# Arctic Biology Field Course Qeqertarsuaq, 2002



# **University of Copenhagen**

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Cover photo:	<i>Angelica archangelica</i> at the warm spring Anguujaartuutit, Disko Fjord. The research vessel Porsild is seen in the background. Photo by J. G. HANSEN.

# Foreword

Reinhardt Møbjerg KRISTENSEN<sup>1</sup> & Poul Møller PEDERSEN<sup>2</sup>

<sup>1</sup> Department of Invertebrate Zoology, Zoological Museum, University of Copenhagen.

<sup>2</sup> Department of Phycology, Botanical Institute, University of Copenhagen.

#### Introduction

The arctic field courses from the University of Copenhagen have been held regularly since 1973 at Arctic Station, Qeqertarsuaq. The Arctic Station is located near the town Qeqertarsuaq (Godhavn) on the south coast of Disko Island, West Greenland. It was founded in 1906 by the Danish botanist Morten Porsild, and the station is thus the oldest research institution north of the Polar Circle. The research vessel "Porsild", named after the founder, was donated by Mærsk McKinney Møller in 1994. Today the station is owned by the Faculty of Science and it is open for international and national scientists as well as graduate students. For a more comprehensive introduction to the Arctic Station, please consult the homepage: <u>http://www.nat.ku.dk/as/</u>.



Arctic Station - Photo by M. Smith



This report summarises the results from the biological field course organised at the Arctic Station, Qegertarsuag and Kangerlussuag from 3 July to 27 July 2002. Twelve students from the University of Copenhagen participated in the field course. Field activities were supervised by senior researchers from three institutes. The botanical teachers were Niels Daugbjerg and Poul Møller Pedersen both from Botanical Institute, the zoological teacher was Reinhardt Møbjerg Kristensen from Zoological Museum, and a special guest teacher in geology was Bjørn Buchhardt from Geological Institute. Furthermore, Martin V. Sørensen (postdoc) and Katrine Worsaae (Ph. D. student), both from Zoological Museum also participated in the field activities and supervision of the students. The station was overbooked during the field course. Several other activities were going on with special emphasis on terrestrial botany supervised by Marianne Philipp, University of Copenhagen (six persons), a French team of four persons working with cormorants in Disko Fjord headed by David Gremillet, a Belgian team of three persons working with soil biology leaded by Louis Beyens and finally a joint team of geologists and geographers working with the surging glacier in Kuannersuit - leading Niels Tvis, Siri Hansen and David Roberts (five persons). In fact, all these scientists were not a disadvantage for the field course, because they all gave lectures in the evenings for the students, so they really felt that Arctic Station is a very dynamic and international place. The terrestrial botanical team jointed us on several excursions and we learned a lot about the unique flora in the neighbourhood of Arctic Station, and finally the Belgian team joined us during the field trip to the glacier of Lyngmarksfjeldet. The scientific leader Bente Graae Jessen borrowed us her laboratory with the new microscopes with digital camera and video-recorder. Without this great gesture we could not have taken all the digital photos shown in this report, and the unique video recording of the new phylum, Micrognathozoa (Limnognathia maerski), now under display at the Zoological Museum in Copenhagen. She also introduced us to her work on dispersal and germination of arctic seeds.

The field course projects addressed terrestrial, limnic and marine issues, but the main effort was clearly marine biology. During the course we visited Diskofjord/Kangerluk, Mellemfjord/Akuliit and Mudderbugten/Aqajarua on board the research vessel "Porsild". A field trip to Isunngua spring and the Lymnaea-Lake in Kvandalen/Sullorsuaq was made the 11 July to 12 July. The course deviated from standard procedures by not using campsites for longer periods of time, as the students needed the laboratory facilities such as microscopes, refrigerators and cooling room more than to stay out in camps. One project, the bird project was based on bird songs recorded in the vicinity of Arctic Station, but all students were allowed to joint several cruises to more remote localities at Disko Island. The two days in Kangerlussuaq were used for a visit to the margin of the ice cap and sampling in the salt lakes.

