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Changing bacterioplankton growth characteristics on a large spatial scale: oligotrophic versus mesotrophic ocean

Hans-Georg Hoppe*, Klaus Gocke, Regine Koppe, Gerd Kraus

Leibniz-Institut für Meereswissenschaften an der Universität Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

ABSTRACT: This study deals with large spatial scale differences in the ratios between bacterial leucine incorporation (TLi: protein synthesis) and thymidine incorporation (TTi: DNA synthesis) in oligotrophic offshore and comparatively more mesotrophic inshore (sub)tropical regions of the Atlantic Ocean. Observations were derived from 2 RV 'Polarstern' cruises, one of which traversed a meridional mid-ocean transect while the other followed the African coast line. Average values (from 42° N to 35° S) of TLi, TTi and chlorophyll *a* (chl *a*) concentration were 40.3 pmol leucine l^{-1} h^{-1}, 1.32 pmol thymidine l^{-1} h^{-1} and 0.18 µg chl *a* l^{-1} along the offshore transect, compared to 51.8 pmol leucine l^{-1} h^{-1}, 2.72 pmol thymidine l^{-1} h^{-1} and 0.29 µg chl *a* l^{-1} along the inshore transect. Mean values of the TLi:TTi ratio (which defines bacterial growth characteristics) were 32.4 in offshore waters and 20.5 in inshore waters. Offshore ratios of TLi:chl *a* or TTi:chl *a* (proxy for bacterial substrate) were 274.1 and 8.5, compared to inshore ratios of 198.7 and 10.0, respectively. This means that, per unit of chl *a*, considerably higher bacterial protein synthesis was supported in water farther from the coast than near the coast, whereas bacterial DNA synthesis per unit chl *a* was slightly higher in the latter. Because temperature variability along the cruise tracts was rather similar (except in the Benguela upwelling region), we assume that substrate supply was mainly responsible for the observed significant differences in bacterial growth characteristics. In addition, the potential different contributions of picocyanobacteria to leucine uptake (TLi) must be considered. We conclude that the different TLi:TTi ratios in (sub)tropical offshore and inshore waters reflect reactions of the relevant bacterial communities to prevailing environmental conditions. Therefore, we did not interpret our results in the context of the currently used terms 'balanced' or 'unbalanced' growth. Bacterial community growth may be balanced in both regions of study, but at different levels of the TLi:TTi ratio.

KEY WORDS: Leucine uptake · Thymidine uptake · TLi:TTi ratio · Balanced growth · Marine bacteria · Cyanobacteria · Primary production · Atlantic

INTRODUCTION

At the ocean-wide scale, bacteria are subjected to different environmental conditions that regulate their growth characteristics. In turn, bacterial growth characteristics reflect prevailing environmental conditions and thus may be appropriate for monitoring the effect of possible changes of these conditions on the microbial level. The growth characteristics of bacteria are determined by the ratio between protein production and DNA synthesis, as measured by tritiated leucine or thymidine incorporation, respectively (TLi:TTi) (Fuhrman & Azam 1982, Kirchman et al. 1985, Simon & Azam 1989). In general, it is assumed that a high TLi:TTi ratio may indicate unfavourable environmental conditions that do not allow bacteria to proceed to cell replication (Gasol et al. 1998). For instance, in the case of low substrate supply bacteria necessarily use the bulk of ingested substrates for respiration, and only a minor portion remains available for cell maintenance by protein production. High TTi (DNA synthesis and cell replication) relative to TLi suggests that substrate levels are sufficient to enable bacterial cell division. However, these responses of bacteria to substrate availability may be moderated by temperature (Middelboe & Lundsgaard 2003), regulating velocities of cell metabolism

*Email: hhoppe@ifm-geomar.de

and substrate cycling within the microbial food web. Furthermore, particularly in oligotrophic regions, the TLi:TTi ratio may be influenced by the occurrence of mixotrophic cyanobacteria that take up leucine, and also thymidine but only to a minor extent (Hietanen et al. 2002).

If cell replication (DNA synthesis) is paralleled by protein synthesis in a constant relationship, this is usually regarded as balanced growth. Unbalanced growth is anticipated when bacteria shift from one growth rate to another in response to changing conditions. This may occur in periods of uncoupling between phytoplankton and bacteria (Chin-Leo 1989). Unbalanced growth of bacteria was observed in offshore regions, in deep samples of vertical profiles and in during pulses of organic matter supply (Chin-Leo 1989) or when bacterial assemblages shifted between growth rates (Chin-Leo & Kirchman 1990). Most studies on the ratio between protein and DNA synthesis (TLi:TTi) are based on experimental or small/medium spatial scale investigations in the temperate climate zone, and the effect of substrate supply rather than that of temperature was the focus. The effects of both substrate supply and temperature on the TLi:TTi ratio were investigated by Shiah & Ducklow (1997) in an experimental approach. It was found that 'the TLi:TTi ratio decreased as temperature increased and correlated negatively with chlorophyll concentrations'. In other words, high temperature and substrate availability stimulated TTi whereas TLi was favoured by the opposite conditions. Generally, measured TLi:TTi ratios from different aquatic habitats varied over a wide range from 2 to 213 (e.g. Tibbles et al. 1992, Mock et al. 1997), with extreme values occurring in more extreme environments.

In the present study we compared the growth characteristics of bacteria (TLi:TTi ratios) observed during 2 cruises in the Atlantic Ocean, one of which went along a meridional mid-ocean transect (ANT X, oligotrophic) while the other followed the African coast line (ANT XI, increasingly mesotrophic) (Fig. 1). Our focus was on the zone with the greatest differences between the TLi:TTi

