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BIOLOGICAL STUDIES IN THE VICINITY OF A SHALLOW-SEA TIDAL MIXING FRONT III. SEASONAL AND SPATIAL DISTRIBUTION OF HETEROTROPHIC UPTAKE OF GLUCOSE

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CONTENTS

Heterotrophic incorporation and respiration of ^{14}C-labelled glucose (tracer approach) and natural concentrations of glucose were measured, as part of a multidisciplinary research project, on five cruises in the western Irish Sea from March to September 1980. The investigations were carried out along a transect across a shallow-sea tidal mixing front and its adjacent stratified and vertically mixed water masses. The spatial

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distribution pattern in relation to hydrographical conditions, diurnal changes observed at drogue stations, and seasonal developments are described. High turnover rates of [^{14}C]glucose were strongly associated with stratification, both spatially as well as seasonally, starting with low rates early in spring at the beginning of stratification, increasing to maximum rates in July after the phytoplankton bloom, and subsequently declining in autumn when stratification weakened. Turnover rates were consistently and significantly higher in the waters above the pycnocline than below it or in the vertically mixed water masses to the east of the front. No distinct diurnal rhythms were recognized. The mixed water column, in particular, was totally uniform in heterotrophic uptake of glucose whereas the surface of the stratified water showed greater variability. A fairly constant proportion of, on average, 32% of the glucose carbon was respired. Natural glucose concentration ranged from less than 30 to 322 nM, mean 116 nM. No particular pattern in its distribution could be detected in the different water masses despite considerable changes in use of glucose. Turnover rates of glucose were unrelated to numbers of bacterial cells or their biomass. Glucose uptake per bacterial cell (uptake index) was estimated and showed pronounced seasonal increase during summer in the surface stratified water mass, especially, in the vicinity of the front. The importance of the surface waters of the stratified water body and the frontal zone in respect to carbon flux and potential bacterial biomass production is discussed.

1. Introduction

Shallow-sea tidal mixing fronts separating vertically mixed and thermally stratified waters in shelf seas (Simpson & Pingree 1978) have been noted as areas of importance for biological activity (Pingree *et al.* 1978; Holligan 1981; review by Owen 1981). The increased phytoplankton standing stock, often observed in connection with frontal zones, suggests that heterotrophic processes may be similarly enhanced and may play an important role in the utilization and recycling of dissolved organic material within this boundary region. Observations at a plume front indicate that the planktonic bacterial population may be influenced by this hydrographical feature (Floodgate *et al.* 1981). Parsons *et al.* (1983), however, found no evidence for enhanced bacterial activity at a tidal front. Although detailed physical and phytoplankton studies have been carried out at these sea water–sea water interfaces, there is an obvious lack of understanding of heterotrophic processes associated with them.

The present investigation was carried out at the tidal front in the western Irish Sea, which has been extensively studied from the oceanographic and phytoplankton point of view (Simpson *et al.* 1978; Simpson & Bowers 1979; Foster *et al.* 1976; Beardall *et al.* 1982; Richardson *et al.* 1985). As long as this seasonal front is stable it separates water masses that are distinct in their chemical and biological characteristics. This provides an opportunity, rare in the dynamic marine environment, to identify the different water régimes and sample them repeatedly to follow the seasonal changes in heterotrophic activity of distinct water masses.

The aim of this investigation was to study the effects of the establishment of the front and the thermocline in the western Irish Sea on heterotrophic activity. The seasonal developments in the adjacent and quite different water masses (stratified and mixed) were followed and compared, and the importance of the frontal zone itself for heterotrophic processes has been considered. This research was carried out as part of a multi-disciplinary research project which included investigation of phytoplankton and nutrient distribution (Fogg *et al.* 1985*a*), bacterial distribution (Egan & Floodgate 1985), zooplankton abundance (Scrope-Howe & Jones 1985), distribution and uptake of urea (Turley 1985), the statistical analysis of the data (Kassab *et al.* 1985) and general discussion by Fogg *et al.* (1985*b*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

2. Methods

Measurements of heterotrophic activity were carried out on six cruises during 1980 on the R.V. *Prince Madog* in the region of the western Irish Sea front. This tidal mixing front and its adjacent mixed and stratified water bodies were sampled along a line of fixed stations, 4–6 km apart, crossing the front at right angles. The dates of cruises, geographical positions of all stations, details of the drogue stations, the general sampling scheme, and all hydrographical, chemical and phytoplankton data referred to here are given by Fogg *et al.* (1985*a*).

(*a*) *Glucose concentration*

Glucose concentration in sea water was measured according to Hicks & Carey (1968). The natural glucose concentration was found to be close to the limit of detection and it was necessary to amend the method slightly. Distilled deionized water, irradiated with high intensity u.v. light for 15 h, was used for the preparation of reagents as well as in the required dilution of samples with distilled water. The analysis of each water sample consisted of triplicate measurement on the sample and on three standards of increasing glucose concentration added to the sample. A blank, routinely determined for each sample, was prepared with filtered sea water u.v.-irradiated as described above and was analysed in the same way as an experimental sample with the full complement of reagents. This blank was more adequate for samples with glucose concentrations close to background levels than a blank that lacks one of the essential enzymes or ATP. Apart from the above the method was as described in the original paper. The natural concentration of glucose in the sample was calculated from the slope of the curve of added standards and the difference between sample and blank. The 95% confidence interval of measurements was 12 nmol l^{-1}; the lower limit of detection was 30 nmol l^{-1}.

(*b*) *Uptake of glucose*

The heterotrophic utilization of ^{14}C-labelled glucose was determined by the tracer approach as developed by Williams & Askew (1968), Williams (1970) and discussed by Wright and Burnison (1979). The method requires the addition of a labelled substrate with high specific activity in trace amounts at a single substrate concentration in order not to alter significantly the natural uptake rate. In the present investigation the amount of labelled glucose added (7.7 nmol l^{-1}) was less than 10% of the average natural glucose concentration measured in the western Irish Sea (116 nmol l^{-1}), thus generally complying with the theoretical methodological requirements. The uptake rates of glucose were found to be linear with incubation time over a period of 8 h (sampled in 30 min intervals) in samples from both the mixed and the stratified waters of the Irish Sea. After this initial period uptake deviated slightly from the linear time course, which was followed up to 24 h (sampled at 5 h intervals). Thus during the initial incubation period no major changes in metabolic activity seem to occur indicating that during the standard incubation time of 4.5 h an uptake rate close to natural conditions was measured (Williams & Askew 1968; Billen *et al.* 1980).

Triplicate samples were collected in sterile 100 ml glass bottles to each of which 0.2 ml of uniformly labelled high specific activity [^{14}C]glucose (260 mCi $mmol^{-1}$) (Amersham) was added. Final concentration of tracer in the sample was 2 μCi l^{-1} and 7.7 nmol l^{-1}. One bottle was fixed immediately with 0.5 ml neutral buffered formalin for blank determination. The samples were incubated in the dark for 4.5 h in a shipboard incubator at sea surface

temperature, they were terminated by addition of 0.5 ml of neutral buffered formalin, and stored at ambient temperature until further processing in the laboratory.

The amount of $^{14}CO_2$ respired by the micro-organisms was determined by a modification of the method described by Hobbie & Crawford (1969). In the laboratory, the fixed sample was transferred to a 500 ml wide-necked screw-top glass bottle. A scintillation vial containing a piece of 2×8 cm accordion-folded chromatography paper (Whatman no. 1) soaked with 0.25 ml of 2-phenolethylamine for CO_2 absorption was suspended in the air space above the sample, 1 ml of 2 M H_2SO_4 was injected into the sample, and the bottle was closed immediately and made airtight. After 24 h the scintillation vial was removed from the bottle and the trapped $^{14}CO_2$ was measured by the liquid scintillation technique with Aquasol (New England Nuclear) as scintillation medium. The channels ratio method was used for quench correction (Herberg 1965). This procedure resulted in a consistent efficiency of $^{14}CO_2$ capture of 53.3% (standard deviation, 2.82) for a wide range of $^{14}CO_2$ concentrations as determined by addition of known amounts of [^{14}C]bicarbonate.

After the CO_2-trapping procedure the samples were filtered through membrane filters (pore size 0.2 μm) under low vacuum (below 8 kPa), which were rinsed with filtered sea water, placed in scintillation vials, and counted as described above. Loss of free low molecular mass intracellular compounds may result from acidification. Yet uptake measurements with [^{14}C]glucose seemed to be less affected (Ramsey 1976) than with other substrates like amino acids (Griffiths *et al.* 1974). It has to be pointed out, though, that the material retained on the membrane filter represents the incorporation into the macromolecular fraction (Baross *et al.* 1975).

The turnover rate (reciprocal of turnover time) is calculated from the proportion of the total added [^{14}C]glucose taken up per day, that is, the sum of labelled substrate retained on membrane filters and captured as CO_2. This is a measure of the fraction of total natural available glucose used by the microbial population per time unit:

$$1/T = \frac{(^{14}CO_2 + {}^{14}C_{inc})}{(^{14}C_{add} \times t)}$$

$^{14}CO_2$ = labelled carbon released as CO_2 (disintegrations per minute); $^{14}C_{inc}$ = incorporated labelled carbon (disintegrations per minute); $^{14}C_{add}$ = labelled glucose added (disintegrations per minute); t = incubation time/day; $1/T$ = turnover rate/d^{-1}.

(*c*) *Derived variables*

Actual glucose uptake is estimated by multiplying the turnover rate by the glucose concentration in the sample. In the present investigation an average constant glucose concentration was assumed for all samples (table 3) (see discussion). Thus, the turnover rate was multiplied by a constant factor (mean value of natural concentration 116 nmol l^{-1} plus added tracer 7.7 nmol l^{-1} = 123.7 nmol l^{-1}), to give a general estimate of the actual amount of glucose turned over in the different water masses.

Glucose uptake index is the quotient of actual glucose uptake and the number of bacterial cells per millilitre. The bacterial counts are taken from Egan & Floodgate (1985).

The stratification parameter, $\bar{V}$, which indicates the energy required per unit depth to bring about complete vertical mixing of the water column, is calculated according to Simpson *et al.* (1977).

(*d*) *Statistical treatment*

With progressive thermal stratification of the water column in summer, different water masses can be distinguished by their densities. Hence, using appropriate density ranges for each cruise (table 1) it was possible to separate the surface layers of the stratified water mass (SSW) from the bottom layers (BSW) and the vertically mixed waters (MW) (see also Fogg *et al.* 1985*a*, figure 3). This method of distinguishing between water masses was found to classify correctly the majority of observations in the stepwise discriminant analysis given in Kassab *et al.* (1985). To test for differences in heterotrophic activity between SSW and the other two water masses untransformed data from SSW and combined BSW and MW were subjected to two-tailed Student's *t*-tests. These tests were carried out for each individual cruise, all data from the transect and accompanying drogue stations being combined.

