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Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic

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Summary

Prokaryotic extracellular enzymatic activity, abundance, heterotrophic production and respiration were determined in the meso- and bathypelagic (sub)tropical North Atlantic. While prokaryotic heterotrophic production (PHP) decreased from the lower euphotic layer to the bathypelagic waters by two orders of magnitude, prokaryotic abundance and cell-specific PHP decreased only by one order of magnitude. In contrast to cell-specific PHP, cell-specific extracellular enzymatic activity (alpha- and beta-glucosidase, leucine aminopeptidase, alkaline phosphatase) increased with depth as did cell-specific respiration rates. Cell-specific alkaline phosphatase activity increased from the intermediate water masses to the deep waters up to fivefold. Phosphate concentrations, however, varied only by a factor of two between the different water masses, indicating that phosphatase activity is not related to phosphate availability in the deep waters. Generally, cell-specific extracellular enzymatic activities were inversely related to cell-specific prokaryotic leucine incorporation. Thus, it is apparent that the utilization of deep ocean organic matter is linked to higher cell-specific extracellular enzymatic activity and respiration and lower cell-specific PHP than in surface waters.

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Introduction

The main source of dissolved organic matter (DOM) in the open ocean is phytoplankton extracellular release and grazing processes either directly on phytoplankton ('sloppy feeding') or within the microbial food web (Nagata, 2000). Also, heterotrophic prokaryotes release copious amounts of DOM via their polysaccharidic envelope surrounding the cells (Heissenberger and Herndl, 1994; Heissenberger *et al*., 1996; Stoderegger and Herndl, 1998). Most of the grazing-related DOM release is in the form of high molecular weight DOM (HMW-DOM, > 1000 Da) and also senescent phytoplankton release mainly HMW-DOM (Lignell, 1990). This HMW-DOM, although comprising only about 20% of the total euphotic DOM pool and even less in the meso- and bathypelagic waters (Benner *et al*., 1992), is preferentially utilized by heterotrophic prokaryotes over the bulk of the low molecular weight DOM (LMW-DOM) (Amon and Benner, 1996). The preferential prokaryotic utilization of HMW-DOM requires cleavage of the substrate prior to uptake as only molecules < 600 Da can be transported through the prokaryotic cell wall (Weiss *et al*., 1991).

Thus, prokaryotic plankton synthesize extracellular enzymes to hydrolyse organic matter (Hoppe *et al*., 2002). Commonly, macromolecules are cleaved by a consortium of hydrolases: endohydrolases cleave off oligomers and exohydrolases cleave the terminal ends of the oligomers to produce monomers (Hoppe *et al*., 2002). Fluorogenic substrate analogues are used to quantify *in situ* extracellular enzymatic activity (EEA) (Hoppe *et al*., 2002). While fluorogenic model substrates lack the chemical complexity of natural substrates (Arnosti *et al*., 2005), they have been commonly applied to determine potential extracellular enzymatic activities over a broad range of aquatic environments (Chrost, 1989; Rath *et al*., 1993; Misic and Fabiano, 2006; Misic *et al*., 2006; Williams and Jochem, 2006), as they were introduced to microbial

ecology (Hoppe, 1983). Most of the previous studies on extracellular prokaryotic activity focused on the euphotic zone and, in the marine environment, on coastal areas (e.g. Sala *et al*., 2001; Zaccone *et al*., 2003; Misic and Fabiano, 2006; Williams and Jochem, 2006). Only very few vertical profiles of extracellular enzymatic activities are available for the open oceans extending from the euphotic zone to the meso- or bathypelagic realm (Rosso and Azam, 1987; Koike and Nagata, 1997; Hoppe and Ullrich, 1999).

Generally, heterotrophic prokaryotes in the meso- and bathypelagic layers of the ocean are limited by the availability of easily metabolizable organic matter. A specific sequence in the utilization of DOM has been shown for the oceanic water column as indicated by the increasing C:N:P ratio of the DOM in different depth layers (Hopkinson and Smith, 2005). From the changes in the elemental composition of the DOM with depth, it appears that the phosphorus compounds are preferentially utilized, followed by nitrogenous compounds (Benner, 2002; Hopkinson and Smith, 2005). Consequently, the deep-ocean DOM is depleted in N and P but rich in C, which is, however, rather refractory as indicated by its average age of about 6000 year (Bauer *et al*., 1992).

Sedimenting particulate organic matter (POM) might represent a major source of organic matter for deep-water microheterotrophic communities (Tamburini *et al*., 2003). As in DOM, POM also exhibits an increase in the C:N ratio with depth (Schneider *et al*., 2003; Baltar *et al*., 2009). In contrast to organic N and P in the deep ocean, inorganic N and P are readily accessible to heterotrophic prokaryotes (Kirchman, 2000). Hence, it is reasonable to assume that deep-water prokaryotic EEA is primarily expressed to target the carbon moieties in the available deep-water organic matter. Hoppe and Ullrich (1999) detected high phosphatase activity in the mesopelagic waters of the Arabian Sea coinciding with high concentrations of phosphate. These authors suggested that heterotrophic prokaryotes might use phosphatase activity to mitigate C limitation.

In this study, we addressed the question whether deep-water prokaryotes maintain the same relation between extracellular enzymatic and metabolic activity as at the base of the euphotic waters (≈ 100 m depth). Therefore, we determined the EEA of two glycolytic enzymes (α-glucosidase and β-glucosidase), one proteolytic enzyme (leucine aminopeptidase) and alkaline phosphatase, along with prokaryotic abundance (PA), production and potential respiration via ETS (electron transport system) measurements at ≈100 m depth, in the mesopelagic (200–1000 m depth) and bathypelagic (1000–4500 m depth) waters of the (sub)tropical Atlantic. We hypothesized that with increasing depth, heterotrophic prokaryotic communities express higher cell-specific EEA, while cell-specific biomass production decreases in response to the increasingly recalcitrant organic matter.

Results

Prokaryotic abundance and prokaryotic heterotrophic production

Total PA (Fig. 1A) decreased exponentially with depth by one order of magnitude in both the eastern and western sections of the transect in the North Atlantic ($R^2 = 0.73$, $n = 65$ and $R^2 = 0.89$, $n = 60$ respectively). In all the water masses, PA was higher in the western than the eastern section (*t*-test; $P = 0.03$, $n = 60$) with the largest difference between the two sections detected in the Antarctic Intermediate Water (AAIW).

As PA, PHP (Fig. 1B) also decreased exponentially with depth ($R^2 = 0.31$, $n = 59$ and $R^2 = 0.52$, $n = 49$ for the eastern and western transects respectively), however, by two orders of magnitude from the subsurface layer (≈ 100 m depth) to the Lower North-east Atlantic Deep Water (LNEADW) (Table 1). No significant difference in the mean PHP was detectable between the individual water masses of the eastern and western transects (*t*-test; $P = 0.53$, $n = 49$). In the subsurface layer, PHP was about two times higher in the eastern than in the western section, probably because of the proximity to the upwelling waters at the continental slope (Fig. 1B). Cell-specific PHP decreased also exponentially with depth ($R^2 = 0.42$, $n = 59$ and $R^2 = 0.57$, $n = 49$ for the eastern and western transects respectively), albeit only by one order of magnitude (Fig. 1C). Mean cell-specific PHP was not significantly different between both transects (*t*-test; $P = 0.30$, $n = 49$).

Potential respiration estimated via ETS measurements

Potential respiration rates as determined by ETS measurements decreased over the sampled depth-range by one order of magnitude (204–30.3 μmol C m^{-3} day^{-1}; Table 1, Fig. 1D). Although ETS activity was higher in the eastern than in the western transect in all the water masses, except in the AAIW, overall, ETS activity was not significantly different between both transects (*t*-test; $P = 0.30$, $n = 60$). Individual water masses, however, exhibited pronounced differences in ETS activity, such as the Middle North-east Atlantic Deep water (MNEADW) and the Mediterranean Sea Outflow Water (MSOW) with 2 and 2.5 times higher ETS activity in the eastern than in the western transect respectively. Although both PA (Fig. 1A) and ETS measurements (Fig. 1D) decreased with depth, cell-specific ETS showed no systematic trend with depth, with the averages of the different water masses ranging from 0.42 to 1.8 fmol C $cell^{-1}$ day^{-1} (Fig. 1E). Cell-specific ETS was significantly higher (*t*-test; $P = 0.00001$, $n = 53$)