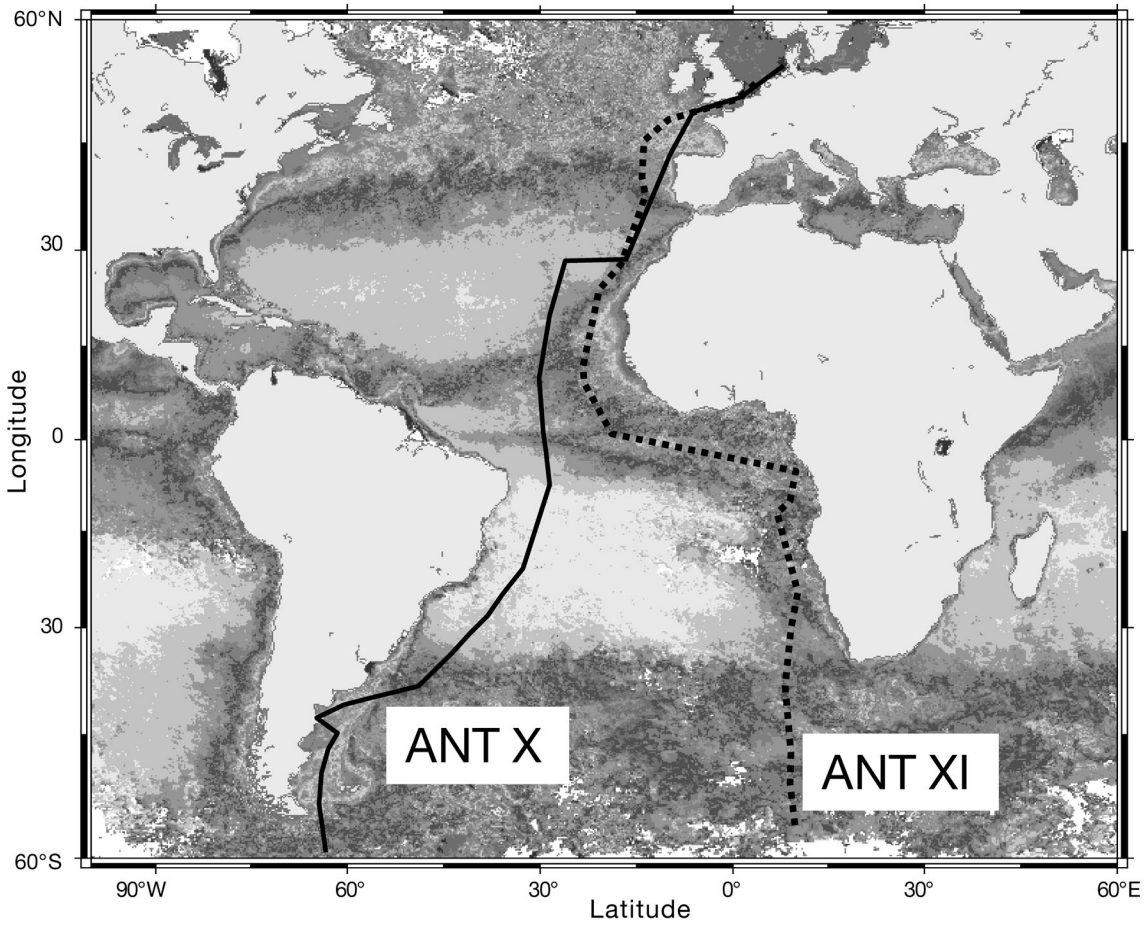

Fig. 1. Cruise tracts of 'offshore' ANT X and 'inshore' ANT XI in the Atlantic. The satellite image from the Coastal Zone Colour Scanner (CZCS) shows average chl *a* distribution over several years. Increasingly dark shading represents higher chl *a* concentrations. Both cruises took place during the same season: November to December-January

ratios of the 2 cruises, which extended from about 42° N to 35° S. According to the biogeographical provinces defined by Longhurst (1998), this zone extends from the northern border of the North Atlantic Subtropical Gyral Province (NAST) to the southern border of the South Atlantic Gyral Province (SATL).

Factors that may be responsible for the divergent behaviour of bacterial growth characteristics in the investigated oceanic provinces are discussed in detail. It was the aim of this study to determine the predominating mode of bacterial growth (TLi:TTi ratios) in relation to oligotrophic and increasingly mesotrophic conditions at a large spatial scale, and to discuss the findings with respect to the current models of 'balanced' and 'unbalanced' growth.

MATERIALS AND METHODS

This study was conducted during 2 cruises aboard RV 'Polarstern' in the Atlantic in 1990-91 and 1992. Both cruises started in November in the north and ended in January (ANT X) or December (ANT XI) in the south. The first cruise (ANT X, 53° N to 65° S) traversed along a mid-ocean profile, crossing the oligotrophic regions of the ocean (Hoppe et al. 2002). The second cruise (ANT XI, 53° N to 58° S) followed the African coastline at a distance of about 200 miles and was characterised by comparatively more mesotrophic conditions (Fig. 1). For practical reasons, we refer to these cruises as 'offshore' or 'oligotrophic' (ANT X) and 'inshore' or 'mesotrophic' (ANT XI). Underway samples were collected at 4 to 8 h intervals (about 140 samples per cruise) by the ship's intake snorkel and a special pump (which avoided breakage of cells and small aggregates) at a water depth of 12 m. Water from the snorkel was tested by parallel sampling with a Niskin sampler, and no significant differences in the measurements of microbial variables were observed. All samples from the 2 cruises were treated using exactly the same methods by the same operators.

Bacterial growth characteristics. Bacterial growth characteristics were derived from [^{3}H]leucine (TLi: protein synthesis) and [^{3}H]methyl-thymidine (TTi: DNA synthesis) incorporation (Fuhrman & Azam 1982, Kirchman et al. 1985, Simon & Azam 1989). A final concentration of 8 nM [^{3}H]leucine (specific activity 126 Ci mmol^{-1}) together with 24 nM unlabelled leucine was added to 20 ml samples (triplicates and 1 blank). The leucine concentration needed for substrate saturation was determined at the beginning of the sampling period. Nevertheless, for a few 'high chl *a*' stations on the Patagonian shelf, the applied leucine concentration may not have been adequate. Treated samples were incubated in the dark for 2 to 4 h in thermo-containers at *in situ* temperature. After fixation with formalin (1%), bacteria were collected on 0.2 µm polycarbonate filters (Poretics) and rinsed 10 times with 1 ml ice-cold 5% trichloroacetic acid (TCA) solution. Samples were radio-assayed in a Packard Tricarb counter with Lumagel as scintillation cocktail. For determining bacterial DNA synthesis, a final concentration of 5.79 nM [^{3}H]thymidine was used. The procedure was similar to that applied for leucine uptake. In the present study, only relationships between TLi and TTi were of interest and thus the question of conversion factors was not critical. Therefore, measurements of bacterial growth are presented in terms of pmol l^{-1} h^{-1} of incorporated [^{3}H]leucine (TLi) or [^{3}H]thymidine (TTi). These variables provide the base for calculation of the TLi:TTi ratio, which is conventionally used for defining the terms 'balanced' and 'unbalanced' bacterial growth.

Chl *a*, *Synechococcus* sp. counts and primary production. Chl *a* was determined fluorometrically according to Holm-Hansen & Riemann (1978). This parameter is conventionally used as a rough measure of algal biomass, which in turn is regarded as a proxy for bacterial substrate. Picocyanobacteria were filtered onto 0.2 µm polycarbonate filters and counted immediately by their specific chl *a* fluorescence (without staining) in the epi-fluorescence microscope (ANT X only). It must be mentioned that mainly organisms from the *Synechococcus* sp. (hereafter *Synechococcus*) fraction of picocyanobacteria were counted, because epi-fluorescence microscopy is not sensitive to *Prochlorococcus* sp. (hereafter *Prochlorococcus*) compared with other techniques e.g. flow cytometry (Zubkov et al. 1998). Primary production was calculated from [^{14}C]-bicarbonate (4 µCi per bottle) uptake under constant light of 150 µE m^{-2} s^{-1} for 8 h. Two replicates and 1 blank (dark) were performed. After incubation, filtered samples (0.2 µm and 2 µm polycarbonate filters) were fumed with HCL and radio-assayed (Packard Tricarb counter, Lumagel scintillation cocktail). Primary production was calculated as mg C m^{-3} d^{-1}. The contribution of photosynthetic picoplankton to total primary production was calculated from the <2 µm fraction of particles in the water samples.

Salinity and temperature. Salinity and temperature data were obtained from continuous measurements by the ship's instrument (Thermosalinograph Seacat SBE 21).

Data analysis. Statistical analysis of data sets was performed using Sigma-stat and STATeasy analytical tools (test of normal distribution by Kolmogorov-Smirnov test, non-random Spearman's rank distribution, Pearson and Spearman correlation, linear regression, Student's *t*-test and Mann-Whitney rank sums, analysis of covariance [ANCOVA] via *F*-test).

