

# **RV Pelagia Cruise Report**

**Cruises 64PE202 & 64PE211**

**Project:**

**Transformation of dissolved organic matter (DOM) in the  
North Atlantic Deep Water and intermediate waters:  
assessing the functional and phylogenetic variability of  
marine bacterioplankton communities in relation to the  
quality of DOM**

**(TRANSAT)**

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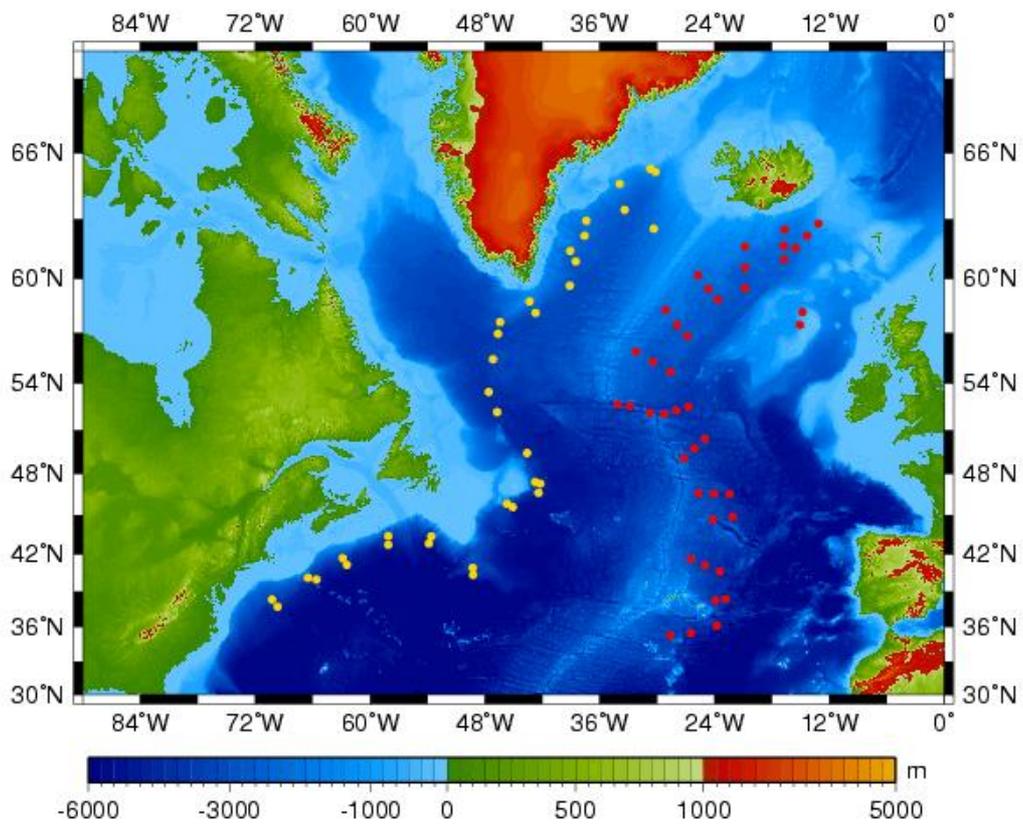
## Scientific background

The cooling of the surface waters in the Greenland-Island-Norwegian (GIN) Sea and the subsequent large scale deep water formation, known as the North Atlantic Deep Water (NADW), is considered the major driving force of the oceanic conveyor belt system which, in turn, influences the earth's climate. Recently, it has been found that the deep water formation in the GIN Sea is more variable than assumed hitherto. Despite this uncertainty, it became clear over the last 2 decades that the conveyor belt system and the NADW are essential elements influencing the global climate and, linked to that, the carbon cycling.

While the turnover of the water masses in the oceanic conveyor belt system is around 1500 years, the turnover of dissolved organic carbon (DOC) in the deep waters is around 6000 years. Thus, on average, the deep water DOC is cycled 4 times within the conveyor belt system before it is completely remineralized. With improved methodology, it is now possible to determine basin-scale variations in the concentrations of deep water DOC. As a consequence of conveyor belt circulation of deep water masses, the deep water DOC concentration decreases from around 45  $\mu\text{M}$  in the NADW to 37  $\mu\text{M}$  in the deep waters of the Pacific, i.e., a decline of 8  $\mu\text{M}$  C or by about 20% of the original DOC concentration. Recently, the existing knowledge on the degradation of deep water DOM within the conveyor belt system has been summarized. It has been concluded that the degradation of the deep water DOC must take place in a non-continuous way involving interactions between abiotic transformation of DOC (chemical, photochemical) and microbial degradation and remineralization.

Despite some recent advances in our understanding of global circulation processes and its potential role for the earth's climate, our knowledge on deep water transformation of DOC and on the bacterioplankton involved is rather poor. Due to this critical lack of a mechanistic understanding of the DOC transformation in the deep sea (which comprises the largest single system in the ocean [ $\approx 80\%$  in terms of volume]), it is impossible to make any educated predictions on the future development of the deep sea as a potential buffer reservoir in the biogeochemical flux of elements in a changing climate. Up to now, this lack of information on deep sea processes could be explained by the lack of proper and sensitive techniques to determine the rather low concentrations of specific organic compounds of the deep sea DOC pool and to determine bacterioplankton activity and composition. However, significant advancements have been made in chemical oceanography and microbial and molecular ecology over the past few years. These advancements allow us now to determine the temporal and spatial variability of the deep sea DOM pool and its major components and to link the characterization of the deep water DOM pool to the composition and activity of the deep water bacterial community, thus to link the biogeochemical aspects with a molecular characterization of the bacterioplankton.

***Thus, the ultimate goal of the 2 TRANSAT cruises was to measure net changes of selected chemical and microbiological parameters in situ in the NADW as it is transported in the conveyor belt system.***



The TRANSAT I and TRANSAT-II cruise tracks in the North Atlantic following the two main branches of the North Atlantic Deep Water. Stations occupied during TRANSAT-I are indicated by red dots, those at TRANSAT-II by yellow dots.

# Hydrography of the Stations occupied during TRANSAT-I and TRANSAT-II

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## General Introduction

In the Polar Ocean and Greenland Sea warm water from the Atlantic inflow as well as shelf waters are cooled and converted to Greenland Sea Deep Water. Thereby it gains an extremely high density. This high-density water is separated from the Atlantic Ocean by the presence of a series of shallow sills, the most important of these sills is the so called Greenland-Scotland Ridge. Through three slightly deeper gaps on this Ridge the water spills into the North Atlantic Ocean. The deepest of these gaps is the Faeroe Bank Channel between the Faeroe Islands and Scotland (~850 m) where cold water (Iceland-Scotland Overflow water or ISOW) from the Norwegian Sea enters the Atlantic. Between the Faeroe and Iceland Additional overflow of ISOW occurs at a depth of ~600 m. In Denmark Strait, between Greenland and Iceland, Denmark Strait Overflow Water (DSOW) enters the north-western Atlantic (Irminger Sea) from the Iceland Sea over a sill of about 650 m. During the overflow process warmer saline so-called Sub-Arctic Mode Water is entrained into the fast flowing, turbulent overflow water, which raises the salinity of both ISOW and DSOW to values above that of the overlying Labrador Sea Water. The latter water mass is formed in winter due to strong cooling and deep convective mixing (>1000 m) in the Labrador Sea between Labrador and Greenland.

The water mass, generated by mixing of ISOW or DSOW with surrounding waters forms the core of the North Atlantic Deep Water (NADW) which finds its course to the Indian and Pacific Oceans in the so-called oceanic conveyor belt circulation or global Thermohaline Circulation (TC). The deep North Atlantic Ocean is divided in an eastern and western Basin by the presence of the shallow Mid-Atlantic Ridge (MAR). In east of these basins a local variety of NADW is found, NEADW and NWADW. The NEADW is assumed to form some sort of shortcut of the THC, upwelling to shallower depths in the eastern basin somewhere between Iceland and the equator. Through a deep gap in the MAR, the Charlie-Gibbs Fracture Zone NEADW flows to the western Basin, and contributes with LSW the formation of NWADW, while mixing with the DSOW.

Bottom water enters the deep North Atlantic from the south. Its main constituent is Antarctic Bottom Water, characterized by a low salinity, high nutrient content (especially dissolved silica, Si), and in the northern hemisphere relatively low oxygen concentrations. The AABW enters the eastern Atlantic basin near the equator, through the Vema Fracture Zone in the MAR. During the inflow it is modified to Lower Deep Water (LDW) by mixing with overlying water. When flowing from the equator to the Iceland Basin LDW is also transformed by sediment water interaction. Among other things this interaction will raise the Si content of the LDW.

In the eastern North Atlantic mixing of ISOW with both LSW and LDW contributes to the formation of NEADW. South of ~45°N the influence of Mediterranean Sea Outflow Water (MSOW) can be perceived. This is a warm and saline water mass formed in the Gulf of Cadiz by mixing of water from the Straits of Gibraltar and overlying thermocline water. This MSOW flows northwards in the boundary current along the European continental Margin, and west to south-westwards in the form of

sub-surface Mediterranean eddies or Meddies. These Meddies, which can survive for over 3 years, have their strongest expression at a depth of about 1000 m.

Everywhere in the North Atlantic Ocean a local oxygen minimum is found in the lower half of the permanent thermocline (thermocline is the layer with a strong downward decrease of temperature from the upper ocean to intermediate depths). The minimum seems to be strongest in areas with a strong supply of particulate organic matter from plankton blooms, especially in the upwelling areas near Northwest Africa and Namibia. The depth and density where the minimum is found changes from location to location. It depends on advection by ocean currents, mixing with better ventilated water layers, and at high latitudes on erosion of the thermocline by deep reaching convection, driven by surface cooling in winter. By the latter process water, saturated in oxygen mixes with the oxygen depleted waters, while nutrients from that layer become available for plankton blooms in the following spring and summer.

### **Description of the water masses encountered along the cruise track of TRANSAT-I**

#### ***Iceland-Scotland Overflow Water and North-East Atlantic Deep Water along the slopes of Iceland and the Mid-Atlantic Ridge.***

##### The $\theta$ -S structure

Iceland-Scotland Overflow Water (ISOW) is formed when deep and intermediate water from the Faroe-Shetland Channel flows across the sill in the Faroe Bank Channel into the Iceland Basin. During that process warmer water from the overlying thermocline is entrained into the turbulent bottom layer where the cold overflow water descends in a thin layer (~100 m) down-slope into the Iceland Basin. In a  $\theta$ -S diagram this process can be followed from the observations carried out during the TRANSAT cruise (Figure 1). A cold core of overflow water, initially with temperatures of about 0°C entered the Iceland Basin across the sill in the Faroe-Bank Channel. By mixing with surrounding water the temperature of the overflow core was raised to about 1.6°C at section A. The entrainment of thermocline water near the Iceland-Faroe Ridge also caused a curvature at an inflection point in the  $\theta$ -S lines near a potential density anomaly of  $\sim\gamma_3 = 41.4 \text{ kg/m}^3$ . This inflection point was maintained further downstream along the eastern slope of the Reykjanes Ridge, where the presence of LSW intervened direct contact between the ISOW and the thermocline waters, until the last section north of the Charlie-Gibbs Fracture Zone (station 19 at section F). The overflow water in the bottom layer below this inflection point generally was nearly homohaline, and increased downstream slightly in temperature and decreased in salinity due to mixing with surrounding fresher and warmer water. At station 20 on the Reykjanes Ridge up-slope of station 19, the  $\theta$ -S structure of the ISOW layer (not shown) was similar to that of station 19, but the salinity of the bottom layer was 0.004 higher, and the bottom temperature was 0.16°C higher. This suggests a stronger influence of entrained thermocline water at the shallower stations over the Reykjanes Ridge (van Aken and de Boer, 1985).