TABLE 1. DENSITY OR TEMPERATURE CHARACTERISTICS FOR EACH PERIOD OF INVESTIGATION, USED TO DEFINE THE WATER MASSES ASSOCIATED WITH THE WESTERN IRISH SEA FRONT

(As depicted in Fogg *et al.* (1985*a*) figure 3).

	water masses separated by density/σ_t	
date	surface stratified (SSW)	bottom stratified and mixed (BSW + MW)
12–13 March	26.717–26.812	26.812–26.966
29 April–1 May	26.436–26.701	26.702–26.932
3–6 June	25.860–26.369	26.370–26.695
15–17 July	25.729–26.001	26.001–26.501

	water masses separated by temperature/°C		
	surface stratified (SSW)	bottom stratified (BSW)	mixed (MW)
23–24 September	13.54–13.78	12.12–12.82	13.81–14.56

The relations between the variables are nonlinear and the variables tend not to follow normal distribution. Thus, Spearman rank non-parametric correlation statistics were applied to all observations for each individual cruise (transect and drogue stations combined) for glucose uptake data and some other relevant measurements kindly supplied by co-workers of the group: density, chlorophyll *a* by Fogg *et al.* (1985*a*); bacterial numbers by Egan & Floodgate (1985); zooplankton numbers by Scrope-Howe & Jones (1985); urea uptake by Turley (1985). A comprehensive statistical treatment is given in Kassab *et al.* (1985).

3. RESULTS

(*a*) *Turnover rates*

A summary of heterotrophic glucose uptake measurements for each cruise is given in table 2. Turnover rates of ^{14}C-labelled glucose measured during the individual cruises show distinct differences along the transect across the front (figure 1*a*–*g*) closely related to the density structure of the water mass (see Fogg *et al.* 1985*a*) (table 5).

In early spring, 12–13 March (figure 1*a*, *b*), when stratification started to develop west of station 6, turnover rates were uniformly low. This was observed on transects carried out on

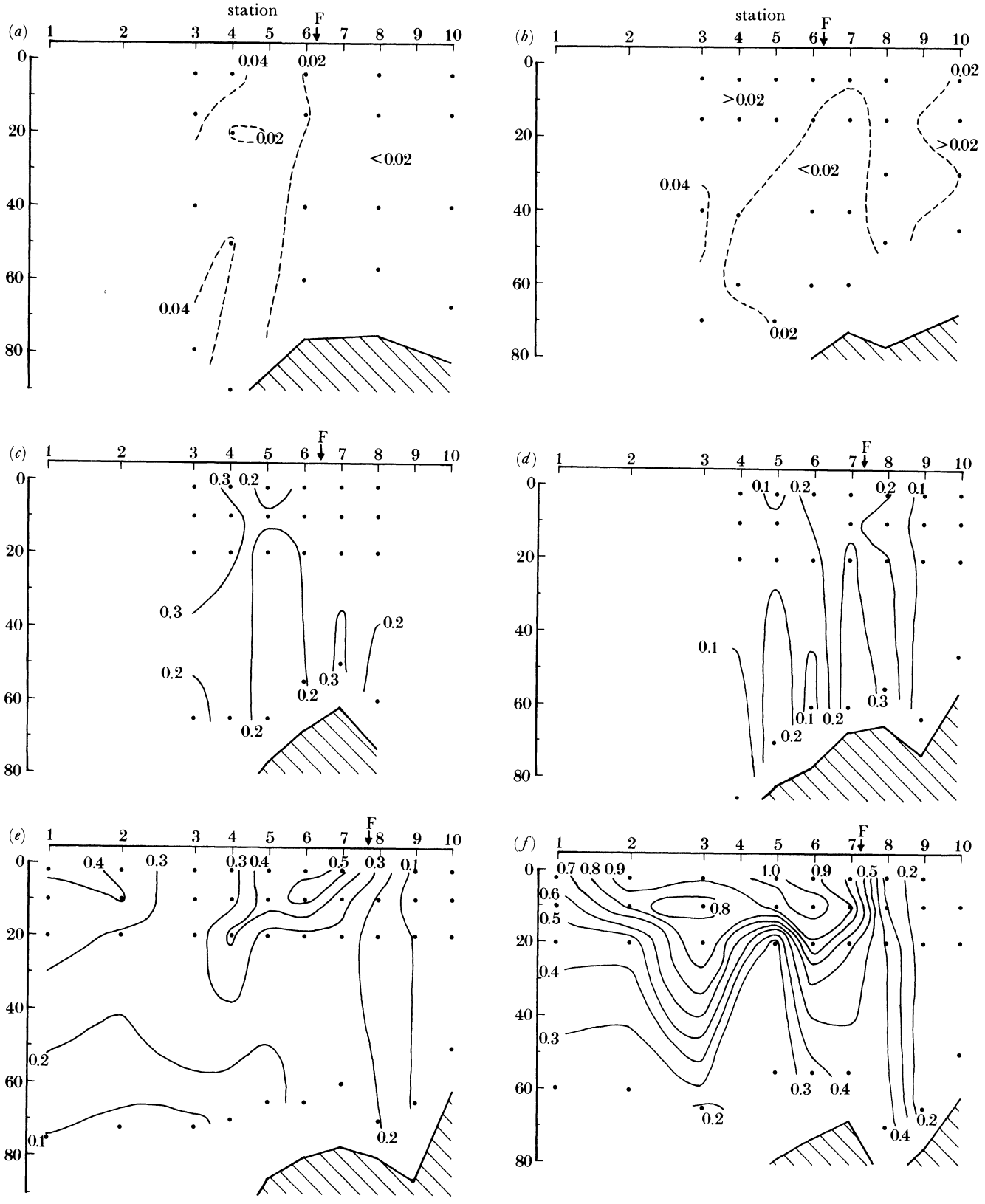

FIGURE 1 (*a–f*). For description see facing page.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

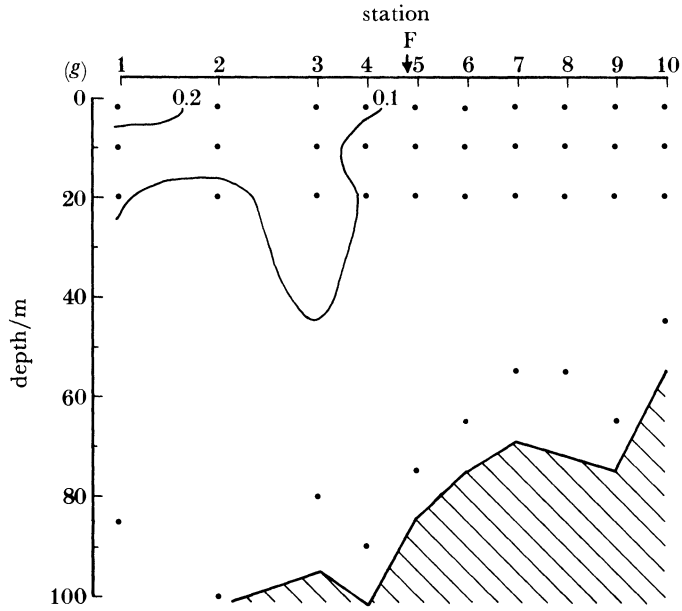

FIGURE 1. Section across the front along the line of stations shown in figure 1 in Fogg *et al.* (1985*a*). Isopleths indicate the distribution of glucose turnover rates (d^{-1}) on (*a*) 12 March, (*b*) 13 March, (*c*) 30 April, (*d*) 1 May, (*e*) 3 June, (*f*) 15 July and (*g*) 23 September. The sampling depth (●), sea bottom (hatching) and position of front (F) are indicated.

two consecutive days. When repetitive transects are run, as during this cruise and the following cruise in April, tidal movements of the water mass probably account for some lateral shift in uptake pattern as no tidal corrections were applied to the data.

One month later, 30 April to 1 May (figure 1*c*, *d*), thermal stratification had strengthened west of a front located between stations 6 and 7 and turnover rates increased by a factor of ten compared with the previous month. The depth profile reveals that the pattern of glucose uptake in both sections across the front was irregular. However, on average, the turnover rates were twice as high in the surface stratified waters than in waters below the pycnocline or in the mixed region (table 2).

At the beginning of June (figure 1*e*), a well-established pycnocline at 30–40 m depth was found west of the front between stations 7 and 8. Turnover rates were closely related to the density structure of the water mass and higher in the surface stratified water. Highest turnover values were found close to the front on the stratified side while the most strongly stratified central parts of the water mass around station 3 seemed less heterotrophically active.

Maximum turnover rates occurred in the subsequent cruise, 15 July (figure 1*f*), again on the stratified side of the front. As observed in June, the turnover in the frontal zone was higher by a factor of 5 than in the bottom and mixed waters, while the rates in the bulk of the stratified surface water were on average only two to three times faster than in the bottom and mixed waters.

Two months later, 23 September (figure 1*g*), the stratification had been partly broken down because of reduced solar surface heat input and increased wind mixing. Hence, only a weak front was observed between stations 4 and 5. Glucose turnover rates were much less and the correlation with density structure is less significant (table 5).

In a cruise attempted later in autumn, 10 October, only station 10 in the mixed water could

be sampled owing to severe gales. However, the turnover rates found at this station (table 2) were similar to values found the previous month and in early spring.