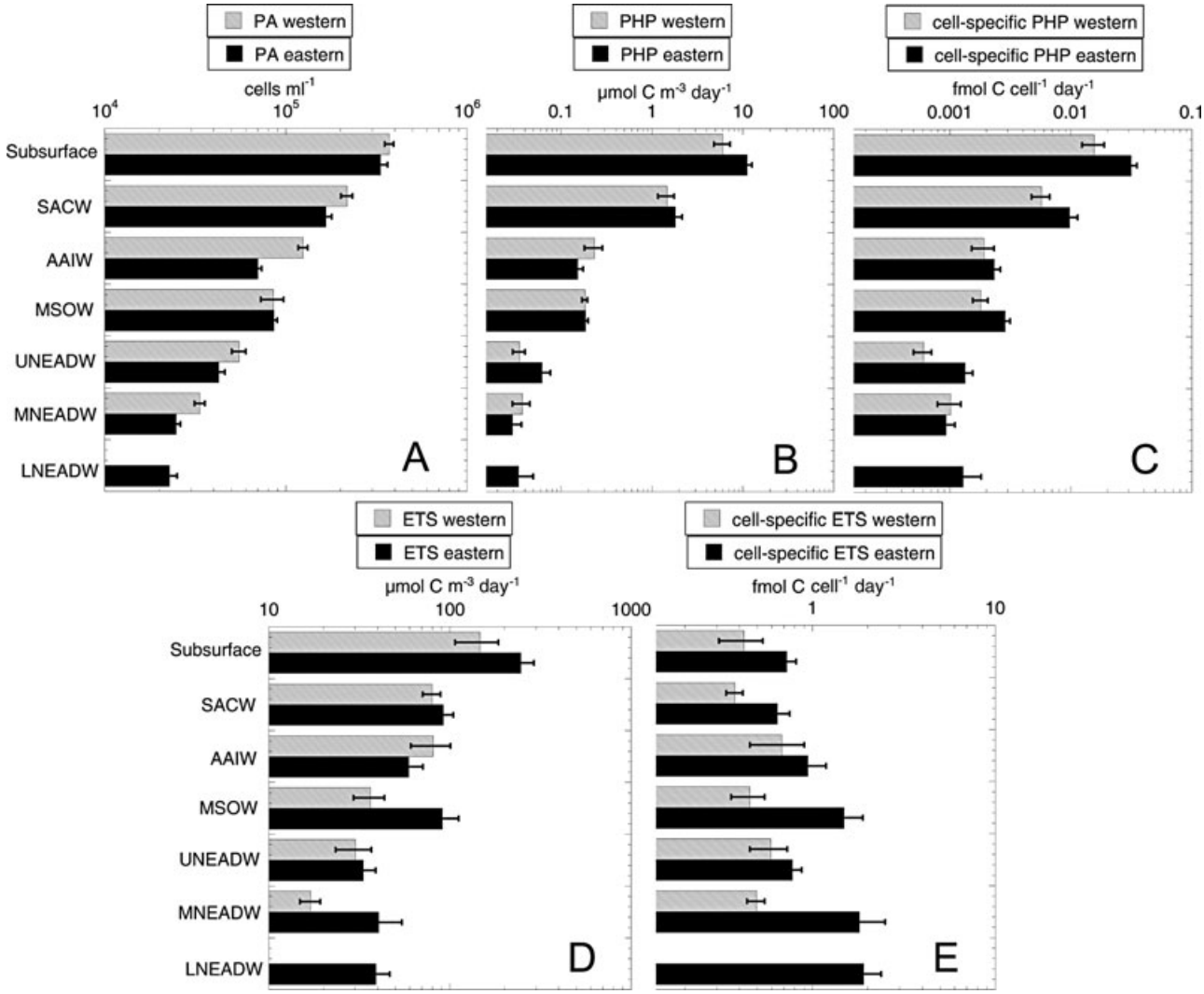

Fig. 1. Distribution of basic prokaryotic parameters in the different water masses through the eastern and western transects (see Table 1 for water depths of each water mass).
A. Prokaryotic abundance (cell^{-1} ml^{-1}).
B. Prokaryotic heterotrophic production (µmol C m^{-3} day^{-1}).
C. Cell-specific heterotrophic prokaryotic production (fmol C cell^{-1} day^{-1}) (D) ETS (µmol C m^{-3} day^{-1}).
E. Cell-specific ETS (fmol C cell^{-1} day^{-1}).
Error bars represent SE of 7–42 measurements.

in all the water masses of the eastern than in the western transect (including the AAIW, in contrast to bulk ETS activity). In the MNEADW and MSOW, cell-specific ETS activity was more than three times higher in the eastern than in the western transect (Fig. 1E).

Extracellular enzymatic activity

In contrast to PHP, EEA decreased only slightly with depth at the two transects (Fig. 2). α-Glucosidase (AGase; Fig. 2A and B) and β-glucosidase (BGase; Fig. 2C and D) activities exhibited higher lateral than vertical variability with maxima and minima of both ectoenzymes at similar locations (compare Fig. 2A and C and Fig. 2B and D). Leucine aminopeptidase (LAPase; Fig. 2E and F) and alkaline-phosphatase (APase; Fig. 2G and H) activities were generally higher than AGase and BGase, particularly at the base of the euphotic layer. LAPase and APase, however, decreased more with depth than AGase and BGase.

In contrast to cell-specific biomass production, cell-specific EEA generally increased with depth at both transects (Fig. 3). On average, cell-specific AGase increased from 0.14 amol cell^{-1} h^{-1} at the base of the euphotic layer to 2.21 amol cell^{-1} h^{-1} in the LNEADW, cell-specific BGase from 0.22 to 1.02 amol cell^{-1} h^{-1}, cell-

Table 1. Mean (± SE) values of physical, chemical and biological properties, characterizing the different water masses sampled during ARCHIMEDES-II.

Water mass	Pressure range (dbar)	Temperature (°C)	Salinity	Oxygen (µmol kg^{-1})	NO_3 (µmol kg^{-1})	PO_4 (µmol kg^{-1})	PA (in 10^5 cells ml^{-1})	PHP (µmol C m^{-3} day^{-1})	ETS (µmol C m^{-3} day^{-1})
Subsurface	96–102 (21)	16.78 ± 1.73 (21)	35.894 ± 0.417 (21)	97.4 ± 31.1 (21)	19.4 ± 7.3 (21)	1.3 ± 0.43 (21)	3.58 ± 0.84 (20)	8.6 ± 4.8 (18)	204 ± 148 (20)
SACW	246–503 (42)	10.52 ± 2.35 (42)	35.120 ± 0.347 (42)	78.5 ± 23.2 (42)	27.8 ± 7.6 (42)	1.8 ± 0.09 (42)	1.92 ± 0.70 (42)	1.66 ± 1.40 (39)	88.7 ± 52.0 (39)
AAIW	748–901 (12)	4.97 ± 0.48 (12)	34.570 ± 0.059 (12)	122.2 ± 14.0 (12)	32.0 ± 5.0 (12)	2.1 ± 0.4 (12)	0.90 ± 0.30 (12)	0.19 ± 0.09 (12)	67 ± 34 (11)
MSOW	851–904 (9)	6.39 ± 0.45 (9)	34.889 ± 0.094 (9)	97.7 ± 6.9 (9)	28.8 ± 8.1 (9)	1.8 ± 0.6 (9)	0.76 ± 0.23 (9)	0.18 ± 0.02 (7)	66.5 ± 45 (9)
UNEADW	1498–2002 (15)	3.74 ± 0.18 (15)	34.981 ± 0.020 (15)	208.0 ± 10.2 (15)	30.2 ± 6.4 (15)	1.9 ± 0.4 (15)	0.52 ± 0.14 (15)	0.045 ± 0.02 (13)	32.1 ± 18 (14)
MNEADW	2100–2752 (19)	2.76 ± 0.14 (119)	34.939 ± 0.015 (19)	215.2 ± 3.2 (19)	22.2 ± 0.3 (19)	1.5 ± 0.03 (19)	0.29 ± 0.72 (19)	0.030 ± 0.019 (16)	30.3 ± 35 (17)
LNEADW	3499–4503 (8)	1.93 ± 0.21 (8)	34.883 ± 0.019 (8)	216.3 ± 3.0 (8)	23.5 ± 0.6 (8)	1.6 ± 0.05 (8)	0.23 ± 0.63 (7)	0.034 ± 0.04 (8)	39.1 ± 21 (8)

Number of measurements given in parenthesis.
Subsurface: ≈ 100 m depth.
PA, prokaryotic abundance; PHP, prokaryotic heterotrophic production; ETS, electron transport system; SACW, South Atlantic Central Water; AAIW, Antarctic Intermediate Water; MSOW, Mediterranean Sea Outflow Water; UNEADW, Upper North-east Atlantic Deep Water; MNEADW, Middle North-east Atlantic Deep Water; LNEADW, Lower North-east Atlantic Deep Water.

specific LAPase from 25.5 to 129.2 amol cell^{-1} h^{-1}, and APase from 3.5 to 28.2 amol cell^{-1} h^{-1}. No significant differences between the eastern and the western transect for any of the cell-specific EEA were detectable (*t*-test; $P = 0.74$, $n = 26$ for AGase; *t*-test; $P = 0.89$, $n = 32$ for BGase; and *t*-test; $P = 0.24$, $n = 49$ for LAPase) except for APase, which was significantly higher in the eastern than in the western transect (*t*-test; $P = 0.0028$, $n = 48$).