At the latitude of the Charlie-Gibbs Fracture Zone, A colder and fresher water mass was found below the density levels of the ISOW core (station 25 at section H in Figure 2). This is the Lower Deep Water (LDW) from the eastern North Atlantic,

which enters the eastern Atlantic Basin at equatorial latitudes and moved from there to the northern North Atlantic Ocean (van Aken, 2000). This water mass owns its low salinity from the contribution of Antarctic Bottom Water (AABW) to its formation. In the northern North Atlantic Ocean LDW circulates in a cyclonic way south of the Rockall-Hatton Plateau and reaches the area of section H from the east (van Aken and Becker, 1996). Due to mixing of the ISOW as it was observed at station 19 with both the fresher overlying LSW and the fresher underlying LDW a water mass is formed which is characterized by a deep salinity maximum, the North East Atlantic Deep Water (NEADW) at sections H to J. The potential density anomaly in the NEADW core at these sections was about  $\gamma_3 = 41.45 \text{ kg/m}^3$ . At sections I and J the salinity of the NEADW core hardly differed (stations 27 and 32 in Figure 2).

When the NEADW moves further south, the influence of the water mass, formed by the outflow of water from the Mediterranean Sea becomes measurable at NEADW levels (van Aken, 2000). This Mediterranean Sea Outflow water (MSOW) is characterized by relatively high salinities and temperatures because of its origin from a sub-tropical enclosed sea with an evaporation excess. South of section J the salinity at the density level of the NEADW core along the Mid-Atlantic Ridge increased to the south because of admixture of this saline lower density MSOW (Figure 3). The highest salinities at the NEADW level were found at station 40, located on section N, south of the Azores. From section J to section L the potential density of the NEADW core shifted upwards from  $\gamma_3 \approx 41.45$  to  $\gamma_3 \approx 41.40 \text{ kg/m}^3$ .

It is assumed that part of the ISOW/NEADW, formed in the eastern Atlantic Basin flows west through the Charlie-Gibbs Fracture Zone (CGFZ). This fracture zone forms a deep gap in the Mid-Atlantic Ridge, which allows inter-basin exchange of deep water between the eastern and western North Atlantic Basins. In the CGFZ Zone also a deep salinity maximum could be observed, with properties similar to those of the NEADW core in the nearby Eastern Basin of the Atlantic Ocean (Figure 4). At the westernmost station in the northern passage of the CGFZ the NEADW (#21) the  $\theta$ -S properties very well agreed with those of the ISOW at station 19 on section F. At station 22, slightly further east the salinity of the NEADW core was lower, similar to the value at Station 25 on section H. Apparently the NEADW flowing west through the CGFZ does not enter this gap from the east, but flows into it directly southwards from the eastern slope of the Reykjanes ridge where the ISOW core is located. In the southern passage in the CGFZ mixing of the NEADW core had progressed further to salinity values below those observed at section H.

### Longitudinal change of salinity, AOU and the inorganic nutrients

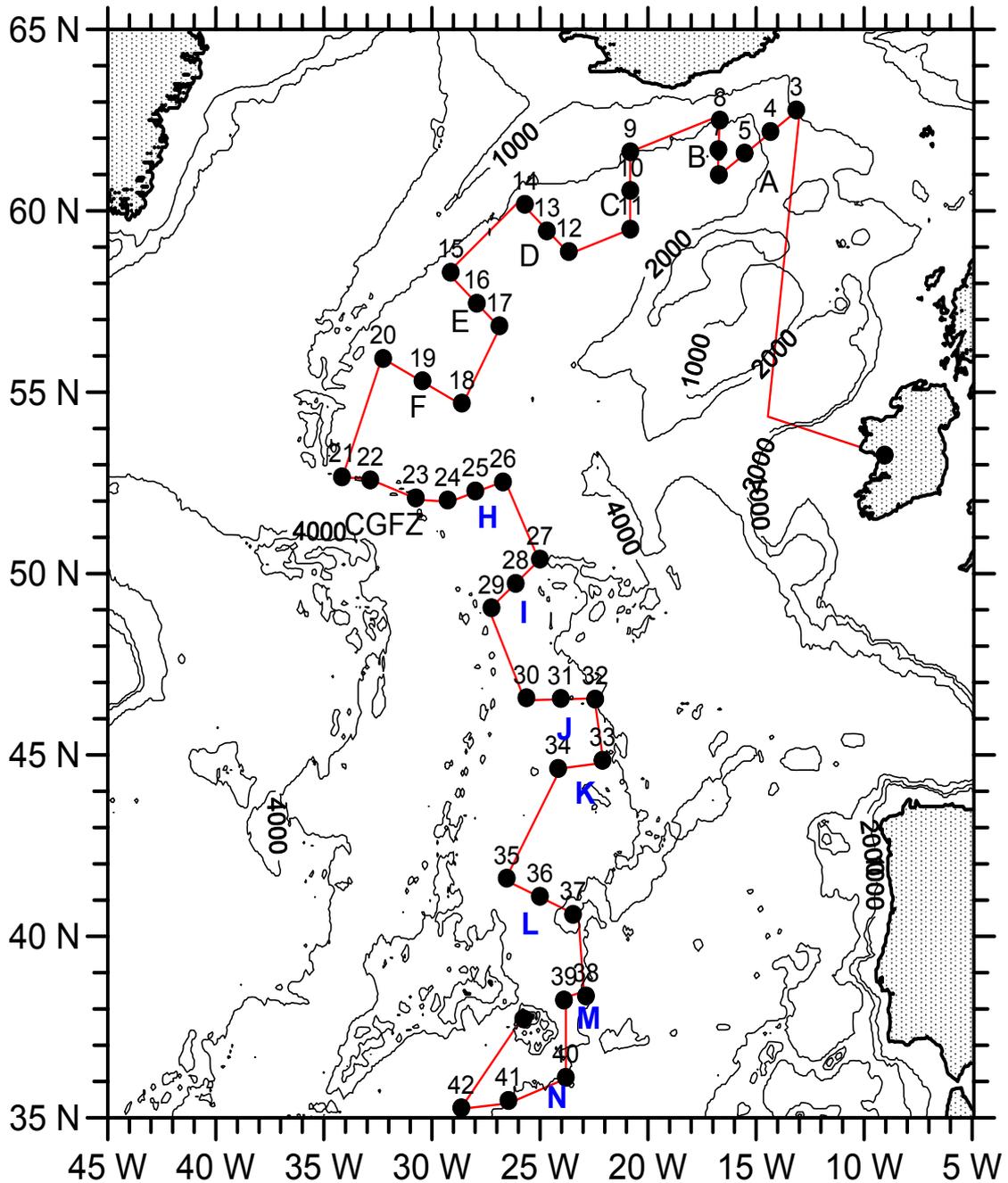
The salinity in the  $\gamma_3 = 41.42 \text{ kg/m}^3$ , characteristic for the NEADW core in the North Atlantic Ocean (van Aken, 2000), follows the development from the overflow near section a to section N near the Azores as described above (Figure 5, open symbols). Because of the changes in the  $\theta$ -S structure from section to section the water samples were not always taken in the same isopycnal, but their salinity more or less followed the development of S in the isopycnal, north of section L at slightly higher salinities (Figure 5, black dots). The longitudinal structure of dissolved AOU and nutrients in the ISOW/NEADW water samples shows a similar structure (Figure 6), which probably also can be attributed to mixing of the southward flowing ISOW core with overlying and underlying water types.

## Suspended matter and bacteria

During its descent in to the Iceland Basin the ISOW flows that fast over the bottom that bottom sediment is stirred up into suspension in the lowest 30 to 100 m above the bottom. The resulting turbidity layer was observed with the optical back-scatter sensor (Figure 7). At section F (station 19) the velocity of the ISOW layer was diminished so far that the turbidity layer apparently was lacking (thick line in Figure 5). With the suspended bottom material both particular organic matter from the sediments as well as bacteria can be introduced into the lower parts of the water column. Bacterial counts have shown that the highest sub-surface amounts of bacteria were found in the ISOW core over the bottom at sections A to E. A mean concentration of  $1.3 \cdot 10^5$  bacteria per millilitre was found, about twice the number observed in the overlying Labrador Sea Water. The HNA percentage of the bacteria was about 20% higher in the ISOW than in the LSW.

The decrease of the total concentration of bacteria and percentage of HNA in the NEADW core continued at a lower pace south of section F where no high turbidity layer was encountered in this core (Figure 8). Also the contrast with LSW was reversed. In the NEADW core the mean total concentration of bacteria was about  $0.33 \cdot 10^5$  /ml,  $0.10 \cdot 10^5$  /ml lower than in the overlying LSW and  $0.02 \cdot 10^5$  /ml higher than in the underlying LDW. The HNA percentage continued to increase downward, being 59% in the NEADW core and 66% in the underlying LDW.

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## References

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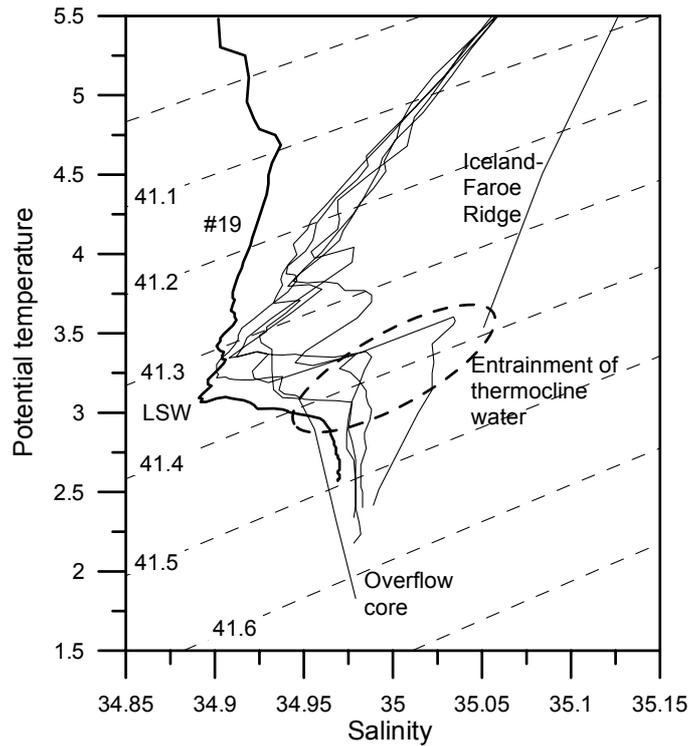


Figure 1. Potential temperature-salinity diagram for stations in the north-eastern Iceland Basin (sections A and B, thin lines) and for station 19 at section F (thick line). The thin dashed lines are isopycnals labelled with the potential density anomaly relative to a reference pressure of 3000 dbar ( $\gamma_3$ ). The curvature of the  $\theta$ -S lines, caused by entrainment of warm and saline water from the thermocline into the overflow water is highlighted with a dashed ellipse.