(*b*) *Glucose concentration*

Glucose concentrations were determined in randomly selected samples in the cruises from June to September (table 3). Measurements carried out in April could not supply absolute

TABLE 2. SUMMARY OF GLUCOSE UPTAKE DATA

(For each period of investigation data were pooled according to water masses, as defined in table 1. SSW is the surface stratified water, BSW is the bottom stratified water, MW is the vertically mixed water mass, n is the number of observations, $\bar{x}$ is the mean, s.d. is the standard deviation, min. is the minimum value, max. is the maximum value, n.a. is not available.)

		glucose turnover rate (d^{-1})					actual glucose uptake ($ng\ l^{-1}\ h^{-1}$)			
date	water mass	n	$\bar{x}$	s.d.	min.	max.	$\bar{x}$	s.d.	min.	max.
12–13 March	SSW	14	0.029	0.010	0.019	0.047	27	9	18	43
	BSW	13	0.025	0.013	0.011	0.048	23	12	10	45
	MW	23	0.016	0.005	0.009	0.025	15	4	8	24
30 April to 1 May	SSW	34	0.46	0.36	0.067	1.357	430	335	62	1259
	BSW	14	0.20	0.14	0.012	0.518	184	127	12	481
	MW	23	0.18	0.10	0.043	0.330	170	95	40	306
3 June	SSW	33	0.32	0.12	0.103	0.529	295	107	96	491
	BSW	19	0.16	0.07	0.053	0.291	147	62	49	270
	MW	30	0.07	0.04	0.033	0.206	67	38	31	191
15 July	SSW	32	0.69	0.23	0.138	1.089	644	213	128	1011
	BSW	15	0.29	0.19	0.134	0.617	271	178	124	746
	MW	18	0.19	0.11	0.098	0.502	178	99	91	466
23 September	SSW	13	0.13	0.06	0.034	0.243	120	57	31	225
	BSW	4	0.06	0.02	0.033	0.077	56	18	30	71
	MW	23	0.04	0.02	0.013	0.064	33	14	12	59
10 October	MW	4	0.08	0.009	0.066	0.088	72	8	62	82

		glucose uptake index (10^{-8} × (nanograms per cell per hour))					respired glucose carbon (percentage of total uptake)				
		n	$\bar{x}$	s.d.	min.	max.	n	$\bar{x}$	s.d.	min.	max.
12–13 March	SSW	14	8.2	4	2	15	14	25.7	4.8	17.5	34.0
	BSW	13	5.8	4	1	16	13	28.3	5.2	19.5	39.0
	MW	22	7.4	7	2	36	23	29.1	4.3	21.0	36.0
30 April–1 May	SSW	33	79.2	107	4	595	34	30.2	6.1	20.5	43.0
	BSW	14	42.8	56	1	214	14	30.4	4.3	24.0	38.0
	MW	23	16.8	29	1	131	23	28.9	3.7	23.5	36.5
3 June	SSW	33	42.2	46	4	212	33	32.0	3.9	25.5	40.5
	BSW	19	18.5	29	2	124	19	34.4	4.4	28.0	45.0
	MW	30	9.3	8	1	28	30	33.2	4.6	22.0	40.0
15 July	SSW	31	131.0	123	20	690	32	30.1	5.1	23.0	45.0
	BSW	15	59.0	53	5	233	15	33.2	5.0	27.5	41.0
	MW	18	70.3	48	7	189	18	31.6	3.9	25.0	37.0
23 September	SSW	13	28.5	32	9	131	13	35.2	2.7	30.0	40.0
	BSW	4	45.5	27	19	80	4	38.4	3.2	34.5	41.0
	MW	23	15.3	15	3	68	23	39.9	4.0	33.0	48.0
10 October	MW	n.a.	n.a.	n.a.	n.a.	n.a.	4	30.9	2.1	29.0	33.5

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

values because of unreliable blank determinations. However, the relative values were uniform indicating that differences in glucose concentration between the various water masses were small on this occasion. Concentrations measured during the other cruises were patchy. They ranged over the whole season from under 30 to 322 nM with a mean of 116 nM, which is similar to concentrations found by Meyer-Reil *et al.* (1979), Billen *et al.* (1980), Bölter (1981), Gocke *et al.* (1981). In July strongest variation in glucose concentration was observed caused by some irregularly high measurements. However, in none of the cruises any particular spatial differences were apparent. There was no significant difference in glucose concentration between different water masses as shown by *t*-tests (table 4) and no significant correlation with turnover rate (April–May cruise: $r^2 = 0.0383$, $n = 13$; June cruise: $r^2 = 0.0063$, $n = 32$). Also during diurnal cycles no relation of glucose concentration to turnover rate (June cruise: $r^2 = 0.312$, $n = 4$, stratified water mass; $r^2 = 0.005$, $n = 4$, mixed water mass) or any of the other measured variables could be found.

TABLE 3. NATURAL GLUCOSE CONCENTRATIONS (NANOMOLES PER LITRE)

(For symbols see heading of table 2).

date	water mass	n	$\bar{x}$	s.d.	min.	max.
3 June	SSW	8	31	1.6	< 30	34
	BSW	3	37	8.7	< 30	47
	MW	4	49	20.7	< 30	77
15 July	SSW	14	122	92	< 30	310
	BSW	5	80	40	40	124
	MW	10	133	108	< 30	322
23 September	MW	4	40	10	31	51

Mean of all analysed samples: 116 nmol l^{-1}.

(*c*) *Glucose uptake index*

The population density of bacteria (Egan & Floodgate 1985) was not always related to glucose uptake (table 5). Thus glucose uptake per cell showed considerable spatial and seasonal variation (figure 2*a–g*, table 2).

In March (figure 2*a*, *b*) the uptake of glucose per bacterial cell was uniformly low throughout the water column on both transects. Owing to great variation in bacterial numbers in April–May uptake indices were very irregular and changed considerably from the first leg of the cruise to the second leg two days later (figure 2*c*, *d*). In June (figure 2*e*) high glucose uptake indices were confined to the surface stratified water. The high values at station 1 in this and the following cruise were related to influence from Irish coastal water (Foster *et al.* 1976; Fogg *et al.* 1975*a*) and it was repeatedly observed that this station differs from the other ones in several biological characteristics (Scrope-Howe & Jones 1985; Turley 1985). Another region of especially high uptake indices was found in the frontal zone. A similar observation was made in July (figure 2*f*) when highest glucose uptake per cell occurred close to the front. The previously low uptake index in the mixed water was increased during this cruise. Much lower and more homogeneous values were observed in September (figure 2*g*) throughout the water masses.

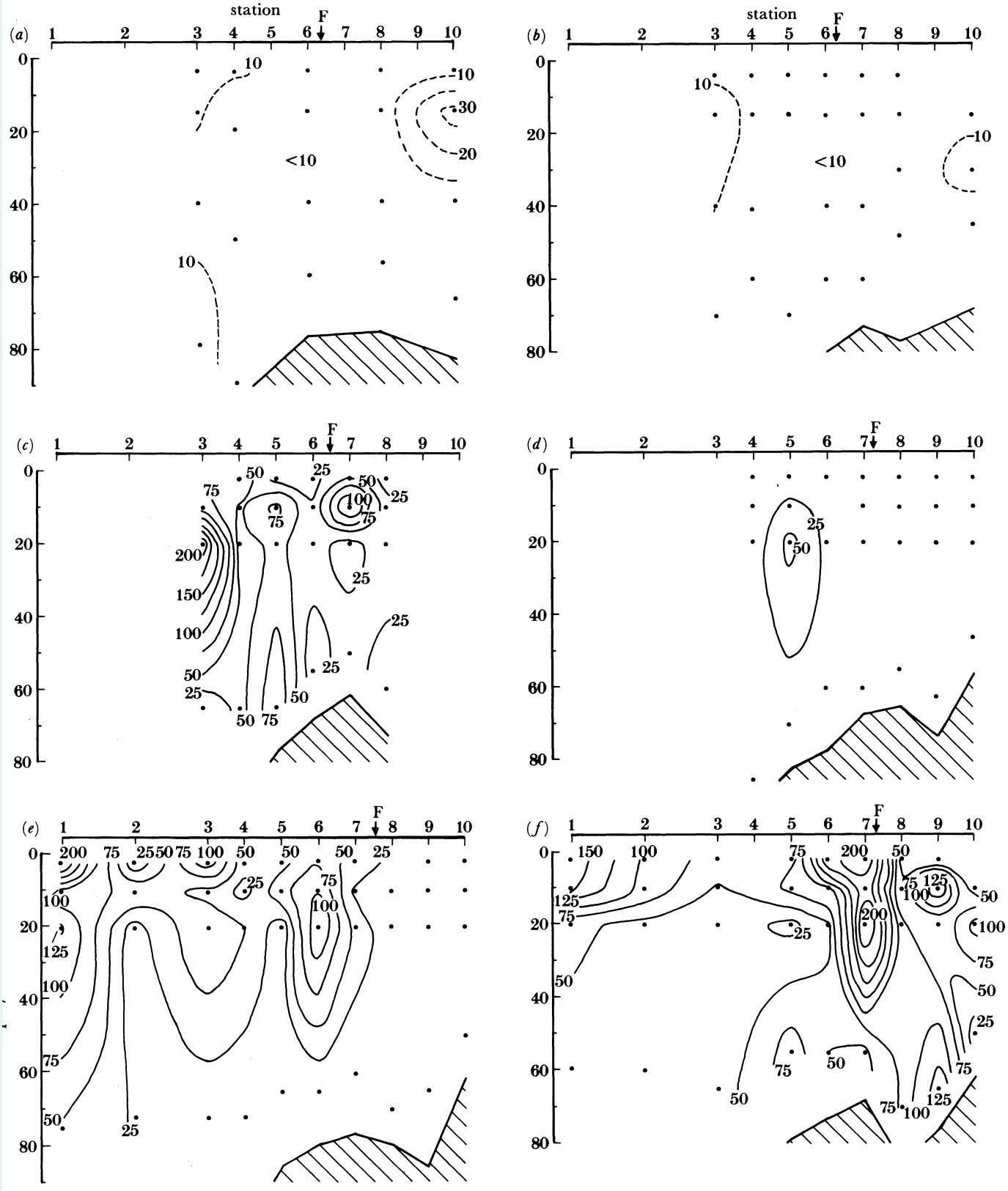

FIGURE 2 (*a–f*). For description see facing page.

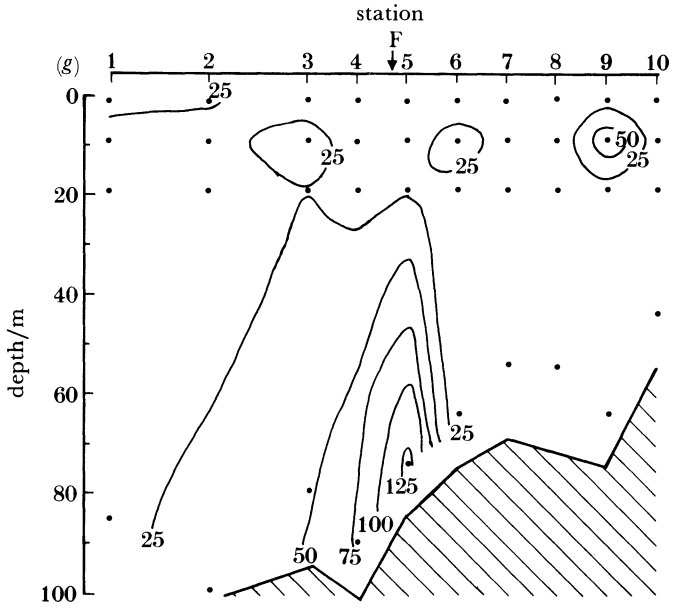

FIGURE 2. Section across the front along the line of stations shown in figure 1 in Fogg *et al.* (1985*a*). Isopleths indicate the distribution of glucose uptake indices $10^{-8} \times$ (nanograms per cell per hour) on (*a*) 12 March, (*b*) 13 March, (*c*) 30 April, (*d*) 1 May, (*e*) 3 June, (*f*) 15 July and (*g*) 23 September. The sampling depth (●), sea bottom (hatching) and position of front (F) are indicated.