Dynamics in kinetics of APase and LAPase

The maximum rates of hydrolysis (V_{max}) of APase and LAPase decreased with depth (Fig. 4A and B). V_{max} of APase was about one order of magnitude lower than the V_{max} of LAPase. No significant difference was discernable between the eastern and western transects for the V_{max} of APase (*t*-test; $P = 0.53$, $n = 50$) and for LAPase V_{max} (*t*-test; $P = 0.54$, $n = 46$). In the AAIW, however, the decreasing trend with depth was not observed, as we measured a lower APase V_{max} and higher LAPase V_{max} in this water mass than in its adjacent water masses (Fig. 4A and B).

The half-saturation constant (K_m) of APase was higher in subsurface waters only in the upwelling regions, showing a relative minimum in intermediate waters (especially in the MSOW for APase and LAPase) and then increasing with depth (Fig. 4C and D). There were no significant differences discernable between the eastern and western transects in the K_m of APase and LAPase (*t*-test; $P = 0.24$, $n = 49$ for APase K_m; and *t*-test; $P = 0.89$, $n = 47$ for LAPase K_m).

Cell-specific potential hydrolytic activity (V_{max} cell^{-1}) increased with depth for both APase and LAPase (Fig. 5A and B), and was significantly higher (cell-specific APase V_{max}: *t*-test; $P = 0.00001$, $n = 62$; cell-specific LAPase V_{max}: *t*-test; $P = 0.00001$, $n = 64$) in the deep-water masses [Upper North-east Atlantic Deep Water (UNEADW), MNEADW and LNEADW] than in the subsurface to intermediate water masses [subsurface, South Atlantic Central Water (SACW), AAIW and MSOW]. Significantly higher cell-specific potential hydrolytic activity was found at the eastern transect (*t*-test; $P = 0.0015$, $n = 49$ for APase V_{max}) but not for cell-specific LAPase V_{max} (*t*-test; $P = 0.11$, $n = 46$). The ratio of cell-specific $V_{max} : K_m$ was significantly higher for both enzymes at the eastern than the western transect (*t*-test; $P = 0.001$, $n = 49$ for APase and $P = 0.038$, $n = 46$ for LAPase). This ratio increased with depth for both enzymes, ranging from 0.018 to 4.25 cell^{-1} h^{-1} for APase (Fig. 5C) and 0.024–0.75 cell^{-1} h^{-1} for LAPase (Fig. 5D), and was significantly higher (cell-specific APase $V_{max} : K_m$: *t*-test; $P = 0.0004$, $n = 56$; cell-specific LAPase $V_{max} : K_m$: *t*-test; $P = 0.0002$, $n = 64$) in the deep water masses (UNEADW, MNEADW and LNEADW) than in the subsurface to intermediate water masses (subsurface, SACW, AAIW

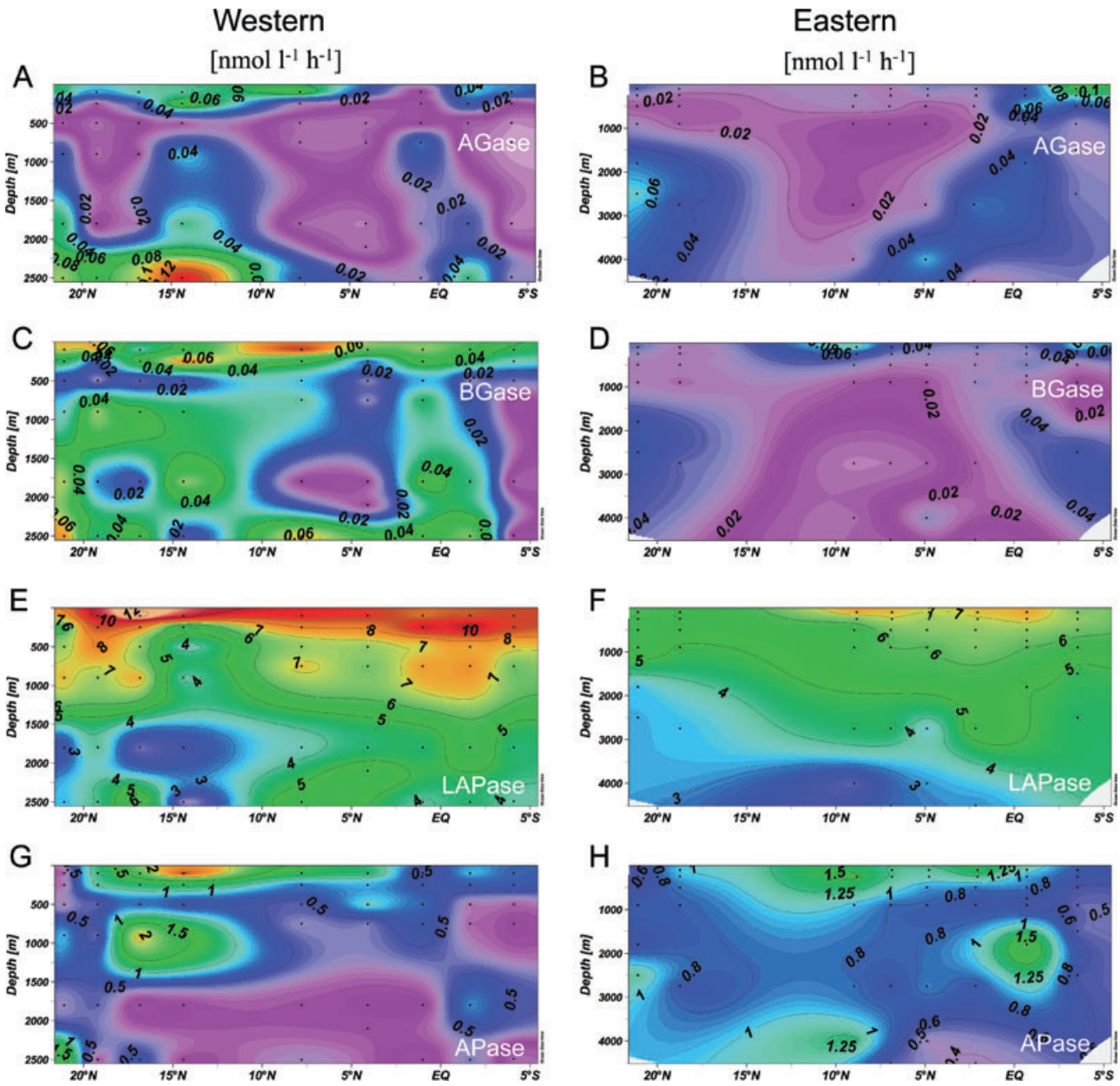

Fig. 2. Distribution of AGase (alpha-glucosidase) (A,B), BGase (beta-glucosidase) (C,D), LAPase (leucine aminopeptidase) (E,F) and APase (alkaline phosphatase) (G,H) activity throughout the water column along the western (left panels) and eastern (right panels) transects in the North Atlantic; values are given in nmol l^{-1} h^{-1}. Note the different depth range in both transects.

and MSOW). Thus, bulk maximum rates of hydrolysis decreased with depth while the half-saturation constant, cell-specific hydrolytic activity and cell-specific V_{max} : K_m ratio increased.

Discussion

Uncertainties in the estimation of the prokaryotic activity rates in the deep ocean

All the methods applied here to determine the different prokaryotic activity parameters have their own inherent limitations. Prokaryotic biomass production was assessed by the incorporation of leucine. While most of the bacterial groups are incorporating leucine (Cottrell and Kirchman, 2003), at least some *Archaea*, reported to be similarly important in terms of abundance in the deep North Atlantic (Teira *et al.*, 2006), might be chemoautotrophic and hence, not take up leucine. However, it has been shown for the North Atlantic and the Arctic Ocean that the percentage of *Archaea* taking up leucine is roughly similar to that of *Bacteria* (Herndl *et al.*, 2005; Kirchman *et al.*, 2007). Moreover, a large variation in the conversion factor

