9.2.9 Biodiversity, phylogeny and trophodynamics of amphipod crustaceans of the Antarctic deep sea

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Objectives

The ANDEEP I & II cruises in 2002 have revealed an overwhelming biodiversity in different faunal components of the Southern Ocean deep-sea ecosystem. The ANDEEP III expedition was planned to test hypotheses resulting from these data, corroborate results of the previous cruises and seek for the potential origin of some of the taxa which seem to have radiated in the Southern Ocean deep sea like others on the shelf.

The peracarid crustaceans, and in particular the Amphipoda, are known to count among the most speciose animal groups in the Antarctic coastal and shelf communities (De Broyer & Jazdzewski 1996). For the Antarctic deep sea, the ANDEEP I-II results showed that Amphipoda contributed up to 32% of the large material collected by the epibenthic sledge (EBS), just after Isopoda (38%), the usual dominant group in the deep sea (Brandt *et al.* 2004). In terms of species richness, the very limited deep-sea investigations in the Southern Ocean before ANDEEP revealed the presence of only 21 benthic amphipod species below 2000 m, all belonging to relatively primitive families characterized by free-swimming males (Thurston 2001). The successful ANDEEP I-II deep-sea amphipod sampling allowed discovering numerous species belonging to at least 28 different families. The ongoing identification work has already shown a high percentage of unknown species in most families (De Broyer *et al.* 2004; Berge pers. comm.; Thurston pers. comm.).

Concerning the origin of the Antarctic deep-sea fauna, pioneer molecular studies (16s rRNA, 18s rRNA and CO1 data) on polar submergence in Antarctic serolid and arcturid isopods (Held 2000) and Asellota Janiroidea indicated multiple colonisation events of the deep sea from the Antarctic shelf (Raupach *et al.* 2004), all of which occurred independently and may be related to the glaciation history in Antarctica. It is presently unknown if the amphipod crustaceans, which present a different evolutionary history, exhibit similar trends.

Investigations on the trophic role of the rich and diverse amphipod taxocoenosis of the Antarctic shelf have revealed a rather large diversity of trophic types (Dauby *et al.* 2001a, b) and a stable isotope approach (Nyssen *et al.* 2002) and a fatty acid analysis (Graeve *et al.* 2001) confirmed the trophic structure. How far can the trophic structure of the deep-sea benthic communities compare with the shelf communities?

The ANDEEP III project aimed at completing the previous results by pursuing the investigations on:

1. Patterns and processes of amphipod biodiversity
- To document species composition and as far as possible ecological traits (e.g. habitats, mode of life) of the Antarctic deep sea amphipod taxocoenoses on latitudinal and bathymetrical scales, and to compare it to the Antarctic shelf fauna and to the fauna of the (south) Atlantic abyssal basins.
- To investigate the determinants of the deep-sea amphipod species richness in comparison to the shelf fauna.
- To contribute by taxonomical material, photographic records, distribution and ecological data to the revision of the Antarctic fauna, the synthesis of its distributional and ecological traits and the preparation of new identification tools by the "Antarctic Amphipodologist Network" (see www.naturalsciences.be/amphi), and to the SCAR Marine Biodiversity Information Network.

2. Molecular phylogeny and phylogeography
- To attempt to establish the phylogeny and phylogeography of selected amphipod taxa (mostly the Lysianassoidea) through parallel molecular and morphological approaches.
- To investigate the role of the Antarctic shelf species pool in the colonisation history of the (Atlantic) deep-sea basins and vice versa.

3. Trophodiversity and trophodynamics
- To characterise the dominant trophic types and to determine the trophodynamic role of the Antarctic deep-sea amphipod biocenosis in comparison with the shelf communities.

The trophic approach relies on digestive tract analyses and ethological observations in aquaria. This will be completed by the use of stable isotope (carbon and nitrogen) ratios and fatty acids as amphipod diet tracers to delineate the trophic relationships involving amphipods in Antarctic deep sea food webs.

Work at sea

Sampling: Amphipods crustaceans were collected from benthic and suprabenthic samples taken at 19 deep-sea stations by using the following gears: epibenthic sledge (EBS), Agassiz trawl (AGT), large box corer (GKG) and autonomous baited trap system (AT). The whole EBS material and selected material from AGT and AT was fixed in cooled ethanol for allowing further DNA analyses.

Sorting and identification: All trap samples, most of the AGT material and part of the EBS material was sorted to the species level and identified as far as time and available documentation permitted.

Photographic documentation: an extensive macro- and micro-photography database focusing on new species was built up during the cruise to feed the existing amphipod database (Ant'phipoda). Over 700 high-quality pictures were taken using either for macrophotography a Nikon D70 (AF 105mm f2.8 Nikkor lense, 2 external electronic flashes (Nikon SB24&SB80),) or for microphotography a Nikon Coolpix 9500 and a Leica binocular microscope.