(*d*) *Respiration of glucose carbon*

The percentage of carbon released as carbon dioxide by respiration of glucose showed only small variation (table 2). On average during the whole investigation 31.7% (total range: 17.5–48.0%) of substrate carbon taken up by micro-organisms is released as CO_2. This means that approximately 68% of glucose carbon was assimilated by the micro-organisms. Similar ranges and constancy of percentage of respired glucose carbon were reported by Hobbie & Crawford (1969), Carney & Colwell (1976), Williams & Yentsch (1976), Billen *et al.* (1980), Bölter (1981), Itturiaga & Zsolnay (1981) and Riemann *et al.* (1982).

There was a slight tendency for a gradual increase in the percentage of respired glucose carbon as the year advances. In March and in September there was a statistically significant difference between the lower respiration in the SSW compared with the higher one in the BSW and MW (table 4). Furthermore, respiration was negatively correlated with chlorophyll *a* during the summer (table 5).

There are some indications that the percentage of respired glucose carbon may be influenced by the amount of nutrients available (Gocke 1976) or sea water temperature (Tison & Pope 1980). This effect may be caused by more rapid isotope equilibrium within the cellular pool at higher metabolic rates, which will be particularly noticeable when short incubation times are used (King & Berman 1984). On the whole, the differences in respiration rate were small and sometimes ambiguous so that any further conclusions regarding their physiological or ecological implications are unjustified.

(*e*) *Drogue stations*

Diurnal changes in glucose uptake were monitored in mixed and stratified water masses which had been marked by drogued buoys (figure 3–7). These measurements were necessary to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

establish the extent of short-term diurnal fluctuations in comparison with the cross-frontal differences in heterotrophic activity.

Although great care was taken to remain within a certain body of water it became obvious from the hydrographical data that this was not successful in all cases. The stratified station 1, which has already been noted as anomalous, occupied on 29 April (figure 3) showed some changes in the salinity of the surface water indicating an influence of less saline inshore water during the first hours of the drogue station, and a decrease in the strength of stratification as shown by the stratification parameter $\bar{V}$. Large variations in turnover rate and in uptake index were encountered on this occasion and very high values were observed during the first hours of sampling. These observations caused the high mean and maximum values for glucose turnover time and uptake index in April shown in table 2.

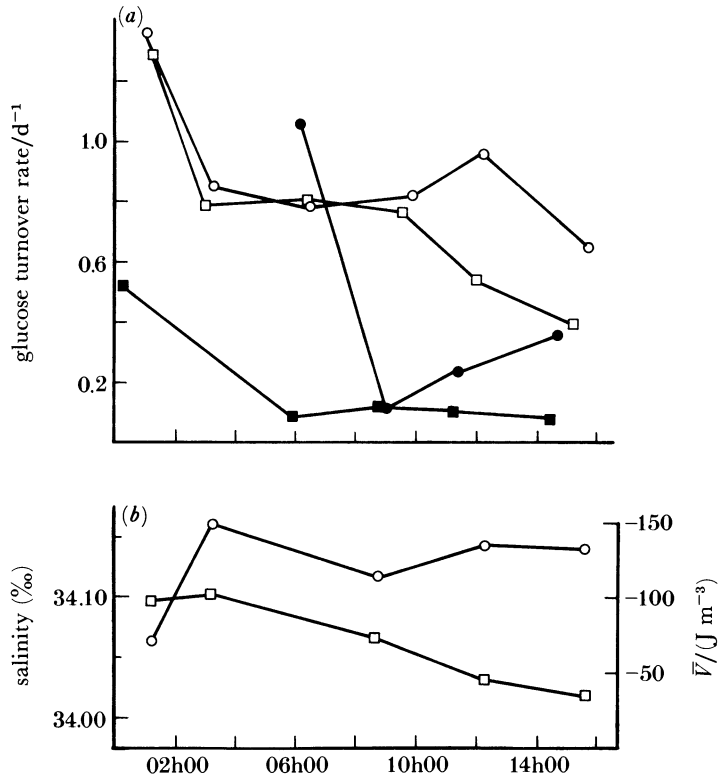

FIGURE 3. Drogue station on 29 April from 00h00 to 16h00, in the stratified water mass (station 1). (*a*) Glucose turnover rate at 2 m (—○—), at 10 m (—□—), at 20 m (—●—), at more than 70 m (—■—); (*b*) salinity at 2 m (—○—) and stratification parameter $\bar{V}$ (—□—).

When it was possible to stay within a certain body of water, indicated by uniform hydrographical variables, heterotrophic turnover of glucose remained relatively constant (figures 4–7). This was especially notable for drogue stations in the vertically mixed water mass (figures 5 and 7) and the deep water samples of the stratified stations. Samples from the surface stratified water tended to vary more strongly by a factor of two maximally. However, distinct diurnal patterns could not be recognized. It should be noted that samples taken at 20 m may be from above, below or within the pycnocline owing to internal waves in this boundary layer and, hence, may show stronger variations than samples from other depths (Fogg *et al.* 1985*a*).

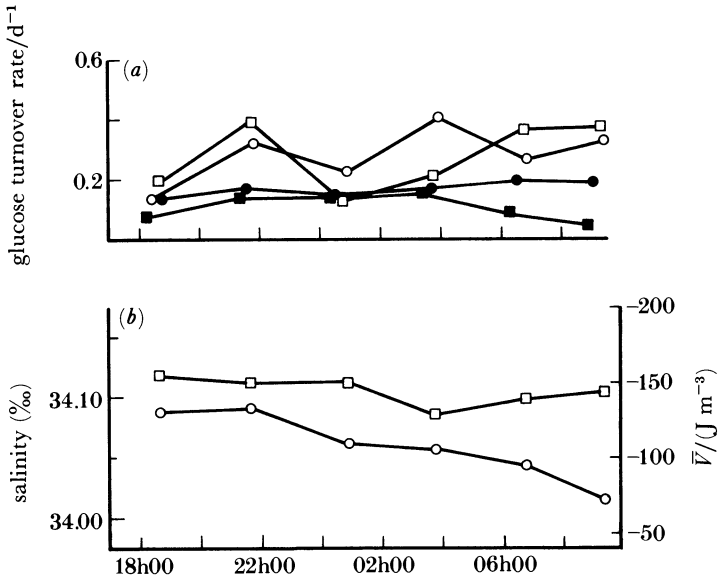

FIGURE 4. Drogue station on 4–5 June from 18h00 to 10h00, in the stratified water mass (station 4). (*a*) Glucose turnover rate at 2 m (—○—), at 10 m (—□—), at 20 m (—●—), at more than 70 m (—■—); (*b*) salinity at 2 m (—○—) and stratification parameter $\bar{V}$ (—□—).

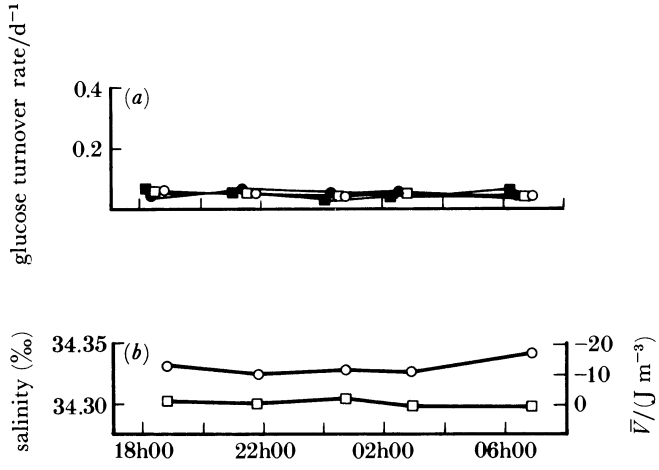

FIGURE 5. Drogue station on 5–6 June from 18h00 to 07h00, in the vertically mixed water mass (station 9). (*a*) Glucose turnover rate at 2 m (—○—), at 10 m (—□—), at 20 m (—●—), at more than 60 m (—■—); (*b*) salinity at 2 m (—○—) and stratification parameter $\bar{V}$ (—□—).

(*f*) *Seasonal changes*

Monthly comparison of surface samples shows the increase in turnover rates of glucose in the stratified water mass bounded by the front during times of strong stratification (June, July) (figure 8). In the mixed water mass turnover remained at low levels throughout the year.

Glucose uptake index (figure 9) also increased during summer showing maximal values in the surface stratified water in July, which were higher by a factor of 20 when compared with measurements in March or September. In contrast to turnover rates uptake indices tended to increase in the vicinity of the front on its stratified side, but they were not uniformly high in the whole of the surface stratified area and were generally much more heterogeneous.

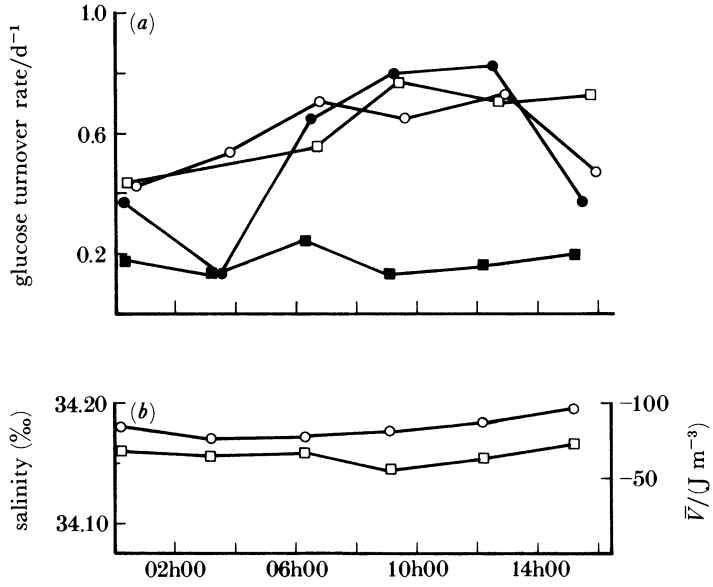

FIGURE 6. Drogue station on 16 July from 00h00 to 16h00, in the stratified water mass (station 1). (*a*) Glucose turnover rate at 2 m (—○—), at 10 m (—□—), at 20 m (—●—), at more than 50 m (—■—); (*b*) salinity at 2 m (—○—) and stratification parameter $\bar{V}$ (—□—).