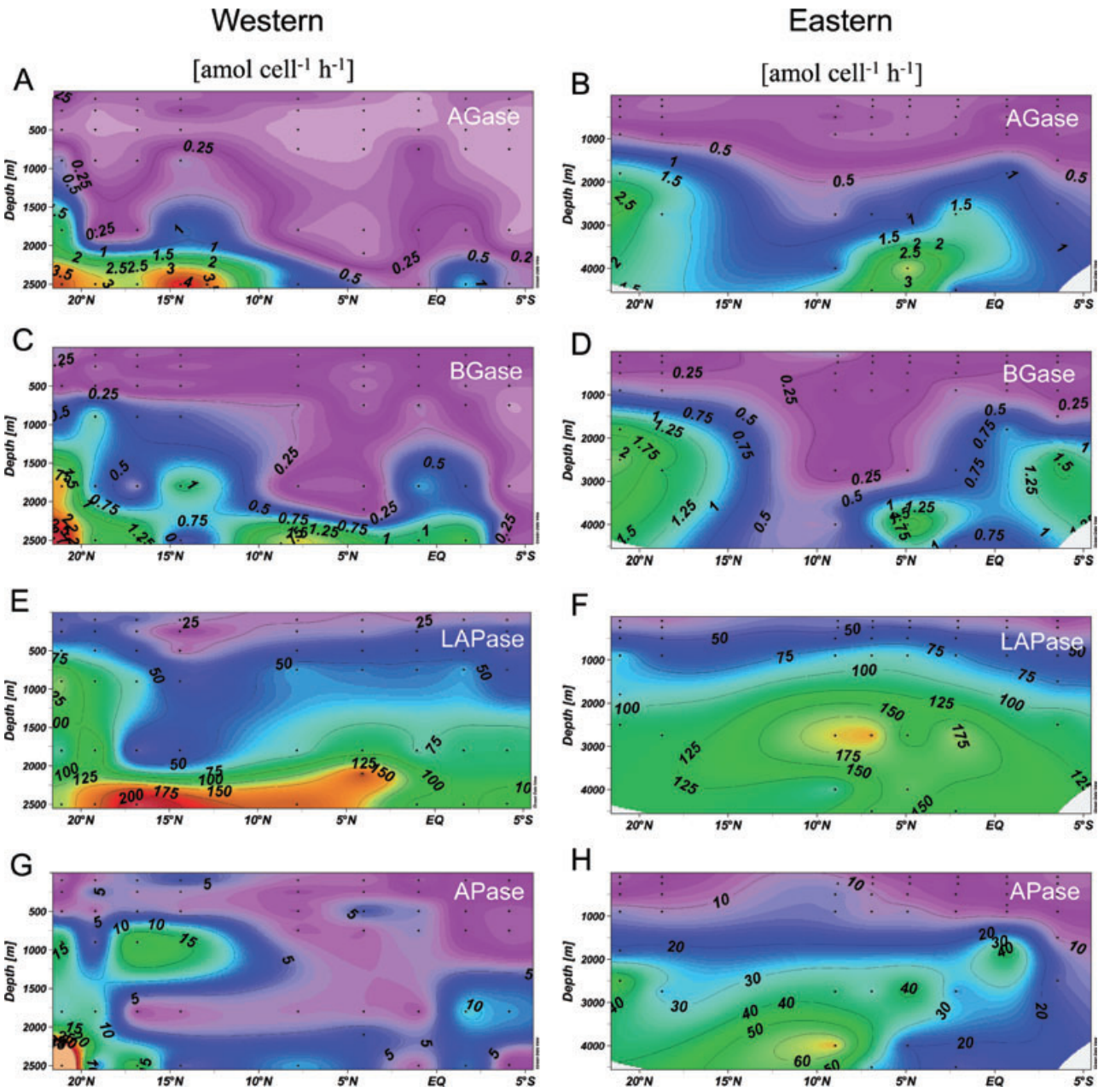

Fig. 3. Distribution of cell-specific AGase (A,B), BGase (C,D), LAPase (E,F) and APase (G,H) activity throughout the water column along the western (left panels) and eastern (right panels) transects in the North Atlantic; values are given in amol cell^{-1} h^{-1}. Note the different depth range in both transects.

of incorporated leucine to produced carbon biomass has been reported for surface Atlantic waters (Alonso-Saéz *et al.*, 2007). Also, the determination of respiration via ETS relies on a conversion factor to convert the measured absorption of the assay into oxygen consumption or carbon dioxide production (Packard *et al.*, 1996).

The use of fluorogenic substrate analogues to estimate EEA bears at least three problems. First, it targets only the exohydrolase activity which cleaves the terminal end of oligomers, while the rate-limiting step in the cleavage of macromolecular DOM is likely the endohydrolase activity cleaving polymers into oligomers (Hoppe *et al.*, 2002). The second problem associated with the fluorogenic substrate analogues is that they are model compounds and do not reflect the enormous diversity of potential extracellular enzyme substrates in nature. Third, with the enzyme assay applied here, we did not discriminate among potentially present different types of each extracellular enzyme.

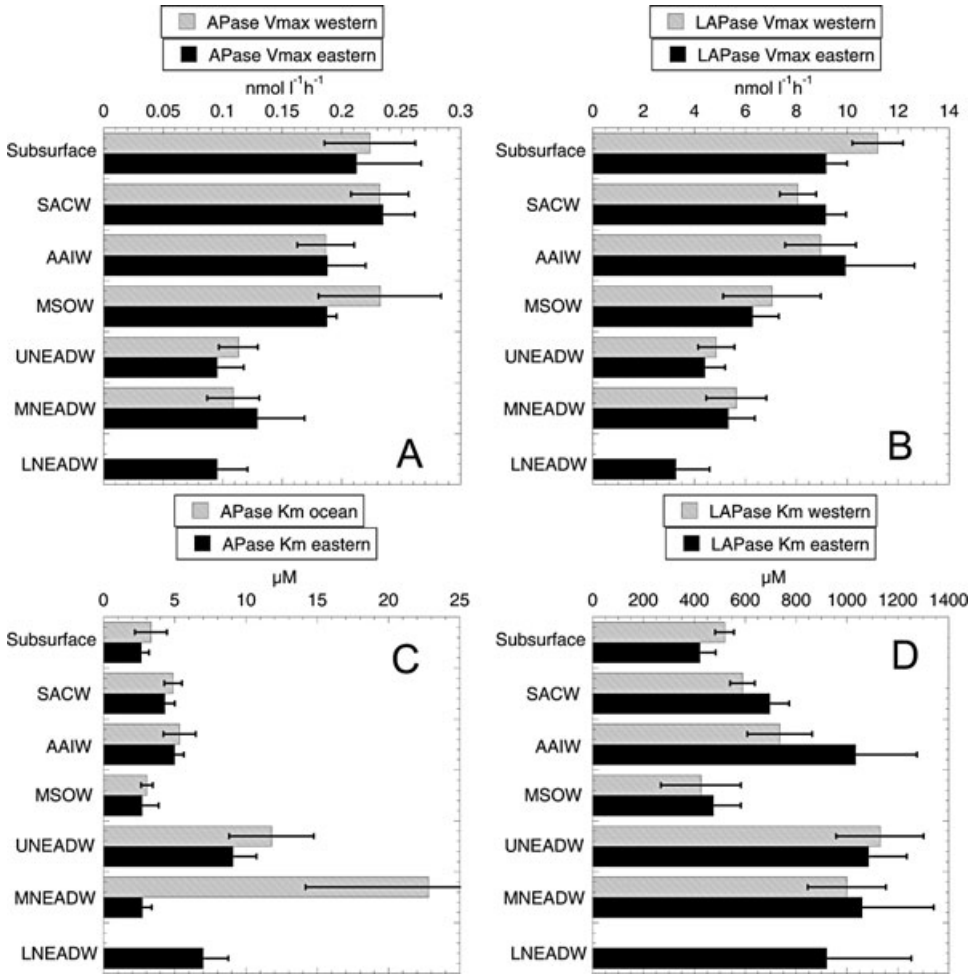

Fig. 4. Ectoenzyme kinetics of APase (A,C) and LAPase (B,D) throughout the water column along the eastern and western transect in the North Atlantic; (A,B) V_{max} (nmol h^{-1}), (C,D) K_m (µM). Error bars represent SE of 6–40 measurements.

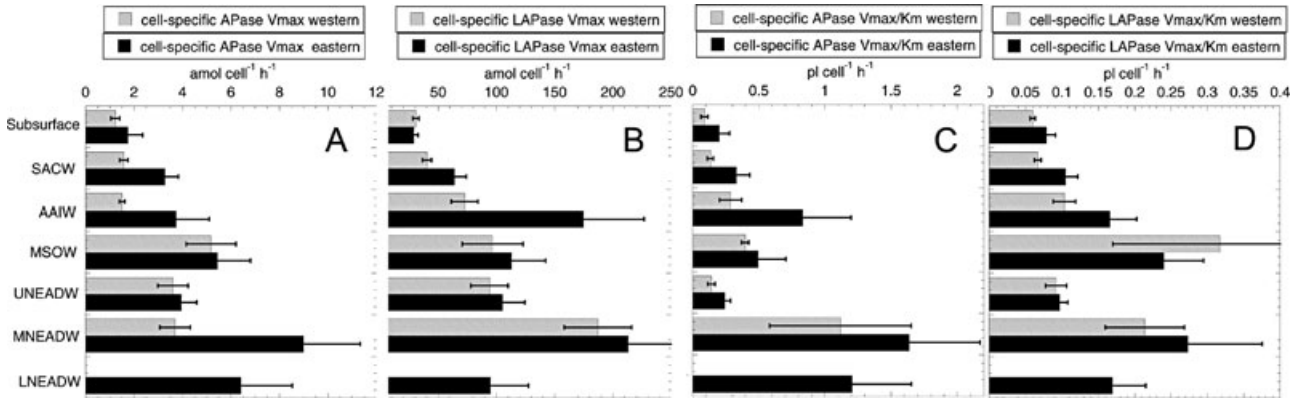

Fig. 5. Cell-specific (A) APase V_{max} (amol $cell^{-1}$ h^{-1}), (B) LAPase V_{max} (amol $cell^{-1}$ h^{-1}), (C) APase V_{max}/K_m (pl $cell^{-1}$ h^{-1}), and (D) LAPase V_{max}/K_m (pl $cell^{-1}$ h^{-1}) in the different water masses of the eastern and western transect in the North Atlantic. Error bars represent SE of 6–40 measurements.

