

NOTE

Determination of conversion factors for estimation of subantarctic marine bacterial production measured by ^3H -thymidine incorporation methodology

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ABSTRACT: In order to determine conversion factors for ^3H -thymidine incorporation to bacterial growth rate, we performed a series of seawater culture experiments on surface mixed layer samples in a subantarctic coastal area. Four different treatments were used: bacterivory reduction (dilution or filtration), nutrient enrichment and temperature increase. Conversion factors were estimated by the 'integration approach': the increase in bacterial biomass (biomass at final time point minus initial value) was divided by incorporation rates integrated over time during the experiment. Both bacterial abundance and mean cell volumes markedly increased in all experiments. The manipulation effects were strongest in the high temperature batch compared with the other experiments. However, the 4 different treatments yielded comparable conversion factors ranging from 9.7×10^5 to 1.5×10^6 cells pmol^{-1} with a mean value of 1.2×10^6 cells pmol^{-1} .

KEY WORDS: Bacterial production · Psychrophilic bacteria · Antarctica

The use of ^3H -thymidine incorporation to derive an estimate of heterotrophic bacterial growth rate has been practiced extensively in aquatic microbial ecology (reviewed by Riemann & Bell 1990). However, bacterioplankton can dissimilate and shunt exogenously supplied thymidine into various macromolecular fractions, rather than use it for DNA synthesis. The extrapolation of ^3H -thymidine incorporation to bacterial growth rate requires several assumptions that apparently are not valid in all areas. Several papers have challenged the validity of ^3H -thymidine assay assumptions. In particular, the extrapolation factor for the conversion of moles of ^3H -thymidine incorporated to bacterial cells produced, which was assumed to be constant, is now known to vary under both laboratory

and field conditions (Kirchman et al. 1982, Bell et al. 1983, Scavia & Laird 1987, Smits & Riemann 1988, Børsheim 1990, Kirchman & Ducklow 1993). Karl et al. 1991 found an empirically determined conversion factor ranging from 2 to 55×10^6 cells pmol^{-1} in the Bransfield Strait (Antarctica). Consequently, the use of a constant value in carbon budgets and models is problematic, and we are not yet able to present a simple solution to account for this variability.

The purpose of the present study is to compare conversion factors deduced from 4 different manipulations (minimizing grazing by dilution or filtration, nutrient addition and temperature increase). The influence of chemical extractions of bacterial material used to determine thymidine uptake rates was also pointed out as a complement.

Materials and methods. This study was conducted from January to April 1992 in the Kerguelen archipelago. In order to determine conversion factors, subsurface seawater was collected in Morbihan Bay (Stn Z; $49^\circ 21.5' \text{ S}$, $70^\circ 13.25' \text{ E}$) using sterile glass bottles at 10 cm depth. Bacterial growth was followed in a number of seawater batch cultures (2 l in 5 l acid-washed sterile glass bottles). Five microcosms were treated as follows: (1) no treatment, (2) filtration through $3 \mu\text{m}$ filters at $<200 \text{ mm Hg}$, (3) filtration through $0.2 \mu\text{m}$ filters and inoculation with ambient seawater to give a 10-fold dilution of the inoculum, (4) peptone addition, (5) high incubation temperature (15°C as compared to 7°C for the other batches). Except for Expt 5, the batch cultures were incubated in the dark close to *in situ* temperature (between 6 and 8°C). With approximately 10 h intervals, 70 ml subsamples were removed from the bottles for estimation of bacterial biomass and production.

Total bacteria were determined by acridine orange direct count (AODC) with an Olympos epifluorescence

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microscope according to the method of Hobbie et al. (1977). Biovolumes were estimated using an ocular micrometer. Viable counts of saprophytic bacteria were made using the spread plate technique on the 2216 E medium (Oppenheimer & ZoBell 1952). Inoculated plates were incubated for 15 d at 7°C. The rate of uptake of tritiated thymidine was measured according to the procedure of Fuhrman & Azam (1982). Samples were incubated for 1 and 2 h with 10 nM ^3H -thymidine (final concentration) under *in situ* conditions. In order to discriminate the fate of assimilated thymidine, 3 different final treatments were used after 1 and 2 h *in situ* incubations and the addition of ice-cold 10% trichloroacetic acid (TCA). (1) Three ml subsamples were extracted in 0.25 N NaOH (final concentration) and chilled on ice. These samples were stored on ice for up to 48 h, then neutralized with ice-cold TCA (final concentration, 20%) and filtered onto 22 mm diameter, 0.2 μm membrane filters. Finally the samples were extracted in the filter holders by rinsing 3 times with 80% ethanol (Wicks & Roberts 1987). (2) Three ml subsamples were filtered onto 22 mm diameter, 0.2 μm membrane filters and then extracted by rinsing the filters over a vacuum 3 times with ice-cold 5% TCA and by rinsing 3 times with 80% ethanol as suggested by Wicks & Roberts (1988). (3) Treatment was similar to that in (2) with a final rinsing with 1 ml of hot 10% TCA.