Table 1. Mean (SD) values of variables from surface water of (sub)tropic zones (ANT X: 42.08° N to 35.26° S; ANT XI: 41.31° N to 34.63° S). *Values are means from ratios of individual data pairs. ANT X: n = 72; ANT XI: n = 90

	Salinity (S)	Temperature (°C)	Chl *a* (µg l^{-1})	Leucine uptake (TLi, pmol l^{-1} h^{-1})	Thymidine uptake (TTi, pmol l^{-1} h^{-1})	TLi:TTi	TLi:chl *a*	TTi:chl *a*
ANT X	36.36 (0.55)	23.9 (3.2)	0.18 (0.13)	40.3 (21.7)	1.32 (0.79)	32.4* (6.7)	274.1* (135.2)	8.45* (3.44)
ANT XI	35.90 (0.90)	23.9 (3.6)	0.29 (0.19)	51.8 (28.2)	2.72 (1.72)	20.5* (4.4)	198.7* (97.8)	10.0* (4.95)

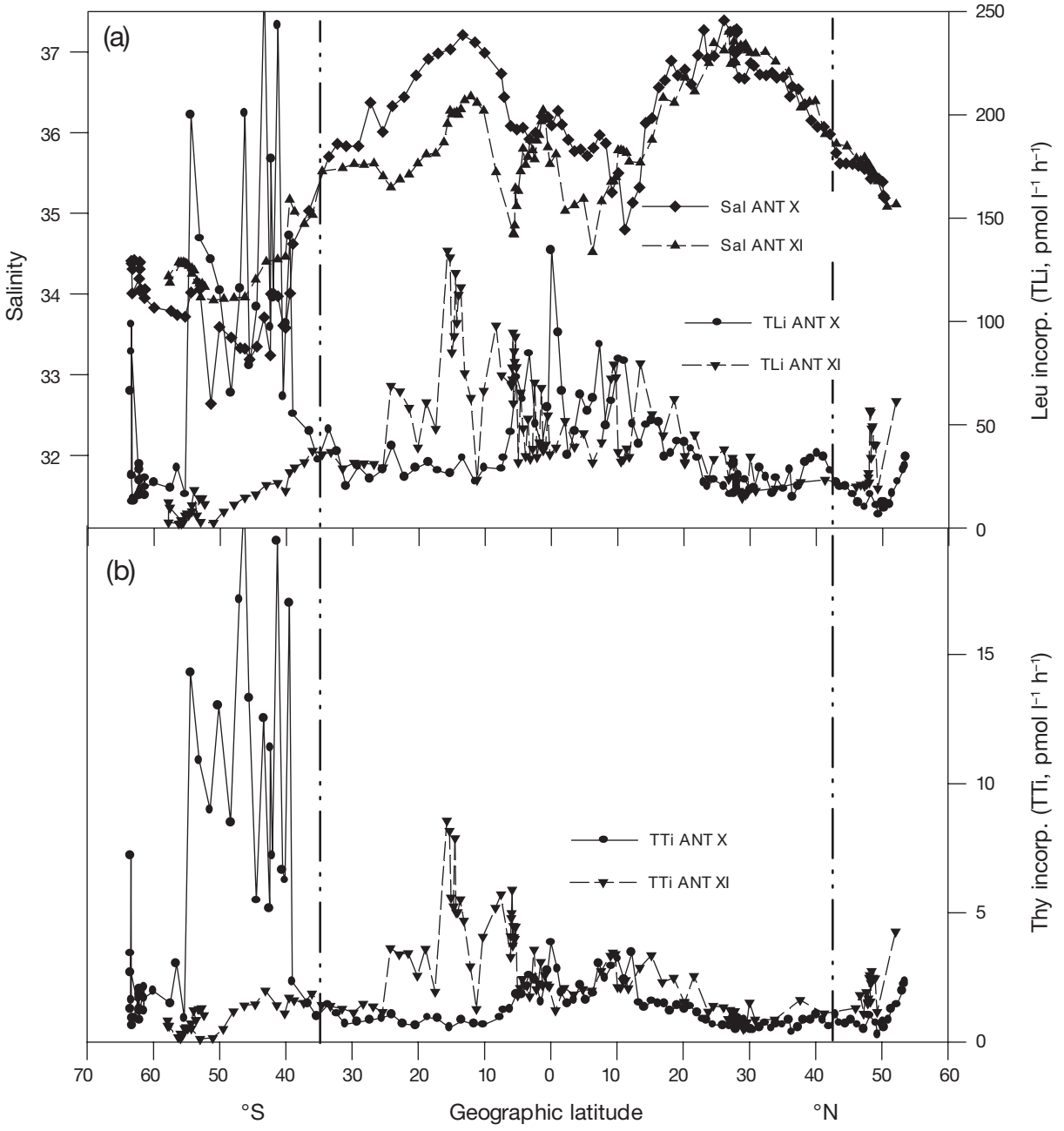

Fig. 2. Distribution of salinity and bacterial growth patterns along transects. (a) Salinity and leucine incorporation (TLi). (b) Thymidine incorporation (TTi). TLi correlated significantly with TTi during both cruises (Pearson's correlation, ANT X, n = 187, r = 0.752, $p < 0.001$; ANT XI, n = 138, r = 0.947, $p < 0.001$) but there was no correlation between salinity and bacterial variables. Zone of highest differences between TLi:TTi ratios (>8) of the cruises indicated by vertical lines

RESULTS

Salinity and temperature along transects

Salinity was generally higher at the offshore cruise than inshore (Table 1, Fig. 2a). At the inter-tropical convergence zones (ITCZ) north and south of the equator, upwelling and heavy rainfall were reflected by relatively low salinities during both cruises. Temperature variability was similar during both cruises from the most northern station to 5° S (Table 1, Fig. 3a). Further south, water was warmer at the offshore cruise than inshore, due to warm water intrusion by the Brazil current into the former and upwelling of cold water from the Benguela region in the latter. There was no correlation between bacterial variables and salinity or temperature along either transect, except for a significant correlation between salinity and *Synechococcus* counts (Pearson's correlation, n = 101, r = –0.51, p < 0.001).