Using a zymography approach and capillary electrophoresis, up to eight different beta-glucosidases were detectable at specific times in the coastal North Sea, all of them with specific enzyme kinetics (Arrieta and Herndl, 2001; 2002).

We have normalized leucine incorporation, respiration and EEA to total PA to compare the cell-specific activity of prokaryotes of subsurface waters with that in deep-waters. This assumes that all the prokaryotic cells have similar activity levels, which is certainly not the case. A part of the enumerated cells might be dormant or dead (Heissenberger *et al*., 1996), and different prokaryotic groups might express different activity levels (Martinez *et al*., 1996; Cottrell and Kirchman, 2003) and even intraspecific differences in activity levels exist (Sintes and Herndl, 2006). For the study area in the North Atlantic, Varela and colleagues (2008) report a contribution of *Bacteria* (determined by FISH using the Eub338I-III oligonucleotide probe) to total picoplankton abundance ranging from about 40–65% with no pronounced trend with depth, while the contribution of *Archaea* was generally below 10% of total picoplankton abundance. Hence, despite all the uncertainties concerning the interspecific and intraspecific variability of the different activity levels measured here, there are no major differences in the contribution of *Bacteria* and *Archaea* to total PA from about 100 m depth to the deep-waters in the study area. Taken together, the contrasting depth-related patterns in cell-specific leucine incorporation versus cell-specific EEA and respiration indicate that these differences are real and not methodological artifacts. The observed contrasting pattern in cell-specific leucine incorporation versus cell-specific EEA and respiration is most likely also not caused by measuring these parameters under surface pressure rather than under *in situ* pressure conditions. While the absolute values of the individual parameters measured at surface pressure conditions might deviated from those *in situ*, there is no reason to assume that prokaryotic biomass production and respiration would show opposite trends under *in situ* than under surface pressure conditions. Otherwise, the measured growth efficiencies for bathypelagic prokaryotic communities of around 2% (Reinthaler *et al*., 2006) would be unrealistically deviating from this value, which seems reasonable considering the comparably low growth efficiencies reported for prokaryotic communities of oligotrophic surface waters (Del Giorgio *et al*., 1997; Del Giorgio and Cole, 1998). Also, until now, there is no consistent trend reported in the literature on the effect of pressure on bathypelagic prokaryotic activity including EEA. There is evidence that deep water prokaryotic activity is overestimated if measured under decompressed conditions (Jannasch and Wirsen, 1982). However, other authors report inhibition of prokaryotic activity because of decompression (Tamburini *et al*., 2003). It is well known that prokaryotic enzymes might be adapted to a specific hydrostatic pressure (Jannasch and Taylor, 1984; Somero, 1992). If this holds true on a community level our bulk EEA would be underestimated under decompressed conditions. In fact, Tamburini and colleagues (2002) showed that LAPase and APase rates measured in samples maintained under *in situ* pressure conditions were around 2.3 times higher than those measured in their decompressed counterparts. Thus, our reported increase in cell-specific EEA with depth might be even higher under *in situ* conditions. Hence, we are confident that the general trend of increasing cell-specific EEA with depth is real, and if there is a bias due to decompressing the samples, the reported rates are likely underestimations of the actual rates under *in situ* pressure conditions.

Alkaline phosphatase utilization in the deep: the paradox of high APase at high end-product concentrations

It is well documented that the expression of many prokaryotic ectoenzymes is regulated by the concentration of its end-product (Chrost, 1991). Chrost and Overbeck (1987) observed in lake Plußsee, that APase significantly decreased when the ambient phosphate concentrations were higher than 0.5 μM. Ammerman and Azam (1991) reported that APase activity was usually low and sometimes undetectable in P-rich waters, and Nausch and colleagues (1998) observed a significant decrease in APase at ambient phosphate concentrations higher than 1 μM in the Baltic Sea. Zaccone and colleagues (2003) also found an inverse relation between APase and phosphate concentrations from the surface to deep waters in the Mediterranean Sea.

We found distinct water mass- and depth-related patterns of cell-specific APase in relation to PO_4 concentrations (Fig. 6). While phosphate concentrations varied only twofold over all the water masses, cell-specific APase activity varied more than sevenfold with lowest average cell-specific APase in the upper and intermediate waters increasing with depth (Fig. 6). This specific pattern indicates that the expression of APase is not regulated by the concentration of its end-product in deep Atlantic waters, a pattern also identified by Hoppe and Ullrich (1999) in the mesopelagic waters of the Arabian Sea. It indicates that shifts in the functioning of APase might take place from the upper mesopelagic waters with their relatively higher contribution of reactive organic matter to the deeper water masses characterized by old, refractory organic matter. As hypothesized by Hoppe and Ullrich (1999), C-limited prokaryotes in the deep strata of the water column might use APase to access organic C moieties from organic matter, rather than phosphate directly.

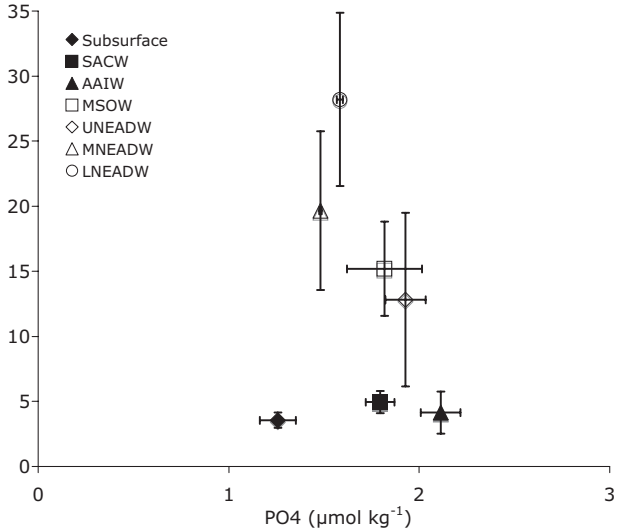

Fig. 6. Cell-specific APase (amol cell^{-1} h^{-1}) versus phosphate concentration (µmol kg^{-1}) in the different water masses. Error bars represent SE of 6–40 measurements.

Relation between EEA and prokaryotic activity parameters in the deep oceanic water column

There are only few reports available on the EEA in the meso- and bathypelagic realm of the open ocean while considerable information is available for open surface waters. The bulk APase activity reported in this study (0.04–3.7 nmol l^{-1} h^{-1}) is in the same range as reported by Hoppe and Ullrich (1999) for the deep Indian Ocean (2–4.5 nmol l^{-1} h^{-1} at 800 m), but is higher than that the range of APase activity (0.03–0.3 nmol l^{-1} h^{-1} at 1000–4000 m) reported by Koike and Nagata (1997) for the deep central Pacific Ocean. The difference between the APase activity reported by Koike and Nagata (1997) and our data might be due to the fact that Koike and Nagata (1997) measured APase activity associated with particles (> 0.2 µm) only and thus, excluding dissolved APase activity. Cell-specific EEA in the meso- and bathypelagic zones have been only reported in a few studies (Table 2). Our ranges of EEA are similar to the data from the northwestern Mediterranean Sea (Tamburini *et al*., 2002; Misic and Fabiano, 2006), the Oman coast and the Arabian Sea (Hoppe and Ullrich, 1999), and the central North Atlantic (F. Baltar, J. Arístegui, J. M. Gasol, E. Sintes, H. M. van Aken, G. J. Herndl, unpublished). In all the studies (except in Misic and Fabiano, 2006), an increase of cell-specific EEA was observed with depth. This increase in cell-specific EEA with depth corresponds to the increase in cell-specific respiration with depth reported for different regions (Table 3). In contrast to that, cell-specific PHP exhibits a larger variability depending on the sampling conditions and location (Table 4). Generally, cell-specific PHP decreases with depth to the bathypelagic layers (Table 4).

Cell-specific LAPase activity decreases exponentially with increasing cell-specific leucine incorporation (Fig. 7). Thus, biomass production decreases with increasing expression of extracellular enzymes.

Generally, the oceanic DOM pool is characterized by increasing C:N:P ratios with depth (Hopkinson and Smith, 2005). Also, it has been observed that the amino acid : carbohydrate ratio of sinking POM decreases with depth (Haake *et al*., 1993), indicating that proteinaceous components of sinking POM are more rapidly degraded than the polysaccharide fraction (Smith *et al*., 1992; Skoog and Benner, 1997). Experiments have consistently shown that bacteria degrade proteins faster than polysaccharides during decomposition of phytoplankton-derived detritus (Skopintsev, 1981) and, thus, that the LAPase: BGase ratios (which can be interpreted as the degradation of protein relative to that of polysaccharides) decrease during phytoplankton decay (Middelboe *et al*., 1995). These general trends suggest that the relative concentration of polysaccharides in sinking POM increases with depth, resulting in an intensive supply of polysaccharide-rich material to deeper waters and a decrease in the LAPase : BGase ratio with depth as observed in the present study (Fig. 8), where the LAPase : BGase ratio was significantly higher (*t*-test; $P < 0.001$, $n = 44$) in the deep water masses (UNEADW, MNEADW and LNEADW) than in the subsurface to intermediate water masses (subsurface, SACW, AAIW and MSOW).