Conversion factors (CF) were estimated by the 'integration approach'; the increase in bacterial biomass (biomass at final time point minus initial value) was divided by incorporation rates integrated over time during the experiment (Kirchman 1992). The rates were calculated according to an equation derived by

Kirchman et al. (1982): $\text{CF} = (\mu N_0)(v_0^{-1})$ where v_0 was the initial thymidine incorporation rate. Growth rate constants were calculated from $\mu = T^{-1}(\ln N_t - \ln N_0)$, where N_t and N_0 were the final and initial total abundance and T the duration of the experiment (Chrzanowski et al. 1993). Bacterial C content was calculated according to the equation: Bacterial C = $90 \times V^{0.6}$ where V was the mean cell volume (Simon & Azam 1989, Bååth 1994).

Results. There was no discernible increase of total bacterial numbers in the control experiment (Fig. 1). In contrast, cell numbers increased in all other microcosms. Both total and saprophytic bacterial abundance markedly increased in the absence of predators. The peptone addition stimulated bacterioplankton growth. However, saprophytic increase was larger than the total increase. High incubation temperature also induced a clear stimulation of bacterial growth but, in contrast with the other microcosms, a lag time was detectable (10 h for total abundance). The high temperature batch was the only experiment where CFU (colony forming units) showed a typical sigmoid growth pattern.

A decrease of mean cell volumes followed by an increase occurred during all experiments (Fig. 2).

As with bacterial abundance, short-term incorporation rates for thymidine increased during all our incubations experiments (Fig. 3). However, the manipulation effect was strongest in the high temperature batch compared with the other experiments. The 3 chemical extraction procedures gave very similar results. The lower values obtained with treatment 3 in the temperature experiment were the only noticeable point of difference.

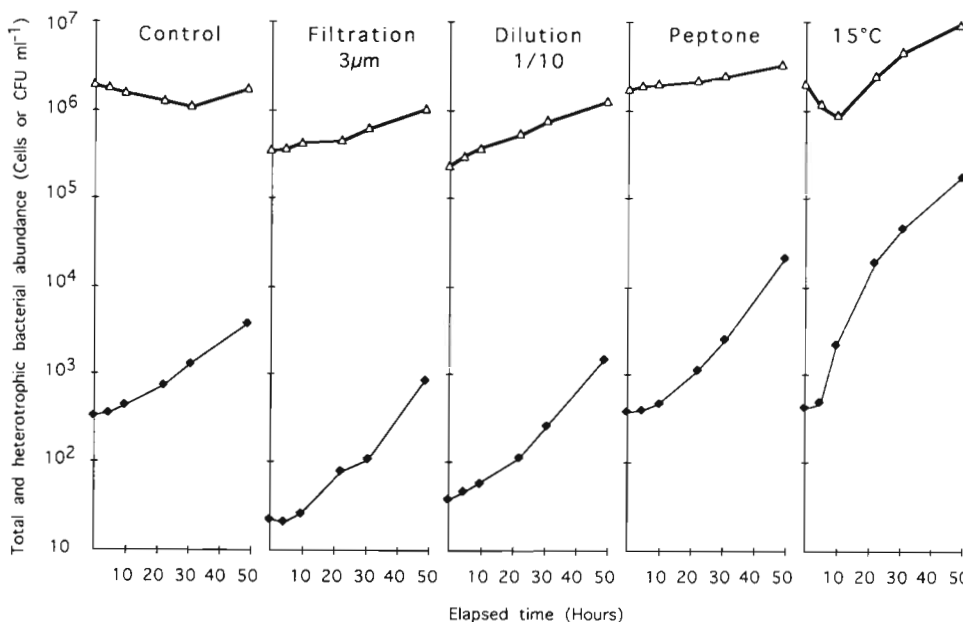


Fig. 1. Time course of total (thick line) and heterotrophic bacterial (thin line) abundance during microcosm experiments