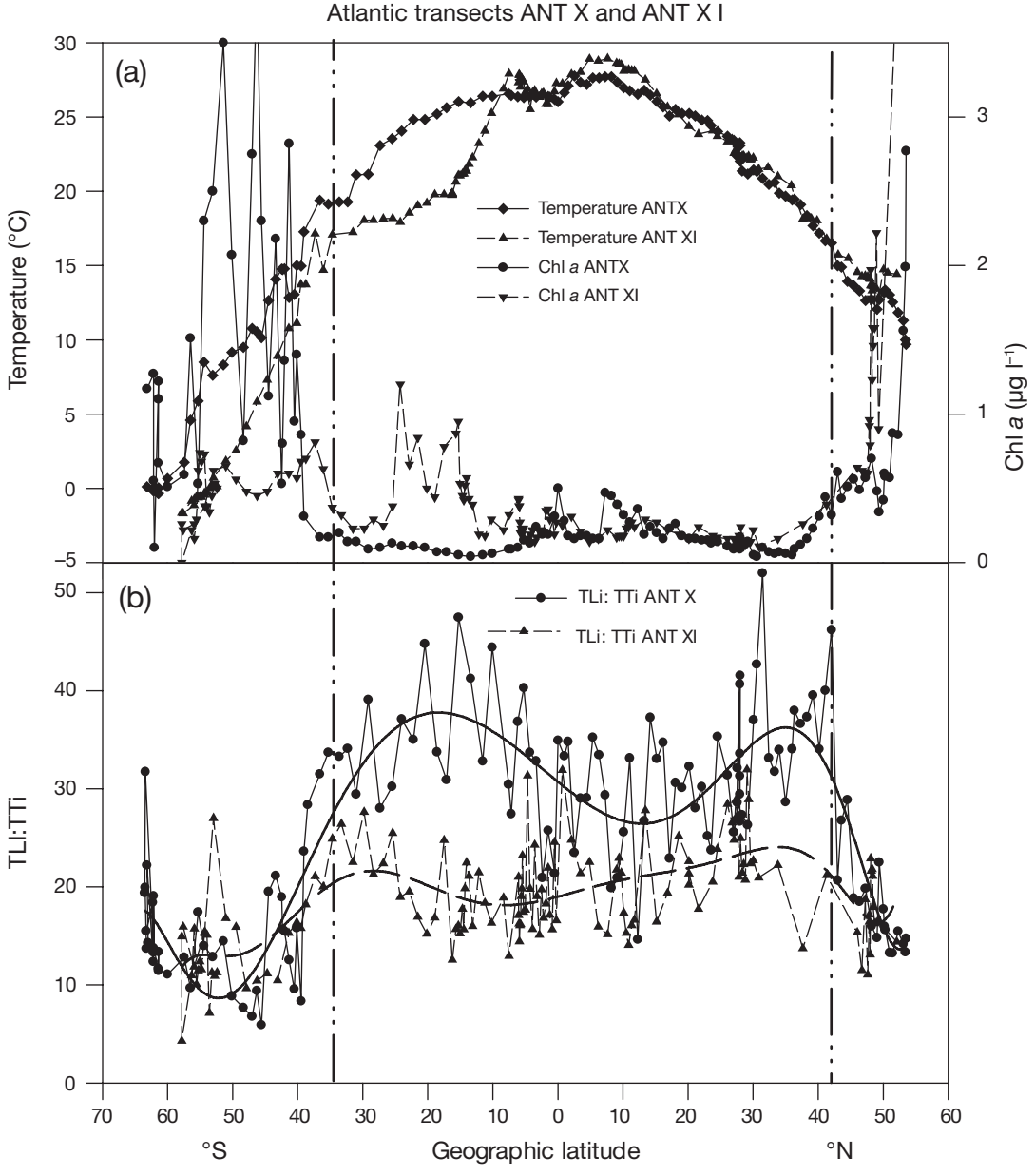

Fig. 3. Temperature, chl *a* concentration and ratios between leucine and thymidine incorporation (TLi:TTi) along transects. (a) Temperature and chl *a* along transects. (b) TLi:TTi ratios along transects. General patterns demonstrated by trend lines. Zone of highest differences between the TLi:TTi ratios (>8) from the cruises indicated by vertical lines. TLi:TTi ratios were different (Mann-Whitney rank sums, n = 68, p < 0.001) within the indicated zone

Bacterial leucine or thymidine incorporation along transects

The meridional patterns of bacterial growth measured via leucine (TLi) (Fig. 2a) or thymidine incorporation (TTi) (Fig. 2b) showed very low values in the (sub)tropical zones during both cruises. As an exception, during ANT XI, both measurements of bacterial growth increased considerably in the Benguela upwelling region. The ITCZ was clearly distinguished from the adjacent (sub)tropical zones by elevated values during both cruises. In the southern temperate latitudes, TLi and TTi values were very high on the shallow Patagonian shelf (ANT X) due to the spring bloom of phytoplankton. Further south, they decreased drastically near the Polar Front and recovered again in the sea ice environment. During ANT XI, both variables decreased continuously after the ship left the continental shelf (Fig. 2). The median values of TLi as well as TTi from both cruises (subtropical zone, Table 1) varied significantly from each other (Mann-Whitney rank sums, $p < 0.001$), with higher values observed during ANT XI compared to ANT X.

Bacterial protein and DNA synthesis ratio (TLi:TTi)

The region of highest differences (>8) between the TLi:TTi ratios of the 2 cruises (indicated by vertical lines in Fig. 3) was situated within the subtropical zone and characterised by low chl *a* concentrations (below 0.5 µg l^{-1} except in the Benguela upwelling region, ANT XI) and relatively high temperatures (between ~17 and 28°C). In this zone, which extended from 42° N to 35° S, a statistically significant difference (Mann-Whitney rank sums, $n = 68$, $p < 0.001$) was found between the TLi:TTi ratios obtained from the 2 cruises (Table 1). The oligotrophic conditions during the offshore cruise were reflected by high TLi:TTi ratios (median value 32), whereas relatively low TLi:TTi values (median value 21) were combined with the more mesotrophic conditions during the inshore cruise. The different trophic conditions during the 2 cruises were documented by the median values of chl *a* (Table 1), which diverged significantly from each other (Mann-Whitney rank sums, $n = 68$, $p < 0.001$). Nevertheless, chl *a* concentrations were rather similar in the northern tropical zone (10° N to 28° N); in this specific zone, TLi:TTi values from the 2 cruises approached each other (Fig. 3b) but they were still distinctly different. Between ~30° N and 38° N the courses of the ship overlapped (Fig. 1); nevertheless, TLi:TTi ratios were much higher during ANT X. When looking closely at chl *a* data in this specific region (Fig. 3a), it is apparent that during ANT X, chl *a* was lower than during ANT XI. In the adjacent temperate climate zones, which were characterised by relatively high chl *a* concentrations and rapidly decreasing temperatures, the ratios of TLi:TTi decreased to around 10 and approached each other during both cruises. Values from the very south beyond 60°, near Antarctica (ANT X), again indicated an increase in the TLi:TTi ratio (Fig. 3b).

Chl *a*, *Synechococcus* and primary production of particle fraction <2 µm

Chl *a* along the transects was significantly different within the subtropical zone (Mann-Whitney rank sums, $n = 58$, $p < 0.001$). In detail, chl *a* distribution did not vary markedly from the northern boundary of the subtropical zone down to 5° S during either cruise (Fig. 3a). Further south, the oligotrophic conditions during ANT X were well reflected by very low chl *a* values, whereas coastal influences and Benguela upwelling were responsible for increasing values of chl *a* during ANT XI mesotrophic conditions. Beyond 40° S (subtropical convergence), chl *a* increased to very high values due to the spring bloom on the Patagonian shelf (ANT X). Strong scatterings of values probably coincided with the meanderings of the Brazil and Falkland currents. During ANT XI, chl *a* increased moderately south of the subtropical convergence.

Synechococcus and primary production <2 µm were investigated (ANT X only) to get an idea of the possible impact of these variables on TLi:TTi ratios. Picocyanobacteria (*Synechococcus* and *Prochlorococcus*) are mainly responsible for primary production in the particle size class <2 µm. *Synechococcus* counts were positively correlated with a high level of significance with primary production <2 µm (Pearson's correlation, $n = 82$, $r = 0.51$, $p < 0.0001$) (Fig. 4). The percentage of total primary production attributed to the particle size class <2 µm was generally high in the entire (sub)tropical zone (about 80%). In the zones beyond the subtropical convergence, the contribution of picoplankton (<2 µm) to primary production decreased rapidly even though their counts increased considerably (Fig. 4). *Synechococcus* counts in the subtropical zone were positively correlated with bacterial leucine (TLi) as well as with thymidine (TTi) incorporation.