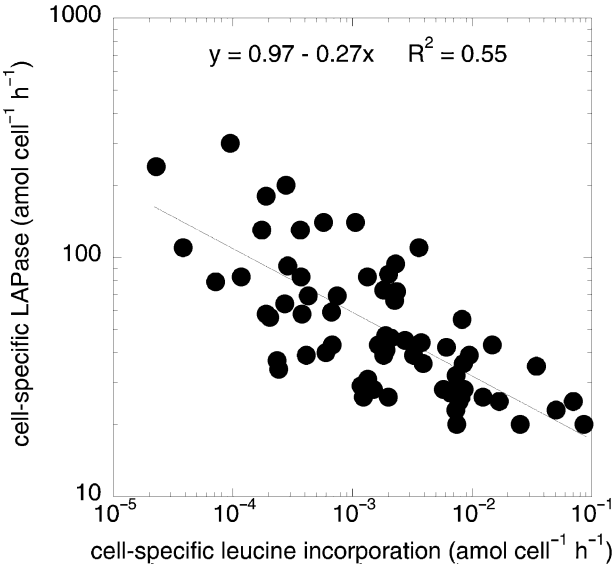

Fig. 7. Relation between cell-specific leucine incorporation and cell-specific leucine aminopeptidase (LAPase) activity. Data are derived from Fig. 2C (for cell-specific leucine incorporation) and Fig. 4E and F (for cell-specific leucine aminopeptidase).

Table 2. Cell-specific extracellular enzymatic activity (amol cell^{-1} h^{-1}) in different marine systems.

Environment	Conditions	LAPase	APase	AGase	BGase	Reference
Baltic Sea	Tank incubations		2.0–14			Nausch (1998)
Baltic Sea	Summer	0.3–5		0.1–3.3	0.2–3.7	Nausch *et al.* (1998)
	Autumn	20–237		0.1–1	0.2–2	
Gulf Aqaba	Euphotic zone	< 0.05–49.2			< 0.1–43.8	Grossart and Simon (2002)
Coastal lagoon	Hypertrophic	188–625			6.9–25	Cunha *et al.* (2000)
San Francisco Bay	Cells < 1 μm	7.2–12			0.16–0.57	Murrell *et al.* (1999)
	All cells	16–31			0.47–1.60	
Uranouchi Inlet, Japan	Surface water	23.2–1017				Patel *et al.* (2000)
	Bottom water	21.1–270				
Florida Bay	Estuarine system	52.2–1571.7	44.5–1029.4	1.3–74.6	0.7–23.6	Williams and Jochem (2006)
Caribbean Sea	Eutrophic	31.6		1.69	0.18	Rath *et al.* (1993)
	Oligotrophic	75.6		0.4	0.06	
Santa Monica Basin	Oligotrophic	78–618				Rosso and Azam (1987)
Adriatic Sea	Marine Snow	432–4996		7–40	6–140	Karner and Herndl (1992)
Selected aggregates	Experimental	av. 242 ± 493				Smith *et al.* (1992)
	Seawater	av. 52.5 ± 15				
Unaltered seawater microcosm	Aggregates	220 ± 98		4.2 ± 0.9	3.0 ± 2.4	Azúa *et al.* (2003)
	Ambient water	560 ± 89		5.0 ± 1.0	6.8 ± 3.4	
Freshly produced phytoplanktonic material microcosm	Aggregates	840 ± 231		12.5 ± 5.6	13.0 ± 3.6	
	Ambient water	170 ± 17		2.0 ± 0.6	4.4 ± 0.9	
California Bight	44 isolates from marine sources	4–3810	0.7–410	0–8	0–35	Martinez *et al.* (1996)
Gulf of Genoa (Ligurian Sea, NW Mediterranean)	Euphotic (0–200 m)	av. 25.3–54.9			av. 1.1	Misic and Fabiano (2006)
	Mesopelagic (200–1000 m)	av. 25.3–38.0			av. 0.7	
DYFAMED station (Mediterranean Sea)	Surface layer (10–200 m)	av. 1.9	av. 1.9			Tamburini *et al.* (2002)
	Deep layer (200– 2000 m)	av. 3.5	av. 7.7			
Oman coast, upwelling	Euphotic zone	12.6–46.9	1.2–8.3		0.02–1.2	Hoppe and Ullrich (1999)
	Deep water	455–1817	10.8–86.2		7.7–52.5	
Arabian Sea	Euphotic zone	6.6–23.2	0.4–3.6		0.16–0.22	
	Deep water	33–118	5.6–23.4		0.27–1.18	
Central Atlantic	Subsurface Layer (100)	5.6–31.4	0.7–2.9	0.1–0.66	< 0.1–0.5	F. Baltar, J. Arístegui, J. M. Gasol, E. Sintes, H. M. van Aken, G. J. Herndl, unpublished
	Mesopelagic zone (200–1000 m)	9.6–39.7	1.3–5.2	< 0.1–1.9	0.17–2.2	
	Bathypelagic zone (1000–7000 m)	5.3–147.2	4.4–20.7	< 0.1–5.20	0.24–6.82	
Sub(tropical) North Atlantic	Subsurface Layer (100)	11.1–43.0	0.4–7.8	< 0.1–0.52	< 0.1–1.1	This study
	Mesopelagic zone (200–1000 m)	4.83–145.5	0.87–27.3	< 0.1–1.58	< 0.1–1.1	
	Bathypelagic zone (1800–4500 m)	16.7–304.5	< 0.1–108.5	< 0.1–5.8	< 0.1–3.9	

av., average; LAPase, leucine aminopeptidase; APase, alkaline phosphatase; Agase, alpha-glucosidase; Bgase, beta-glucosidase.

Table 3. Cell-specific respiration (R) (fmol C cell^{-1} day^{-1}) in different marine systems.

Environment	Conditions	Cell-specific R	Reference
Gulf of Mexico	< 0.8 μm	0.39	Jørgensen *et al.* (1999)
Santa Rosa Sound estuary	< 0.8 μm	0.17	
Aberystwyth (Cardigan Bay, Wales, UK)	Coastal water	4.28 ± 1.12	Mukhanov *et al.* (2003)
Sevastopol Bay (SB; Black Sea, Ukraine)	Coastal water	6.63 ± 4.51	
North Wales	< 0.8 μm	0.4–6.8	Blight *et al.* (1995)
Louisiana (USA) shelf waters	< 1 μm	2.4–8.7	Biddanda *et al.* (1994)
Southern North Sea	Seasonal Cycle	0.3–3.6	Reinthaler *et al.* (2005)
Elbe Estuary, Germany	Aggregate on sampling day	22.0	Ploug *et al.* (2002)
	Aggregate on day 6 of incubation	2.6	
Kerguelen Plateau (Southern Ocean)	Mean value upper 100 m of < 0.8 μm	0.5–1.9	Obernosterer *et al.* (2008)
	Bathypelagic zone (1000–4500 m)	0.23–6.90	
Temperate North Atlantic	Subsurface Layer (100–135 m)	av. 0.85	Reinthaler *et al.* (2006)
	Oxygen minimum (402–725 m)	av. 1.67	
	Bathypelagic zone (1800–3000 m)	av. 2.74	
Subtropical North Atlantic	Subsurface Layer (100)	0.04–3.82	Baltar *et al.* (2009)
	Mesopelagic zone (200–1000 m)	0.12–5.23	
	Bathypelagic zone (1000–5000 m)	0.43–7.66	
Sub(tropical) North Atlantic	Subsurface Layer (100)	0.17–1.18	This study
	Mesopelagic zone (200–1000 m)	0.14–2.88	
	Bathypelagic zone (1800–4500 m)	0.23–6.90	

av., average.

Table 4. Cell-specific prokaryotic heterotrophic production (PHP) (fmol C cell^{-1} day^{-1}) in different marine systems.