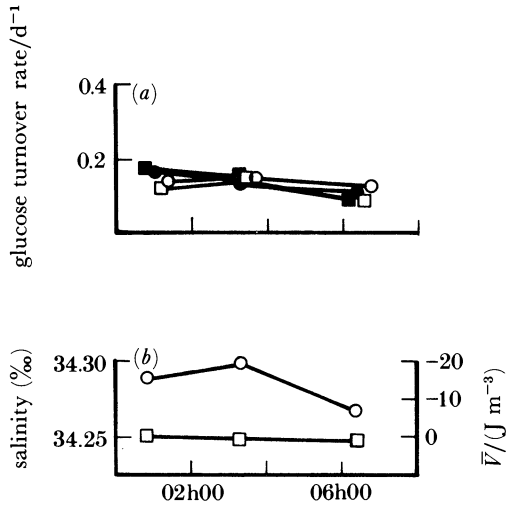

FIGURE 7. Drogue station on 17 July from 00h00 to 07h00, in the vertically mixed water mass (station 10). (*a*) Glucose turnover rate at 2 m (—○—), at 10 m (—□—), at 20 m (—●—), at more than 40 m (—■—); (*b*) salinity at 2 m (—○—) and stratification parameter $\bar{V}$ (—□—).

(*g*) *Separation into water masses and relation to other variables*

A comprehensive statistical analysis will be given in Kassab *et al.* (1985). Therefore, only a few important points will be mentioned here.

Student's *t*-tests applied to glucose uptake data showed consistently highly significant difference in glucose turnover rates between SSW and BSW plus MW (table 4). This is corroborated by one-way analysis of variance given for this variable by Kassab *et al.* (1985). Thus, the SSW as a whole is characterized by significantly higher turnover throughout the

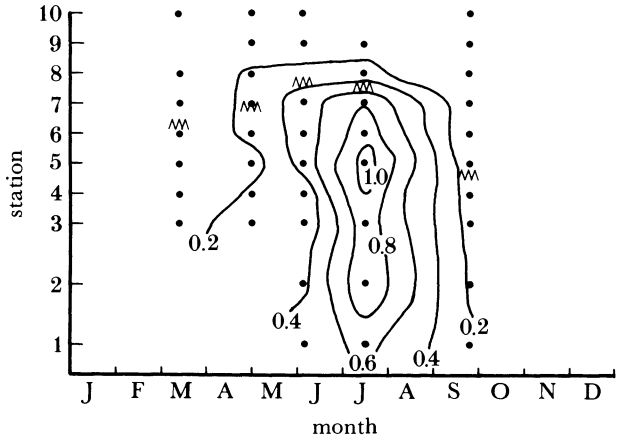

FIGURE 8. Seasonal distribution of glucose turnover rates (d^{-1}) of surface water samples (2 m). Stations are as shown in figure 1 in Fogg *et al.* (1985*a*). The position of the front (⋀⋀) is indicated for each cruise.

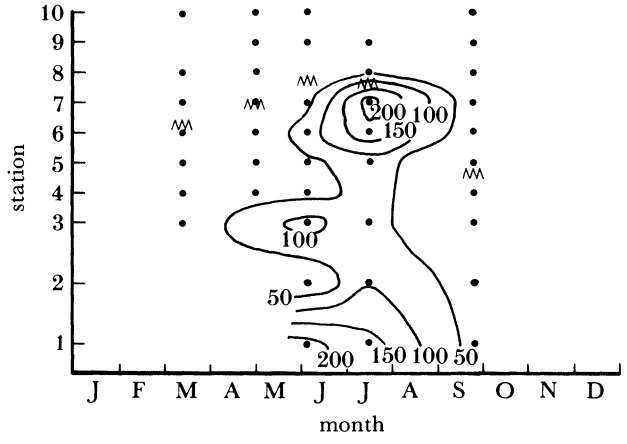

FIGURE 9. Seasonal distribution of glucose uptake indices $10^{-8} \times$ (nanograms per cell per hour) of surface water samples (2 m). Stations are as shown in figure 1 in Fogg *et al.* (1985*a*). The position of the front (⋀⋀) is indicated for each cruise.

season. Glucose uptake indices showed significant differences between water masses only during the summer.

Spearman rank correlations between glucose uptake variables and some selected physical and biological variables are given in table 5. Glucose turnover rates were highly significantly associated with density on all cruises except for the September cruise when wind mixing had eroded the density structure of the water column. Owing to their more patchy distribution glucose uptake indices only correlate with physical data during the summer months when stratification was well developed. Glucose turnover rates do not usually correlate with the number or biomass of bacteria although uptake of glucose can be attributed nearly exclusively to the activity of bacteria (Williams 1970; Azam & Hodson 1977; Hoppe 1978). The variables of glucose uptake correlate well with urea uptake, which indicates phytoplankton utilization of this nitrogenous compound (Turley 1985). Furthermore, zooplankton abundance shows very consistent highly significant positive correlations to all glucose uptake variables during the summer months (Scrope-Howe & Jones 1985). The correlations do not imply direct causal

TABLE 4. TWO-TAILED STUDENT'S *t*-TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN SURFACE STRATIFIED WATER (SSW) AND COMBINED BOTTOM STRATIFIED AND VERTICALLY MIXED WATER MASS (BSW + MW)

(Water masses are as defined in table 1. Based on the significance of the *F*-test either pooled variance estimates (*) or separate variance estimates (†) were used. $\bar{x}$, mean; d.f., degrees of freedom; *p*, probability; n.a., not available.)

	12–13 March	30 April to 1 May	3 June	15 July	23 September
			glucose turnover rate/d^{-1}		
$\bar{x}$ SSW	0.028	0.46	0.32	0.62	0.13
$\bar{x}$ BSW + MW	0.019	0.19	0.11	0.24	0.04
t-value	−3.16*	−4.26†	−9.50†	−6.85†	−5.21†
d.f.	49*	40.62†	46.62†	56.55†	12.94†
p	0.003*	0.000†	0.000†	0.000†	0.000†
			respired glucose carbon (%)		
$\bar{x}$ SSW	25.5	30.1	32.0	30.6	35.2
$\bar{x}$ BSW + MW	28.8	29.0	33.7	32.4	39.6
t-value	2.34*	−0.88†	1.72*	1.58*	3.74*
d.f.	49*	58.55†	80*	68*	38*
p	0.023*	0.384†	0.090*	0.118*	0.001*
			glucose uptake index/10^{-8} × (nanograms per cell per hour)		
$\bar{x}$ SSW	8.1	77.1	42.2	118.3	28.5
$\bar{x}$ BSW + MW	6.8	26.7	12.9	65.2	19.8
t-value	−0.76*	−2.58†	−3.49†	−2.46†	−0.90†
d.f.	48*	42.62†	39.64†	47.86†	16.46†
p	0.453*	0.014†	0.001†	0.018†	0.380†
			natural glucose concentration/(nmol l^{-1})		
$\bar{x}$ SSW	n.a.	n.a.	32	122	n.a.
$\bar{x}$ BSW + MW	n.a.	n.a.	42	115	n.a.
t-value	n.a.	n.a.	−1.47*	0.20*	n.a.
d.f.	n.a.	n.a.	13*	27*	n.a.
p	n.a.	n.a.	0.150*	> 0.500*	n.a.

TABLE 5. SPEARMAN RANK CORRELATION COEFFICIENTS

(Glucose turnover rate, glucose uptake index and respired glucose carbon with density (σ_t) and chlorophyll *a* (from Fogg *et al.* 1985), bacterial numbers (from Egan & Floodgate 1985), zooplankton numbers (from Scrope-Howe & Jones 1985), urea uptake (from Turley 1985). The level of significance is indicated by *** ⩽ 0.001, ** ⩽ 0.01, * ⩽ 0.05, n.a. is not available.)

	σ_t	chlorophyll *a*	bacterial numbers	zooplankton numbers	urea uptake in the dark	urea uptake in the light
12–13 March						
glucose turnover rate	−0.613***	n.a.	0.317*	0.900***	0.599***	0.685***
glucose uptake index	−0.201	n.a.	—	0.319	0.092	0.208
percentage respired glucose carbon	−0.294*	n.a.	−0.030	0.130	−0.400**	−0.320*
30 April–1 May						
glucose turnover rate	−0.562***	0.167	−0.178	0.665***	0.355**	0.562***
glucose uptake index	−0.570***	−0.129	—	0.533***	0.445***	0.526***
percentage respired glucose carbon	0.046	−0.013	−0.018	−0.109	0.148	−0.098
3 June						
glucose turnover rate	−0.648***	0.502***	−0.007	0.647***	0.633***	0.628***
glucose uptake index	−0.416***	0.349**	—	0.400***	0.430***	0.453***
percentage respired glucose carbon	0.208	−0.347**	0.080	−0.167	−0.167	−0.122
15 July						
glucose turnover rate	−0.553***	0.101	0.517***	0.542***	0.562***	0.641***
glucose uptake index	−0.447***	−0.082	—	0.382***	0.268*	0.432***
percentage respired glucose carbon	0.130	−0.543***	−0.222	−0.154	0.089	−0.129
23 September						
glucose turnover rate	0.297	0.367*	0.383*	n.a.	0.362*	0.323
glucose uptake index	0.299	0.001	—	n.a.	−0.095	−0.109
percentage respired glucose carbon	−0.245	−0.345*	−0.230	n.a.	−0.178	−0.177

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

relationships but are rather evidence of indirect effects within a highly interdependent biological system induced by stratification and progressively developing over the summer season.

4. Discussion

(a) *Methodological considerations*

Glucose has commonly been used as a substrate in the estimation of heterotrophic activity in sea waters (Williams & Askew 1968; Wood & Chua 1973; Meyer-Reil *et al.* 1979; Billen *et al.* 1980; Bölter 1981; Gocke *et al.* 1983, etc.). This sugar was found to be a dominant constituent of particulate carbohydrates in marine offshore waters, which were probably derived from storage polysaccharides in phytoplankton (Ittekkot *et al.* 1982). It is also the major portion of dissolved free carbohydrates often observed in association with phytoplankton (Liebezeit *et al.* 1980; Mopper *et al.* 1980; Ittekkot *et al.* 1981). Thus, glucose is likely to be an important intermediate in the flux of organic carbon from phytoplankton via the dissolved organic carbon pool (DOC) to bacterioplankton (Hodson & Azam 1979; Bölter 1981). Dissolved free amino acids are also important labile substances in sea water (Liebezeit *et al.* 1980; Mopper & Lindroth 1982) and have frequently been used as tracers with heterotrophic uptake rates of the same order as glucose (Dawson & Gocke 1978; Billen *et al.* 1980). However, ^{14}C-labelled phytoplankton exudates were found to be taken up much faster or in larger quantities than glucose or other commonly used substrates (Lancelot 1979; Wolter 1982; Riemann *et al.* 1982). These substances are probably more easily available to heterotrophic uptake either because of their chemical nature or close spatial links which cannot be imitated by the addition of tracers. However, monitoring the turnover of glucose as an example of a labile compound may yield useful information about the relative level of heterotrophic activity, assuming that this substrate is utilized by the bacterial population in approximately constant proportion to other *in situ* available substrates. In a wider sense, these assumptions seem to be justified by correlations of glucose turnover with the turnover of other labelled substrates (Dawson & Gocke 1978; Billen *et al.* 1980; Bölter 1981; Riemann *et al.* 1982), with the number of active bacteria determined by microautoradiography (Bölter *et al.* 1981) and with thymidine incorporation measurements (Fuhrman *et al.* 1980).