Selection of material for DNA analyses, bacterial gut content studies, contaminant biokinetics studies, and trophic characterization by stable isotopes was done. DNA extractions were performed on selected species.

Maintenance and observation of living animals: Living amphipods and isopods were kept in different aquaria (of 6 to 30 l) aerated with air bubblers and provided with artificial substrate (nylon gauze). Some ethological observations (mostly on mobility and locomotion mode) were performed as well as systematic gut clearance observations on selected species.

Preliminary results

1. Faunistic survey

Material collected: Among the collected amphipod specimens, 7297 (Gammaridea and Corophiidea) were sorted.

EBS. Thirteen stations (of 19) provided 3286 specimens. Part of the samples have been sorted to the family level, and in few cases to the genus and species level. The most common families represented were: Ampeliscidae, Corophioidea (mostly Ischyroceridae and Podoceridae, few Caprellidae), Eusiridae, Hyperiopsidae, Lepechinellidae, Liljeborgiidae, Lysianassoidea (adeliellids, Lysianassidae, Uristidae), Melphidippidae, Oedicerotidae, Pardaliscidae, Phoxocephalidae, Stegocephalidae, Stilipedidae, Synopiidae, and Urothoidae.

AGT. Sixteen AGT samples provided 225 specimens from at least 12 families.

GKG. Three specimens were recorded so far (partial results).

AT. Fourteen baited trap deployments allowed collecting 3783 amphipod specimens as well as specimens from 2 cirolanid isopods (table 9.15) belonging to 30 species from at least 9 families (table 9.16). In most cases, these species were not collected by the other gears.

Table 9.15: Summary of ANDEEP III trap samples. For the different taxa, numbers indicate the number of species, numbers in parentheses correspond to the number of individuals.

Station	Depth (m)	Soak Time (hrs)	Amphipoda	Isopoda	Mysidacea	Pisces
057 AT	1831	64	4 (108)			
059 AT	4553	30	11 (687)	1 (1)	1 (2)	
074 AT	927	9	6 (132)	1 (11)		
078 AT	2123	17	4 (397)			
080 AT	2880	22	12 (445)			1 (1)
081 AT	4439	28	8 (653)			
088 AT	4934	27	5 (180)			1 (1)
094 AT*	4850	34	4 (65)			
102 AT*	4754	34	4 (23)			
110 AT	4587	27	4 (350)			
142 AT	3337	18	4 (16)			
150 AT	1125	34	5 (714)	1 (82)		
153 AT	2001	15	1 (10)	1 (4)		
154 AT	3689	17	2 (3)			
Total			**30 (3783)**	**2 (98)**	**1 (2)**	**1(2)**

* bait in container

Table 9.16: List of amphipod and isopod species collected in the baited traps

Species	Stations
AMPHIPODA	
Abyssorchomene plebs	150
Abyssorchomene cf scotianensis	57, 59
Abyssorchomene sp.1	59
Abyssorchomene sp.2	78, 80, 81
Abyssorchomene sp.3	80, 81, 110
Eurythenes gryllus	57, 59, 78, 80, 81, 88, 94, 110, 142
Lepedipecreellid n.gen. n.sp.1	80
Lysianassoidea gen. sp.1	59
Lysianassoidea gen. sp.2	59, 110
Lysianassoidea gen. sp.3	59
Lysianassoidea gen. sp.4	59
Lysianassoidea gen. sp.5	74
Lysianassoidea gen. sp.6	80
Lysianassoidea gen. sp.7	81
Orchomenopsis cf cavimanus	57, 80, 150
Orchomenopsis sp.1	80, 150
Paracallisoma n.sp.1	59, 80, 88, 94, 110, 154
Paralicella cf fusiformis	110
Paralicella sp.1	59, 80, 81, 154
Parschisturella simplex	74, 150
Pseudorchomene cf coatsi	57
Pseudorchomene n.sp.1	150
Stegocephalidae gen.sp.1	78
Stegocephalidae gen.sp.2	88, 94
Stegocephalidae gen.sp.3	81, 110
Stilipedidae gen.sp.1	59
Tryphosinae gen. sp.1	59
Tryphosinae gen.sp. 2	74
Tryphosinae gen.sp. 3	80
Tryphosinae gen.sp. 4	81
Valettiopsis n.sp.1	153
ISOPODA	
Natatolana albinota	150
Natatolana intermedia	74, 153

2. Molecular phylogeny and phylogeography

Selected samples for molecular phylogeny studies comprise, in addition to the rich and diverse EBS samples, 34 lysianassoid species (including several new species) belonging to 18 genera and 10 families and 10 species from 5 other amphipod families provided by AGT and AT. Particular attention was paid to the selection of potential cryptic species.

Specimens for molecular analyses were preserved in 96 % cooled ethanol as soon as possible after sampling, preferably live, in order to avoid DNA degradation by enzymatic activity. As a rule, a little part of each specimen was taken for DNA extraction, usually the pereopod 6 as a whole or a part of it, depending of the size of the animal. About 50 DNA extractions and purifications were carried out using QIAamp DNA Mini Kit (Qiagen), from specimens belonging to 44 different morphospecies from 23 genera and 17 families.

All this material will be processed at the Royal Belgian Institute of Natural Sciences (IRSNB), in order to obtain DNA fragment sequences of at least 18S, CO1 and possibly ITS2 genes. These genes have proven to be useful for different phylogenetic levels and to give complementary information.

3. Maintenance of living amphipods and isopods and observations of feeding eco-ethology

Living specimens of 11 amphipod and 2 isopod species collected mostly by traps and occasionally by AGT and EBS were kept in a cool container at a temperature of +1°C: the scavenging lysianassoids *Abyssorchomene plebs, A. scotianensis, A.* sp.1, *A.* sp 2, *A.* sp.3, *Orchomenopsis* sp.1, *Eurythenes gryllus, Parschisturella simplex, Pseudorchomene coatsi,* and *Valettiopsis* n.sp.1, the detritivore *Paraceradocus cf gibber,* and the scavenging cirolanid isopods *Natatolana intermedia and N. albinata.* Some ethological observations (mostly mobility and locomotion mode) were carried out.

4. Gut clearance follow-up

Several experiments have been conducted on 3 living crustacean species (*E. gryllus*, *P. simplex*, *N. intermedia*) maintained in cooled aquaria, in order to estimate gut clearance rates. Stomach content analyses were also carried out in order to extend the existing database on Antarctic amphipod feeding habits.

Gut clearance was followed in a total of 40 individuals, at 8 different times (table 9.17). Interesting differences were spotted between the three considered species. At the end of the observations (42 d), the gut content of *E. gryllus* was still important, though well homogenized. Dissection showed that the guts were still relatively full and that the gut content (fish from the baits) had the form of a homogenous white paste. At the end of the experiment (39 d), the gut content of *P. simplex* had almost entirely disappeared, in most individuals. *P. simplex* was found to produce a black oily substance (which has been isolated for further study), which is formed during the

digestion process. Along time, this substance spread through the whole length of the digestive tube. This substance was not found in the other considered species. Finally, individuals of *N. intermedia* showed a very high variability in the digestion process, some individuals having almost emptied their gut at the end of the experiment, while others were still displaying the same content as that of the beginning of the experiment.

In order to quantify the remaining proportion of gut content in the individuals, digital pictures were taken at various intervals. Image analyses software (NIH Image©) will be used to measure the variations in gut content along time.

Table 9.17: Gut clearance follow-up. Number of individuals considered in the experiment (n) and duration of the experiment.

Species	n	Time (d)
E. gryllus	10	42
P. simplex	20	39
N. intermedia	10	39

5. Gut content bacteriology (B. Danis).

At three stations, the gut content of detritus-feeding amphipods was sampled for bacteria population analyses. The study of these samples will bring important information about barophilism in intestinal bacteria from deep-sea organisms, and on their role in the nutrition, which still is not well understood. For details, see Danis in the present cruise report.

6. Selected heavy metal levels and biokinetics in deep-sea Antarctic amphipods (B. Danis).

A total of 275 individuals of *Eurythenes gryllus* collected by traps at stations 81, 94 and 102 were immediately frozen for subsequent heavy metal analyses. Amphipods belonging to the species *Abyssorchomene plebs* (Amphipoda, Lysianassidae, n=250) and *Parschisturella simplex* (Amphipoda, Lysianassidae, n=250) were captured using AT at station 150 and kept alive to be sent to the International Atomic Energy Agency facilities (IAEA-MEL, Monaco). For details, see Danis in the present cruise report.

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9.2.10 Selected heavy metal levels and biokinetics in deep-sea Antarctic amphipods

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Background and objectives

Pollution of the ocean by heavy metals such as Co, Zn, Ag, Cu, Pb and Cd is a major environmental problem in many parts of the world (Clark 2001). When reaching marine waters, heavy metals mainly concentrate in the sediments due to their generally low solubility in sea water and their tendency to adsorb onto particles. Sediments therefore constitute the bulk of anthropogenic contaminants in the coastal environment and may be a major source of contamination for numerous organisms living in or close to them (Stebbing et al. 1992, NSTF 1993, Karbe et al. 1994, Alzieu & Michel 1998). Heavy metals reach the environment via natural sources (which account for a background exposure), increased by anthropogenic inputs. To differentiate between natural and anthropogenic metal inputs, which is one of the main objectives in biomonitoring, natural background concentrations of chemicals in organisms and their fluctuations have to be well established (Fialkowski et al. 2000). In this respect, investigations in remote areas such as the Antarctic Ocean are extremely interesting (Bargagli et al. 1996, 1998, Duquesne et al. 2000) because in these areas anthropogenic metal inputs are considered to be of minor importance. Among candidate bioindicator organisms in these areas, amphipods are particularly interesting, being widespread and key components of Antarctic marine ecosystems (Rainbow 1995).

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