: evaluation and field results. Mar. Biol. 62: 1–12

Fuhrman, J. A., Ammerman, J. W., Azam, F. (1980). Bacterioplankton in the coastal euphotic zone: distribution, activity and possible relationships with phytoplankton. Mar. Biol. 60: 201–207

Hagström, Å., Larsson, U., Höstedt, P., Normark, S. (1979). Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl. environ. Microbiol. 37: 805–812

Harder, W., Dijkhuizen, L., Veldkamp, H. (1984). Environmental regulation of microbial metabolism. In: Kelly, D. P., Carr, N. G. (ed.) The microbe 1984: II. Prokayotes and eukaryotes. Cambridge Univ, Press, Cambridge, p. 51–95

Hobbie, J. E., Daley, R., Jasper, S. (1977). Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. environ. Microbiol. 33: 1225–1228

Joint, I. R., Pipe, R. K. (1984). An electron microscope study of a natural population of picoplankton from the Celtic Sea. Mar. Ecol. Prog. Ser. 20: 113–118

Joint, I. R., Pomroy, A. J. (1983). Production of picoplankton and small nanoplankton in the Celtic Sea. Mar Biol. 77: 19–27

Joint, I. R., Pomroy, A. J. (1986). Photosynthetic characteristics of nanoplankton and picoplankton from the surface mixed layer. Mar. Biol. 92: 465–474

Joint, I R., Owens, N. J. P., Pomroy, A. J. (1986). The seasonal production of picoplankton and nanoplankton in the Celtic Sea. Mar. Ecol. Prog. Ser. 28: 251–258

Kana, T M., Glibert, P. M. (1987). Effect of irradiances up to 2000 µE $m^{-2} s^{-1}$ on marine *Synechococcus* WH 7803. Growth, pigmentation, and cell composition. Deep Sea Res. 34: 479–495

Karl, D. M., Winn, C. D., Wong, D. C. L. (1981a). RNA synthesis as a measure of microbial growth in aquatic environments. I. Evaluation, verification and optimization of methods. Mar. Biol. 64: 1–12

Karl, D. M., Winn, C. D., Wong, D. C. L. (1981b). RNA synthesis as a measure of microbial growth in aquatic environments. II. Field applications Mar Biol. 64: 13–21

Lancelot, C., Billen, G. (1984). Activity of heterotrophic bacteria and its coupling to primary production during the

spring phytoplankton bloom in the southern bight of the North Sea. Limnol. Oceanogr. 29: 721–730

Larsson, U., Hagström, Å. (1982). Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic eutrophication gradient. Mar. Biol. 67: 57–70

Lee, S., Fuhrman, J. A. (1987). Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. environ. Microbiol. 53: 1298–1303

Linley, E. A. S., Newell, R. C., Lucas, M. I. (1983). Quantitative relationships between phytoplankton, bacteria and heterotrophic microflagellates in shelf waters. Mar Ecol. Prog. Ser. 12: 77–89

Malone, T C., Kemp, W. M., Ducklow, H. W., Boynton, W. R., Tuttle, J. H., Jonas, R. B. (1986). Lateral variation in the production and fate of phytoplankton in a partially stratified estuary. Mar. Ecol. Prog. Ser. 32: 149–160

Moriarty, D. J. W., Pollard, P. C. (1981). DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser. 5: 151–156

Newell, S. Y., Christian, R. R. (1981). Frequency of dividing cells as an estimator of bacterial productivity. Appl. environ. Microbiol. 42: 23–32

Norland, S., Heldal, M., Tumyr, O. (1987). On the relation between dry matter and volume of bacteria. Microb. Ecol. 13: 95–101

Pingree, R. D. (1980). Physical oceanography of the Celtic Sea and English Channel. In: Banner, F. T., Collins, M. B., Massie, K. S. (ed.) The north-west European shelf seas: the sea bed and the sea in motion. II. Physical and chemical oceanography and physical resources. Elsevier, Oceanography Series 24B, Amsterdam, p. 415–465

Porter, K. G., Feig, Y S. (1980). The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25: 943–948

Sieracki, M. E., Sieburth, J. McN. (1986). Sunlight-induced growth delay of planktonic marine bacteria in filtered seawater. Mar. Ecol. Prog. Ser. 33: 19–27

Väätänen, P. (1980). Effects of environmental factors on microbial populations in brackish waters off the southern coast of Finland. Appl. environ. Microbiol. 40: 48–54

Warwick, R. M., Joint, I. R. (1987). The size distribution of organisms in the Celtic Sea: from bacteria to Metazoa. Oecologia (Berl.) 73: 185–191

Wright, R. T., Coffin, R. B. (1983). Planktonic bacteria in estuaries and coastal waters of northern Massachusetts: spatial and temporal distribution. Mar. Ecol. Prog. Ser. 11: 205–216

This article was submitted to the editor; it was accepted for printing on September 25, 1987