The seasonal and spatial differences in the temperature of the sea water directly affect glucose turnover rates. Samples from the investigated area were incubated with [^{14}C]glucose at temperatures of 9 and 15 °C, the temperatures of bottom and surface stratified water during summer (unpublished data). From the Arrhenius equation an activation energy of 55 255 J mol^{-1} was calculated which corresponds to a Q_{10} value of 2.3 for the temperature dependency of glucose uptake (Takahashi & Ichimuro 1971; Burnison & Morita 1974; Toerien & Cavari 1982; Laake *et al.* 1983; Hodson & Azam 1979). Thus, the mean glucose turnover rate in the surface stratified water was calculated to rise from 0.029 d^{-1} in March to 0.043 d^{-1} in July with a rise in water temperature of 4.96 °C. Glucose uptake index, which takes into account any concurrent change in bacterial abundance, is calculated to rise from 8.2×10^{-8} to 12.2×10^{-8} ng glucose per cell per hour for the same water mass and period of year. These figures are clearly exceeded by the observed average values of 0.69 d^{-1} and 131.0×10^{-8} ng glucose per cell per hour, respectively, measured in the surface stratified water in July (table 2). In fact, the water temperature was highest in September when turnover rates and uptake indices

were decreasing rapidly. Furthermore, all samples in this investigation were incubated in deck incubators at sea surface temperature, thus bottom as well as surface samples had the same incubation temperature. Despite this there was a highly significant difference in glucose turnover rate between bottom and surface stratified waters (table 4). Hence, temperature influences, where they apply, are small and cannot account for the observed spatial and seasonal changes, which are higher by an order of magnitude than the calculated temperature-based differences.

(*b*) *Drogue stations*

Diurnal fluctuations in uptake of glucose and fructose have been shown by Meyer-Reil *et al.* (1979) in a uniform sea water body. But Riemann (1980) found irregular variations in glucose uptake probably caused by sampling different bacterial communities which would mask diurnal rhythms. Observations of marked changes in concentrations of free amino acids, dissolved carbohydrates or DOC (Sieburth *et al.* 1977; Hammer *et al.* 1981; Sellner 1981; Burney *et al.* 1982; Mopper & Lindroth 1982) also led to the conclusion that the heterotrophic utilization of these substrates is likely to occur in diel rhythms. As the stations of the cross-frontal transect were sampled as different times of the day it was necessary to establish the extent of short-term diurnal variations in glucose uptake in each of the water masses associated with the front.

On our drogue stations, when a particular surface water mass was successfully tracked as indicated by relatively constant hydrographic measurements, glucose turnover rates showed some degree of variability in the surface stratified water (figure 4 and 6). However, on the basis of these limited data no distinct consistent diurnal pattern could be recognized. Variations in glucose concentration in these samples were irregular and showed no correlation to uptake rates. The drogues only indicate currents in the water depth in which they are deployed (here 4–7 m), hence currents below the thermocline are ignored. Despite the possibility of sampling different bottom waters the unchanged turnover rates indicated that the deep waters of the stratified part of the western Irish Sea are very homogeneous in respect to heterotrophic activity. Also the mixed water mass showed no indications of diel rhythms (figures 5 and 7). This may be indirect evidence for lack of diurnal or vertical patterns in DOC release. As shown by the stratification parameter $\bar{V}$ these water columns were completely vertically mixed which probably prevents the evolution of distinct diurnal patterns.

The strongest short-term variations in the surface stratified water were maximally 30% of the average cross-frontal differences in glucose uptake. These drogue stations demonstrated that diurnal fluctuations in these waters, although probably present, were of minor importance when compared with the large and consistent differences in heterotrophic activity between water masses.

(*c*) *Glucose concentration*

The presently employed method of determination of glucose is clearly too insensitive and inadequate to handle the large amounts of samples required for ecological surveys. Thus, more glucose data would be desirable to complement the turnover rate measurements. Recently developed more sensitive methods (see, for example, Liebezeit *et al.* 1980; Mopper *et al.* 1980) may in future enable more detailed insights into the relation between natural substrate concentration and its heterotrophic utilization.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Despite the methodological limitations it could be established that glucose concentration did not correlate with glucose turnover rates nor with physical data. Similar observations of rates of heterotrophic utilization unrelated to the *in situ* substrate concentration have been reported by Andrews & Williams (1971), Wood & Chua (1973), Billen *et al.* (1980), Liebezeit *et al.* (1980) and Gocke *et al.* (1981, 1983). Consequently, hypotheses for steady-state conditions in the oceanic environment characterized by low constant standing stock of substrates but high fluxes have been put forth (Smith & Higgins 1978; Billen *et al.* 1980; Burney *et al.* 1982). They, however, are based upon measurements of time scales and volumes of water large in relation to the temporal and spatial scale of microbial events. This neglects the problem of biological availability of substrates and small scale diffusion gradients (Azam & Hodson 1981; Gocke *et al.* 1981; Williams & Muir 1981). As the actually available fraction of natural glucose and its microdistribution completely eludes the presently used methods it appears to be a less misleading approach to assume an average steady-state constant glucose concentration to obtain some order of magnitude estimations of actual glucose uptake.

(*d*) *Glucose turnover rates*

The development of the seasonal pycnocline and frontal zone in the western Irish Sea had a strong influence on glucose turnover rates (figure 1*a–g*, figure 8) as shown by the close correlation with density (table 5) and the significant differences between water masses (table 4). Neither the higher water temperatures in the surface stratified water, diurnal fluctuations nor concurrent changes in natural glucose concentration (s.a.) could account for the observed spatial or seasonal patterns in glucose uptake rate. These observations, therefore, show that, in general, higher heterotrophic activity and turnover of DOC occurred in the surface stratified waters relative to both bottom and mixed waters, particularly during the summer months with strong stratification.

Parsons *et al.* (1983) measuring glucose uptake at a frontal zone in Saanich Inlet also found high uptake in the stratified water and low uptake in the vertically mixed water. But in contrast to the observations reported here, the frontal zone itself showed relatively low uptake values. However, since Saanich Inlet receives terrestrial input the nutrient characteristics are likely to be very different to those in the western Irish Sea frontal area which is not terrestrially influenced (Foster *et al.* 1976) and depends upon *in situ* produced and recycled nutrients.

The seasonal cycle of glucose uptake rates in the stratified waters of the western Irish Sea may be considered in various steps:

(i) before the phytoplankton bloom, heterotrophic uptake is very low;

(ii) at the beginning of the spring phytoplankton bloom, uptake rates increase irregularly;

(iii) during the summer months after the spring phytoplankton bloom, high uptake rates occur in the surface stratified water;

(iv) when the pycnocline breaks down in autumn, uptake rates decrease again.

During these seasonal stages the relative importance of the different sources and sinks of DOC will progressively change from a phototrophically dominated system to a more heterotrophically oriented one (Williams 1981). It is evident from the observations reported here that bacterial activity is progressively gaining importance in the transformation of organic matter in the surface stratified water after the spring phytoplankton bloom.

Turnover rates of glucose correlated positively with high statistical significance with turnover rates of urea (table 5), which can be considered as indication of phytoplankton metabolic

activity (Turley 1985), but there was no consistent correlation with chlorophyll *a*. There are two possible ways by which the heterotrophic and phototrophic activity may be linked. First, Turley showed that the high rate of degradation of urea in the surface stratified waters requires a similarly high production of urea and argues that bacterial transformation of more complex nitrogenous compounds may be to some extent responsible for this production. Secondly, uptake of urea may be indicative of primary production and, thus, of phytoplankton exudate release (Ittekkot *et al.* 1981; review by Fogg 1983) representing important bacterial nutrition (Iturriaga & Hoppe 1977; Lancelot 1979; Larssen & Hagström 1979; Iturriaga 1981; Wolter 1982). Similarly, Smith & Higgins (1978); Nalewajko *et al.* (1976); Bell & Sakshaug (1980) and Burney *et al.* (1982) found evidence for close links between DOC release and its uptake and observed a balance in the resulting DOC concentration when phototrophic and heterotrophic species interacted. The consistent positive correlation with zooplankton abundance (table 5) suggests that their grazing and excretion also affects the DOC pool and, hence, the bacterial activity (Eppley *et al.* 1981).

(*e*) *Glucose uptake index*

Heterotrophic glucose uptake usually did not correlate with bacterial numbers or biomass (table 5). Thus, changes in uptake are not accounted for by proportional changes in bacterial numbers. Similar observations were made by Wright (1978); Ferguson & Palumbo (1979); Valdes & Albright (1981) and Gocke *et al.* (1983). Possible explanations for this phenomenon are:

(i) uptake of glucose by organisms other than bacteria;

(ii) uptake of other substrates in different proportion to glucose;

(iii) differences in the metabolic activity of the total bacterial population either by changes in the metabolism of individual bacterial cells or by changes in the percentage of actively metabolizing cells.

Firstly, a number of investigations (Williams 1970; Gocke 1975; Herbland & Pages 1976; Azam & Hodson 1977; Hoppe 1978; review by Sepers 1977) showed that uptake of glucose in the pelagic system is mostly attributable to bacterial activity. Therefore, it seems unlikely that uptake by other organisms could actually cause such large differences in glucose uptake index. Secondly, on the basis of the present knowledge it is difficult to evaluate whether marked differences in uptake of specific substrates occur in planktonic microbial populations. It has as yet to remain an assumption that in a constantly physically mixed and relatively homogeneous environment any such variations are likely to be small. Thirdly, there are numerous indications, obtained by quite different methods, of changes in metabolic activity of bacteria in response to nutritional conditions. The existence of dormant cell stages in bacterial populations of nutrient-limited marine ecosystems has been postulated by Stevenson (1978). Torrella & Morita (1982) demonstrated, in a marine *Vibrio* strain, that short-term starvation induced morphological changes, motility and chemotactic responses; Karl *et al.* (1981) showed a rapid response of RNA synthesis to nutrient enrichment; Torrella & Morita (1981) followed microscopically the widely differing growth responses of different bacterial cell types to nutrient enrichment; radioautographic studies (Hoppe 1978) indicate that the proportion of actively metabolizing cells within a population varies according to the nutrient level of the water mass; Hagström *et al.* (1979) and Krambeck *et al.* (1981) observed that the percentage of dividing cells within a bacterial population was related to primary productivity. Thus, as suggested by

Wright (1978) uptake indices may be a valid indication of the physiological state of the microorganisms.