TLi:TTi ratios in relation to chl *a* along transects

It was anticipated that substrate supply (chl *a*, a proxy of bacterial substrate) had a strong influence on bacterial growth characteristics (TLi:TTi ratios). Taking into account the total range of chl *a* in the (sub)tropical

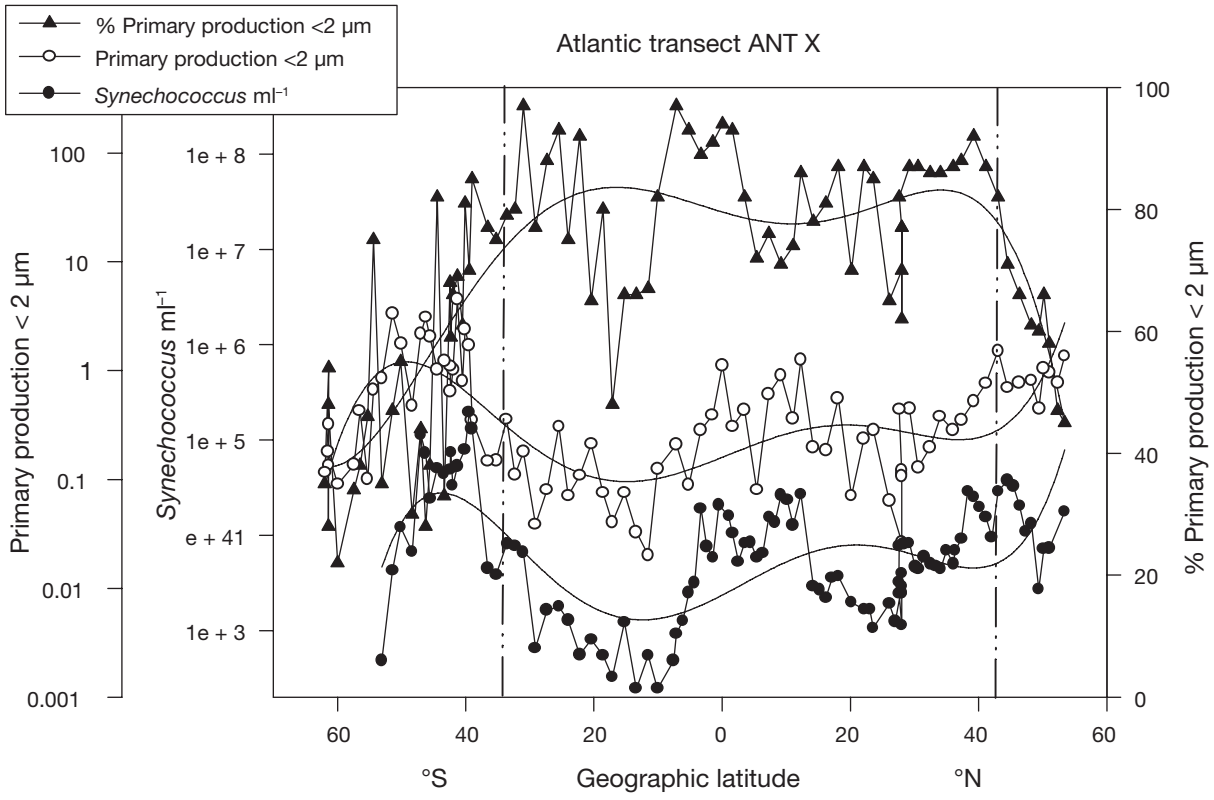

Fig. 4. Distribution of *Synechococcus*, primary production of particle size class <2 µm and contribution (%) of <2 µm particle size class to total primary production along ANT X transect. General patterns demonstrated by trend lines. Vertical lines as for Figs. 2 & 3

zones of both cruises (Fig. 5a), the slopes of the regression lines between the TLi:TTi ratio and chl *a* differed significantly from each other (ANCOVA via *F*-test, *F* critical value 3.89 < *F*-test value 6.35, p = 0.05). Furthermore, the distance between the regression lines was different from 0 (*F*-test, *F* critical value 6.78 < *F*-test value 171.69, p = 0.01) and the mean values of TLi:TTi ratios were significantly different (*t*-test, p < 0.001). When exclusively considering the chl *a* range from 0 to 0.5 µg l^{-1} (Fig. 5b), the slopes of the regression lines did not differ significantly from each other (*F*-test, *F* critical value 6.80 > *F*-test value 0.08, p = 0.01). The distance between the regression lines differed significantly from 0 (*F*-test, *F* critical value 6.80 < *F*-test value 138.09, p = 0.01) and the mean values of TLi:TTi ratios from the 2 cruises were distinctly different (Mann-Whitney rank sums, p < 0.001). The different levels of the TLi:TTi ratio regression lines at a similar chl *a* range are surprising, but they may provide a key for interpreting the different growth characteristics of bacteria in offshore and inshore oceanic waters.

The individual parameters (TLi or TTi) were positively correlated with chl *a* in the (sub)tropical zone (Pearson's correlation: TLi versus chl *a*, ANT X, n = 80, r = 0.532, p < 0.001; ANT XI, n = 94, r = 0.596, p < 0.001; TTi versus chl *a*, ANT X, n = 80, r = 0.533, p < 0.001; ANT XI, n = 94, r = 0.566, p < 0.001).

Ratios of TLi:chl *a* and TTi:chl *a* along transects

The ratios between bacterial protein synthesis (TLi) and chl *a* were significantly higher during ANT X compared to ANT XI (Mann-Whitney rank sums, n = 68, p < 0.004, Table 1, Fig. 6a). In detail, differences between the TLi:chl *a* ratios from the 2 cruises were much greater in the south than in the north. This may be attributed to differences in chl *a* concentrations (substrate) between the cruises. Chl *a* concentrations in the (sub)tropical zones of ANT X were on average 0.185 µg chl *a* l^{-1} (north) and 0.117 µg chl *a* l^{-1} (south). Corresponding values for ANT XI were 0.193 µg chl *a* l^{-1} (north) and 0.339 µg chl *a* l^{-1} (south). The slightly lower chl *a* values in the north during ANT X were reflected by slightly higher TLi:chl *a* ratios compared to ANT XI. In the south, chl *a* was very low during ANT X but was considerably higher during ANT XI, leading to a wide difference in the TLi:chl *a* ratios between the cruises (Fig. 6a).

The TTi:chl *a* ratios of the cruises (Table 1, Fig. 6b) did not vary significantly from each other (*t*-test, n =