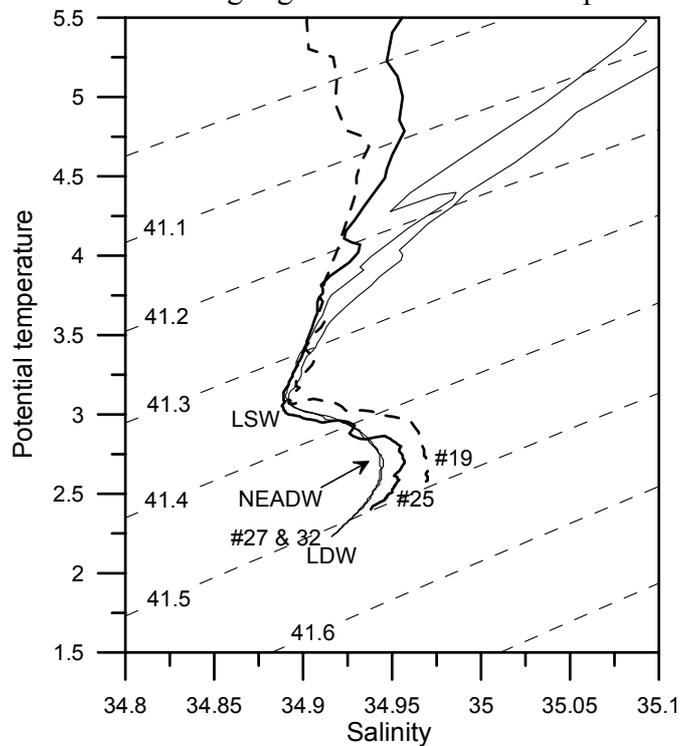


Figure 2. Potential temperature-salinity diagram for hydrographic stations on the sections F (thick dashed line), H (thick full line), I and J (thin full lines). The thin dashed lines show the isopycnals labelled with the potential density

anomaly relative to a reference pressure of 3000 dbar ( $\gamma_3$ ). The arrow points at the deep salinity maximum characteristic for the North East Atlantic Deep Water (NEADW)

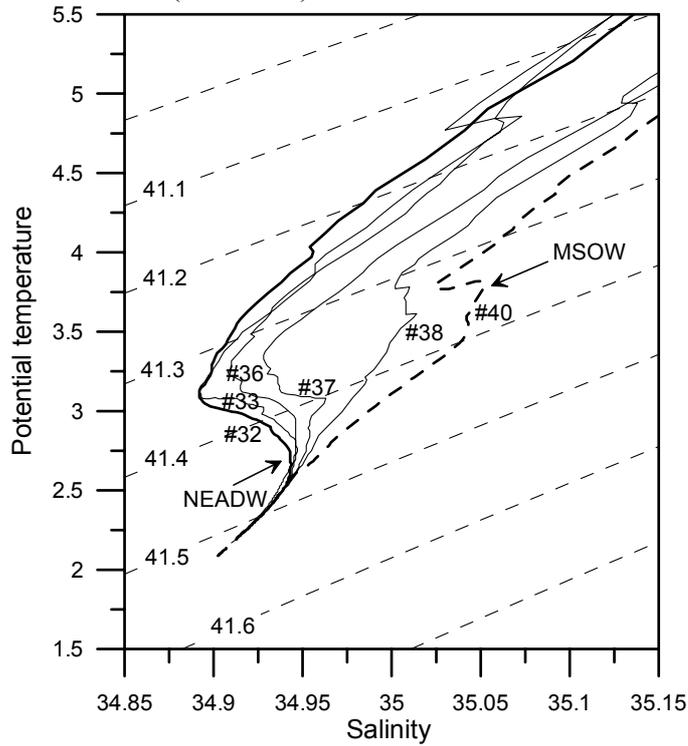


Figure 3. Potential temperature-salinity diagram for hydrographic stations on sections J (thick full line), K, L, and M (thin full lines) and section N (thick dashed line). The thin dashed lines show the isopycnals labelled with the potential density anomaly relative to a reference pressure of 3000 dbar ( $\gamma_3$ ). The arrow points at the deep salinity maximum characteristic for the North East Atlantic Deep Water (NEADW) and of Mediterranean Sea Outflow Water (MSOW).

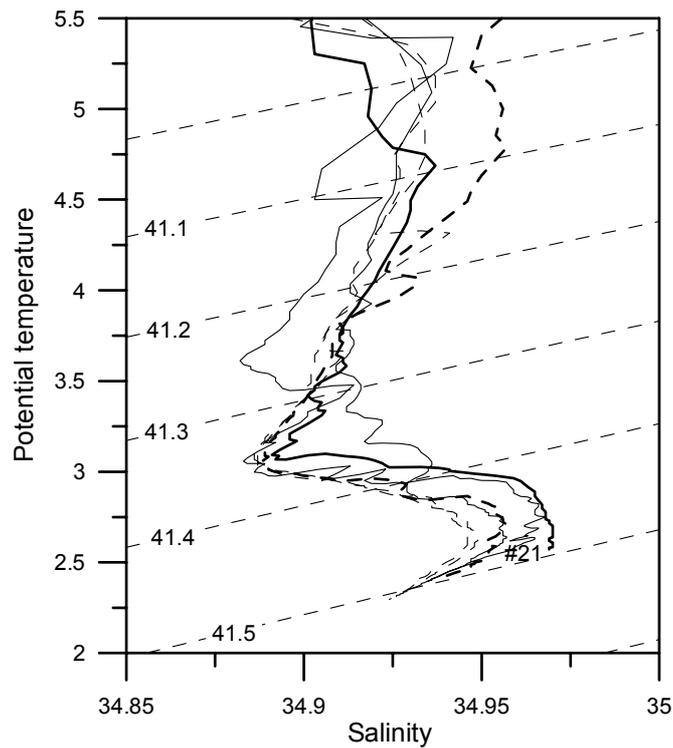


Figure 4. Potential temperature-salinity diagram for hydrographic stations in the northern (thin full lines) and southern (thin dashed lines) of the Charlie-Gibbs Fracture Zone. For comparison the  $\theta$ -S lines for station 19 on section F (thick full line) and station 25 on section H (thick dashed line) have been added. The thin dashed lines show the isopycnals labelled with the potential density anomaly relative to a reference pressure of 3000 dbar ( $\gamma_3$ ).

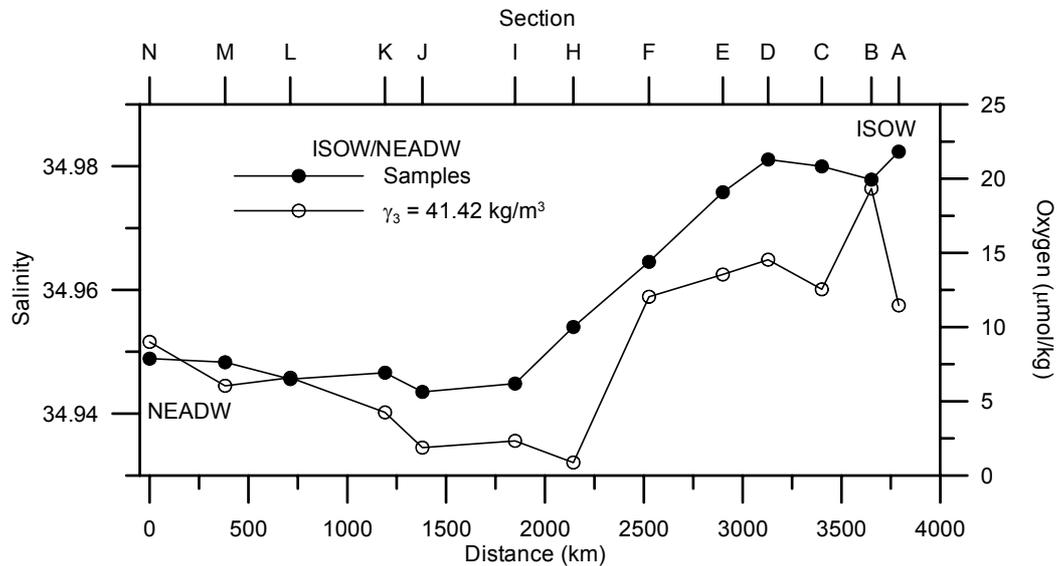


Figure 5. The longitudinal development of the salinity of the ISOW/NEADW core from the overflow area near section A to section N near the Azores. The black dots give the salinity of the water samples taken in the water mass core, the open symbols the salinity in the  $\gamma_3 = 42.42 \text{ kg/m}^3$  isopycnal.

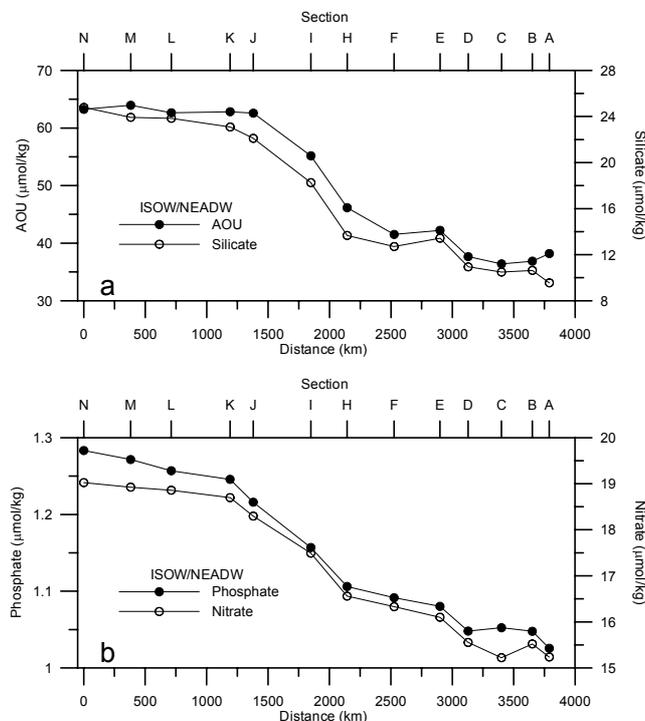


Figure 6. The longitudinal development of (a) AOU and dissolved silicate and (b) dissolved phosphate and nitrate in the water samples taken near the ISOW/NADW core.

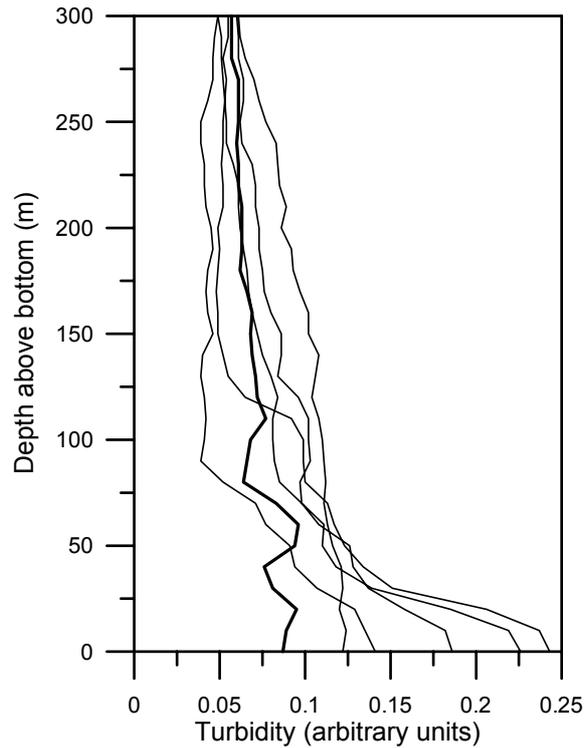


Figure 7. The turbidity of the water in the lowest 300 m above the bottom, measured with an optical back-scatter sensor. The thin lines show the profiles for section A to E, while the thick line depicts the turbidity profile of station 19 on section F.

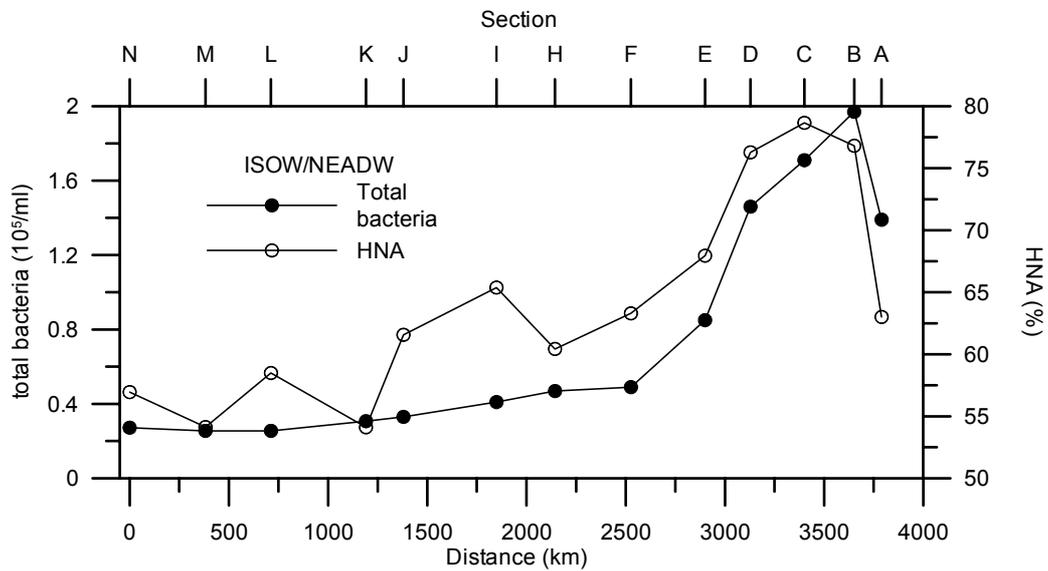


Figure 8. Longitudinal distribution of the concentration of bacteria and percentage HNA in the water samples taken from the ISOW/NADW core.