The observations reported here also imply a relationship between uptake index and environmental conditions. The frontal zone, which showed high glucose uptake indices, was previously noted for characteristically high phytoplankton standing stock (Pingree *et al.* 1976; Beardall *et al.* 1982; Richardson *et al.* 1984; Fogg *et al.* 1985*a*). In the present study, however, chlorophyll *a* did not correlate with glucose uptake index (except in June) (table 5), which at first sight appears to be contradictory to the above notion. Yet, as suggested by the positive correlations with urea uptake, primary productivity may be a better indicator of flux of organic matter and may yield closer relationships with heterotrophic activity than phytoplankton standing stock. Furthermore, the highly significant positive correlations with zooplankton numbers (table 5) indicate that nutrients for the microheterotrophs may be recycled in a more indirect way via grazing and excretion of the zooplankton (Eppley *et al.* 1981; Azam *et al.* 1983). Pomeroy *et al.* (1983) found, near a shelf sea front, distinct microbial communities dominated either by autotrophic or by heterotrophic biomass, which also may be indirect evidence that bacteria responded to nutrients released both by phytoplankton and zooplankton.

Since respiration rates remained constant, the widely differing uptake indices imply a net increase in assimilated glucose per cell in the SSW during summer. Thus, if other DOC compounds show similar uptake characteristics, increased bacterial biomass production is likely to occur in waters with high uptake indices. Based upon the relative differences between the indices three to five times more biomass may potentially be produced per unit time in the SSW than the BSW or MW during summer. Especially, the frontal zone may show rapid bacterial growth. If this were found to be the case, then bacterial turnover of DOC and biomass production may indeed be of great importance in these waters (Turley & Lochte 1985; Lochte & Turley 1985). However, unequivocal corresponding increases in bacterial numbers of this magnitude were not seen (Egan & Floodgate 1985). This may well be the consequence of rapid removal of bacterial biomass by grazing (Fenchel 1982*a*, *b*; Azam *et al.* 1983).

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References

Andrews, P. & Williams, P. J. LeB. 1971 Heterotrophic utilization of dissolved organic compounds in the sea. III. Measurement of glucose oxidation rates and concentrations of glucose and amino acids in sea water. *J. mar. biol. Ass. U.K.* **51**, 111–125.

Azam, F. & Hodson, R. E. 1977 Size distribution and activity of marine heterotrophs. *Limnol. Oceanogr.* **22**, 492–501.

Azam, F. & Hodson, R. E. 1981 Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. *Mar. Ecol. Prog. Ser.* **6**, 213–222.

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L.-A. & Thingstad, F. 1983 The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* **10**, 257–263.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Baross, J. A., Hanus, F. J., Griffiths, R. P. & Morita, R. Y. 1975 Nature of incorporated ^{14}C-labelled material retained by sulfuric acid fixed bacteria in pure cultures and in natural aquatic populations. *J. Fish. Res. Bd Can.* **32**, 1876–1879.

Beardall, J., Foster, P., Voltolina, D. & Savidge, G. 1982 Observations on the surface water characteristics in the western Irish Sea: July 1977. *Estuar. coast. Shelf Sci.* **14**, 589–598.

Bell, W. H. & Sakshaug, E. 1980 Bacterial utilization of algal extracellular products. 2. A kinetic study of natural populations. *Limnol. Oceanogr.* **25**, 1021–1033.

Billen, G., Joiris, C., Wijnant, J. & Gillain, G. 1980 Concentration and microbiological utilization of small organic molecules in the Scheldt Estuary, the Belgian coastal zone, the North Sea, and the English Channel. *Estuar. coast. mar. Sci.* **11**, 279–294.

Bölter, M. 1981 DOC-turnover and microbial biomass production. *Kieler Meeresforsch., Sonderheft* **5**, 304–310.

Bölter, M., Meyer-Reil, L.-A., Dawson, R., Liebezeit, G., Wolter, K. & Szwerinski, H. 1981 Structure analysis of shallow water ecosystems: Interaction of microbial, chemical and physical characteristics measured in the overlying waters of sandy beach sediments. *Estuar. coast. Shelf Sci.* **13**, 579–589.

Burney, C. M., Davis, P. G., Johnson, K. M. & Sieburth, J. McN. 1982 Diel relationships of microbial trophic groups and *in situ* dissolved carbohydrate dynamics in the Caribbean Sea. *Mar. Biol.* **67**, 311–322.

Burnison, B. K. & Morita, R. Y. 1974 Heterotrophic potential for amino acid uptake in a naturally eutrophic lake. *Appl. Microbiol.* **27**, 488–495.

Carney, J. F. & Colwell, R. R. 1976 Heterotrophic utilization of glucose and glutamate in an estuary: Effect of season and nutrient load. *Appl. environ. Microbiol.* **31**, 227–233.

Dawson, R. & Gocke, K. 1978 Heterotrophic activity in comparison to the free amino acid concentrations in Baltic seawater samples. *Oceanologica Acta* **1**, 45–54.

Egan, B. & Floodgate, G. D. 1985 Biological studies in the vicinity of a shallow-sea tidal mixing front. II. The distribution of bacteria. *Phil. Trans. R. Soc. Lond.* B **310**, 435–444.

Eppley, R. W., Horrigan, S. G., Fuhrman, J. A., Brooks, E. R., Price, C. C. & Sellner, K. 1981 Origins of dissolved organic matter in southern California coastal waters: experiments on the role of zooplankton. *Mar. Ecol. Prog. Ser.* **6**, 149–159.

Fenchel, T. 1982*a* Ecology of heterotrophic microflagellates. II. Bioenergetics and growth. *Mar. Ecol. Prog. Ser.* **8**, 225–231.

Fenchel, T. 1982*b* Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. *Mar. Ecol. Prog. Ser.* **9**, 35–42.

Ferguson, R. L. & Palumbo, A. V. 1979 Distribution of suspended bacteria in neritic waters south of Long Island during stratified conditions. *Limnol. Oceanogr.* **24**, 697–705.

Floodgate, G. D., Fogg, G. E., Jones, D. A., Lochte, K. & Turley, C. M. 1981 Microbiological and zooplankton activity at a front in Liverpool Bay. *Nature, Lond.* **229**, 133–136.

Fogg, G. E. 1983 The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica marina* **26**, 3–14.

Fogg, G. E., Egan, B., Hoy, S., Lochte, K., Scrope-Howe, S. O. V. & Turley, C. M. 1985*a* Biological studies in the vicinity of a shallow-sea tidal mixing front. I. Physical and chemical background. *Phil. Trans. R. Soc. Lond.* B **310**, 407–433.

Fogg, G. E., Floodgate, G. D., Jones, D. A., Kassab, J. Y., Lochte, K., Rees, E. I. S. & Turley, C. M. 1985*b* Biological studies in the vicinity of a shallow-sea tidal mixing front. VII. The frontal ecosystem. *Phil. Trans. R. Soc. Lond.* B **310**, 555–571.

Foster, P., Savidge, G., Foster, G. M., Hunt, D. T. E. & Pugh, K. B. 1976 Multivariate analysis of surface water characteristics in the summer regime of the western Irish Sea. *J. exp. mar. Biol. Ecol.* **25**, 171–185.

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.

Gocke, K. 1975 Untersuchungen über die Aufnahme von gelöster Glukose unter natürlichen Verhältnissen durch grössenfraktioniertes Nano- und Ultraplankton. *Kieler Meeresforsch.* **31**, 87–94.

Gocke, K. 1976 Respiration von gelösten organischen Verbindungen durch natürliche Mikroorganismen-Populationen. Ein Vergleich zwischen verschiedenen Biotopen. *Mar. Biol.* **35**, 375–383.

Gocke, K., Dawson, R. & Liebezeit, G. 1981 Availability of dissolved free glucose to heterotrophic microorganisms. *Mar. Biol.* **62**, 209–216.

Gocke, K., Hoppe, H. G. & Bauerfeind, S. 1983 Investigations on the influence of coastal upwelling and polluted rivers on the microflora of the northeastern Atlantic off Portugal. *Botanica marina* **26**, 189–199.

Griffiths, R. P., Hanus, F. J. & Morita, R. Y. 1974 The effects of various water-sample treatments on the apparent uptake of glutamic acid by natural marine microbial populations. *Can. J. Microbiol.* **20**, 1261–1266.

Hagström, A., Larsson, U., Hörstedt, P. & Normark, S. 1979 Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. *Appl. envir. Microbiol.* **37**, 805–812.

Hammer, K. D., Brockmann, U. H. & Kattner, G. 1981 Release of dissolved free amino acids during a bloom of *Thalassiosira rotula*. *Kieler Meeresforsch., Sonderheft* **5**, 101–109.

Herberg, R. J. 1965 Channels ratio method of quench correction in liquid scintillation counting. *Packard Tech. Bull.* **15**, 1–8.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Herbland, A. & Pages, J. 1976 Note on the variability of heterotrophic activity measurements by ^{14}C-method in seawater. *Mar. Biol.* **35**, 211–214.

Hicks, S. E. & Carey, F. G. 1968 Glucose determination in natural waters. *Limnol. Oceanogr.* **13**, 361–363.

Hobbie, J. E. & Crawford, C. C. 1969 Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. *Limnol. Oceanogr.* **14**, 528–532.

Hodson, R. E. & Azam, F. 1979 Occurrence and characterization of a phosphotransferase system in a marine bacterium, *Serratia marinorubra*. *Appl. environ. Microbiol.* **38**, 1086–1091.

Holligan, P. M. 1981 Biological implications of fronts on the northwest European continental shelf. *Phil. Trans. R. Soc. Lond.* A **303**, 547–562.

Hoppe, H.-G. 1978 Relations between active bacteria and heterotrophic potential in the sea. *Neth. J. Sea Res.* **12**, 78–98.

Ittekkot, V., Brockmann, U., Michaelis, W. & Degens, E. T. 1981 Dissolved free and combined carbohydrates during a phytoplankton bloom in the northern North Sea. *Mar. Ecol. Prog. Ser.* **4**, 299–305.

Ittekkot, V., Degens, E. T. & Brockmann, U. 1982 Monosaccharide composition of acid-hydrolyzable carbohydrates in particulate matter during a phytoplankton bloom. *Limnol. Oceanogr.* **27**, 770–776.

Iturriaga, R. 1981 Phytoplankton photoassimilated extracellular products; heterotrophic utilization in marine environment. *Kieler Meeresforsch., Sonderheft* **5**, 318–324.

Iturriaga, R. & Hoppe, H.-G. 1977 Observations of heterotrophic activity on photoassimilated organic matter. *Mar. Biol.* **40**, 101–108.

Iturriaga, R. & Zsolnay, A. 1981 Transformation of some dissolved organic compounds by a natural heterotrophic population. *Mar. Biol.* **62**, 125–129.