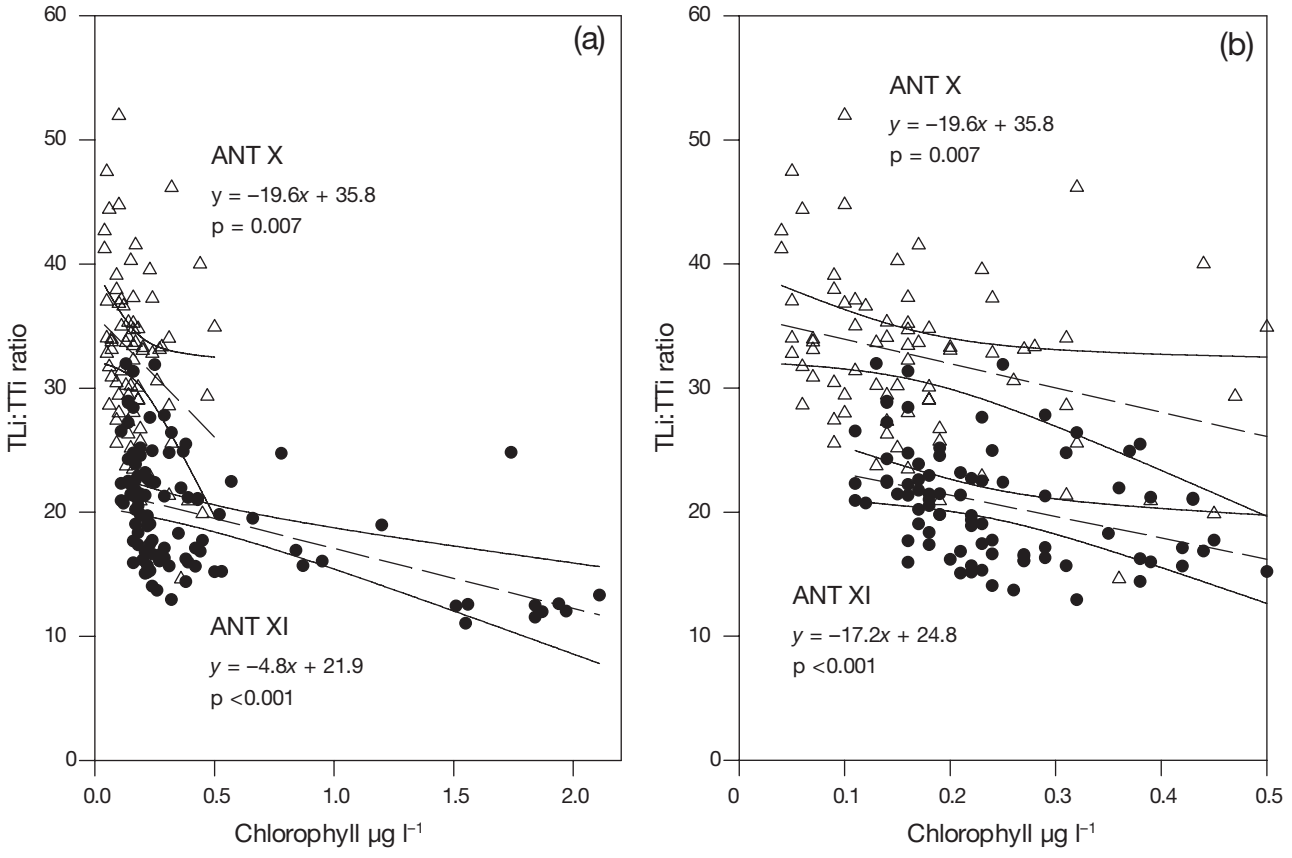

Fig. 5. Relationships between chl *a* and TLi:TTi ratio in (sub)tropical regions during ANT X and ANT XI. Regression lines are shown together with 99% confidence intervals. (a) TLi:TTi ratio plotted against total ranges of chl *a* along transects (0 to 0.5 µg for ANT X; 0.5 to 2.2 µg for ANT XI). (b) TLi:TTi ratio plotted exclusively against chl *a* range 0 to 0.5 µg, which is characteristic for oligotrophic conditions

128, p = 0.592). Nevertheless, they were frequently higher along the inshore transect than offshore except in the region south of 15° S. In this region, the TTi:chl *a* ratios were surprisingly low during ANT XI despite relatively high chl *a* concentrations. However, it has to be considered that during ANT XI, temperatures south of 15° S were lower than during ANT X (Fig. 3a). If bacterial degradation of substrate and thus cell replication (TTi) was retarded by low temperatures during ANT XI, this could have influenced the discrepancy between the 2 cruises in the south.

DISCUSSION

Growth characteristics of bacteria in terms of bacterial protein production (TLi) or DNA synthesis (TTi) reflect prevailing environmental conditions. The results of our 2 cruises in the Atlantic show that ratios between TLi and TTi were different between offshore (oligotrophic) and inshore (more mesotrophic) conditions. In particular, in the warm (sub)tropical climate zone, the TLi:TTi ratios were significantly higher in offshore (average 32.4) than in inshore waters (20.5), but both were within the range generally reported for aquatic ecosystems (Table 2). Several factors may have been responsible for the different levels of the TLi:TTi ratios observed during the 2 cruises.

Temperature variability, which is regarded as a regulating factor of bacterial growth, was similar in the (sub)tropical zones along both transects (except in the Benguela upwelling region), and thus temperature cannot explain the differences in ratios between the oligotrophic offshore and the more mesotrophic inshore cruise (Table 1, Fig. 3b). Nevertheless, changes in the TLi:TTi ratios during each cruise may have been due to temperature variability at the meridional scale. When investigating the effect of temperature on the incorporation of leucine and thymidine by bacteria in marine environments, Tibbles (1996) observed a positive relationship between temperature and the TLi:TTi ratio. This was also observed during the 2 cruises within the temperature ranges of about 9 to 18°C in the north and 5 to 24°C in the south. How-

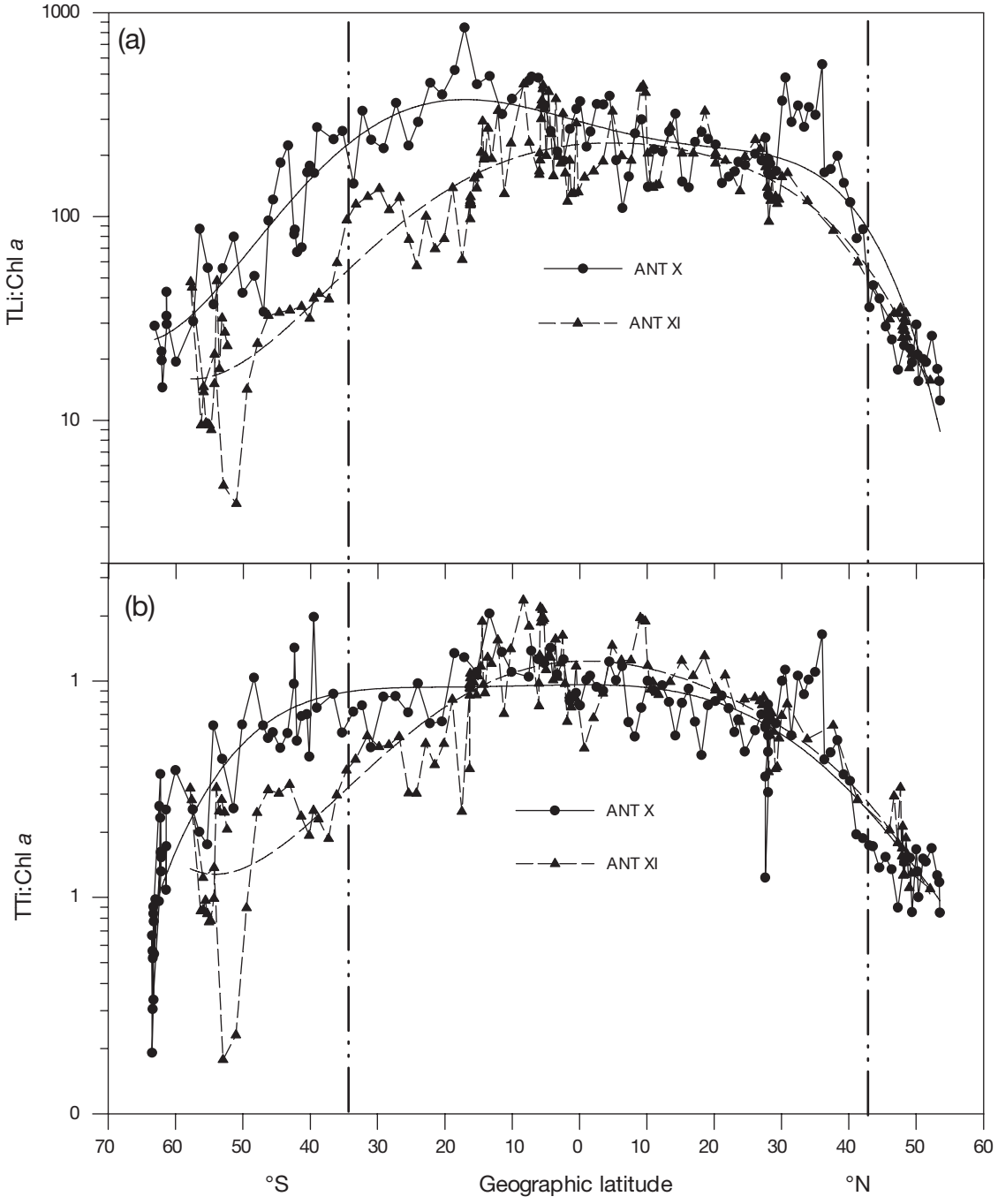

Fig. 6. Ratios between bacterial leucine (TLi) or thymidine (TTi) incorporation and chl *a* along the transects. (a) TLi:chl *a*, ANT X compared to ANT XI. According to Mann-Whitney rank sums, median values differed significantly at $p < 0.001$. (b) TTi:chl *a* along both transects. According to *t*-test ($p = 0.592$), median values did not vary significantly from each other. Trend lines were applied to get a better overview of the partly overlapping data curves. Vertical lines as for Figs. 2 to 4