Environment	Conditions	Cell-specific PHP	Reference
Rhone river plume	Euphotic zone (0–100 m)	0.34–0.40	Kirchman *et al.* (1989)
	Mesopelagic zone (150–700 m)	0.14–0.34	
Natural diatom aggregates in Øresund (Denmark)	Natural diatom aggregates	18.0–51.8	Grossart *et al.* (2003)
	Surrounding waters	4.3–16.3	
Dona Paula bay (west coast of India)	13-month field observations from surface waters (1 m)	0.26–3.9	Bhaskar and Bhosle (2008)
Coastal transition zone	Surface layer (5–82 m)	0.57–4.68	Baltar *et al.* (2007)
North Atlantic	Mesopelagic zone (200–760 m)	0.016–0.50	
	2000 m	0.020–0.33	
SE Mediterranean Sea	Euphotic zone (0–100 m)	0.03–0.09	Robarts *et al.* (1996)
	Mesopelagic zone (100–1000 m)	0.03–0.20	
Gulf of Mexico	0–300 m	0.01–1.33	Skoog *et al.* (1999)
Tasman Sea	Euphotic zone (0–100 m)	0.01–0.086	Moriarty and O'Donohue (1995)
	Mesopelagic zone (100–1200 m)	0.002–0.091	
East Sea, Korea	Euphotic zone (0–200 m)	0.028–1.75	Cho *et al.* (2000)
	Mesopelagic zone (200–1000 m)	0.014–0.23	
Southern North Sea	Seasonal Cycle	0.41–3.0	Reinthaler *et al.* (2005)
Global data set	Epipelagic (0–200)	av. 0.199 ± 0.018	Arístegui *et al.* (2009)
	Mesopelagic zone (200–1000 m)	av. 0.112 ± 0.017	
	Bathypelagic zone (1000–4000 m)	av. 0.076 ± 0.010	
Subarctic Pacific	Euphotic zone (0–100 m)	0.0016–0.402	Nagata *et al.* (2000)
	Mesopelagic zone (100–1000 m)	0.0005–0.0050	
	Bathypelagic zone (1000–5834 m)	0.0003–0.0011	
Indian Ocean	Euphotic zone (0–200 m)	0.007–0.308	Ducklow (1993)
	Mesopelagic zone (200–1000 m)	0.002–0.007	
Southern Ocean	Euphotic zone (0–200 m)	0.005–0.21	Simon *et al.* (2004)
	Mesopelagic zone (200–1000 m)	0.003–0.56	
Temperate North Atlantic	Subsurface Layer (100–135 m)	0.004–0.12	Reinthaler *et al.* (2006)
	Oxygen minimum (402–725 m)	0.002–0.14	
	Bathypelagic zone (1800–3000 m)	0.0005–0.26	
Subtropical North Atlantic	Subsurface Layer (100)	0.01–0.07	Baltar *et al.* (2009)
	Mesopelagic zone (200–1000 m)	0.001–0.05	
	Bathypelagic zone (1000–5000 m)	0.0004–0.01	
Central Atlantic	Subsurface Layer (100)	0.26–1.61	F. Baltar, J. Arístegui, J. M. Gasol, E. Sintes, H. M. van Aken, G. J. Herndl, unpublished
	Mesopelagic zone (200–1000 m)	0.012–1.33	
	Bathypelagic zone (1000–7000 m)	0.0017–0.046	
Sub(tropical) North Atlantic	Subsurface Layer (100)	0.01–0.05	This study
	Mesopelagic zone (200–1000 m)	0.0005–0.03	
	Bathypelagic zone (1800–4500 m)	0.0001–0.004	

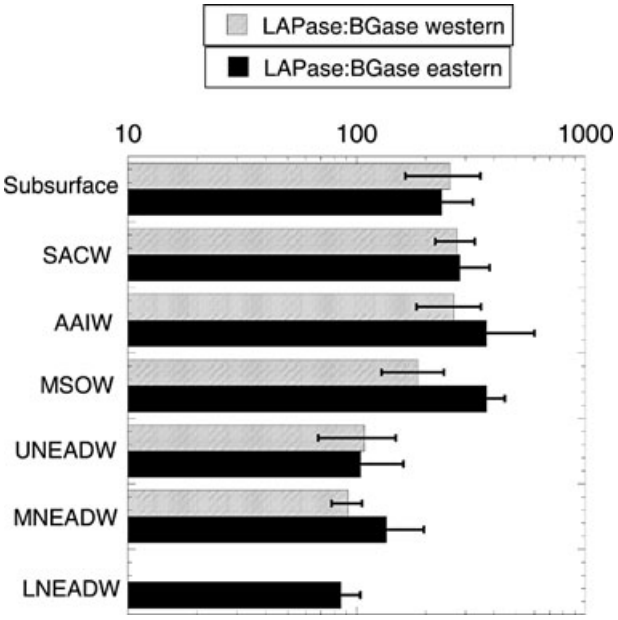

Fig. 8. Ratio of LAPase:BGase in the different water masses of the eastern and western transect in the North Atlantic. Error bars represent SE of 3–10 measurements.

Davey and colleagues (2001), in the only other study where extracellular enzyme kinetics have been measured below the euphotic zone, report an increase in the K_m with depth in distinct profiles of LAPase to 200 m depth in the North Atlantic, while the V_{max} decreased. This trend is in agreement with our study for the full depth range (Fig. 4). Azúa and colleagues (2003) studied the influence of the quality of the organic matter on the hydrolysis of polymers by marine prokaryotes in microcosms. They found that the cell-specific V_{max}/K_m ratio, which describes the ability of enzymes to compete at low substrate concentration (Healey, 1980), was higher in the unamended seawater microcosm than in seawater enriched in easily utilizable DOM. These experimentally obtained results agree with our findings from the meso- and bathypelagic waters of the Atlantic characterized by major differences in the DOM reactivity between surface and deep waters. Deep-water prokaryotes require apparently a high K_m and a high cell-specific V_{max}/K_m ratio because of the refractory nature of the organic matter present in the deep ocean.

In summary, the decrease in the availability of labile organic matter (dissolved and particulate) with increasing depth leads to an increase in cell-specific extracellular enzymatic expression and concomitantly, to a reduction in the prokaryotic growth yield. The increase in the K_m and the V_{max}/K_m ratio of LAPase and APase with depth further indicates an adaptation of the extracellular prokaryotic enzymes to the refractory nature of the deep-water organic matter.

Experimental Procedures

Study site and sampling

During the ARCHIMEDES-2 cruise with R/V *Pelagia* (November to December 2006), EEA was measured on 16 out of 20 stations occupied in total in the eastern (sub)tropical Atlantic along an eastern and a western N-S transect (Fig. 9). Meso- and bathypelagic samples were taken from the main water masses down to 4500 m depth and additionally, one subsurface sample per station was taken at the base of the euphotic layer at around 100 m depth. Sampling was performed with 22 10 l NOEX bottles mounted in a frame also holding sensors for conductivity, temperature, depth, chlorophyll fluorescence and optical backscattering. Subsurface waters consisted of the more saline subtropical and the less saline tropical subsurface waters. The waters below the thermocline (250–500 m depth) were formed by either North Atlantic Central Water (NACW; only found at the northernmost station – St. 50) or South Atlantic Central Water (SACW; rest of the stations). Underneath these central waters, the high salinity MSOW at the northern stations and the low salinity AAIW at the southern stations (from St. 13–37) were found between 700 and 900 m depth. Deeper in the water column, UNEADW, MNEADW and LNEADW were flowing southwards with cores at around 1800, 2500–2750 and 4000 m in depth respectively. The specific water masses were identified based on their distinct temperature-salinity characteristics, oxygen content and inorganic nutrient signatures (Table 1).

Water from the NOEX bottles was collected in acid-rinsed (0.1 N HCl) polycarbonate flasks rinsed three times with sample water prior to collecting samples for PA, PHP, potential respiration and extracellular enzymatic activities. The samples were either immediately processed for specific measurements aboard the ship or stored for subsequent analyses in the home lab as described below.

Prokaryotic abundance determined by flow cytometry

Prokaryotic plankton collected from the different depth layers of the water column were enumerated using flow cytometry. Samples (2 ml) were fixed with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 5 min and stored at −80°C (Kamiya *et al*., 2007). Picoplankton cells were stained with SYBR-Green I and enumerated with a FACSCalibur flow cytometer (Becton Dickinson) within 2 months. Immediately before analysis, the thawed picoplankton samples were stained with SYBR-Green I at room temperature in the dark for 15 min. Fluorescent microspheres (Molecular Probes) with a diameter of 1 μm were added to all samples as an internal standard. Counts were performed with an argon laser at 488 nm wavelength. Prokaryotic cells were enumerated according to their right angle scatter and green fluorescence. The counting window of the flow cytometer was set to exclude eukaryotic picoplankton.

