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Bacterial Standing Stock, Activity, and Carbon Production during Formation and Growth of Sea Ice in the Weddell Sea, Antarctica†

SÖNNKE GROSSMANN* AND GERHARD S. DIECKMANN

Alfred-Wegener-Institut für Polar- und Meeresforschung, 27515 Bremerhaven, Germany

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Bacterial response to formation and growth of sea ice was investigated during autumn in the northeastern Weddell Sea. Changes in standing stock, activity, and carbon production of bacteria were determined in successive stages of ice development. During initial ice formation, concentrations of bacterial cells, in the order of 1×10^8 to 3×10^8 liter^{-1}, were not enhanced within the ice matrix. This suggests that physical enrichment of bacteria by ice crystals is not effective. Due to low concentrations of phytoplankton in the water column during freezing, incorporation of bacteria into newly formed ice via attachment to algal cells or aggregates was not recorded in this study. As soon as the ice had formed, the general metabolic activity of bacterial populations was strongly suppressed. Furthermore, the ratio of [^{3}H]leucine incorporation into proteins to [^{3}H]thymidine incorporation into DNA changed during ice growth. In thick pack ice, bacterial activity recovered and growth rates up to 0.6 day^{-1} indicated actively dividing populations. However, biomass-specific utilization of organic compounds remained lower than in open water. Bacterial concentrations of up to 2.8×10^9 cells liter^{-1} along with considerably enlarged cell volumes accumulated within thick pack ice, suggesting reduced mortality rates of bacteria within the small brine pores. In the course of ice development, bacterial carbon production increased from about 0.01 to 0.4 μg of C liter^{-1} h^{-1}. In thick ice, bacterial secondary production exceeded primary production of microalgae.

In the course of current discussion on possible global climate change, biogeochemical carbon cycles in the Southern Ocean have received increasing attention (36, 39). A dominating feature of this marine environment is the annual formation and decline of sea ice, which at its maximum extent during winter covers an area of approximately 20×10^6 km^2 (59). A widely branched channel system, often containing high saline brine solution, pervades sea ice (51). Rich and divers microbial communities composed of various groups of autotrophic and heterotrophic organisms live within this unique habitat (25, 38). Actively metabolizing bacterial populations can produce high biomass, even though environmental conditions within sea ice are remarkably different and more variable than those in open waters (34, 47). Analyses of microbial carbon cycling have elucidated the ecological significance of bacterium-mediated food webs in the ice (32, 34, 42).

The predominant process of pack ice development in the Weddell Sea has been identified as a succession of several characteristic stages of ice growth, described as a "pancake" ice cycle (35, 50). It has a severe impact on planktonic organisms (10). In a recent study, Gleitz and Thomas (18) described the responses of phytoplankton populations to sea ice formation and analyzed the physiological characteristics of microalgae in successive stages of ice growth. Less attention has been paid to reactions of bacterial populations to formation and growth of new sea ice. Taxonomic studies indicated that bacterial communities inhabiting sea ice differ from those of the water column (7, 58), but how populations in these habitats interact during the annual cycle of ice formation and melting prevalent in most regions of the Antarctic ocean is largely unknown.

Microalgae may be physically enriched within newly forming sea ice by the "scavenging" effects of frazil ice crystals (1, 13, 15) or by wave fields which pump water through grease ice, causing algae to become trapped between the ice crystals (2, 51). Laboratory freezing experiments revealed that enrichment of bacterial cells in new ice occurred in conjunction with certain algal species, suggesting "coincorporation" of bacteria attached to algal cells and aggregates (22). However, little information is available on the fate of incorporated bacteria during ice growth. Activity measurements in the laboratory suggest a change in bacterial populations during the course of ice development, as indicated by initial metabolic suppression followed by subsequent recovery (22). Microautoradiographic determinations of individual, metabolically active cells revealed reduced biomass-specific activity of sea ice bacteria compared with bacteria living in ice-free surface water (21).

The aim of this study was to investigate bacterial responses to formation and growth of sea ice under natural conditions in the field and to relate open-water populations to those of thick pack ice. In addition to the study of biomass distribution patterns, we focus mainly on changes of bacterial growth and activity during different developmental stages of the ice and provide information on production rates of bacterial carbon in relation to primary production by microalgae. Seasonal fluctuations in the ratio of algal primary to bacterial secondary production in sea ice are discussed.

MATERIALS AND METHODS

Sample collection. This study was conducted during the cruise *ANT X/3* (March to May 1992) of the R.V. *Polarstern* to the northeastern Weddell Sea, Antarctica (20). Different

* Corresponding author. Mailing address: Alfred-Wegener-Institut für Polar- und Meeresforschung, Sektion Biologie II, Postfach 12 01 61, 27515 Bremerhaven, Germany. Phone: 49-471-4831471. Fax: 49-471-4831425.

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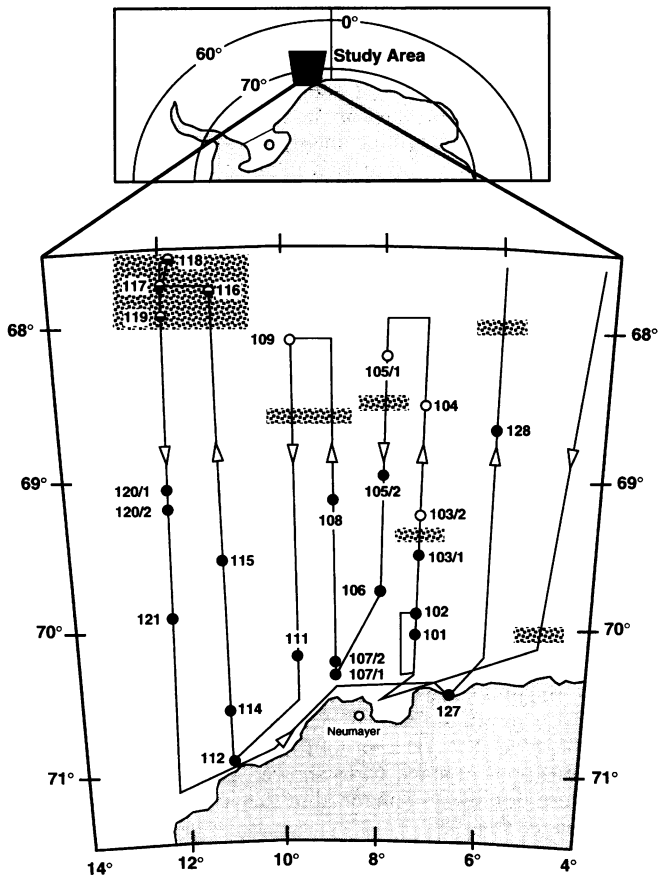

FIG. 1. Study area and cruise track of the R.V. *Polarstern* during expedition *ANT X/3*. Station numbers denote dates of sampling (Julian days). ▥, approximate location of ice edge; ○, open water station; ●, ice station; ◒, ice edge station.

stages of sea ice as well as ice-free surface water were sampled by crossing the transition zone between open water and thick pack ice several times (Fig. 1). An initial layer of "grease ice" consisting of separate frazil ice crystals was forming at the sea surface under turbulent conditions. During further freezing, decimeter-sized, roughly circular ice "pancakes" consolidated and merged into meter-sized "superpancakes." These finally accreted into a closed pack ice cover. Accordingly, the ice and water samples were grouped into five successive classes: open water (OW), grease ice (GR), young pancake ice (PC I), old pancake ice (PC II), and closed pack ice sheet (CPIS) (Table 1).

Water and ice were collected as described by Gleitz and Thomas (18) in a companion paper. Briefly, water samples were obtained with 12-liter PVC Niskin bottles (General Oceanics) or by bucket from the ship. Grease ice and small pancakes were collected from a dinghy. Both ice types were gently scooped from the sea surface with a rectangular coal scuttle-like sampler made of stainless steel. These samples were immediately drained over a plastic sieve to separate ice crystals from interstitial pore water. Entire large pancakes were gathered with an ice basket operated by the ship's crane. The basket was designed so that the pancakes remained floating in a layer of seawater in order to avoid brine drainage during sampling. Old, large superpancakes, often consisting of smaller pancakes frozen together, were sampled by coring (ice

TABLE 1. Description of sample types collected during the cruise *ANT X/3*

Sample type	Description	Estimated age
Open water (OW)	Ice-free surface water	
Grease ice (GR)	Frazil ice layer accumulating at the sea surface	Hours
Young pancake ice (PC I)	Small individual pancakes, <1 m in diam, <20 cm thick No snow cover	Days
Old pancake ice (PC II)	Larger pancakes, 1 m to several meters in diam, 20–50 cm thick; often consisting of smaller pancakes frozen together 1–20-cm snow cover	1–2 wk
Closed pack ice sheet (CPIS)	Floes of thick pack ice, >50 cm thick 10–50-cm snow cover	>2 wk

auger, 7.6 cm in diameter; Cold Regions Research and Engineering Laboratory). Brine was separated from the ice matrix by centrifuging pieces and core segments of pancake ice at 275 × *g* for 5 min at −2°C (51). Brine from thick pack ice floes was obtained from dead-end "sack holes": several minutes after removing short cores (30 to 40 cm) from the ice sheet, brine collected in these holes and could be scooped out for subsequent analyses.

In order to maintain conditions in the brine as unchanged as possible, care was taken to prevent further freezing or dilution through melting of ice crystals by storing samples in insulated containers. Salinity increased from about 34‰ in open water to an average of 47‰ in older ice stages, the maximum observed being 90‰ (see reference 18 for a comprehensive discussion of physical and chemical conditions in successive ice stages). A total of 16 open water, 7 grease ice, 10 pancake ice, and 8 thick pack ice, samples were analyzed. However, not all parameters were determined for every sample.

Bacterial cell concentration and biomass. Acridine orange direct counts were determined by epifluorescence microscopy (6, 24). The fixed samples (0.4% formaldehyde, final concentration; stored at 0°C) were filtered, in the laboratory, onto 0.2-μm-pore-size Nuclepore filters and subsequently stained with 0.01% acridine orange. For statistical accuracy of acridine orange direct count data, two filters (subsamples) were prepared from each sample (29). Microscopic analyses were carried out with a Zeiss Axioskop 20 epifluorescence microscope (filter system BP450-490/FT510/LP520, oil immersion objective, Plan-Neofluar 100). Bacterial cell concentration was calculated from ca. 400 cells recorded in 15 fields of a counting grid (9.8×10^3 μm^2) on each of the two filters. Estimates of bacterial biovolume were based on measurements of bacterial cell sizes and calculations of individual cell volumes, as described by Grossmann and Reichardt (23). Total biovolume of bacteria per liter of sample was converted into bacterial carbon, using a factor of 3.0×10^{-13} g of C μm^{-3} (4).

As shown by Turley and Hughes (48), storage of samples can reduce cell counts. However, the main purpose of this study was to investigate relative changes of bacterial characteristics during successive development of sea ice, assuming that effects of storing were similar for all samples of different ice stages. Furthermore, the sizing of bacterial cells under the microscope for biomass estimates cannot be carried out on board due to

movement and vibrations of the ship, which also reduce counting accuracy.

Turnover rates. Uptake rates of organic compounds were determined with trace amounts of [^{3}H]leucine as substrate (19, 54). Triplicate 20-ml water or brine samples were incubated with 1.85 MBq of L-[4,5-^{3}H]leucine liter^{-1}(≙0.4 nM; specific activity, 5.2 TBq mmol^{-1} [Amersham Buchler]) for 90 to 100 min at −1°C. The dependence of [^{3}H]leucine uptake on the incubation time was checked by a time series and found to be linear for up to 5 h (data not shown). Incubations were stopped with formaldehyde (0.4% final concentration). Triplicate formaldehyde-prefixed samples were treated identically and used as blanks. The samples were filtered onto 0.2-μm-pore-size Nuclepore filters, rinsed twice with filtered seawater (0.2-μm-pore-size filter), and radioassayed in a scintillation counter (Packard Tri-Carb 1900). Quench correction was performed by automatic external standardization. Since natural levels of leucine were not measured, uptake rates are given as the percentage of initial [^{3}H]leucine concentration turned over per hour. Specific leucine uptake per unit of bacterial biomass was calculated as turnover rate per respective biomass concentration of bacteria (55).

Incorporation of [^{3}H]leucine into hot TCA precipitates. Subsamples of water and brine were taken to determine rates of incorporation of [^{3}H]leucine into the hot trichloroacetic acid (TCA)-insoluble fraction (28, 31). Triplicate 20-ml samples were incubated with 10 nM L-[4,5-^{3}H]leucine (diluted with cold L-leucine to a specific activity of 1.3 to 2.6 TBq mmol^{-1}) for 90 to 100 min at −1°C. Samples were extracted for 30 min in boiling TCA (5% final concentration). After cooling in an ice bath, the TCA-insoluble fraction was collected on 0.2-μm-pore-size Nuclepore filters, rinsed twice with filtered seawater (0.2-μm-pore-size), and radioassayed in the scintillation counter. Otherwise, the procedure paralleled that described above for determination of turnover rates.

Incorporation of [^{3}H]thymidine into cold TCA precipitates. Determination of the incorporation rate of [^{3}H]thymidine as a measure of bacterial multiplication of nucleic acids was carried out according to the method of Fuhrman and Azam (12). Triplicate 20-ml water and brine samples were incubated with 10 nM [*methyl*-^{3}H]thymidine (diluted with cold thymidine to a specific activity of 1.6 TBq mmol^{-1}; Amersham Buchler) for 90 to 100 min at −1°C. After being filtered through 0.2-μm-pore-size Nuclepore filters, samples were extracted for 5 min in 5% ice-cold TCA and subsequently rinsed five times with the ice-cold TCA solution. Further processing of samples was conducted as described above for [^{3}H]leucine incorporation.

Growth rates and carbon production. Calculation of bacterial carbon production was based on [^{3}H]thymidine incubations. Conversion factors to estimate bacterial cell production from thymidine incorporation rate were calculated from time course experiments in which the increase of bacterial cell concentration was followed by epifluorescence microscopy parallel to one initial measurement of thymidine incorporation (26). To account for possible changes of bacterial thymidine metabolism during ice growth, two calibration experiments were carried out separately for young and old ice stages. Particulate matter (including bacteria) in unfiltered inocula of 50 ml was diluted 10-fold with 450 ml of filter-sterilized seawater prior to the onset of the time series. Growth of bacteria was followed in 10 steps over a period of 190 h and was found to be exponential in both incubations (r^2 = 0.95 [young ice] and 0.89 [old ice]), suggesting that grazing on bacteria was minimal (30). Exponential regressions of cell concentrations versus time resulted in conversion factors of 1.77×10^{18} cells for grease and pancake ice and 1.89×10^{18} cells for thick pack ice per mol of thymidine. Cell production in open water was estimated by using a thymidine conversion factor of 1.1×10^{18} cells mol^{-1} determined by Bjørnsen and Kuparinen (3) from water samples of the Southern Ocean. Bacterial carbon production in ice and water samples was calculated from thymidine incorporation rate, using the respective conversion factor and mean cell volume of sample, which was converted into units of carbon by the factor applied for acridine orange direct count determinations.

Specific bacterial growth rates (μ) were estimated from the production/biomass ratio. Corresponding generation times (G) of bacterial populations were calculated according to the formula: $G = \ln 2/\mu$.

Statistical considerations. Medians were calculated for grouped ice and water samples in order to indicate central tendencies of the data sets as well as to avoid impacts of extreme values. This statistical treatment was applied to the larger data sets making up bacterial cell concentrations and biomasses, leucine turnover rates, and specific turnover rates per biomass.

RESULTS

Bacterial standing stock. Median bacterial cell concentrations increased from the open water through the different developmental stages of sea ice, reaching maximum values in the pore water of old pancake ice and in the brine of thick pack ice (Fig. 2a, PC II and CPIS). The wide range of cell concentrations at stage CPIS showed that bacterial cell numbers were not always enhanced in this ice type compared with open water samples.

Distribution spectra of cell volumes changed towards larger cell sizes in the growing ice (Fig. 3). Bacterial populations in the two thickest pack ice floes sampled (thickness, >1 m) were characterized by considerably larger cells than in the other ice types, resulting in a higher median value ($CPIS_b$). In one sample of grease ice (GR), an assemblage of extremely large bacteria which differed clearly in cell size from those usually found in this ice type was recorded. As depicted in Fig. 3, the respective median volume of these large cells, when sized separately, amounted to 0.43 μm^3, which was comparable to the largest bacterial cells of thick pack ice. This extreme value was not considered for median calculations of biomass and leucine turnover.

Generally, biomass of bacteria increased in older ice stages (PC II and CPIS) because of both higher cell concentrations and larger cell sizes (Fig. 2b). In some PC II and CPIS samples, bacterial biomass exceeded that in open water by more than one order of magnitude.

Activity measurements. Turnover rates of [^{3}H]leucine in successive stages of ice development differed substantially from patterns of bacterial standing stock (Fig. 4a). A broad range of values was recorded for open water samples. During formation and growth of young ice (GR to PC II), turnover rates decreased strongly. Also, the very large cells found in one grease ice sample (plus symbol) exhibited lower values than recorded for several samples of open water. In the brine of thick pack ice (CPIS), turnover rates rose again, and values covered a wide range comparable to that of open water samples. Specific turnover rates per unit of bacterial biomass showed an even stronger reduction between open water and pancake ice samples (Fig. 4b). The specific turnover rates of the large cells in grease ice did not differ from those of "normal" sized bacteria in this ice stage. At stage CPIS, the biomass-specific turnover rates increased similarly to the ab-

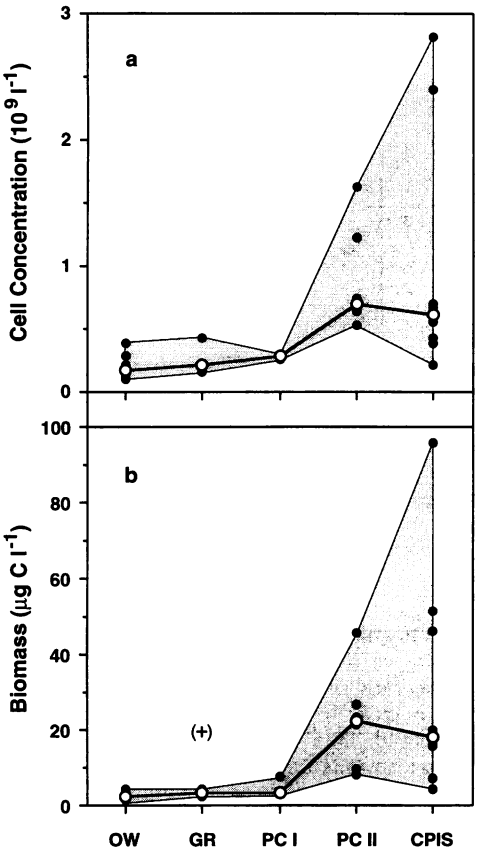

FIG. 2. Bacterial cell concentrations (a) and biomasses (b) in open water (OW) and in four successive stages of sea ice (GR, PC I, PC II, and CPIS). Open circles denote median of each data set. Shaded area indicates range of datum points (●). (+) Biomass of conspicuously large bacteria recorded in one grease ice sample (see text) was not considered for median calculation.

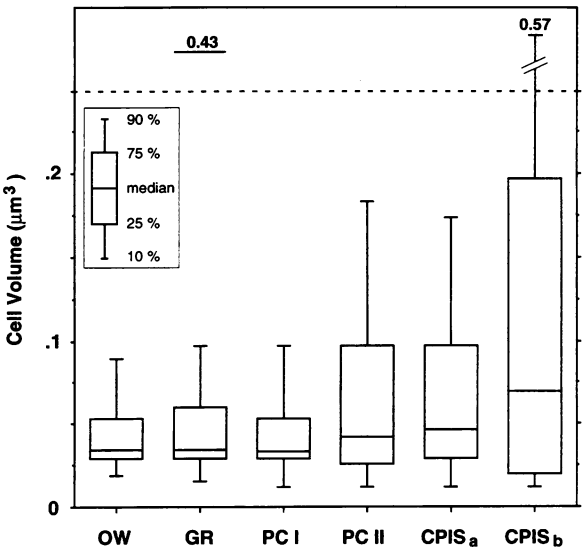

FIG. 3. Box plots of bacterial size spectra in open water and in successive stages of sea ice. Horizontal lines within boxes indicate the median of the spectrum; boxes and vertical bars represent percentiles. The size spectrum of bacterial cells in the two thickest pack ice floes sampled was plotted separately (CPIS$_b$; thickness of ice sheet, >1 m). The median of conspicuously large bacteria recorded in one grease ice sample (see text) is shown separately.

solute values. However, the median of the specific rates remained lower than that of open water.

Rates of [^{3}H]leucine incorporation into the hot TCA-insoluble fraction showed a trend similar to that of the turnover rates of this amino acid during ice succession (Fig. 5). Relatively high values in open water were followed by a reduction in those in young ice stages. At stage CPIS, incorporation of [^{3}H]leucine exceeded that in open water samples. This was also recorded in one sample of old pancake ice, while in two other cases incorporation rates were low in this stage.

Rates of [^{3}H]thymidine incorporation into the cold TCA-insoluble fraction were low in open water samples (Fig. 5). During formation of new ice, incorporation rates decreased only slightly. At stages PC II and CPIS, [^{3}H]thymidine incorporation was high and in all cases exceeded that in open water samples.

Specific growth rates of bacteria in open water were in the order of 0.06 to 0.2 day^{-1}, corresponding to generation times of 3.5 to 12 days. A similar range of values was obtained for the ice populations. The highest growth rates, 0.4 to 0.6 day^{-1}, were recorded in two samples of older ice stages (PC II and CPIS).

Carbon production. Production of bacteria in different stages of ice development was compared with that of microalgae determined in the same sample (Fig. 6). In open water prior to ice formation, both algal and bacterial productivities were low. In the developing young ice, primary production increased relatively to secondary production of bacteria. In brine of thick pack ice, bacterial production exceeded primary production, which, in turn, was lower than in pancake ice.

DISCUSSION

An outstanding feature of sea ice communities is the accumulation of high algal and bacterial biomass (16, 47). Enrichment of algal cells is often initiated during the transition from open water to early stages of new ice. This has been attributed to physical concentration mechanisms such as scavenging by frazil ice crystals or wave fields pumping water through the new ice layer, causing cells to become attached to or trapped between ice crystals (2, 15). In newly formed ice, Gleitz and Thomas (18) recorded enhanced algal concentrations exceeding that in the open water by a factor of 1.5 to 2.5, while concentration factors of up to 70 have been reported for the foraminifer *Neogloboquadrina pachyderma* (9). Our results for bacteria revealed that their biomass was not substantially enhanced in the grease ice compared with the respective concentrations in open water samples. Simulation of sea ice formation in the laboratory (22), as well as observations in the field (21), revealed an enrichment of bacterial cells in new ice only in conjunction with algae. No enrichment was observed when the concentration of phytoplankton was low. The authors hypothesized that the predominant process for bacterial enrichment in new ice is coincorporation of bacteria attached to algal cell surfaces or aggregates. During the present study, the concentration of algal cells was always low in open surface waters. Chlorophyll concentrations did not exceed 0.4 µg liter^{-1} (8). Consequently, bacterial cells were not enriched in the newly formed grease ice due to lack of "carrier" algae. Apparently, physical concentration mechanisms are not effective below a certain threshold size such as that of planktonic

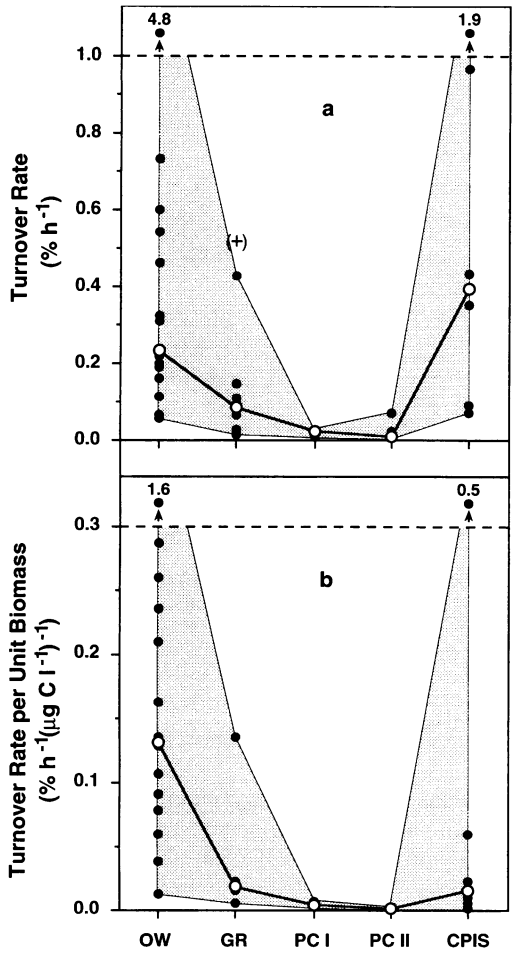

FIG. 4. Turnover rates of [^{3}H]leucine (a) and specific turnover rates of [^{3}H]leucine per bacterial biomass (b) in open water and in successive stages of sea ice. Symbols and significance of shaded areas are given in the legend to Fig. 2.

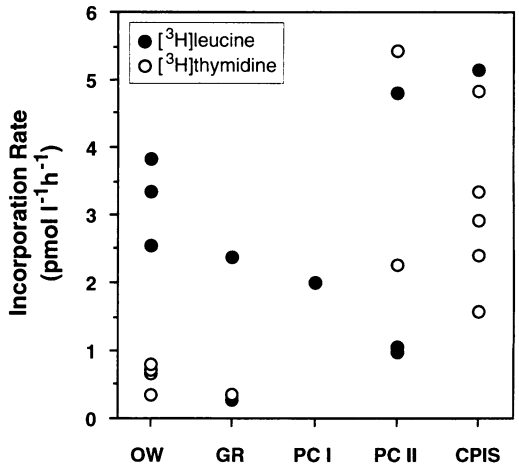

FIG. 5. Rates of incorporation of [^{3}H]leucine and [^{3}H]thymidine into the hot and cold TCA-insoluble fractions, respectively, determined in open water and in successive stages of sea ice.

bacterial cells. Physical enrichment, however, might have been responsible for the occurrence of extremely large bacterial cells of up to 1 μm^3 in one sample of grease ice which reached a median cell volume about 10 times that of bacteria normally found in this ice type (Fig. 3). It is unlikely that growth accounted for the large cells, because this ice type was formed within a time span of hours (Table 1) and the specific metabolic activity of these bacteria did not differ from values determined for bacterial populations in other grease ice samples (Fig. 4b).

As soon as the ice had formed, the activity of bacteria was substantially reduced in all samples. This was indicated by turnover rates of the amino acid leucine (Fig. 4a), which served as a measure for general heterotrophic activity of bacteria (19, 56). An even stronger decline in the specific activity of bacteria between stages OW and PC II (Fig. 4b) indicates substantial deterioration of metabolic activity in bacterial assemblages (55). The change in bacterial activity in young sea ice is corroborated by results from freezing experiments under controlled conditions in the laboratory which also showed that, despite enhanced biomass, activity of bacteria in newly formed ice was reduced (22).

In addition to the suppression of bacterial activity, the ratio of leucine to thymidine incorporation also changed during the course of sea ice development (Fig. 5). In open water, the average molar ratio of Leu/Thy incorporation was of the order of 5, which is close to values determined in various aquatic environments (40, 43, 57). In the growing young ice, however, both were incorporated at a molar ratio of approximately 1, suggesting frequent division of cells but low rates of protein synthesis (31, 40). Changes of thymidine incorporation during ice succession were paralleled by the development of bacterial cell concentration (Fig. 2a and 5). In contrast, the incorporation rate of leucine as a measure for bacterial protein synthesis (28, 31) was low in the growing ice and did not match distribution patterns of bacterial cell sizes (Fig. 3 and 5). It is therefore possible that the 10 nM [^{3}H]leucine used was insufficient to suppress bacterial utilization of organic com-

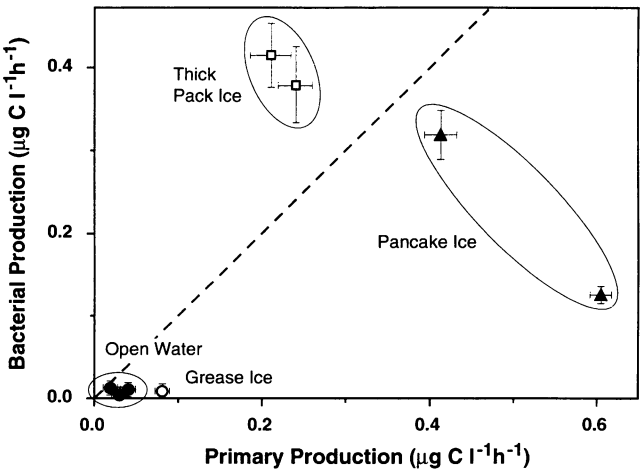

FIG. 6. Primary production of microalgae versus bacterial production in samples of open water and successive ice types, with production rates determined simultaneously. Primary production data at these stations were provided by M. Gleitz and D. Thomas (see also reference 18). The broken line represents the 1:1 ratio. Error bars denote standard errors of three replicates radioassayed each way. Circles depict the range of data found for different ice types.

pounds other than the radioactive substrate. Consequently, (total) leucine incorporation may be underestimated due to isotope dilution of the added tracer (44). On the other hand, Bjørnsen and Kuparinen (3), who measured protein synthesis by Antarctic planktonic bacteria at similar [^{3}H]leucine concentrations, argued that the use of high concentrations increases the risk that organisms other than bacteria (preferentially microalgae) may incorporate the labelled amino acid. Furthermore, we would assume that substantial utilization of alternative, nonradioactive substrate by the bacteria is correlated with high concentrations of dissolved organic matter. Since dissolved organic matter is thought to accumulate along with microalgal biomass, reaching highest concentrations in old, thick pack ice (18, 38), we expect a potential underestimate of leucine incorporation especially in old ice stages. However, the incorporation of leucine was lower than that of thymidine, particularly in early stages of ice development (Fig. 5). Also, the turnover rate of this amino acid was extremely low in pancake ice (Fig. 4a). Using microautoradiography, Grossmann (21) showed that only 3 to 5% of bacteria in this ice type utilized [^{3}H]leucine. Apparently, leucine metabolism by bacteria in young sea ice is substantially suppressed and has not yet been fully explained.

In thick pack ice (CPIS), bacterial activity increased. Specific growth rates as high as 0.6 day^{-1} indicated rapidly dividing populations. This value was lower than growth rates found for highly productive communities in surface ponds of pack ice during autumn in the Weddell Sea (34) but similar to those determined in McMurdo Sound during summer for active bacterial populations inhabiting the bottom 5 cm of congelation ice (32). Turnover of leucine showed that this amino acid was extensively metabolized by the bacteria. Biomass-specific turnover rates indicated, however, that the average metabolic activity of those bacteria which developed in the ice did not reach that of open water populations (55). As yet, we have no conclusive explanation for this low specific activity. Due to preferential utilization of polymeric compounds compared with free amino acids (27), an underestimate of total heterotrophic activity using turnover rates of leucine cannot be ruled out. On the other hand, reduced specific activity of sea ice bacteria may be caused by a lower substrate affinity of the cells, which in turn is a consequence of high organic nutrient concentrations within the brine system of thick ice floes (38). Bacteria adapted to oligotrophic conditions (as assumed for those of the open water samples) were shown to consume a considerable proportion of metabolic energy to achieve high substrate affinity (57). Alternatively, it is reasonable to assume that populations in the ice do not maintain diversified enzyme systems required for highly effective nutrient uptake and, therefore, utilize a higher proportion of the assimilated substrate for biomass production.

Since generation times of bacteria at later ice stages were usually much lower than the estimated age of these ice types, the observed accumulation of bacterial biomass in the ice is likely to be the result of growth rather than of concentration processes caused by the removal of water due to freezing. Enhanced biomass of bacteria in older ice was due to high concentrations as well as large volumes of the cells. About 25% of bacterial cells in brine of thick pack ice floes were larger than 0.2 μm^3 (Fig. 3, $CPIS_b$). The occurrence of large bacteria has frequently been observed in sea ice (37, 47). Kottmeier and Sullivan (34) found average cell volumes of about 0.3 μm^3 for bacterial populations of various sea ice habitats. In the bottom section of thick fast ice, cell volumes of up to 10 μm^3 were recorded (47). This enlargement of bacteria during ice development contrasts with observations made for algal cells, which showed a species succession towards smaller forms (18). The reduction of algal cell volumes was explained by confined pore space available for colonization within the brine channel system. In the range of bacterium-sized organisms, restriction due to pore space is not likely to be a regulating factor. Enlarged cell volumes of Arctic bacteria have been attributed to both low temperature and high nutrient concentration (53). The development of large bacterial cells can also be the result of low grazing pressure (49). Potential consumers of bacteria are proto- and metazoa, which occur frequently within the ice (14). On the other hand, considerably high amounts of bacterial biomass accumulated in some samples of older ice stages, indicating that, in these cases, growth rates of the bacteria surpassed grazing rates. Presumably, bactivoral organisms were restricted to larger brine channels and had no access to small pores of only a few micrometers in diameter (51) inhabited by bacterial cells. We hypothesize that, in contrast to conditions of open water, bacteria living within thick pack ice are temporarily protected from grazing as long as the isolated brine system persists.

Results of a microautoradiographic investigation carried out in different, successive developmental stages of sea ice suggested a change in the composition of bacterial populations during the course of ice development (21). The present study indicates that this may be a general response of bacteria to sea ice formation in the Weddell Sea. We hypothesize that alterations of environmental conditions accompanying the formation and growth of sea ice suppress bacterial populations originating from open water and induce the development of new assemblages adapted to the ice situation. This suggestion is in accordance with taxonomic investigations using morphological and biochemical tests on bacterial strains isolated from ice and water habitats of the western Weddell Sea, which revealed that bacterial communities inhabiting pack ice differ from those of underlying seawater (7, 58).

Carbon production of bacteria increased from about 0.01 μg of C liter^{-1} h^{-1} in open water to 0.4 μg of C liter^{-1} h^{-1} in the brine of thick pack ice (Fig. 6). Assuming a mean brine content of ca. 10% (52), bacterial production in the ice was approximately four times higher than that in an equivalent volume of open water. These calculations were based on determinations of thymidine incorporation. Since leucine metabolism of bacteria was substantially suppressed, especially at young ice stages, and did not reflect development of bacterial biovolume during ice succession (see above), we propose that calculations based on incorporation rates of thymidine constitute more reliable estimates of bacterial carbon production in the sea ice investigated. Theoretically, a conversion factor for calculating biomass production of bacteria from incorporation rates of leucine would have to vary over about two orders of magnitude between open water and different ice stages in order to match thymidine-based production rates, which seems to be unlikely.

In thick pack ice, bacterial secondary production exceeded primary production by microalgae (Fig. 6). The decrease of the latter in this stage has been explained by reduced light supply due to thickening of the ice sheet and increasing snow accumulations in conjunction with low temperatures and high salinities (18). Assuming a bacterial growth yield of 40% (3), carbon demand of bacteria would outweigh primary production also in samples of pancake ice. For planktonic communities of Antarctic regions, the ratio of algal primary production to bacterial secondary production exhibits a strong seasonality throughout the year (41). During bloom conditions in late austral spring and summer, bacterial production is often low relative to that of phytoplankton (11, 46). At the end of a bloom as well as at low levels of primary production during

autumn and winter up to early spring, bacterial production can approach or even exceed that of algae (5, 17, 41). A similar picture emerges for microbial communities in the ice. During an early summer bloom, bacterial production in thick sea ice constituted only 9% of microalgal production (32). A similar low ratio was reported for Arctic sea ice communities throughout the spring bloom (45). In ice communities studied between autumn and late winter, however, the consumption rate of organic carbon by bacteria in some cases exceeded its production by the algae (34, 42). On the other hand, Kottmeier and Sullivan (33) recorded substantial rates of primary production in sea ice also outside the bloom season during late austral winter. In this case, bacterial production was only about 10% that of the microalgae. In the autumnal ice sampled during this study, algal production was considerably lower than rates usually reported for spring and summer communities (18). Furthermore, light conditions during autumn limited primary production to a few hours per day. Therefore, daily carbon consumption by bacterial populations that developed in this ice clearly exceeded that produced by the algae. Considering the isolated nature of the brine channel system, these results suggest a negative carbon balance for ice communities during autumn as soon as bacterial populations have established themselves within the ice sheet. We conclude that metabolic activity of bacteria contributes substantially to overall heterotrophy of sea ice microbial communities. Significant amounts of organic carbon within the ice environment and/or in ice-related, cryopelagic food webs are thus channelled through the "microbial loop."

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