ever, the increase in the TLi:TTi ratios with temperature was much steeper offshore than inshore. Furthermore, the ratios decreased despite temperatures increasing up to 28°C, particularly in the less oligotrophic region of the ITCZ (around 10° N, ANT X, Fig. 3). This irregularity, together with the different TLi:TTi ratios of the 2 cruises, suggests that another factor (most likely substrate availability) may interfere with the influence of temperature on TLi:TTi ratios on a large spatial scale.

In our study, chl *a* was regarded as a proxy for phytoplankton biomass and, ultimately, bacterial substrate

Table 2. Observed molar leucine:thymidine incorporation ratios (TLi:TTi) from different aquatic environments

Environment	TLi:TTi	Comment	Source
Chesapeake Bay	10.5–25	Depth profile 4–24 m	Chin-Leo & Kirchman (1988)
Estuary	<5–>70	Bacteria in seawater culture	Chin-Leo & Kirchman (1990)
Weddell Sea	55	Experimental approach with Reduced dissolved inorganic carbon (DIC)	Bjørnsen & Kuparinen (1991) re-calculated
General, limnetic, marine	7–9.5	Average molar ratios	Bjørnsen (1992)
Lagoon, South Africa	2–39 51–113	Water Sediment	Tibbles et al. (1992)
Diverse aquatic ecosystems	7–9.5	Average	Servais (1992)
Eastern Baltic Sea	5–30	Nutrient addition experiments	Heinaenen & Kuparinen (1992)
NE Atlantic	18.9 10.4	Large attached bacteria Small free living bacteria	Turley (1993)
Western North Atlantic	~7–33	Spring bloom condidions	Li et al. 1993, re-calculated
Baltic Sea, Gulf of Finland	7.7 3.0	Upper mixed layer Below thermocline	Heinaenen et al. (1995)
Kiel Bight	25–213	Sea ice (brine)	Mock et al. (1997)
Salt marsh tidal creek Mesocosms	5.6–29.5 16–50	Different seasons Effect of temperature and substrate	Shiah & Ducklow (1997)
Mediterranean Sea	~30–170 ~30–65	Depth profiles Diel variation	Gasol et al. (1998)
Arabian Sea	35	Average, SW monsoon	Pomroy & Joint (1999)
Mesotrophic lake Pareloup	34.5 11.5	Draining period After filling	Petit et al. (1999)
North and South Atlantic	~4 ~52 ~18	Surface, 50° N 4° S 40° S	Zubkov et al. (2000) re-calculated
Northeast Pacific	28 ± 8 18 ± 11 41± 22 8.4 ± 2.9	0–30 m, midshelf 0–30 m, slope 0–30 m, gyre 50–80 m, gyre	Sherr et al. (2001)
Schlei Fjord, Baltic Sea Coastal zone, Baltic Sea	17.4 ± 6.4 20.6 ± 13.2	Hypertrophic, annual mean Mesotrophic, annual mean	Gocke, unpubl. data
sub/tropic Atlantic	32.4 20.7	Offshore surface water, mean Inshore surface water, mean	Present study
Patagonian shelf	~10	High chlorophyll, spring bloom	
Antarctic sea ice environment	40–80	Ice pond, sea ice border	Unpublished

supply. There was a statistically significant difference between the TLi:TTi values scaled to chl *a* during the 2 cruises in the (sub)tropical zone (Fig. 5a). This also held true if only the low chl *a* (0 to 0.5 µg l^{-1}) regions of both cruises were compared (Fig. 5b). In this case, the slopes of the regression lines were similar but the distance between the upper regression line (ANT X) and the lower line (ANT XI) was significantly different from 0 (*F*-test). This may suggest that the quality of organic matter combined with chl *a* was more nutritious near the shore (causing higher TTi and thus lower TLi:TTi values) than offshore, and/or that organic matter from allochthonous sources supported bacteria near the shore. Our assumption is supported by the study of Raymond & Bauer (2001), who found that in Atlantic water, much of the young dissolved organic carbon was selectively degraded over the residence times of coastal waters 'leaving an older and more refractory component for oceanic export'. Furthermore, different contributions of picocyanobacteria to leucine uptake may have influenced the patterns of TLi:TTi ratios (cf. below).

In detail, there were differences between the northern and southern subtropical zones with regard to the TLi:TTi versus chl *a* ratios as well as to the relationships between individual parameters and chl *a* (Figs. 5 & 6). These are inherent with the courses of the cruises, which partly overlapped in the north but were distinctly different in the south (Fig. 1). Nevertheless, TLi:TTi ratios were considerably higher during ANT X compared to ANT XI not only in the south but also in the north. When looking closely at the chl *a* data, par-

ticularly in the region between 28 and 38° N (overlapping cruise tracts, Fig. 3a), it is apparent that chl *a* was lower during ANT X than ANT XI (due to interannual variability), leading to higher TLi:TTi (Fig. 3b) and TLi:chl *a* ratios (Fig. 6a) in the former.

Taking temperature as well as substrate supply (chl *a*) into consideration as factors that influence bacterial growth characteristics, Shiah & Ducklow (1997) found in estuarine habitats that TLi:TTi ratios (which varied between 5.6 and 29.5; Table 2) were lower during periods of high temperature and high chl *a*. This trend was supported only by our large scale oceanic results for chl *a*; it did not hold for the temperature response because we found an increase in the ratio for temperatures up to about 24°C combined with low chl *a* (Fig. 3). Thus, we suggest that substrate supply dominates over temperature in the regulation of TLi:TTi ratios. Finally, Shiah & Ducklow (1997) proposed that changes toward less favourable conditions (low temperature or substrate supply) reduce bacterial protein and DNA synthesis rates simultaneously, but that protein synthesis may be favoured, resulting in high TLi:TTi ratios. Conversely, when conditions become favourable, bacteria favour DNA synthesis, resulting in low TLi:TTi ratios. With respect to conditions in the (sub)tropical zones of our cruises, one of the factors was favourable (temperature) while the other (low substrate) was not. This combination of factors led to generally high TLi:TTi ratios (Figs. 3b & 5) but the increase in ratios was less pronounced during the inshore cruise, likely due to a relatively better substrate supply that included allochthonous organic matter.