Karl, D. M., Winn, C. D. & Wong, D. C. L. 1981 RNA synthesis as a measure of microbial growth in aquatic environments. II. Fixed applications. *Mar. Biol.* **64**, 13–21.

Kassab, J. Y., Whitaker, C. J. & Floodgate, G. D. 1985 Biological studies in the vicinity of a shallow-sea tidal mixing front. VI. A general statistical study. *Phil. Trans. R. Soc. Lond* B **310**, 521–555.

King, G. M. & Berman, T. 1984 Potential effects of isotopic dilution on apparent respiration in ^{14}C heterotrophy experiments. *Mar. Ecol. Prog. Ser.* **19**, 175–180.

Krambeck, C., Krambeck, H. J. & Overbeck, J. 1981 Microcomputer-assisted biomass determination of planktonic bacteria on scanning electron micrographs. *Appl. environ. Microbiol.* **42**, 142–149.

Laake, M., Dahle, A. B. & Hentzschel, G. 1983 Productivity and population diversity of marine organotrophic bacteria in enclosed planktonic ecosystems. *Mar. Ecol. Prog. Ser.* **14**, 59–69.

Lancelot, C. 1979 Gross excretion rates of natural marine phytoplankton and heterotrophic uptake of excreted products in the southern North Sea, as determined by short-term kinetics. *Mar. Ecol. Prog. Ser.* **1**, 179–186.

Larsson, U. & Hagström, A. 1979 Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Mar. Biol.* **52**, 199–206.

Liebezeit, G., Bölter, M., Browne, J. F. & Dawson, R. 1980 Dissolved free amino acids and carbohydrates at pycnocline boundaries in the Sargasso Sea and related microbial activity. *Oceanologica Acta* **3**, 357–362.

Lochte, K. & Turley, C. M. 1985 Heterotrophic activity and carbon flow via bacteria in waters associated with a tidal mixing front. *J. mar. biol. Ass. U.K.* (In the press.)

Meyer-Reil, L.-A., Bölter, M., Liebezeit, G. & Schramm, W. 1979 Short-term variations in microbiological and chemical parameters. *Mar. Ecol. Prog. Ser.* **1**, 1–6.

Mopper, K., Dawson, R., Liebezeit, G. & Ittekkot, V. 1980 The monosaccharide spectra of natural waters. *Mar. Chem.* **10**, 55–66.

Mopper, K. & Lindroth, P. 1982 Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. *Limnol. Oceanogr.* **27**, 336–347.

Nalewajko, C., Dunstall, T. G. & Shear, H. 1976 Kinetics of extracellular release in axenic algae and in mixed algal–bacterial cultures: significance in estimation of total (gross) phytoplankton excretion rates. *J. Phycol.* **12**, 1–5.

Owen, R. W. 1981 Fronts and eddies in the sea: mechanisms, interactions and biological effects. In *Analysis of marine ecosystems* (ed. A. R. Longhurst), pp. 271–291. London and New York: Academic Press.

Parsons, T. R., Perry, R. I., Nutbrown, E. D., Hsieh, W. & Lalli, C. M. 1983 Frontal zone analysis at the mouth of Saanich Inlet, British Columbia. *Mar. Biol.* **73**, 1–5.

Pingree, R. D., Holligan, P. M., Mardell, G. T. & Head, R. N. 1976 The influence of physical stability on spring, summer and autumn phytoplankton blooms in the Celtic Sea. *J. mar. biol. Ass. U.K.* **56**, 845–873.

Pingree, R. D., Holligan, P. M. & Mardell, G. T. 1978 The effects of vertical stability on phytoplankton distributions in the summer on the northwest European Shelf. *Deep Sea Res.* **25**, 1011–1028.

Pomeroy, L. R., Atkinson, L. P., Blanton, J. O., Campbell, W. B., Jacobsen, T. R., Kerrick, K. H. & Wood, A. M. 1983 Microbial distribution and abundance in response to physical and biological processes on the continental shelf of southeastern U.S.A. *Continental Shelf Res.* **2**, 1–20.

Ramsey, A. J. 1976 The effect of acidification on measurements of microbial uptake of radioactive glucose in freshwater. *Limnol. Oceanogr.* **21**, 922–926.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Richardson, K., Lavin-Peregrina, M. F., Mitchelson, E. G. & Simpson, J. H. 1985 Seasonal distribution on chlorophyll *a* in relation to physical structure in the western Irish Sea. *Oceanologica Acta.* **8**, 77–86.)

Riemann, B. 1980 Diurnal variations in the uptake of glucose by attached and free living microheterotrophs in lake water (Lake Mossø, Denmark). In *Shallow lakes. Contributions to their limnology* (ed. M. Dokulil, H. Metz & D. Jewson), pp. 153–160. The Hague: Dr W. Junk.

Riemann, B., Søndergaard, M., Schierup, H.-H., Bosselmann, S., Christensen, G., Hansen, J. & Nielsen, B. 1982 Carbon metabolism during a spring diatom bloom in the eutrophic lake Mossø. *Int. Revue ges. Hydrobiol.* **67**, 145–185.

Scrope-Howe, S. O. V. & Jones, D. A. 1985 Biological studies in the vicinity of a shallow-sea tidal mixing front. V. The vertical distribution of zooplankton. *Phil. Trans. R. Soc. Lond* B **310**, 501–519.

Sellner, K. G. 1981 Primary productivity and the flux of dissolved organic matter in several marine environments. *Mar. Biol.* **65**, 101–112.

Sepers, A. B. J. 1977 The utilization of dissolved organic compounds in aquatic environments. *Hydrobiologia* **52**, 39–54.

Sieburth, J. McN., Johnson, K. M., Burney, C. M. & Lavoie, D. M. 1977 Estimation of *in situ* rates of heterotrophy using diurnal changes in dissolved organic matter and growth rates of picoplankton in diffusion culture. *Helgoländer wiss. Meeresunters.* **30**, 565–574.

Simpson, J. H., Hughes, D. G. & Morris, N. C. G. 1977 The relation of seasonal stratification to tidal mixing on the continental shelf. In *A voyage of discovery* (ed. M. Angel), pp. 327–340. Oxford and New York: Pergamon Press.

Simpson, J. H., Allen, C. M. & Morris, N. C. G. 1978 Fronts on the continental shelf. *J. geophys. Res.* **83**, 4607–4614.

Simpson, J. H. & Bowers, D. 1979 Shelf sea fronts' adjustments revealed by satellite IR imagery. *Nature, Lond.* **280**, 648–651.

Simpson, J. H. & Pingree, R. D. 1978 Shallow sea fronts produced by tidal stirring. In *Oceanic fronts in coastal processes* (ed. M. J. Bowman & W. E. Esaias), pp. 29–42. Berlin, Heidelberg and New York: Springer Verlag.

Smith, D. F. & Higgins, H. W. 1978 An interspecies regulatory control of dissolved organic carbon production by phytoplankton and incorporation by microheterotrophs. In *Proceedings in life sciences. Microbial ecology* (ed. M. W. Loutit & J. A. R. Miles), pp. 34–39. Berlin, Heidelberg and New York: Springer Verlag.

Stevenson, L. H. 1978 A case for bacterial dormancy in aquatic systems. *Microbial Ecol.* **4**, 127–133.

Takahashi, M. & Ichimuro, S. 1971 Glucose uptake in ocean profiles with special reference to temperature. *Mar. Biol.* **11**, 206–213.

Tison, D. L. & Pope, D. H. 1980 Effect of temperature on mineralization by heterotrophic bacteria. *Appl. environ. Microbiol.* **39**, 584–587.

Toerien, D. F. & Cavari, B. 1982 Effect of temperature on heterotrophic glucose uptake, mineralisation and turnover rates in lake sediments. *Appl. environ. Microbiol.* **43**, 1–5.

Torrella, F. & Morita, R. Y. 1981 Microcultural studies of bacterial size changes, microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. *Appl. environ. Microbiol.* **41**, 518–527.

Torrella, F. & Morita, R. Y. 1982 Starvation induced morphological changes, mobility and chemotaxis patterns in a psychrophilic marine vibrio. *Publ. CNEXO (Actes Collog.)* **13**, 45–60.

Turley, C. M. 1985 Biological studies in the vicinity of a shallow-sea tidal mixing front. IV. Seasonal and spatial distribution of urea and its uptake by phytoplankton. *Phil. Trans. R. Soc. Lond.* B **310**, 471–500.

Turley, C. M. & Lochte, K. 1985 A direct measurement of bacterial productivity in stratified waters close to a front in the Irish Sea. *Mar. Ecol. Prog. Ser.* **23**, 209–219.

Valdes, M. & Albright, L. J. 1981 Survival and heterotrophic activities of Fraser River and Straits of Georgia bacterioplankton within the Fraser River plume. *Mar. Biol.* **64**, 231–241.

Williams, P. J. LeB. 1970 Heterotrophic utilization of dissolved organic compounds in the sea. I. Size distribution of population and relationship between respiration and incorporation of growth substrates. *J. mar. biol. Ass. U.K.* **50**, 859–870.

Williams, P. J. LeB. 1981 Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. *Kieler Meeresforsch., Sonderheft* **5**, 1–28.

Williams, P. J. LeB. & Askew, C. 1968 A method of measuring the mineralization by micro-organisms of organic compounds in sea-water. *Deep Sea Res.* **15**, 365–375.

Williams, P. J. LeB. & Muir, L. R. 1981 Diffusion as a constraint on the biological importance of microzones in the sea. In *Ecohydrodynamics* (ed. J. C. Nihoul), Elsevier Oceanography Series 32, pp. 209–218. Amsterdam, Oxford and New York: Elsevier Scientific Publ. Co.

Williams, P. J. LeB. & Yentsch, C. S. 1976 Photosynthetic production, excretion of photosynthetic products and heterotrophic utilization of dissolved organic compounds with reference to results from a coastal subtropical sea. *Mar. Biol.* **35**, 31–40.

Wolter, K. 1982 Bacterial incorporation of organic substances released by natural phytoplankton populations. *Mar. Ecol. Prog. Ser.* **7**, 287–295.

Wood, L. W. & Chua, K. E. 1973 Glucose flux at the sediment–water interface of Toronto Harbour, Lake Ontario, with reference to pollution stress. *Can. J. Microbiol.* **19**, 413–420.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Wright, R. T. 1978 Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. *Appl. environ. Microbiol.* **36**, 297–305.

Wright, R. T. & Burnison, B. K. 1979 Heterotrophic activity measured with radiolabelled organic substrates. In *Native aquatic bacteria: enumeration, activity and ecology* (Proceedings, A.S.T.M. Symposium, 25 June 1977, Minneapolis) (ed. J. W. Costerton & R. R. Colwell), pp. 140–155. Philadelphia: American Society for Testing and Materials.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES