# Bacterioplankton processes off the Galician shelf

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## 1. INTRODUCTION

The recognition of the importance of pico- and nanoplankton in the sea led to the reformulation of the classic concept of a linear food chain based on algae grazing by zooplankton into a more dynamic and complex model of a microbial food web, which incorporates DOC cycling via bacteria and phagotrophic protists (Pomeroy, 1974; Azam *et al.*, 1983; Legendre and Rassoulzadegan, 1995). C-cycling by bacteria (uptake and respiration), the extent of coupling of bacterial biomass and production with phyto- and protistoplankton biomass and production and DOC and DON are important timely questions. To what extent bacteria represent a sink for carbon and how the importance of this sink varies in time and space in the Iberian Margin is not known. It is therefore the aim of UAl-a to develop an understanding of the temporal (short-term and seasonal) and spatial variability of bacterioplankton biomass and activity and to assess the role of bacterioplankton in the carbon fluxes on and off the Galician shelf under different hydrographic conditions.

Within the framework of the OMEX II-II project, UAl-a contributes to *Work Packages I, II and IV* and has specific objectives (in brackets) included in the following tasks (in italic):
***Task I.4*** *Zooplankton and microbial cycling* (bacterial biomass, production, respiration, growth efficiency and grazing)
***Task I.7*** *3-D nested model for the Galician shelf*
***Task II.5.5*** *Biomass carbon* (Bacterial C-biomass)
***Task II.6.3*** *Dissolved organic carbon* (Bacterial utilisation of DOC)
***Task II.9.1*** *Distribution of bacteria and microzooplankton* (Distribution of bacteria biomass)
***Task II.9.2*** *Nitrogen and $CO_2$ regeneration by bacteria, micro- and mesozooplankton* (Bacterial respiration)
***Task IV.2*** *Carbon sources, cycling and fates* (contribution to bacteria biomass and production)
***Task IV.3*** *Nutrients, trophodynamics and fertility* (contribution to trophodynamics)

## 2. MATERIALS AND METHODS

### 2.1 General sampling strategy

Samples have been collected on a total of 5 cruises. Samples were collected for UAl-a by IIM during *CD110b* cruise. A compilation of cruises, number of stations sampled, number of samples taken, number of experiments performed and variables determined for each task is given in Table 1. Samples were mostly obtained from CTD water bottle casts in vertical profiles (see Annex). During *CD110b*, *BG9815* and *OMEX-0898* WP II cruises the sampling strategy was concentrated on the N, P, and S reference lines and directed towards the evaluation of meso-scale spatial variability. During *CD114* WP I cruise two Lagrangian experiments were performed along the shelf-edge and along a filament in order to evaluate short-term variability. Due to shortage of berths in this cruise it was not possible to set up large bottle experiments to evaluate bacterial growth efficiency and bacterivory, which are WP I UAl-a subtasks. However, these experiments were performed on board a simultaneous WP II cruise (*OMEX-0898*). In the absence of a WP I cruise during 1999, UAl-a participated in the first semester of 1999 (May 1999) in a WP II cruise (*Almeida Carvalho*) cruise, which is included in Table 1 although data will not be included in this report.

**Table 1:** Contribution of UAl-a to Workpackages I, II and IV: cruises, samples/variables analysed and respective tasks. BA: bacterial abundance; BB: bacterial biomass; BG: bacterial grazing; BGE: bacterial growth efficiency; BP: bacterial production; BR: bacterial respiration; BU: bacterial DOC uptake rates; ctd: samples from CTD stations; ICE: intercalibration exercise; FDC: frequency of dividing cells as a proxy of production; uw: samples from underway stations

| Cruise | Date | WP | Nr. Stations | Nr. samples/ Nr. experiments | Variables | Tasks |
|---|---|---|---|---|---|---|
| *Charles Darwin CD110b* | January 98 | II | 16 | 46 (12 uw + 24 ctd) | BA, BB, FDC | **II.5.5**; **II.9.1** |
| *Belgica BG9815* | July 1998 | II | 13 | 77 (ctd) | BA, BB, BP, BR | **II.5.5**; **II.9.1**; **II.9.2** |
| *Charles Darwin CD114a* and *b* | August 1998 | I, IV | 27 | 171 (ctd) | BA, BB, BP, BR, ICE | **I.4**; **I.7**; **IV.3** |
| *Prof. Shtokman OMEX-0898* | August 1998 | II, I | 18 | 51 (ctd)<br>4 (BU; BGE)<br>2 (BG) | BA, BB, BP, BR, BG, BGE, BU, FDC | **I.4**; **II.5.5**; **II.6.3**; **II.9.1**; **II.9.2** |
| *Almeida Carvalho* | May 1999 | I, II | 8 | 46 (ctd)<br>8 (BG) | BA, BB, BP, BR, BG | **I.4**; **II.5.5**; **II.9.1**; **II.9.2** |

2.2 Bacterial abundance and biomass (***Tasks I.4**, **I.7**, **II.5.5**, **II.9.1** and **IV.2***)
Bacterial abundance, mean cell volume, frequency of dividing cells (only in *CD110b* samples) and bacterial biomass were determined after filtration of glutaraldehyde fixed water samples through 0.2µm polycarbonate filters (within 8 h of sample collection) and staining with acridine orange, according to Hobbie *et al.* (1977). Cellular carbon content was determined according to Simon and Azam (1989). The microscopic analyses were completed in the first semester of 1999.

2.3 Bacterial production (*Tasks **I.4**, **I.7** and **IV.2***)
Bacterial production rates were measured with the addition of saturating concentrations of $^{14}$C leucine followed by incubation in a water bath (modified from Kirchman *et al.*, 1989). Analysis of saturation kinetics was performed during *BG9815* and *OMEX-0898* cruises (5 experiments). The effect of incubation time (2-6 h) was tested at several stations during *CD114a*. Final filtration and/or scintillation counting of samples were undertaken at IIM and UAl-a laboratories. Bacterial carbon production rates were based on an empirical conversion factor of 0.44 kg C.(mol leucine)$^{-1}$. This value represents the average value obtained during experiments with unamended diluted seawater cultures from subsurface waters at Stations P100, P2800, S150 and S1000 during *OMEX-0898* cruise.

2.4 Bacterial respiration (***Tasks I.4**, **I.7** and **II.9.2***)
Bacterial respiration was based on the recovery of $^{14}CO_2$ produced after additions of $^{14}$C-leucine. Additionally, total microbial respiration and bacterial respiration (<0.8 µm) were determined using the Winkler technique in *Almeida Carvalho* cruise in May 1999 to help interpret fraction of respired leucine in the contest of total respiration. However, this information will be incorporated only in the final scientific report.

2.5 DOC uptake by bacteria (***Task II.6.3***)
Large polycarbonate bottle experiments (in replicates) were set up with 0.8 µm filtered and diluted samples to estimate rates of bacterial biomass increase in the absence of grazers and DOC uptake. Samples were collected every 6 h along each experiment (ca. 36 h duration). Microscopic analyses of bacterioplankton were concluded in the first semester of 1999. DOC analyses were performed by PML-a (Axel Miller).

2.6 Bacterial growth efficiency (***Tasks I.4** and **I.7***)
Large polycarbonate bottle experiments (in replicates) were set up with 0.8 µm filtered and diluted samples to estimate rates of bacterial biomass increase and DOC uptake in the absence of grazers. Samples were collected every 6 h along the experiment (ca. 36 h duration). Bacterial growth efficiency was estimated as 100*(C-produced)/C-consumed (see Carlson and Ducklow, 1996).

2.7 Grazing on bacteria (***Tasks I.4**, **I.7** and **IV.3***)
Bacterial abundance in 2 l polycarbonate bottles with (<10 µm fraction) and without grazers (0.8 µm) was determined every 6h throughout each experiment (ca. 36 h duration). Grazing rates will be calculated as the difference between bacterial specific growth rate (<0.8µm) and bacterial apparent growth rate (<10 µm) according to Wright and Coffin (1984). In addition, during *Almeida Carvalho* cruise a total of 8 grazing experiments were carried out with the dilution technique (Landry and Hassett, 1982) with C, N and P additions.

3. RESULTS

3.1 Bacterial Abundance and Biomass *(**Tasks I.4**, **I.7**, **II.5.5**, **II.9.1** and IV.2)*
Table 2 summarises bacterial biomass data from the four 1998 cruises (*CD110b*, *BG9815*, *CD114a* and *b*, *OMEX-0898*) giving minimum and maximum values for samples (5-200 m) collected at offshore and onshore stations along N, P and S reference lines. Typical vertical profiles of bacterial biomass are illustrated in Figure 1 for onshore and offshore stations during winter (*CD110b*) and

summer (*BG9815*) situations. Data collected for bacterial abundance during the Lagrangian drift experiment along shelf-edge (*CD114a*) are depicted in Figure 2.

**Table 2 -** Range of bacterial biomass (BB, µg $C.l^{-1}$) and bacterial production (BP, µg $C.l^{-1}.d^{-1}$) for on-shore and off-shore stations during OMEX II-II cruises. * data set not yet fully processed.

| Cruise | BB On-shore | BB Off-shore | BP On-shore | BP Off-shore |
|---|---|---|---|---|
| *CD100b* | 1.9-6.3 | 1.3-3.4 | - | - |
| *BG9815* | 4.0-31.8* | 6.0-24.2* | 0.03-3.64 | 0.003-0.94 |
| *CD114a* and *b* | 0.9-22.2 | 2.6-16.5 | 0.02-3.22 | 0.02-0.75 |
| *OMEX-0898* | 5.0-37.8 | 1.9-36.1 | 0.06-13.26 | 0.003-1.09 |

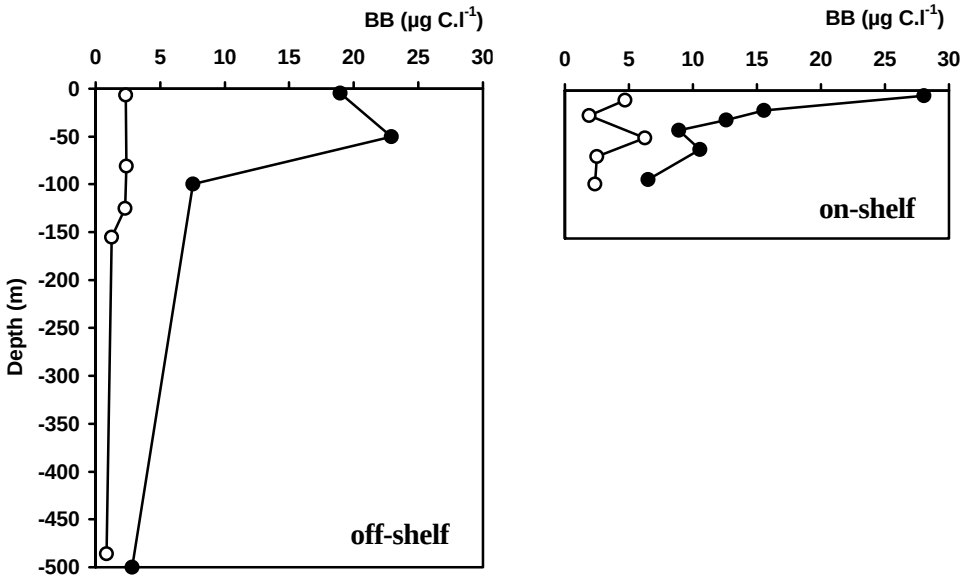


**Figure 1** - Example of vertical distribution of bacterial biomass (µg $C.l^{-1}$) during winter poleward flow conditions (*CD110b* cruise: ❍) and summer upwelling conditions (*OMEX-0898*: ●) at shelf (*CD110b*: P100; *OMEX-0898*: P100) and slope stations (*CD110b*: P2800; *OMEX-0898*: P2000).

3.2 Bacterial Production ***(Tasks I.4, I.7 and IV.2)***

Table 2 summarises data from three 1998 cruises (*BG9815*, *CD114a* and *b*, *OMEX-0898*) giving minimum and maximum values for samples (5-200 m) collected at offshore and onshore stations along N, P and S reference lines. Typical vertical profiles of bacterial production are illustrated in Figure 3 for offshore and onshore stations during summer conditions (*BG9815* and *OMEX-0898* cruises). Data for specific bacterial production determined during Lagrangian drift experiment along the shelf-edge (*CD114a*) are also depicted in Figure 4.

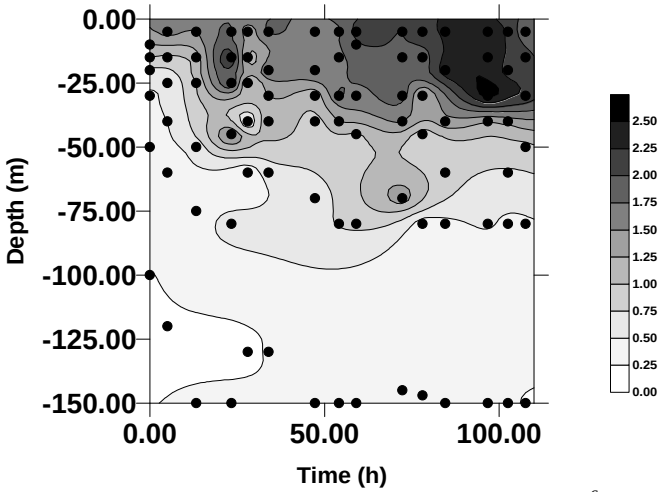


**Figure 2** - Short-term variation of bacterial abundance ($10^6$ cells.ml$^{-1}$) during a Lagrangian drift experiment along shelf-edge (*CD114a*).

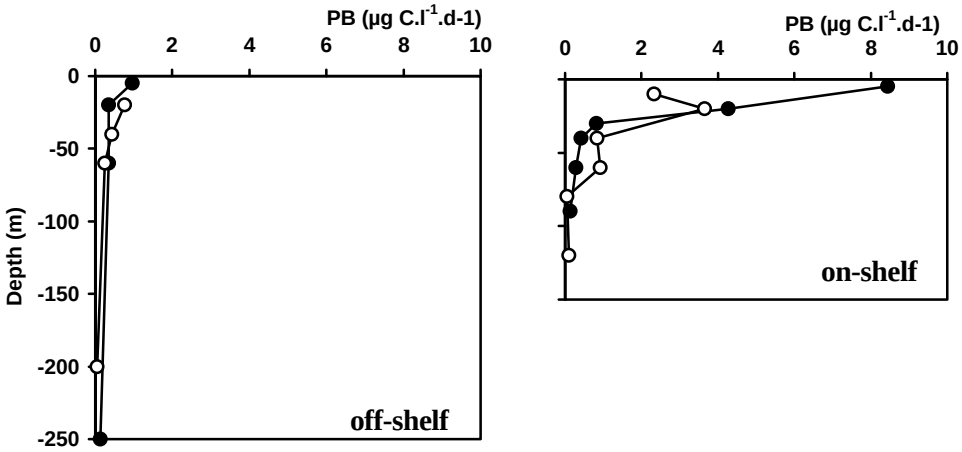


**Figure 3** - Example of vertical distribution of bacterial production (µg C.l$^{-1}$.d$^{-1}$) during early summer (*BG9815*) and mid-summer conditions (*OMEX-0898*) at shelf (*BG9815*: P20; *OMEX-0898*: P100) and slope stations (*BG9815*: P26; *OMEX-0898*: P2800).

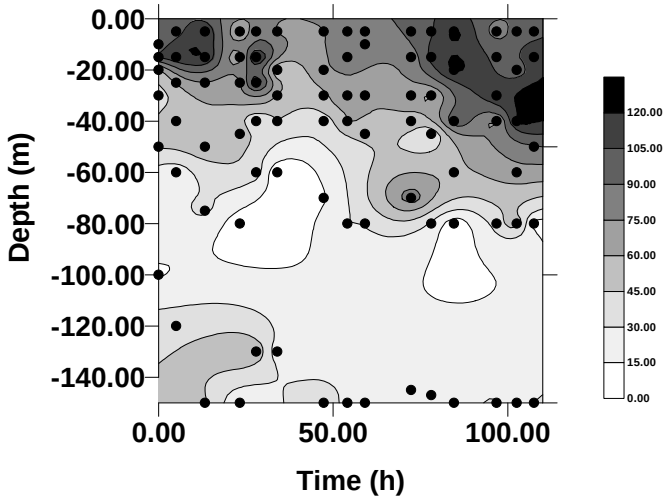


**Figure 4** - Short-term variation of specific bacterial production (x10$^{-9}$ nmol leuc. cell$^{-1}$. h$^{-1}$) during Lagrangian drift experiment along shelf-edge (*CD114a*).

3.3 Bacterial Respiration ***(Tasks I.4** and **II.9.2)***
Table 3 summarises data from three 1998 cruises (*BG9815*, *CD114a* and *b*, *OMEX-0898*) giving minimum and maximum values for samples (5-200 m) collected at offshore and onshore stations along N, P and S reference lines.

**Table 3 -** Range of fraction of respired leucine (%) for on-shore and off-shore stations during OMEX II-II cruises.

| Cruise | On-shore | Off-shore |
|---|---|---|
| *BG9815* | 39.5-95.4 | 32.5-100.0 |
| *CD114a* and *b* | 3.0-35.9 | 45.1-95.1 |
| *OMEX-0898* | 21.1-33.9 | 58.1-60.4 |

3.4 DOC Uptake ***(Task II.6.3)*** and Bacterial Growth Efficiency ***(Tasks I.4** and **I.7)***
DOC uptake by bacteria in surface waters ranged from 0.23 to 0.46 µMC.$h^{-1}$ at stations P100 and S1000 (*OMEX-0898* cruise), respectively. These values corresponded to bacterial growth efficiencies of 19.3 and 20.3 %, respectively. DOC samples of one experiment (station S150) were lost due to bad weather conditions so DOC uptake and bacterial growth efficiency could not be estimated for this particular experiment. A few DOC samples from experiment P2800 are still in the process of re-analyses due to possible contamination. Additional experiments will be performed during *Belgica* 09/99 and, possibly, *Thalassa* 10/99.

3.5 Grazing on bacteria ***(Task I.4** and **IV.3)***
Although two experiments were performed on board *OMEX-0898* to determine grazing rates and grazing impact on bacteria by nanoplankton, grazing rates could not be determined due to stringent carbon and nutrient limitation in predator free treatments. However, growth rates of nanoplankton were determined. The information of 8 serial dilution experiments with C, N and P additions performed in the 1999 *Almeida Carvalho* cruise is still being processed.

4. DISCUSSION

4.1 Bacterial abundance, biomass and production
Lagrangian sampling during an upwelling-relaxation period (*CD114a*) revealed an enhancement in both bacterial abundance and specific production (< 140 $10^{-9}$ nmol leucine.$cell^{-1}$.$h^{-1}$) during the transition between mixed and stratified conditions coinciding with increased primary production (PML-c). This contrasted with further sampling of an upwelling filament (*CD114b*) where stratified waters yielded much lower values of specific production (< 60 $10^{-9}$ nmol leucine.$cell^{-1}$.$h^{-1}$).

Vertical profiles observed during summer upwelling (*Prof. Shtokman*) in N, P and S reference transects showed a sub-surface (5 to 50 m) maximum in bacterial abundances (and biomass) of 3-4.5 $10^6$ cells.$ml^{-1}$. Values decreased rapidly with depth reaching a minimum of ca. 0.5 $10^6$ cells.$ml^{-1}$ below 250 m. During winter downwelling (*CD110b*), bacterial abundances were generally much lower (<0.6 $10^6$ cells.$ml^{-1}$). Very low frequency of dividing cells (<0.8%) were associated to downwelling conditions. Vertical structure in bacterioplankton distribution was much more apparent during upwelling than downwelling situations. Although bacterial abundances in surface waters did not vary significantly along an onshore-offshore gradient, surface bacterial production decreased markedly from 3.5-13.3 µgC.$l^{-1}$.$d^{-1}$ onshore to 0.6-1.1 µgC.$l^{-1}$.$d^{-1}$ offshore. This resulted in a marked offshore

decrease of specific bacterial production, which suggests substantial enrichment in DOC on the Rias' shelf.

4.2 Grazing on bacteria

During grazing experiments using <10 µm and <0.8 µm size fractions, bacterial growth rates were not significantly higher in the grazer free treatment suggesting more stringent substrate limitation in the absence of primary producers and predators. Furthermore, the relationship between primary production and specific bacterial production, combined with the significant correlation between bacterial production and bacterial abundance observed during Lagrangian sampling, are indicative of bottom-up control through DOC limitation. However, the importance of top-down control will be evaluated from the results of dilution experiments performed in *Almeida Carvalho* cruise.

4.3 Fraction of respired leucine, DOC uptake and bacterial growth efficiency

In general, the fraction of respired $^{14}$C-leucine varied from 11 to 68 % in surface waters with an increasing offshore trend. Bottom waters always exhibited higher values reaching a maximum of 100 % at an offshore station. The fact that large variations in the fraction of respired leucine were observed along horizontal and vertical profiles leads to the conclusion that single average values for BGE should not be used when modelling carbon fluxes in the area. Furthermore, the fraction of respired leucine was indicative of increasing DOC limitation offshore and in bottom waters. Routine screening of respired fraction may be warranted if accurate estimates of bacterial production are to be obtained in oligotrophic waters.

Finally, measurements of DOC uptake with concomitant increase in bacterial biomass yielded direct estimates of bacterial growth efficiency of ca. 20% at two intermediate stations, P100 and S1000 (*OMEX-0898* cruise). Although, these estimates are in agreement with those reported in the literature (*e.g.*, Carlson and Ducklow, 1996), more variation should be expected further onshore and offshore. More DOC uptake experiments will be carried out at stations located further apart along reference transects in future cruises (*Belgica* 09/99 and *Thalassa* 10/99) to provide a better assessment of spatial and temporal variability.

5. REFERENCES


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## 6 - ANNEX

Summary of stations (cast reference) and depth levels. Additional surface underway samples were collected during *CD110b* at S (1000, 2000, 2250, 2550, 2600) and N reference lines (100, 220, 1600, 2000, 2300, 3100, 3300). Depth levels in bold represent samples used for BG, BGE and BU experiments. Depth levels in italic represent samples with BB only.

| Cruise | Station (Cast reference) | Depth levels (m) |
|---|---|---|
| *CD110b* | P100 | 10, 25, 48, 67, 95 |
| | P200 | 8, 25, 52, 76, 101, 175 |
| | P1000 | 9, 29, 52, 78, 102, 201, 296 |
| | P2800 | 7, 81, 125, 155, 486, 635 |
| *BG9815* | S2 | 10, 20, 40, 60, 125 |
| | S3 | 10, 20, 40, 60, 80, 150 |
| | S4 | 10, 40, 100, 400, 1000, 1250 |
| | P20 | 10, 20, 40, 60, 80, 120 |
| | P21 | 10, 20, 40, 60, 100, 200 |
| | P23 | 20, 60, 100, 400, 1000, 1600 |
| | P24 | 20, 40, 100, 400, 1000, 1720 |
| | P26 | 20, 40, 60, 200, 900, 1750 |
| | N33 | 10, 20, 40, 60, 80, 120 |
| | N34 | 10, 20, 40, 60, 100, 200 |
| | N35 | 20, 40, 100, 400, 1000, 1500 |
| | N37 | 20, 60, 150, 400, 800, 900 |
| | N38 | 20, 40, 200, 400, 1000, 1750 |
| *OMEX-0898* | S90 | 5, *20*, *40*, 60 |
| | S150 | **5**, 20, 30, 40, 50 |
| | S300 | 5, *10*, *20*, *30*, *75*, 150 |
| | S1000 | **5**, *30*, *60*, *150*, *500*, 705 |
| | S2000 | 5, *30*, *50*, *100*, *200*, 500 |
| | S2550 | 5, *30*, *50*, *100*, *200*, 500 |
| | P100 | **5**, 20, 30, 40, 60, 90 |
| | P200 | 5, *10*, *30*, *50*, *100*, 150 |
| | P1000 | 5, *30*, *50*, *150*, *500*, 900 |
| | P2000 | 5, *50*, *100*, *500*, *1000*, 1500 |
| | P2550 | 5, *20*, *60*, *150*, *500*, 1500 |
| | P2800 | **5**, 20, 60, 250, 700, 2000 |
| | N30 | 5, 15, 30 |
| | N100 | 5, *10*, *20*, *50*, *75*, *100* |
| | N220 | 5, *10*, *30*, *50*, *100*, 150 |
| | N1600 | 5, *20*, *60*, *150*, *700*, 1500 |
| | N2300 | *5*, *30*, *50*, *200*, *700*, *1000* |
| | N3100 | 5, *20*, *60*, *150*, *700*, 2000 |
| | N3300 | 5, 20, 60, 250, 700, 2000 |

Summary of stations (cast reference) and depth levels (continued). Stations in bold represent samples used for grazing experiments.

| Cruise | Station (Cast reference) | Depth levels (m) |
|---|---|---|
| *CD114a* | 09 | 10, 15, 20, 30, 50, 100 |
| | 10 | 5, 15, 25, 40, 60, 120 |
| | 11 | 5, 15, 25, 50, 75, 150 |
| | 13 | 5, 15, 25, 45, 80, 150 |
| | 14 | 5, 15, 25, 40, 60, 130 |
| | 15 | 5, 20, 30, 40, 60, 130 |
| | 19 | 5, 20, 30, 40, 70, 150 |
| | 20 | 5, 15, 30, 40, 80, 150 |
| | 21 | 5, 10, 30, 45, 80, 150 |
| | 24 | 5, 15, 30, 40, 70, 145 |
| | 25 | 5, 15, 30, 45, 80, 147 |
| | 26 | 5, 20, 40, 60, 80, 150 |
| | 30 | 5, 15, 30, 40, 80, 150 |
| | 31 | 5, 20, 40, 60, 80, 150 |
| | 32 | 5, 15, 30, 50, 80, 150 |
| | 37 | 5, 15, 40 |
| | 38 | 5, 30, 50 |
| | 39 | 5, 25, 40 |
| | 40 | 5, 25, 40 |
| | 41 | 5, 15, 30 |
| *CD114b* | 57 | 10, 20, 30, 40, 50, 100 |
| | 58 | 10, 20, 30, 50, 250, 500 |
| | 60 | 10, 15, 20, 30, 50, 100 |
| | 61 | 20, 30, 40, 50, 60, 500 |
| | 63 | 10, 20, 30, 40, 50, 150 |
| | 64 | 10, 20, 45, 50, 200, 500 |
| | 66 | 10, 20, 30, 45, 60, 100 |
| | 67 | 10, 30, 50, 55, 100, 500 |
| | 69 | 20, 50, 55, 60, 65, 150 |
| | 70 | 10, 30, 50, 55, 60, 500 |
| | 72 | 15, 330, 40, 45, 70, 150 |
| *Almeida Carvalho* 05/99 | **P26, P30, P31** | 10 |
| | **93, 115, 127, 141, 147** | 10 |
| | 1 | 5, 10, 15, 20, 25 |
| | 3 | 10, 26, 51, 96 |
| | 4 | 5, 10, 20, 30, 50, 75, 140 |
| | 6 | 5, 10, 25, 35, 50, 100, 500, 1116 |
| | 8 | 5, 10, 25, 35, 50, 100, 500, 1119 |
| | 11 | 5, 10, 25, 35, 50, 100, 500, 1000 |
| | 79 | 5, 1450 |
| | 43 | 5, 63 |