## **Description of the water masses encountered along the cruise track of TRANSAT-II**

### **Description stations #01 to #36 from Denmark Strait to Bermuda**

#### ***Station #36 on the lower west flank of the Reykjanes Ridge***

$\theta$ -S properties near the bottom close to the saline North-East Atlantic Deep Water (NEADW) core. No Labrador Sea Water (LSW) at ~1500 to 2000 dbar. Instead between 1200 and 1800 dbar a salinity maximum (~34.94). Near 1100 dbar a salinity minimum. Surface water ~8.0°C, 35.10.

#### ***Stations #33 to #35 near the overflow sill in Denmark Strait***

The cold (~0.8 to 1.2°C) core of Denmark Strait Overflow Water (DSOW) was found at station #34 between 1100 dbar and the bottom at 1550 dbar. From 1050 to 1100 dbar an interface with strong temperature and salinity gradients was present. In the DSOW layer itself a vertical salinity stratification was present with  $S \approx 34.81$  in the upper half, and  $S \approx 34.87$  in the lower half of the DSOW layer. These salinity layers were separated by a thin interface with strong salinity gradients. These layers are assumed to have different origins in the Nordic seas. At stations #33 the interface above the DSOW layer could be recognized in the near bottom layers. The lowest water sample at #33 came from that interface. At #35 no DSOW was encountered. Instead the  $\theta$ -S properties followed those of #36 reasonably well. In the thermocline above the interface near 1100 m, the  $\theta$ -S properties followed those of #36, that applied also for #33 and #34. The surface temperatures varied from 7.6 to 8.1°C, the surface salinity from 35.1 to 35.15.

#### ***Stations #31 and #32***

At station #31 cold (~1.1°C) and relatively fresh ( $S \approx 34.89$ ) water, derived from DSOW, is found in the near bottom layer at ~2790 dbar. At the shallower (1360 dbar) station #32 the cold bottom layer was warmer (~2.6°C) and slightly fresher ( $S \approx 34.87$ ). Above the cold bottom layer a salinity maximum was found, probably reflecting the presence of NEADW. Another set of sub-surface salinity maxima were found at 1400 and 1100 dbar at respectively #31 and #32., salinity minima at ~1000 and ~850 dbar. The overlying thermocline is slightly less saline than at the CTD stations at Denmark Strait (#33 to 35), with surface temperatures of 7.0 to 7.8°C and surface salinity of ~35.1.

#### ***Stations #29 and #30***

Near the bottom the temperature at station #29 was ~1.40°C at #29, and ~1.80°C at #30, with bottom salinities of ~34.89. The presence of NEADW was less outspoken as at #31 and #32. But at both stations a secondary salinity maximum could be observed between 1000 and 1500 dbar. the thermocline water was colder and less saline than for #31 and #32, leading to surface temperatures of ~6.5 to 6.8°C and surface salinities of 35.0 to 35.6.

#### ***Stations #26, #27 and #28***

The  $\theta$ -S structure is similar to #29 and #30, but with a bottom temperatures and salinities of 1.0 to 1.1°C and ~34.89. The surface temperature and salinity were ~5.8 to 6.6°C and ~34.86 to 34.95.

#### ***Stations #24 and #25 east of Cape Farewell***

The main  $\theta$ -S characteristics near Cape Farewell were similar to those further north. The bottom temperature was 1.3°C for the deepest station, and 2.1°C for the shallower one, with salinities ~ 34.89 at both stations. That salinity profile was irregular with a lot of fine-structure. At the shallowest station (#24) a sub-surface high salinity core was observed between 50 and 450 dbar ( $S_{\max}$  ~35.01), however with salinities less than found in the thermocline at the CTD-stations further north.. characteristic of the re-circulation of Atlantic water in Irminger Current. The surface temperature was 5.1 to 5.9°, the salinity 34.89 to 34.90.

#### ***Stations #23 & #22, south of Cape Farewell***

The main  $\theta$ -s characteristics are similar to those observed east of Cape Farewell. The bottom temperature was ~1.2°C at 3620 dbar, and 1.45°C at 2970 dbar both with  $S = 34.886$ , characteristic for the DSOW. Above the DSOW layer a salinity maximum ( $S \sim 34.92$ ), connected with the re-circulating NEADW was observed, followed by an S-minimum near ~1800 dbar, connected with an old and deep LSW core, and an S maximum near ~1400 which separated the old LSW from the overlying new LSW. The salinity minimum, connected with the latter was found near ~900 dbar. In the upper 800 dbar a sub-surface salinity maximum (~34.92), representing the Atlantic water core from the Irminger current, was observed at the station closest to the continental slope (#23). Further offshore at #22, the salinity decreased more or less monotonously from ( $S \sim 34.85$ ) to the surface ( $S \sim 34.79$ ), where a thin warm surface layer (4.2°C) was found.

#### ***Stations #19-#21, SE boundary of the Labrador Sea.***

The bottom temperature varied from 2.05°C at 3300 dbar, at #21 to ~1.5°C at pressure over 4000 dbar at #19 and #20. The bottom salinity was ~34.90 at #21 and ~34.89 at #19 and #20. The dissolved silicate concentration about 200 dbar above the bottom decreased from 1.8 at #21 to 13.6 and 13.1  $\mu\text{mol/kg}$  from #21 via #20 to #19. This indicates that the purest DSOW was encountered near #19, while at #21 NEADW had mixed with measurable amounts into the bottom water. Probably the DSOW core in the Labrador Sea was below 3300 dbar. Between 2750 and 2900 dbar a salinity maximum was found, connected with the NEADW, with the highest salinity at #21, closest to Greenland. Above the NEADW core the alternation of low salinity old LSW, the intermediate S maximum between 1500 and 1700 dbar and the S minimum of the new LSW was observed the latter was less outspoken at #21 ( $S \sim 34.85$ ). In the upper 600 dbar the  $\theta$ -S structure differed for the different stations. While at #21 the sea surface temperature and salinity were low (5.1°, 34.82) they were significantly high at the more southern stations #19 and #20 (6.8°C, ~34.94). At station #21 a sub-surface temperature and salinity minimum near 250 to 300 dbar was found (3.16°C,  $S \sim 34.76$ ). At station #20 a pycnostad was present between 100 and 410 dbar ( $\theta = 6.2^\circ\text{C}$ ,  $S \sim 34.94$ ). And the southernmost station #19, density stratifications was constant in the upper 500 m with a salinity minimum of 34.79 near 470 dbar. both

stations #19 and #21 showed considerable thermohaline fine structure in the upper 500 dbar.

### ***Stations #15 to #19 north and east of Flemish Cap***

At these stations near the SE exit of the Labrador Sea the bottom temperatures were close to 1.6°C and the bottom salinities approached 34.88, reminiscent of ISOW core water. At all 4 stations a salinity maximum, connected with the NEADW core was encountered near 2750 dbar. The lowest salinity at the S maximum level was encountered at station #17, closest to the continental slope near Flemish Cap. Near 1850 dbar a local salinity minimum was encountered at all 4 stations, the old LSW, with an overlying salinity maximum between 1250 and 1600 dbar. Above that level on average the salinity decreased to a minimum near ~ 400 dbar, but with thermohaline fine-structure which easily surpassed the large-scale vertical structure in magnitude. Above ~400 dbar the potential temperature increased strongly to the surface, where temperatures of ~11 to 12°C were encountered. In the upper 400 dbar large-amplitude thermohaline fine-structure dominated the salinity profiles, leading to surface salinities between 35.01 and 35.23. Apparently some Gulfstream water reached this region.

### ***Stations #13 & #14, south of Flemish Cap***

Also at these stations, south of Flemish Cap low bottom temperatures were encountered (~1.7°C), and the bottom salinity was about 34.885, both slightly above the bottom water encountered east of Flemish Cap. Near 2800 dbar an overlying salinity maximum (S = 34.903) represents the NEADW. Near 1800 dbar and 950 dbar salinity minima were encountered, representing different LSW vintages (before and after 1996?), separated by an intermediate salinity maximum (S ~ 34.904). Above 300 dbar, in the shallow thermocline, the temperature gradient was definitely less than at the stations east and north of Flemish Cap, while a saline surface layer was absent. The resulting surface temperature and salinity were 6.3 to 7.4°C and 34.77 to 34.89 respectively., definitely lower than at stations #15 to #19.

### ***Stations #11 and #12, South of the Grand Banks***

At these stations the bottom temperature was ~1.8 and 2.1°C at respectively 4120 and 3160 dbar, with salinities of ~34.90. Near ~2500 dbar a salinity maximum of ~ 34.94 was found (NEADW), definitely higher than further north. Near 2000 dbar the salinity minimum (#34.91) connected with a single older LSW core was encountered. Above that level a lot of large amplitude thermohaline fine-structure was encountered, with the largest salinity and temperature in sub-surface maxima, while near the sea surface low salinities (~33.0) and lower temperatures (~7.5°C) were encountered, characteristic of slope water or Labrador Current water. Above ~2500 dbar the  $\theta$ -S structure was dominated by thermohaline fine structure.

### ***Station #09 & #10, SW of the Grand Banks***

Whereas the bottom temperature were close to those for #11 and #12, the salinity maximum of the NEADW core was less saline (~34.93) than at #11 and #12. Below

~1500 dbar the temperature is larger than at #11 and #12. Above that level intensive thermohaline fine-structure is observed, reaching to the surface. The sea surface temperature amounted to 6.6 and 10.8°C, while the surface salinity ranged from 33.3 to ~34.3.

***Station # 08 #07 near the Nova-Scotia Slope***

Deep and bottom water as at #09 & #10, but with weaker salinity minimum (~34.93) at the LSW core. At the level of the NEADW the salinity was ~34.935. A fresher subsurface water mass with shallower isopycnals was encountered in the upper 1000 m, apparently influenced by water from a northern origin, probably transported south by the Labrador current inshore of the Gulf Stream.

***Station #05 & #06, The southern Nova-Scotia slope***

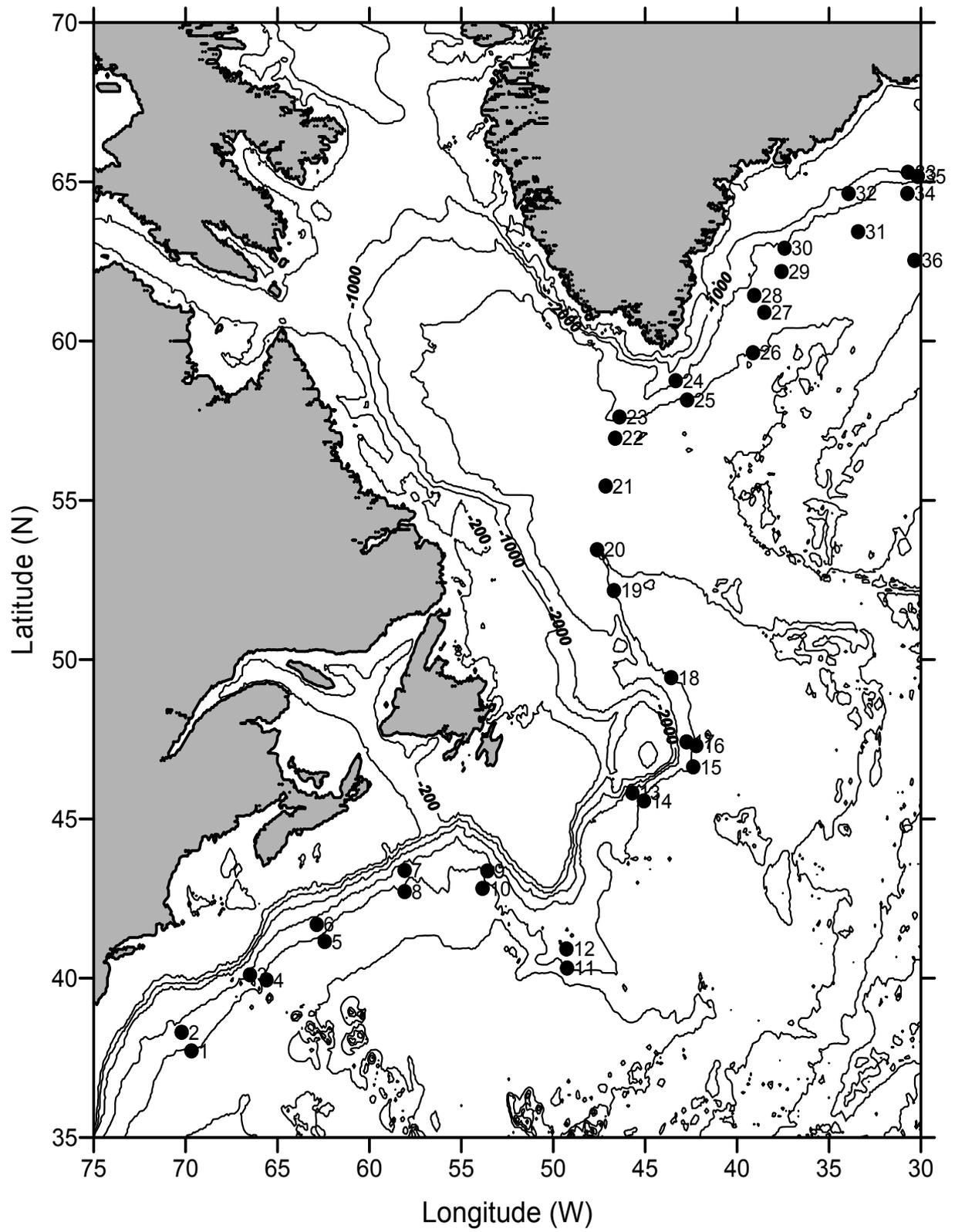
The bottom water is identical as further north. At the level of the NEADW salinity was ~34.934 to 34.945 at a temperature of ~2.95 to 3.05°C. at the LSW level the salinity minimum amounted to 34.93 to 34.94. At station and #05 a pycnostad with homogeneous STMW from the Gulfstream (18 degrees water) was encountered between 100 and 200 m. The typical Gulf Stream character is also reflected in the high temperature and salinity (~18.5°C and ~36.6). At station #06 the near surface water had more the characters of the cold Labrador Current ( $\Theta$  ~5.39°C, S ~32.5).

***Station #03 & #04, Gulf of Maine?***

At the NEADW level the salinity maximum amounted 34.94 to 34.948. The salinity minimum at the LSW core was ~34.94. At both stations a subsurface pycnostad was encountered with salinity ~36.6 and temperature ~18.6°C, characteristic for the STMW from the Gulfstream.

***Station #01 & #02, near Bermuda***

The bottom water ( $\Theta$ =1.80°C, S=34.884 still reminding of DSOW. The dissolved silica concentration is enhanced relative to the overlying water, indicative for either ageing, or admixture of AABW. At the NEADW level still a salinity maximum is encountered (S ~34.95). At shallower levels the relatively fresh LSW core (S ~ 34.935 to 34.940 and overlying levels show influence of saline intrusions, possible Mediterranean Outflow influence? At station #01 the most saline Gulfstream water of the cruise (S ~36.74) was encountered a ~80 dbar. At stations #02 the near surface the water had more a shelf water character,  $\Theta$  = ~21.6, S = ~36.05), with at 50 dbar cold subducted water (Labrador Current or winter shelf water) with  $\Theta$  and S in a minimum with 10.8°C and 34.6, respectively.



## ***Key dates and list of scientific crew on TRANSAT-I***

### **Key dates:**

29 Aug 2002: loading of R/V *Pelagia* at the NIOZ harbor and transit to Galway

5 Sept 2002: Galway (Ireland), bunkering,

6 Sept 2002: sailing from Galway to first station, then transect to the Azores

4 Oct 2002: disembarkation at Punta Delgada (Azores)

### **List of scientific crew:**

<b>Name</b>	<b>Affiliation</b>	<b>function on cruise</b>
Gerhard J. Herndl	NIOZ-BIO	chief scientist, prokaryotic production
Hendrik v. Aken	NIOZ-FYS	water mass identification
Txetxu Arrieta	NIOZ-BIO	capillary electrophoresis, T-RFLP, ectoenzymes
Markus Weinbauer	LOV-CNRS (France)	viruses,
Thomas Reinthaler	NIOZ-BIO	ultrafiltration of DOM, prokaryotic respiration
Eva Teira	NIOZ-BIO	uptake of D/L asp by bacteria, MICRO- CARD-FISH
Geraldine Kramer	NIOZ-BIO	DOC, DON, DOP, sampling for D/L-DAA
Cecilia Alonso	MPI-Bremen	cloning, sequencing, probe design
Annelie Pernthaler	MPI-Bremen	isolation of bacteria, incorporation experiments with BrdU,
Philippe Catala	OOB-CNRS (France)	cell sorting, uptake of radiolabeled substrates of sorted cells
Jeff Ghiglione	OOB-CNRS (France)	air sampling for bacteria,
Jan Hegeman	NIOZ-BIO	bicarbonate uptake of Archaea, radioactivity officer
Karel Bakker	NIOZ-MRF	inorganic nutrients, oxygen determination
Henk Franken	NIOZ-MT	electronics
Margriet Hiehle	NIOZ-MRF	CTD operator, data management

## ***Key dates and list of scientific crew on TRANSAT-II***

### **Key dates:**

22-23 April 2003: loading of R/V *Pelagia* at the NIOZ harbor

24 April 2003: transit of R/V *Pelagia* from Texel to Bermuda

8 May 2003: at 3 PM R/V *Pelagia* arrives at St. George, Bermuda

9 May 2003: embarking and start sailing in the evening to first station

6 June 2003: disembarkation at Peterhead (Scotland)

20-21 June: unloading of R/V *Pelagia* at the NIOZ harbor

### **List of scientific crew:**

<b>Name</b>	<b>Affiliation</b>	<b>function on cruise</b>
Gerhard J. Herndl	NIOZ-BIO	chief scientist, prokaryotic production
Cees Veth	NIOZ-FYS	identification of water masses, data management
Txetxu Arrieta	NIOZ-BIO	capillary electrophoresis, T-RFLP, ectoenzymes
Thomas Reinthaler	NIOZ-BIO	ultrafiltration of DOM, respiration
Denise Cummings	PML, Plymouth, UK	electron transport system assay
Markus Weinbauer	LOV-CNRS, France	viral abundance, production, diversity
Eva Teira	NIOZ-BIO	uptake of D/L asp by bacteria, MICRO-FISH
Martha Schattenhofer	MPI-Bremen	collection of FISH samples
Philippe Catala	OOB-CNRS, France	cell sorting, uptake of radiolabeled substrates of sorted cells
Jan Hegeman	NIOZ-BIO	bicarbonate uptake by Archaea, radioactivity officer
Evaline v Weerlee	NIO-MRF	inorganic nutrients, oxygen determination
Sven Ober	NIOZ-FYS	CTD operator
Jan Derksen	NIOZ-MT-DEL	electronics, CTD operator
Arjan Smit	NIOZ-BIO	ultrafiltration of DOM

## Field work of the individual participants during TRANSAT-I

### CTD observations by Margriet Hiehle, Henk Franken, Hendrik van Aken

CTD observations were carried out with a SBE9/11+ CTD. The CTD with sensors for the determination of pressure, temperature, and electrical conductivity was mounted in a rack, fitted with 22 NOEX 12 dm<sup>3</sup> water sample bottles. Additional sensors were mounted for the measurement of the dissolved oxygen concentration, fluorescence, photosynthetic active radiation (PAR) and turbidity (See Configuration Table). Three sampler bottles were fitted with SIS electronic reversing pressure samplers for calibration purposes. Additional to the continuous recording of the temperature, temperature was also recorded with a high accuracy SBE 35 temperature sensor when each sampler was closed. These data will be used for calibration purposes.

The NOEX sampler bottles were cleaned with bleach at the beginning of the cruise, as well as halfway. Water samples were taken for biological analysis as well as for the calibration of the salinity (conductivity) and oxygen sensors. The calibration samples were processed on board. The salinity was determined by means of a Guildline 8400B salinometer in a temperature controlled laboratory container. The oxygen concentration was determined by means of a spectro-photometric method.

The data have been processed preliminarily with a vertical resolution of 1 dbar. For all water samples the CTD readings have been processed too. After the cruise these data will be corrected for the final calibration of the sensors. A preliminary analysis suggests that a linear correction of the manufacturers calibration will yield an accuracy of the pressure, temperature and salinity measured by the CTD (standard deviation) of respectively 0.7 dbar, 0.001°C and 0,001. The newly acquired SBE 43 oxygen sensor behaved quite reproducible. A calibration to an accuracy well within 2 µmol/kg by means of a linear algorithm appeared to be possible. Final calibrations of the CTD sensors will be determined after the cruise.

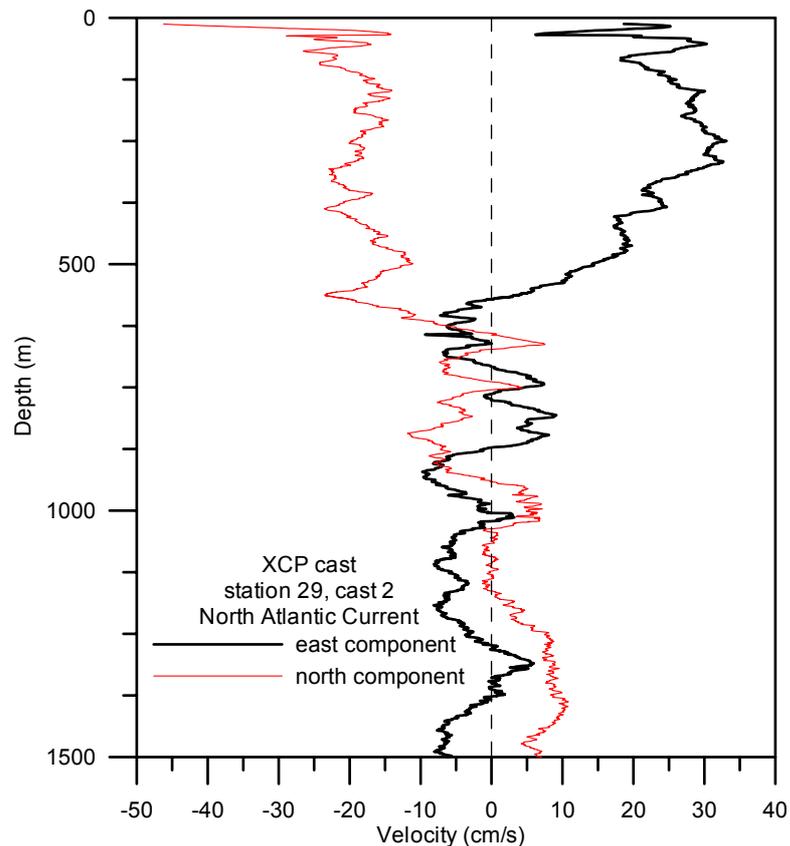
During part of the CTD casts sampler 1 was fitted with an experimental lid, in order to test its performance. The results of this test were negative. In about half of the cases the lid did not close properly. After several failures it was decided to replace the experimental lid by a standard one.

### Configuration of the CTD sensors

Type	serial nr
SBE temperature sensor	1197
SBE conductivity sensor	2142
SBE pressure sensor	53978
Chelsea fluorometer	88/725/026
SBE 43 oxygen sensor	0234
Irradiance sensor (PAR)	4410
Seapoint turbidity meter	1737

## XCP and XCTD measurements by Margriet Hiehle

On request of Prof. Dr. Toshiyuki Hibiya of the Department of Earth and Planetary Science of the University of Tokyo, expendable current profilers (XCP) have been launched at 22 different positions, following a CTD cast. The XCPs were Sippican Mark 10A probes, recording the current profile to a depth of about 1500 m. An example of the resulting current profiles for the North Atlantic Current is shown here.



Additionally at 5 stations Tsurumi-Seiki expendable CTD's (XCTD) were deployed in order to determine their performance. All 5 XCTD launches succeeded.

## **Total and Inorganic Nitrogen and Phosphorus analyses**

*Karel Bakker (Dept. of Marine Chemistry and Geology, Royal Netherlands Institute for Sea Research – NIOZ)*

A Traacs 800 autoanalyzer is used for spectrophotometric determination of the different nutrients using the classic methods:

### **Phosphate:**

Ortho-phosphate is measured by formation of a blue reduced molybdophosphate-complex at pH 0.9-1.1 at a wavelength of 880nm. Potassium antimonyltartrate is used as a catalyst and Ascorbic acid as the reductant.

Described by Murphy and Riley, 1962

### **Ammonium:**

Ammonium is measured as the indo-phenolblue-complex, using phenol and sodium hypochlorite at a pH of 10.5 using citrate as a complexant for calcium and magnesium at this pH. The resulting color is measured at 630nm.

Described by Koroleff, 1969, and optimized by Helder and de Vries, 1979

### **Nitrate and Nitrite:**

Diazotation of nitrite with Sulfanylamide and N-(1-Naphtyl)-Ethylene Diamonium Dichloride to form a reddish-purple dye measured at 550nm. Nitrate is separately first reduced in a copperized Cd-coil using Imidazole as a Buffer and is then measured as Nitrite

Described by Grasshoff, 1983

### **Dissolved silica:**

Measured as a blue reduced silicon-molybdenum-complex at 880nm. Ascorbic acid is used as reductant and oxalic acid is used to prevent interference of phosphate.

Described by Strickland and Parson, 1972

### **Total N and Total P:**

Destructions were carried out by G. Kramer in a pressure-cooker using Teflon bombs and a buffered persulfate reagent.

Total N is measured as nitrate and nitrite being the oxidation products after destruction for N using the method as mentioned above.

Total P is measured after diluting the destructed samples three times with a mixture of ascorbic acid added to the seawater used as the blank for the autoanalyser.

### **DIC:**

DIC will be measured afterwards on the lab, using an autoanalyzer method dialyzing the bicarbonate as CO<sub>2</sub> through a silicon-membrane, detecting the CO<sub>2</sub> as a decoloring of a phenolphthalin-solution at 550nm.

Described by Stoll and Bakker, 2001

### **Sample handling:**

All samples were filled into high-density 125ml polyethylene sample bottles after rinsing three times with sample water. The samples were stored in the dark at 4°C and analyzed within 12 hours for the parameters PO<sub>4</sub>, NH<sub>4</sub>, NO<sub>3</sub> and NO<sub>3</sub>. Samples for SiO<sub>2</sub> were kept at 4°C in the dark for analyses within a week.

The samples for DIC were all filtered over Acrodisc 0.2µm and filled in 5 ml glass-vials containing 15 µl saturated HgCl<sub>2</sub> as a preservative.

### **Calibration and Standards:**

Calibration curves were daily produced by diluting stock standards in plastic calibration-flasks. All calibrated laboratory glassware was calibrated at the lab before

the cruise. Nutrient depleted aged surface ocean water was used to dilute the standards to determine the calibration-lines and as water for the baseline of the autoanalyzer. As a daily check of the calibration, a lab-made cocktail-standard containing all nutrients was measured in every run. This cocktail was diluted 100-fold in the same ocean water, as a reference standard.

For TN and TP, a mixture of 10 organic compounds was treated in the same way as the samples as a recovery check of the method.

Performances:

Reproducibility of 10 replicates from one bottle within a run:

	mean $\mu\text{M}$	SD	cv. % of level	or	% off full scale
SiO <sub>2</sub>	14.99	0.11	0.7		0.25
PO <sub>4</sub>	0.875	0.004	0.5		0.15
NH <sub>4</sub>	0.84	0.03	3.4		0.7
NO <sub>3</sub>	14.07	0.03	0.18		0.06

For the CTD station 2 all 22 NOEX bottles were closed at the same depth and analyzed. The resulting statistics of this analysis are:

	mean $\mu\text{M}$	SD	cv. % of level	or	% off full scale
PO <sub>4</sub>	0.817	0.006	0.7		0.25
NH <sub>4</sub>	0.09	0.01	14		3
NO <sub>3</sub>	12.55	0.03	0.23		0.07

For almost all samples during TRANSAT-I, ammonium concentrations were around the baseline-value containing a background of  $\approx 0.09\mu\text{M}$  NH<sub>4</sub>. They NH<sub>4</sub> was not measured as peaks, thus undetectably low. Only the surface samples produced peaks above the baseline.

For the first few files a hand correction is necessary because of an unstable spectrophotometer generating long waves of the baseline.

For TP and TN the average recovery for TOP was 88% and for TON 96% based on the recovery of the different model compounds used and calculating their organic P and N content.

### **DOC sampling**

*Geraldine Kramer (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)*

Water samples for DOC were taken directly from the NOEX bottles into combusted glass ampoules acidified with conc. phosphoric acid, sealed and stored at  $-20^{\circ}\text{C}$ . DOC analyses are done back in the lab on a Shimadzu TOC-5000 analyzer operated by Santiago Gonzalez and Geraldine Kramer.

### **Prokaryotic abundance and cell sorting by flow cytometry**

*Philippe Catala, Jeff Ghiglione (Observatoire Oceanologique de Banyuls-Marine Microbial Ecology laboratory)*

The major task was to determine the abundance of bacteria in the water column for all the sampled stations. This determination was available almost immediately or one day after the sampling time for the others participants. Some others bacterial abundance estimations were also done for the participant who needed for individual scientific purpose during the cruise. The number of bacteria was measured by flow cytometry (FACS Calibur, Beckton Dickinson) after cell staining with SyBR-Green I. Our results showed that the abundance of bacteria decrease with depth (from  $10^6$  to  $10^4$  bacteria.ml<sup>-1</sup>) and remained stable in each specific different current followed (even for the North East Atlantic Deep Water current). Further analysis will be performed after the cruise to correlate these estimations to others parameters measured by the others participants.

In parallel to bacterial abundance estimation, the percentage of cells with an High Nucleic Acid (HNA) content in the total population was determined by flow cytometry for all the sampled stations. We previously demonstrated that HNA cells are responsible of the bacterial production in coastal seawater (Lebaron et al., 2001). In order to verify this characteristic in oligotrophic environment, samples from the water column at different stations (stations 8, 14, 16, 22, 29, 34, 38) were re-analyzed after [<sup>3</sup>H] Leucine incorporation by sorting the HNA cells by flow cytometry. HNA cells production to the total cell production ratio will be further determined in collaboration with Gerhard Herndl (Royal NIOZ) after the cruise.

A general overview of our results revealed that the percentage of cells with HNA content in the total population decrease from the surface to the oxygen minimum level (from 60 to 50%) and increase with depth (from 50 to 70-80%). Even if the bottom layer should influence the percentage of cells with HNA content because of re-suspension of organic material, a closer look will be taken after the cruise to correlate these results with others parameters (POM/LOM ratio, nutrient content, diversity, etc.) to understand the determinism of fluctuations of this parameter between the different currents (55 to 65% in the North East Atlantic Deep Water, far from the bottom layer).

At stations 22 and 29, HNA cells were also sorted at all the sampled depths by flow cytometry in order to determine the percentage of HNA cells belonging to the Archaeobacteria group in the water column. For this purpose, at least two different 16S rRNA probes for Euryarchaeota and Crenarchaeota kingdoms will be further used for Fluorescent In Situ Hybridization (FISH) experiment, in collaboration with Annelie Pernthaler (MPI Marine Microbiology, Germany).

In order to isolate bacteria still unknown and of phenotypical interest, a dilution culture method was used at stations 9 (100 and 2070 meter depths), station 25 (150 and 3716 meter depths) and station 38 (150m and 4120 meter depths). For this purpose, 1 to 10 or 10 to 100 bacteria were inoculated in fresh oligotrophic 30kDa filtered water in 3 different conditions : non-treated water, water with nutrients and vitamins, and water with multi-enrichments. Phylogenetic and phenotypic characteristics of the isolated strains will be performed after the cruise.

Finally, in order to estimate the influence of air born bacteria to the surface water bacterial composition, air samples and surface samples were taken at different stations (stations 5, 14, 15, 18, 27, 30, 38, 40) depending on the weather. Air samples were taken by flushing air on a special polymer (Sampl'air, Chemunex, France) soluble in

sterile seawater. The comparison between air and surface bacterial community structure will be performed after the cruise.

### **Fluorescence in situ hybridization and probe design**

*Annelie Pernthaler, Cecilia Alonso (Max-Planck-Institute for Marine Microbiology, Bremen, Germany)*

Samples for fluorescence in situ Hybridization (FISH) were taken at all station and all depths together with Cecilia Alonso. FISH samples will be processed immediately after arrival with probes specific for *Bacteria*, *Crenarchaea* and *Euryarchaea*. FISH with more specific probes will be done as soon as TRFLP data and clone sequences are available.

To detect DNA synthesis in bacterial and archaeal populations, incubations with the tracer bromodeoxyuridine were done once for each section (stations 4, 9, 16, 19, 21, 24, 30, 33, 35, 40; all depths). The evaluation of these samples will be done as soon as specific probes are available.

For the isolation of marine prokaryotes, dilution cultures at selected stations and depths were done (station 7 from oxygen minimum; station 11 from NADW; station 20 from 5 m depth; station 36 from NADW; station 40 from 5 m depth). As a growth medium I used 0.1  $\mu\text{m}$  filtered seawater (provided by Markus Weinbauer and Txetxu Arrieta) which was either unamended or amended with glucose, fractionated DOM, or different marine algae cultures. The evaluation of the dilution cultures will start immediately after arrival in Bremen.

To investigate the effect of light on the activity of phototrophic bacteria (SAR86 clade, *Roseobacter*), Cecilia Alonso and I sampled a day-night cycle of surface water between stations 38 and 42. We also performed a light pulse experiment using artificial light and different wavelengths. Activity measurements will be done using radioactively labeled thymidine (Micro-FISH) and bromodeoxyuridine (immunocytochemistry and FISH) as tracers for DNA synthesis and in situ hybridization of proteorhodopsin mRNA in SAR86 cells.

Samples for cloning and subsequent probe design were taken from all stations and depths. The processing of samples will start as soon as other data on prokaryotic communities structures (FISH, T-RFLP) are available.

Another aspect of the work was the characterisation of prokaryotic communities from an functional point of view. Incubations with radiolabeled thymidine were performed which will be analyzed with the Micro-FISH technique. The samples for Micro-FISH were taken at the following stations: 9,14, 19, 22, 23, 28, 30, 33, 37 and 40.

Incubations with tritiated thymidine were done for 3 selected depths: bottom of euphotic zone, oxygen minimum and North Atlantic Deep Water. For stations 30 and 37 the incorporation of thymidine was done for all depths as changes in water masses were expected.

Using the same approach, also an experiment was done to test differences in the degradation of potentially labile and refractory substrate by the microbial communities in the mesopelagic and deep layers. Incubations with thymidine for water from the bottom of the euphotic zone and the North Atlantic Deep Water in the presence of either algae lysate or concentrated dissolved organic matter from the NADW layer were performed at Sts 30 and 40.

The processing of samples for Micro-FISH will start immediately after arrival to the MPI.

## **D- vs. L- Aspartic acid uptake/MICRO-CARD-FISH and CDOM extraction**

*Eva Teira & Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

The objectives of this study were:

- 1) to determine whether there are shifts in the utilization of D- vs. L- amino acids between surface and deep water layers, and as NADW ages whether these shifts could be related to changes in the composition of the prokaryotic plankton community. Thus, we measured the rates of D- vs. L- Aspartic acid uptake by the prokaryotic plankton, and combined the classic microautoradiography to detect uptake of D- vs. L- Aspartic acid with fluorescence in situ hybridization (MICRO-CARD-FISH).
- 2) to elucidate whether there is a gradual shift in the source of dissolved organic matter (DOM) from phytoplankton in the surface waters and in the initially formed NADW to a bacterial-derived DOM as NADW ages by determining the enantiomeric ratio (D/L ratio) of amino acids in the CDOM fraction extractable with C18 mini-columns.

### **Sampling and Methods:**

D- vs. L- Aspartic acid uptake and MICRO-CARD-FISH: water samples were collected at 23 out of 42 stations for incubations with either D-[2, 3-<sup>3</sup>H] Aspartic acid or L-[2, 3-<sup>3</sup>H] Aspartic acid. At each station, 20-40 mL subsamples were collected at every depth, inoculated with either D- or L- radiolabelled Aspartic acid and incubated at *in situ* temperature for 6 hours to estimate D- vs. L- uptake rates by the bulk prokaryotic community or, for MICRO-FISH 8-10 h. Thereafter, samples were fixed with formalin and filtered through 0.2 µm cellulose nitrate filters. Samples for MICRO-CARD-FISH were fixed by adding particle-free paraformaldehyde (f.c. 2%), incubated in the dark for about 18h, filtered onto 0.2 µm Millipore polycarbonate GTTP filters and stored at -20°C for further processing ashore.

CDOM extraction: water samples were collected at 40 out of 42 stations. At each station, water samples from every depth were filtered through 0.2 µm polycarbonate GTTP filters and 20 mL subsamples were taken and acidified for CDOM extraction. C18 mini-columns were rinsed with methanol and 0.3 N HCl before running the sample. Finally the columns were cleaned with Milli-Q water and the sample was eluted with 4 mL of methanol. Samples were kept at -20°C for further processing ashore.

### **Prokaryotic production measurements**

*Gerhard J. Herndl & Jan Hegeman (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

**Prokaryotic production** was measured via [3H]-leucine (20nM final conc., SA 151 and 160 Ci mmol<sup>-1</sup>) and/or [3H]-thymidine (10nM final conc.) incorporation into bacterial protein and DNA, respectively. All the samples (10-40 ml) were done in duplicate with one formaldehyde-killed (3% final conc.) blank. Incubation temperature was close to *in situ* temperature (±1°C) and incubation period varied between 4 and 8 h, depending on the expected general activity. The filters were rinsed twice with ice-cold 5% trichloroacetic acid and transferred to scintillation vials which were stored frozen until the radioactivity was assessed in the radioisotope lab of the NIOZ.

On selected samples, prokaryotic production was also measured on the 0.6 µm filtered fraction for water column in order to allow direct comparison with the prokaryotic respiration of this fraction. Fractionation over 0.6 µm filters was done to separate free prokaryotic plankton from larger protists.

**Archaeal production** was estimated via the incorporation of <sup>14</sup>C-bicarbonate into archaea. To 40 ml samples (each in duplicate with one formalin-fixed blank), 40-100 µCi of <sup>14</sup>C bicarbonate was added and incubated at in situ temperature for 60-84 h. Thereafter, the samples were collected on a 0.2 µm polycarbonate filter, fumed over conc. HCl for 8 h and then, the filters were stored in scintillation vials and frozen until the radioactivity was counted back at the NIOZ.

### **Prokaryotic growth efficiencies**

*Thomas Reinthaler & Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

Bacterioplankton are acknowledged to play an important role in the remineralization of dissolved organic matter (DOM). Bacterial growth efficiencies – calculated by  $BGE = BP / (BP + BR)$  – serve as proxy to estimate the amount of DOM taken up and remineralized by the bacterial compartment. Generally for the whole water column, a BGE of 30% is applied to model biotic organic carbon fluxes. There is quite some debate in the literature concerning the extent and variation of bacterial growth efficiencies.

Bacterial secondary production measurements are done frequently on field campaigns but data on bacterial respiration (BR) are scarce. Current estimates of bacterial respiration are available almost exclusively for the euphotic zone of some parts of the oceans.

We measured bulk prokaryotic respiration rates and prokaryotic secondary production on several stations in the mesopelagic (around 150m depth), the oxygen minimum zone (500 to 800m), the Labrador sea water (LSW) at around 1000 to 2000m and the North Atlantic deep water (NADW) below 2000m. We will investigate the vertical variation of prokaryotic growth efficiencies through the water column but also the horizontal change following the NADW from northern to southern sections.

### **Methods:**

Samples from around 100m, 500m, 1000m and lower than 2000m depth were tapped from CTD-mounted NOEX bottles into acid rinsed flasks. The sample water was filtered over 0.6µm polycarbonate filters and transferred to flasks maintained at reach in situ temperature and shaken vigorously to saturate the oxygen content of the water. Subsequently biological oxygen demand (BOD) bottles (nominal volume of 120 cm<sup>3</sup>) were filled, t<sub>0</sub> bottles were stopped immediately by adding the Winkler reagents. Both, the t<sub>0</sub> and t<sub>1</sub> BOD-bottles were submersed in temperature-controlled water baths in the dark. T<sub>1</sub> BOD-bottles were stopped after 48 and 96 h. All time points were done in triplicates.

We used a spectrophotometric approach based on the classical Winkler method for oxygen measurements. Measurements were done on a HITACHI U-3010 spectrophotometer with a flow-through cuvette. T<sub>0</sub> and t<sub>1</sub> bottles were measured in the same run.

From the 0.6µm filtrate, samples were also drawn for prokaryotic abundance and bacterial secondary production measurements.

Preliminary results:

Back at the NIOZ, prokaryotic respiration rates were calculated and used along with the prokaryotic production data to calculate prokaryotic growth efficiencies (PGE). A PGE of around 2 % was obtained for the water column below the euphotic layer. A publication on the PGE in the main water masses of the North Atlantic have been submitted to *Limnology & Oceanography*.

### **Prokaryotic remineralization of different molecular size fractions of the DOM of the North Atlantic Deep Water**

*Thomas Reinthaler & Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

At 2 stations, we conducted an experiment with water from the North Atlantic Deep Water (NADW) to assess remineralization rates of deep sea prokaryotes growing on high molecular weight (HMW) and low molecular weight (LMW) dissolved organic matter (DOM).

Methods:

Twenty L of NEADW water was pre-filtered over 0.8 $\mu$ m. Pre-filtered water was subsequently filtered with a 0.2 $\mu$ m Pellicon cassette thereby concentrating the bacteria. Part of the 0.2 $\mu$ m fraction was ultrafiltered with an Amicon cartridge of a nominal pore size of 1000 Dalton. Dilution cultures were made in 2L Erlenmeyer flasks by adding bacterial concentrate to the 0.2 $\mu$ m and the 1000 Dalton size fractions. The final bacterial abundance was similar to the original sample water as assessed by flow cytometry. One flask of 0.2 $\mu$ m filtered water without addition of bacteria served as control. Over 6 days, the development of the prokaryotes in the cultures was followed under in situ temperature (2 - 3°C) held in the dark. TOC, amino acid samples and samples for bacterial production were taken every other day. To follow a possible community change also samples for fluorescence in situ hybridization (CARD-FISH) were fixed. Part of the dilution cultures was filled in bacterial oxygen demand bottles and incubated for up to 96 hours to assess bacterial respiration rates of prokaryotes growing on different molecular weight fractions.

Results:

Analyses of the samples are performed at the NIOZ.

### **Viral abundance and production, isotopic analysis of selected DOM compounds**

*Markus Weinbauer (Laboratory of Oceanography Villefranche sur mer, CNRS, France)*

Concentrates of the viral and prokaryotic size fraction were obtained from ca. 120 L of seawater at selected stations covering the NADW and source waters. These samples will be used to perform a metagenome analysis of the viral and prokaryotic community from a bathypelagic water mass and its major contributory water masses. In addition, carbon isotopes of amino acids in these size fractions will be analyzed as parameters to estimate carbon flow.

### **Determining prokaryotic ectoenzymatic activity**

*Jesus Maria Arrieta (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

**Bulk bacterial ectoenzyme activities** (aminopeptidase,  $\alpha$ - and  $\beta$ -glucosidase, phosphatase) were measured by means of methylumbelliferyl-substrates (Hoppe 1983) in triplicate 5 ml subsamples containing the corresponding substrate at 100  $\mu\text{mol L}^{-1}$  final concentration. The increase in fluorescence ( $\lambda_{\text{ex}}=360 \text{ nm}$ ,  $\lambda_{\text{em}}=445 \text{ nm}$ ) was monitored during incubations at in situ temperature every 2 h for up to 8 h until a significant increase was measured. When no significant fluorescence increase was measured after 8 h, the ectoenzyme activity was considered to be 0.

**Concentration of bacterial biomass by tangential flow filtration for zymography and DNA fingerprinting.** Large seawater samples (150-200 L) were filtered through 0.8  $\mu\text{m}$ -pore-size polycarbonate filters (142 mm diameter, Millipore) to exclude most of the eukaryotic organisms. To minimize clogging, the filter was replaced every 25 L. Bacteria in the filtrate were concentrated to a final volume of about 0.5-L using a Pellicon (Millipore) tangential flow filtration system equipped with a 0.2  $\mu\text{m}$  pore-size filter cartridge (Durapore, Millipore). Bacteria in the retentate of the Pellicon system were further concentrated by centrifugation (20,000g; 30 min; 4° C). The resulting pellet was washed 3 times with 0.2  $\mu\text{m}$ -filtered seawater and split into aliquots for subsequent analysis (16S rDNA fingerprinting, zymography).

### **16S rDNA fingerprinting of the prokaryotic community using T-RFLP, and cloning and sequencing**

*Jesus Maria Arrieta (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis of bacterial communities is performed using the methods previously used in our lab (Moeseneder et al, 1999). Briefly, DNA is extracted from an aliquot of the bacterial concentrate and subsequently amplified by PCR using the bacteria-specific forward primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the universal reverse primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). The forward primer (27F) is 5'-labeled with 5-carboxy-fluorescein and the reverse primer (1492R) with 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein. All primers are synthesized by Interactiva (Ulm, Germany). With this technique two labeled fragments can be obtained from each PCR product after the restriction digest, readily distinguishable by their fluorescence emission wavelength using an ABI Prism 310 capillary sequencer (Moeseneder et al. 2001). Two different restriction enzymes *HhaI* and *MspI* are used independently on each sample to generate the restriction patterns. Only those peaks with a peak area > 1% of the total peak area of the electropherograms were counted. Once phylotypes of interest are identified they are cloned and sequenced, so that oligonucleotide probes can be designed to be used for the CARD-FISH assay in future studies on the deep ocean.

**Field work of individual participants during TRANSAT-II** (only work is described in this chapter which has not already been mentioned under TRANSAT-I activities; all the activities described under TRANSAT-I activities were also performed on TRANSAT-II)

**Fluorescence in situ hybridization on selected members of the prokaryotic community**

*Martha Schattenhofer (Max-Planck-Institute for Marine Microbiology, Bremen, Germany)*

The aim of the work was to collect samples for FISH analysis. The samples were taken the same way for every station. Water from specific depths and water masses were filled into glass bottles, roughly 500 ml each depth. After this, the water was accurately filled into another set of glass bottles, the volume depending on the depth (see table below). Then the samples were fixed with formaldehyde solution (conc. 36%) to a final concentration of 1% (see again table below). The fixed samples were kept at room temperature for 2 h and thereafter filtered. Finally, the filters were marked, stored in Petri-dishes and kept frozen at  $-80^{\circ}\text{C}$ .

Depth	Water volume	Volume of Formaldehyde
100 – 150 m	100 ml	2.8 ml
500 – 600 m	150 ml	4.2 ml
~ 1000 m	150 ml	4.2 ml
~ 2000 m	200 ml	5.6 ml
~ 3000 – 4000 m	250 ml	6.9 ml

Another part of the work during the cruise was the sampling of surface probes for mRNA hybridization of the bacterial *Proteorhodopsin* gene. This was sampled by collecting water from the surface with a glass bottle tied to a rope. Tubes were prepared with formaldehyde prior to adding the water. The end volume was 40 ml and the fixation again 1%.

The fixed samples were stored for 10 – 15 h in the fridge at  $4^{\circ}\text{C}$ , after that they were frozen in liquid nitrogen for 1 – 2 min and stored in the  $-80^{\circ}\text{C}$  freezer. Samples were taken from every station and also additionally during night. All samples were taken in duplicate.

**Enumeration of picoplankton, Crenarchaeota and Euryarchaeota**

*Eva Teira (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

Water masses were identified based on their salinity-temperature characteristics. Water from the distinct water masses was collected with NOEX-bottles mounted on a CTD frame. Samples were taken for enumeration of total picoplankton, Bacteria and Archaea and fixed instantly with formaldehyde (2% final conc.). Heterotrophic picoplankton are enumerated after DAPI staining, Bacteria and Archaea by catalysed reporter deposition fluorescence in situ hybridisation (CARD-FISH) under the epifluorescence microscope. For enumeration of Bacteria the oligonucleotide probe Eub338 are used, for Crenarchaeota Cren537 (5'-TGACCACTTGAGGTGCTG-3'),

and for Euryarchaeota Eury806 (5'-CACAGCGTTTACACCTAG-3'). All the probes were tested for their specificity prior to the study. Cell walls are permeabilized for Eub338 with lysozyme (Sigma; 10 mg ml<sup>-1</sup> in 0.05 M EDTA, 0.1 Tris-HCl [pH 8]) or with proteinase-K for Eury806 and Cren537 ([1844 U mg<sup>-1</sup>, 10.9 mg mL<sup>-1</sup>, Sigma]; 0.2 µl ml<sup>-1</sup> in 0.05 EDTA, 0.1 Tris-HCl [pH 8]) at 37°C for 1 h. Probe working solution (50 ng µl<sup>-1</sup>) are added at a final concentration of 2.5 ng µl<sup>-1</sup>. Hybridisation is done at 35°C for 8-12 h.

#### **Microautoradiography combined with CARD-FISH (MICRO-CARD-FISH).**

*Eva Teira & Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)*

To 30-40 ml samples [<sup>3</sup>H]-leucine (SA 157 Ci mmol<sup>-1</sup>, Amersham) at a final concentration of 20 nM or [<sup>14</sup>C]-bicarbonate (100 µCi, Amersham) was added and incubated in the dark at in situ temperature for 8-10 h or 60-72 h, respectively. Controls were fixed with 2% paraformaldehyde final concentration. Incubations were terminated by adding paraformaldehyde (2% final concentration) and storing the samples in the dark at 4°C for 12-18 h. The autoradiographic development is conducted in the home lab by transferring previously hybridized filter sections onto slides coated with photographic emulsion (type NTB-2, melted at 43°C for 1h). Subsequently, the slides are placed in a dark-box with a drying agent and exposed at 4°C for 36-48 h. The slides are developed and fixed using Kodak specifications (Dektol developer [1:1 dilution with Milli-Q water] for 2 min, a rinse with Milli-Q water for 10 s, in fixer for 5 min followed by a Milli-Q water rinse for 2 min). Cells are counter-stained with a DAPI-mix (5.5 parts of Citifluor, 1 part Vectashield and 0.5 parts of PBS amended with DAPI at a final concentration of 1 µg ml<sup>-1</sup>). The silver grains in the autoradiographic emulsion are detected by switching to the transmission mode of the microscope. More than 700 DAPI-stained cells are regularly counted per sample.

#### **Sampling to assess the activity of the bacterial electron transport system (ETS)**

*Denise G. Cummings (Plymouth Marine Laboratory, Plymouth, England)*

Two long-term respiration experiments were carried out and 6 respiration experiments with unfiltered seawater were carried out to compare with bacterial respiration of the 0.6 µm filtered seawater fraction.

Also, samples were taking for ETS to be measured by Javier Aristegui Ruiz at Universidad de Las Palmas de Gran Canaria. For the ETS measurements, approximately 10 L of seawater were filled into aspirators from the NOEX bottles via silicon tubing. These were then filtered onto 47mm GFF. Once filtered they were folded and put into cryovials and then placed into a liquid nitrogen dewar. At the end of the cruise these were put into a liquid nitrogen dry shipper and sent to Javier. See table for ETS collection:

**Samples collected for ETS  
measurements:**

<b>Date</b>	<b>Station</b>			<b>Depths (m)</b>		
10/5/03	1	3500	2450	1000	500	100
12/5/03	2	3000	2450	1000	500	100
13/5/03	3	3000	2450	1000	500	100
14/5/03	5	3700	2750	1000	570	100
14/5/03	6	3000	2500	1000	210	100
15/5/03	7	3000	2600	1000	200	100
16/5/03	8	3300	2580	1000	320	100
17/5/03	9	3000	2500	1000	260	100
17/5/03	10	3800	2700	1700	480	100
18/5/03	11	3500	2450	850	240	100
20/5/03	13	3000	2250	1500	750	100
21/5/03	14	3550	2700	1000	240	100
22/5/03	16	3500	2500	1150	265	100
23/5/03	18	3500	2400	1100	750	100
24/5/03	19	3800	2000	1100	700	100
24/5/03	20	3800	2060	1100	620	100
25/5/03	21	3000	1780	1100	520	87
25/5/03	22	2800	1900	1300	500	100
26/5/03	24	1850	1400	1000	550	100
26/5/03	25	3000	2500	1700	800	100
28/5/03	27	2700	2150	1687	700	100
28/5/03	29	2400	1770	850	500	100
29/5/03	31	2730	2400	1900	1000	100
29/5/03	32		1320	1000	600	100
30/5/03	35	1500	1200	1000	700	100
31/5/03	36	2100	1500	1100	600	100

**Publications resulting from work done during the TRANSAT cruises thus far:**

***Abstract of publication in Appl. Environ. Microbiol., 2004: 70: 4411-4414***

**Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean.**

Eva Teira, Thomas Reinthaler, Annelie Pernthaler<sup>1</sup>, Jakob Pernthaler<sup>1</sup> & Gerhard J. Herndl

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The recently developed CARD-FISH protocol was refined for the detection of marine Archaea by substituting the lysozyme permeabilization treatment with proteinase-K. This modification resulted in about 2-times higher detection rates of Archaea in deep waters. Using this method in combination with microautoradiography, we found that Archaea are more abundant than Bacteria (42% vs. 32% of DAPI counts) in the deep waters of the North Atlantic and that a larger fraction of Archaea takes up L-aspartic acid than Bacteria (19% vs 10%).

***Abstract of publication in Appl. Environ. Microbiol., 2005: 71: 2303-2309***

**Contribution of Archaea to Total Prokaryotic Production in the Deep Atlantic Ocean**

Gerhard J. Herndl<sup>1</sup>, Thomas Reinthaler<sup>1</sup>, Eva Teira<sup>1</sup>, Hendrik van Aken<sup>2</sup>, Cornelis Veth<sup>2</sup>, Annelie Pernthaler<sup>3</sup> & Jakob Pernthaler<sup>3</sup>

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Fluorescence in situ hybridization (FISH) in combination with polynucleotide probes revealed that the two major groups of planktonic Archaea (*Cren-* and *Euryarchaeota*) exhibit a different distribution pattern in the water column of the Pacific subtropical gyre and in the Antarctic Circumpolar Current system. While *Euryarchaeota* were found to be more dominant in nearsurface waters, *Crenarchaeota* were relatively more abundant in the meso- and bathypelagic waters. We determined the abundance of Archaea in the meso- and bathypelagic North Atlantic along a S-N transect of more than 4000 km. Using an improved catalyzed reporter deposition-FISH (CARD-FISH) method and specific oligonucleotide probes, Archaea were found to be consistently more abundant than Bacteria below 100 m depth. Combining microautoradiography with CARD-FISH (MICRO-CARD-FISH) revealed a high fraction of metabolically active cells in the deep ocean. Even at 3000 m depth, about 16 % of the Bacteria were taking up leucine. The percentage of *Eury-* and *Crenarchaeota* taking up leucine did not follow a specific trend with depth ranging from 6 – 35 % and 3 – 18 %, respectively. The fraction of *Crenarchaeota* taking up inorganic carbon increased with depth, while *Euryarchaeota* taking up inorganic carbon decreased from 200 m to 3000 m depth. The ability of Archaea to take up inorganic carbon was used as a proxy

to estimate archaeal cell production and to compare this archaeal production with total prokaryotic production measured via leucine incorporation. We estimate that archaeal production in the meso- and bathypelagic North Atlantic contributes between 13-27 % to the total prokaryotic production in the oxygen minimum layer, 41-84 % in the Labrador Sea Water and declining to 10-20 % in the North Atlantic Deep Water. Thus, planktonic Archaea are actively growing in the dark ocean although at lower growth rates than Bacteria and might play a significant role in the oceanic carbon cycle.

***Abstract of publication accepted in Limnol Oceanogr in May 2005***

**Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the North Atlantic**

Eva Teira<sup>1,3</sup>, Hendrik van Aken<sup>2</sup>, Cornelis Veth<sup>2</sup>, Gerhard J. Herndl<sup>1</sup>

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In the oceanic realm, amino acids are produced and released into the dissolved organic matter pool predominately as L-amino acids. The only significant source of D- amino acids is thought to be the bacterial cell wall containing four enantiomeric amino acid species (alanine, aspartic acid, serine, glutamic acid). Recently, it has been found that the D-/L-aspartic acid (Asp) uptake ratio of the bulk prokaryotic community increases by 2-3 orders of magnitude from the surface to the deep mesopelagic waters in the North Atlantic. In this study, we determined the contribution of the three major prokaryotic groups (Bacteria, *Cren-* and *Euryarchaeota*) on the uptake of D-/L-Asp in the major water masses of the North Atlantic (from 100 m to 4,000 m depth) using microautoradiography combined with catalyzed reporter deposition fluorescence in situ hybridization (MICRO-CARD-FISH). In the meso- and bathypelagic waters of the North Atlantic, Archaea are more abundant (42±2% of DAPI stained cells) than Bacteria (30±1% of DAPI stained cells) and more archaeal than bacterial cells are actively incorporating D-Asp (62±2% vs 38±2% of total D-Asp active cells). In contrast, Bacteria and Archaea almost equally contribute to L-Asp utilization in the deep waters of the N Atlantic (47±2% vs 53±2% of total L-Asp active cells). The increase in the D-/L-Asp uptake ratio of the prokaryotic community with depth appears to be driven by the efficient uptake of D-Asp by especially the *Crenarchaeota* in the deep waters. As Archaea, and particularly *Crenarchaeota*, commonly dominate the prokaryotic communities in the ocean's interior, we suggest that they represent a, thus far unrecognized, sink of D-amino acids in the deep ocean.

***Abstract of publication submitted to Limnol Oceanogr in May 2005***

**Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin**

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The meso- and bathypelagic realm comprises more than 70% of the volume of the global ocean and harbors about half of the prokaryotic plankton. Yet, direct measurements of the dark ocean's prokaryotic production and respiration are scarce. We measured prokaryotic production and respiration in the major water masses of the North Atlantic down to a depth of ~4000 m by following the progression of the two branches of North Atlantic Deep Water (NADW) in the oceanic conveyor belt. Prokaryotic abundance decreased exponentially with depth from ~0.4–3.0 x 10<sup>5</sup> cells ml<sup>-1</sup> in the eastern and from ~0.3–3.6 x 10<sup>5</sup> cells ml<sup>-1</sup> in the western North Atlantic basin. Prokaryotic production measured via [<sup>3</sup>H]leucine incorporation showed a similar pattern as prokaryotic abundance and ranged from ~1.1–9.2 μmol C m<sup>-3</sup> d<sup>-1</sup> in the eastern and from ~1.2–20.6 μmol C m<sup>-3</sup> d<sup>-1</sup> in the western North Atlantic basin. Prokaryotic respiration, measured via oxygen consumption, ranged from 60–300 μmol C m<sup>-3</sup> d<sup>-1</sup> from ~100 m depth to the NADW. Prokaryotic growth efficiencies in the deep North Atlantic (depth range ~1200–4000 m) of ~2% indicate that the prokaryotic carbon demand exceeds recent estimates of dissolved organic matter input and surface primary production by ~2 orders of magnitude. Cell-specific prokaryotic production was rather constant throughout the water column ranging from 15–32 amol C cell<sup>-1</sup> d<sup>-1</sup> in the eastern and from 35–58 amol C cell<sup>-1</sup> d<sup>-1</sup> in the western North Atlantic basin. Along with increasing cell-specific respiration towards the deep water masses and the relatively short turnover time of the prokaryotic community in the dark ocean (34–54 days), prokaryotic activity in the meso- and bathypelagic North Atlantic is higher than previously assumed. The apparent discrepancy between the prokaryotic carbon demand and the flux of organic carbon in the dark ocean represents a major challenge for our understanding of the oceanic carbon cycle and highlights the need to study deep water prokaryotic activity and organic carbon fluxes more intensively.

*Several other manuscripts are currently in preparation by several participants of the two TRANSAT cruises.*

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