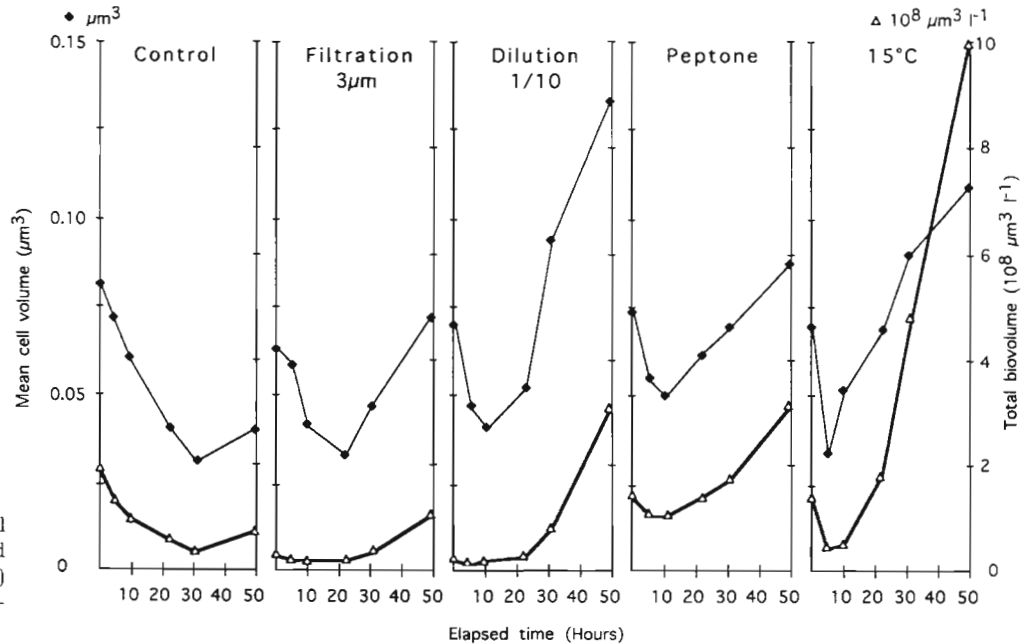


Fig. 2. Time course of total biovolume (thick line) and mean cell volume (thin line) during microcosm experiments

Calculated conversion factors are shown in Table 1. The 4 different treatments yielded comparable conversion factors ranging from 9.7×10^5 to 1.5×10^6 cells pmol^{-1} and from 15 to 30 ng C pmol^{-1} .

Discussion. Because the rates and patterns of nutrient utilization can be highly variable, elucidating the factors regulating bacterial growth and its concomitant influence on elemental fluxes is central to understanding biogeochemical cycles in the marine environment (Ducklow 1994). Predation and mortality due to viruses can partly regulate bacterial standing stock

(Azam et al. 1983, Kuosa & Kivi 1989, Bratbak et al. 1992, 1994, Shiah & Ducklow 1995, Weinbauer & Peduzzi 1995, Steward et al. 1996). A number of observations suggest that the microbial loop is also controlled by the interaction of 2 other main factors, temperature and substrate supply. These 2 factors clearly induced a strong stimulation of subantarctic bacterioplankton in our study. Based upon the analysis of 66 published studies, Rivkin et al. (1996) reported a weak relationship between specific growth rate of bacterioplankton and temperature with a $Q_{10} = 1.5$.