In addition to substrate supply, the possible impact of picocyanobacteria (*Synechococcus* and *Prochlorococcus*) on TLi:TTi ratios must be considered (Zubkov et al. 2000, 2003, Zubkov & Tarran 2005). Several species of cyanobacteria have been reported to incorporate leucine but not thymidine (Bern 1985, Kamjunke & Jähnichen 2000, Hietanen et al. 2002). However, the behaviour of *Synechocoocus* and *Prochlorococcus*, particularly with respect to thymidine uptake, remains unclear (Torreton & Dufour 1996). *Synechococcus* counts during ANT X correlated significantly with primary production of the <2 µm particle class (Fig. 4). This suggests that *Prochlorococcus* (which were not detected) had a meridional distribution similar to that of *Synechococcus*; however, this does not correspond to the observations of Zubkov & Tarran (2005) from the Southern Atlantic Gyre. In detail, counts of *Synechococcus* were very low (average 7700 ml^{-1}, SD 10.9) in subtropical regions, whereas they increased in the adjacent tropical and temperate climate zones (28 450 ml^{-1}, SD 42.2). Nevertheless, organisms <2 µm (mainly picocyanobacteria) contributed 80.6% (SD 10.5) to total primary production in the subtropical region, while this percentage was only 55.2 (SD 18.1) in the adjacent, more mesotrophic zones (Fig. 4), which were dominated by eucaryotic algae. However, as long as the relationship between leucine uptake and primary production by cyanobacteria (*Synechococcus*) remains unclear, we cannot derive the contribution of these organisms to total bacterial leucine uptake from their production patterns.

With respect to the possible impact of *Prochlorococcus*, it must be considered that these organisms may account for about 5 to 20% of total bacterioplankton consumption of amino acids in the southern Atlantic oligotrophic gyre (Zubkov & Tarran 2005). If *Prochlorococcus* contributed up to 20% of total leucine uptake (but not to thymidine uptake), this might have affected the measured TLi:TTi ratios. Nevertheless, the relationship between the TLi:TTi ratios of the 2 cruises would only be influenced if the abundance of picocyanobacteria during the 2 cruises differed greatly. This was probably not the case, as suggested by Partensky et al. (1999), who observed during several Atlantic transects that picocyanobacteria were nearly as frequent in coastal regions as in the oligotrophic open sea. We therefore infer that, even if picocyanobacterial leucine incorporation is taken into account, heterotrophic bacteria contribute significantly to the shift in TLi:TTi ratios between oligotrophic and mesotrophic marine regions.

Besides these major factors, other factors such as grazing, bacterial activity state, community composition and, particularly, methodological constraints may have affected our results. With respect to grazing pressure, Zubkov et al. (2000) reported that heterotrophic nanoplankton can tightly control the growth of active bacteria in oligotrophic as well as in more productive oceanic regions. We assume that even if active bacteria were controlled by nanoplankton grazing, the ratios between active and total bacteria were different during our 2 cruises and thus likewise was the contribution of active bacteria to observed TLi:TTi ratios.

Furthermore, it is reasonable to assume that different bacterial communities existed in the 2 regions of observation. These communities may be adapted to the prevailing substrate regimes and characterized by specific patterns of TLi:TTi ratios. The interaction between leucine or thymidine uptake, organic matter supply and community structure was studied by Cottrell & Kirchman (2003, 2004) in an estuarine salinity gradient. It was observed that with increasing salinity, the percentages of [^{3}H]thymidine-labelled and [^{3}H]leucine-labelled bacteria changed within the α-, β- and γ-Proteobacteria and *Cytophaga*-like bacteria. This was attributed to dissolved organic matter (DOM) supply that affects differences in biomass production by bacterial groups. We speculate that differences in DOM supply may have influenced the variability within the group-specific por-

tions of thymidine- and leucine-incorporating cells during our cruises. However, this is a topic for future research, which should include molecular biology.

Methodical constraints may have influenced our results via unspecific labelling of bacteria by leucine, which means that incorporated leucine was converted to other compounds by cell metabolism. Furthermore, different levels of external and internal isotope dilution must be considered. External isotope dilution can be a problem in eutrophic coastal waters where concentrations of leucine and thymidine may be high. This was most likely not the case in the (sub)tropical regions sampled during our cruises. Internal isotope dilution with thymidine may have been a problem because of the anticipated low concentrations of DNA precursors in the water. In this case, we argue that the applied concentration of [^{3}H]thymidine was high enough to minimise thymidine synthesis by cellular metabolism. According to Kirchman (1992), isotope dilution in oceanic water was low if a concentration of 5 nM thymidine was used (we used 5.8 nM).

The discussions above lead to controversy regarding the definition of the terms 'balanced growth' (steady-state) and 'unbalanced growth' (non steady-state) of bacteria. One may argue that — in a strict sense — these terms can only be used for describing changes in the growth characteristics of bacterial pure cultures during a period of incubation. In multi-species natural bacterial communities, changes in environmental conditions may lead to adaptation of the total community or of a selection of species, causing variability in growth characteristics. This imparts a wider and more ecological interpretation of the terms 'balanced' and 'unbalanced' growth. Under natural conditions, some parts of the mixed community may replicate while others just survive, whether they are in balanced or unbalanced growth state, respectively. Depending on the relationship between these 2 groups and their TLi:TTi ratios, growth of the total community may appear more or less (un)balanced. However, we conclude from our results that if this is a permanent state valid for entire oceanic provinces, a high TLi:TTi ratio may also indicate balanced growth that is adequate with regard to prevailing environmental conditions.

In the literature, the terms 'balanced' and 'unbalanced' have been used to describe changes in bacterial growth over time periods or between different regions. With regard to the combined temporal/spatial aspect, Chin-Leo (1989) observed an increase in the TLi:TTi ratio in deep water of Chesapeake Bay during summer in comparison to the surface, 'indicating unbalanced growth in the former'. This was attributed to episodic pulses of DOM that lead to a transient response in bacterial growth (Chin-Leo 1989, Chin-Leo & Kirchman 1990). These findings on transient fluctuations of bacterial growth state due to substrate supply may help to explain the small/medium spatial scale variability of the TLi:TTi ratio (Fig. 3b) during our cruises. Concerning regional differences in the growth state of bacteria, Chin-Leo (1989) observed balanced growth in the eutrophic Chesapeake Bay and unbalanced growth in the oligotrophic Mid-Atlantic Bight. Unbalanced growth in the latter was attributed to an uncoupling of phytoplankton and bacteria. In contrast, remarkably balanced growth was noted by Zubkov et al. (2000) across the whole Atlantic. However, this general statement was curtailed by the fact that TLi:TTi ratios suggested that bacterial communities in oligotrophic gyres 'are growing somewhat differently from communities inhabiting more productive mesotrophic waters'. This observation has now been confirmed and specified by the consistently different TLi:TTi ratios observed under oligotrophic and more mesotrophic conditions of our 2 cruises.

We conclude from our results that

(1) Substrate supply is more important than temperature in the regulation of bacterial growth characteristics (ratio between leucine and thymidine incorporation, TLi:TTi) on a large spatial scale

(2) Variability of bacterial growth characteristics (TLi:TTi ratio) is influenced by picocyanobacteria. While heterotrophic bacteria incorporate leucine and thymidine at a site-specific ratio, the leucine component may be supplemented by picocyanobacterial leucine uptake

(3) The thymidine component of the ratio is regulated by heterotrophic bacteria, depending on the amount and quality of organic matter and additional allochthonous organic matter

(4) Heterotrophic bacteria may favour the leucine component within the TLi:TTi ratio if conditions of substrate supply are unfavourable for cell replication, e.g. in the most oligotrophic South Atlantic gyre, where *Synechococcus* was not abundant.

(5) The terms 'balanced' and 'unbalanced' growth are not suitable for describing bacterial growth state if picocyanobacteria are abundant, or for characterising heterotrophic bacterial growth in oceanic provinces with constantly unfavourable environmental conditions

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