Prokaryotic heterotrophic production by [^{3}H] leucine incorporation

Bulk PHP was measured by incubating triplicate 10–40 ml samples and formaldehyde-killed blanks (2% final concentra-

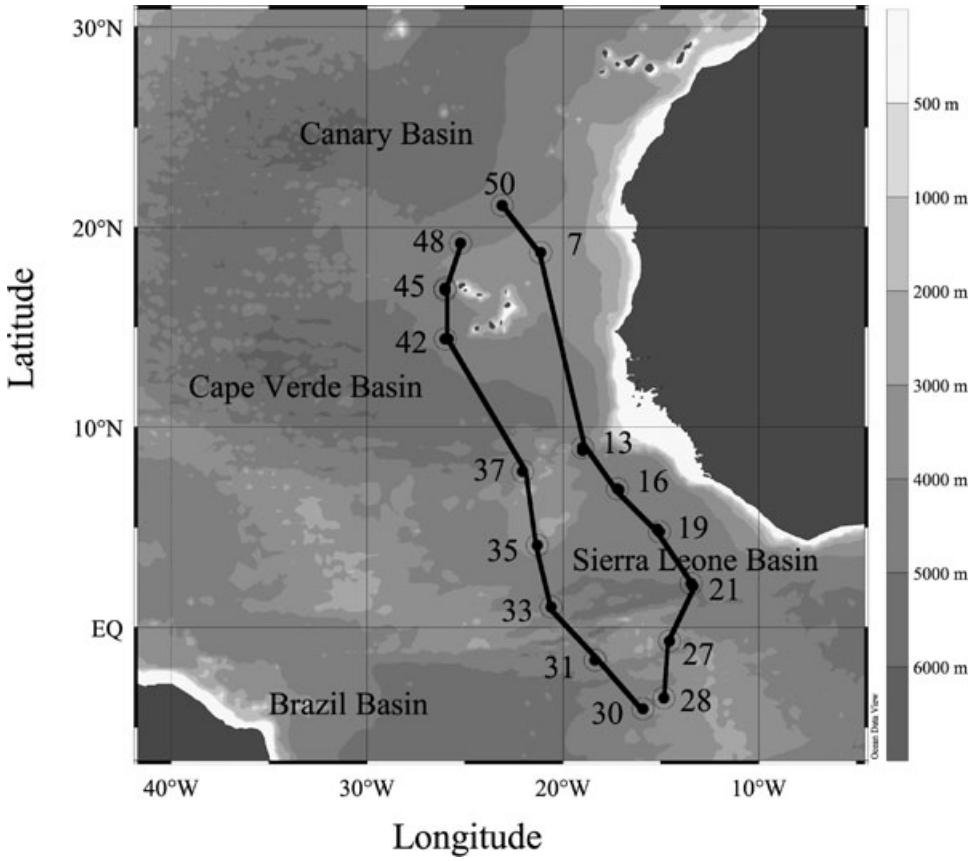

Fig. 9. Stations occupied during ARCHIMEDES-II. Both transects (eastern and western) began at Station 50, but the eastern transect finished at St. 28 and the western at St. 30.

tion) with 10 nM [^{3}H]-leucine (final concentration, specific activity 160 Ci mmol^{-1}; Amersham) in temperature-controlled incubators in the dark at *in situ* temperature for 4–10 h (Kirchman *et al.*, 1985). Incubations were terminated by adding formaldehyde (2% final concentration) before filtering the samples and the blanks through 0.2 μm polycarbonate filters (25 mm filter diameter; Millipore). Subsequently, the filters were rinsed three times with 5% ice-cold trichloroacetic acid, dried and placed in scintillation vials. Scintillation cocktail (8 ml Canberra-Packard Filter Count) was added, and after 18 h, counted in a liquid scintillation counter (LKB Wallac model 1212). The mean disintegrations per minute (DPM) of the formaldehyde-fixed blanks were subtracted from the mean DPM of the respective samples, and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using a conservative theoretical conversion factor of 1.55 kg C mol^{-1} Leu assuming no internal isotope dilution (Kirchman and Ducklow, 1993).

Activity of the ETS

Electron transport system activity was measured following the modifications of the tetrazolium reduction technique as described earlier (Arístegui and Montero, 1995). Some minor modifications of the method were made to increase its sensitivity. Briefly, about 10 l of sample was filtered through a Whatman GF/F filter (47-mm diameter). Filters were folded into cryovials and immediately stored in liquid nitrogen until analysis in the laboratory. Back in the laboratory, the filters with the collected material were homogenized in 2.5 ml phosphate buffer with a Teflon-glass tissue grinder at 0–4°C for 1.5 min. A 0.9 ml aliquot of the crude homogenate was incubated in duplicate with 0.5 ml of substrate solution (NADH, NADPH) and 0.35 ml of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 18°C for 20 min. The reaction was quenched by adding 0.25 ml of a mixture of formalin and phosphoric acid. The quenched reaction mixture was centrifuged at 4000 *g* at 4°C for 20 min, and the absorbance of the particle-free solution measured in a Beckman DU-650 spectrophotometer at 490 and 750 nm wavelength after adjusting the sample to room temperature. Readings at 750 nm, to correct for turbidity, were always negligible. In addition to the samples, duplicate controls were run by replacing the crude extract with a clean Whatman GF/F filter homogenized in phosphate buffer. ETS activity was calculated using the equation given in Packard and Williams (1981):

$$\mathrm{ETS}_{\mathrm{ASSAY}}(\mathrm{mmol\ O_2\ m^{-3}\ h^{-1}}) = H \times S \times (OD_{\mathrm{corr}})/(1.42 \times V \times f \times t/60) \times 22.4$$

where H is the volume of the homogenate (in ml), S is the volume of the quenched reaction mixture (in ml), OD_{corr} is the

absorbance of the sample measured at 490 nm wavelength and corrected for blank absorbance, *V* is the volume (in l) of the seawater filtered through the Whatman GF/F filter, *f* is the volume of the homogenate used in the assay (in ml), *t* is the incubation time (in min), 60 converts minutes to hours, the factor 1.42 converts the INT-formazan formed to oxygen units (in μl) and 22.4 converts the μl O_2 to μmol O_2. ETS activity was corrected to *in situ* temperature using the following equation:

$$ETS_{IN\ SITU} = ETS_{ASSAY} \times e^{[Ea/R\times(1/Tass-1/Tis)]}$$

where *Ea* is the Arrhenius activation energy (in kcal mol^{-1}), *R* is the gas constant, and *Tass* and *Tis* are the temperatures (in degrees Kelvin) in the assay and *in situ* respectively. A calculated activation energy of 16 kcal mol^{-1} was used (Arístegui and Montero, 1995). The final oxygen consumption rates were converted to carbon units using a respiratory quotient of 1. We are aware of the fact that the respiratory quotient might vary from about 0.6–1.3 depending on the substrate used, however, the general conclusions of this paper are not affected by the choice of the respiratory quotient.

Measurements of prokaryotic extracellular enzymatic activity

The hydrolysis of the fluorogenic substrate analogues 4-methylcoumarinyl-7-amide (MCA)-L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-α-D-glucoside, 4-MUF-β-D-glucoside and MUF-phosphate was measured to estimate potential activity rates of α-, β-glucosidase, aminopeptidase and alkaline phosphatase (Hoppe, 1983). All the chemicals were obtained from Sigma and appropriate stocks prepared in methyl-cellosolve. The activity of the enzymes was linearly related to the fluorescence resulting from the cleavage of MUF or MCA and was detected spectrofluorometrically using a Fluorolog-3 fluorometer with a MicroMax 384 microwell plate reader (Horiba) at an excitation and emission wavelength of 365 and 445 nm respectively. The samples (145 μl) were incubated in microwell plates, in the dark and at *in situ* temperature for 24 h. The linearity of the increase in fluorescence upon addition of fluorogenic substrate over time was checked by incubating samples up to 48 h. The increase in the relative fluorescence over time was transformed to cleavage activity using a standard curve established with different concentrations of the fluorochromes MUF and MCA added to 0.2 μm filtered sample water. In order to determine enzyme kinetics, model substrate concentrations should cover a sufficiently wide range. In this study, 12 different concentrations were used for the different substrates ranging from 0.6 to 1200 μM. For routine measurements of α- and β-glucosidase activities, a final concentration of 10 μM was applied, previously determined as saturating concentration. As a consequence of adding saturating concentrations, the rates measured with this technique are potential rates.

Profiles of enzyme kinetics for alkaline phosphatase and leucine-aminopeptidase were determined at each station. The saturation curves were transformed into a Lineweaver–Burk or Hanes–Woolf plot to reveal maximum enzyme activity (V_{max}, nmol l^{-1} h^{-1}) and substrate affinity (Michaelis–Menten half saturation constant; K_m, μM). Cell-specific ectoenzyme activity (amol $cell^{-1}$ h^{-1}) was calculated by dividing V_{max} by PA. The ratio cell-specific V_{max}/K_m, which is the slope of the Michaelis–Menten equation at low substrate concentrations, was also calculated (Healey, 1980). This ratio is an indicator of the ability of prokaryotes to attain a high hydrolysis rate at low substrate concentrations, as organisms might compensate for a high K_m by a high V_{max}.

Statistical analysis

Data were log transformed to attain normality before the *t*-student test was applied. To check the normality of the individual data sets, the Kolmogorov–Smirnoff test was used. For calculating uncertainties on ratios, error propagation was taking into account; standard deviations were calculated using the formula for the propagation of error (Bevington and Robinson, 2003) as follows:

$$\Delta z/z = \sqrt{\left[\left((\Delta x)^2/x\right)+\left((\Delta y)^2/y\right)\right]}$$

where *z* is the per cent abundance or the per cent assimilating and is equal to *x* divided by *y*, as described above. Δx and Δy are the standard deviations associated with *x* and *y* respectively. Δz is the standard deviation calculated for *z*. Standard deviations were then converted to standard errors for each calculation.

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