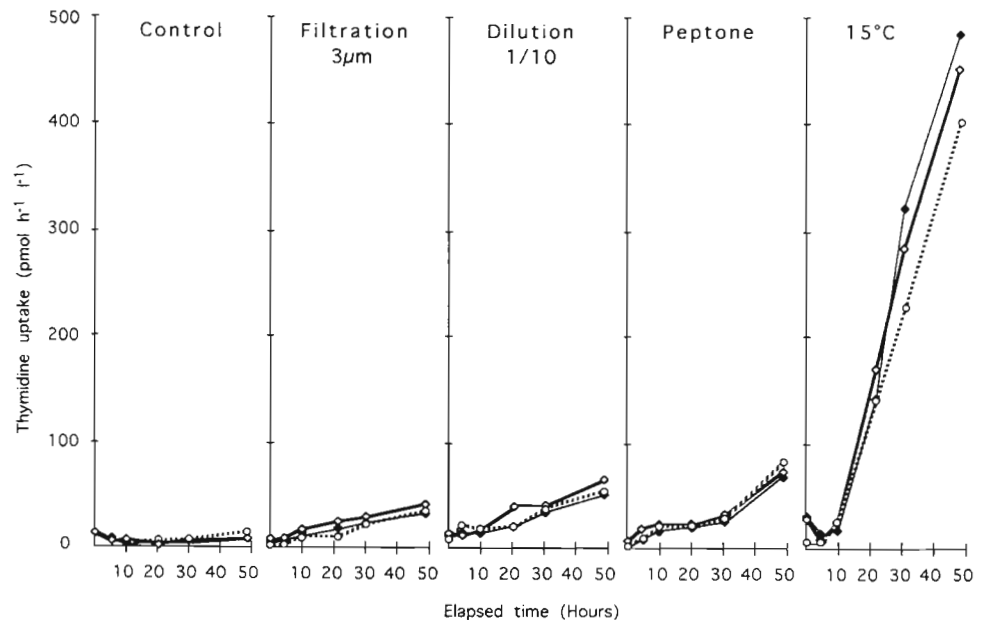


Fig. 3. Time course of thymidine incorporation (thick line: extraction treatment 1, thin line: extraction treatment 2, dotted line: extraction treatment 3) during microcosm experiments

Table 1. Conversion factors deduced from microcosm experiments

Method	Extraction procedure	Conversion factors	
		10^6 cells pmol^{-1}	ng C pmol^{-1}
Filtration	1	0.97	15
	2	0.98	15
	3	1.3	20
Dilution	1	1.2	26
	2	1.4	30
	3	1.3	28
Peptone	1	1.2	21
	2	1.4	23
	3	1.2	20
15°C	1	1.2	25
	2	1.3	25
	3	1.5	29
Mean		1.2	23

They also found that the specific growth rate of bacteria from cold ($<4^\circ\text{C}$) and warm ($>4^\circ\text{C}$) waters were not significantly different. In contrast, in this study a 10°C increase induced a more than 10-fold increase in thymidine uptake. This positive influence of temperature is in agreement with numerous observations that reported significant correlations between bacterial production and temperature (Morita et al. 1977, Hanson & Pope 1981, Delille et al. 1988, Hoch & Kirchman 1993, Shiah & Ducklow 1994). However, such large increases in temperature (10°C) are rarely found under natural subantarctic conditions. In any case, these data confirm previous studies in the same area which demonstrated that a large majority of the subantarctic and antarctic heterotrophic bacterial isolates have substantial growth rates for a wide range of temperatures (0 to 20°C ; Straka & Stokes 1960, Herbert & Tanner 1977, McMeekin & Franzmann 1988, Delille & Perret 1989, Nedwell & Rutter 1994, Helmke & Weyland 1995). Over large scales the best predictors of bacterial biomass and productivity may be factors related to the total resource level in an ecosystem. In addition, bacterial resources are probably influenced by food-web interactions (Pace & Cole 1994).

When bacterial growth is unbalanced, different estimating methods may yield different values for conversion factors because cell numbers, mean cell volumes and isotope incorporation change at different rates (Ducklow et al. 1992). The empirically determined thymidine conversion factors can vary >500 fold (Kirchman et al. 1982, Coveney & Wetzel 1988, Smits & Riemann 1988, Ducklow et al. 1992). Despite these observations the conversion factors derived from the 4 experimental methods are homogenous (Table 1). Furthermore, these factors agree well with the factor of 1.25×10^6 cells pmol^{-1} evaluated in mixed cultures of

bacterial assemblages from the Weddell Sea by Bjørnsen & Kuparinen (1991).

For the microbial assemblages present in the western Bransfield Strait during the 1986–1987 austral summer, Karl et al. (1991) reported that ^3H -thymidine appeared to have been extensively incorporated into macromolecules other than DNA; the total radioactivity incorporated into DNA as a percentage of total acid insoluble material ranged from $<10\%$ at selected stations in December to $>50\%$ later in the season. However, despite the fact that the 3 extraction procedures used in the present study must correspond to different fractions of the synthesized bacterial material, they yield relatively similar results.

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