Our stay on Disko Island was generally successful from a scientific point of view. Some of the results in this report are outstanding and three contributions are currently being prepared for publication in international journals. These are:

• HANSEN, J. G. & KATHOLM, A. K. (in prep): A study of the genus *Amphibolus* from Disko Island with special attention on the life cycle of *Amphibolus nebulosus* (Eutardigrada: Eohypsibiidae)

- KIRKEGAARD, M. & KNUDSEN, S. (in prep): First report on a new tantulocaridan (Crustacea: Maxillopoda) parasitic on harpacticoid copepods found off the coast of West Greenland.
- JENSEN, K. G., DAUGBJERG, N. & THOMSEN, H. A. 200X. Diversity and succession of planktonic and sea ice diatoms from the Disko Bugt, West Greenland. Submitted to MoG, Bioscience.

All the contributions published in this report are also available as PDF-files at the homepage of the Arctic Station: <u>http://www.nat.ku.dk/as/.</u>

Following the diversity studies of marine protists in July 2002 Niels Daugbjerg established a homepage for his DATMAP-D project (DAtabase of MArine Protists from Disko Bugt, West Greenland). This homepage can be visited at <u>http://www.bot.ku.dk/disko/index.asp</u> and it lists all records of protists from the area and attempts to include micrographs of all species and previous records.

Acknowledgements. First we want to thank the entire staff at the Arctic Station for providing logistic support, especially the crew onboard "Porsild", who also learned us that there is nothing as "Greenlandic time" at the sea. Thanks are also given to a number of colleagues at the University of Copenhagen for assisting the students to process field data and samples etc.: Torben Dabelsten (Zoological Institute), Bjarne Bisballe (Zoological Museum), furthermore Rony Huys (British Museum) is acknowledged for help with the systematic and molecular data of the new species of Tantuloracida.

The Botanical Institute is acknowledged for financial support that allowed us to include Niels Daugbjerg in the marine biological team of teacher, as well as the Geological Institute for financial support that allowed us to have guest teacher Bjørn Buchardt in geology and water analysis. The Faculty of Science covered travel expenses for 12 students and two teachers and additional support was given by the Arctic Station. Substantial support to students' accommodation was given by the Danish Botanical Society (Mikroorganismefonden) and the Carlsberg Bequest to the Memory of Brewer J.C. Jacobsen for financial support. Finally, we are indebted to Jesper G. Hansen for editing this report.



Photo of the participants of the Arctic Biology Field course, 2002 and the technical staff of the Arctic Station



1. B. Thygesen 2. Wife of F. Grønvold 3. K. Worsaae 4. F. Grønvold 5. M. V. Sørensen 6. A. K. Katholm 7. L. S. Andersen 8. P. M. Pedersen 9. B. Christensen 10. N. Daugbjerg 11. K. A. Jønsson 12. S. W. Knudsen 13. J. G. Hansen 14. F. Nielsen 15. R. M. Kristensen 16. A. Geisler 17. Son of A. Geisler 18. A. M. Nielsen 19. M. T. Jensen 20. M. Smith 21. L. Munk 22. M. Kirkegaard 23. B. B. Westergaard 24. S. Fisker 25. Wife of S. Fisker 26. Wife of F. Nielsen 27. Daughter of F. Nielsen



Fairy shrimp (Branchinecta paludosa). Drawing by S. W. Knudsen

# Oxygen Isotope Composition of Seawater and Spring Water at Disko Island: Implications for Origin of Water

Bjørn Buchardt WESTERGAARD

Geological Institute, University of Copenhagen, Øster Voldgade 10, DK-1350 Copenhagen K, Denmark e-mail: bjornb@geol.ku.dk

Abstract. More that 100 samples of natural waters from Disko Island, North West Greenland have been sampled and analysed for their oxygen isotope composition. Marine waters were sampled in Disko Bay and adjacent waters at varying depths from surface to 300 m. Fresh-water samples come from springs, rivers, lakes and glaciers on Disko. Oxygen isotope compositions of marine waters are strongly correlated to salinity, and the results suggest that mixing between Atlantic water and glacial melt water is the most important oceanographic process in Disko Bay. Spring water analyses clearly demonstrate a meteoric water origin for the homothermic spring on Disko. The oxygen isotope data presented in this report are the first from the Disko Bay area, and further isotope studies of Disko area waters are recommended.

#### Keywords.

#### **1. INTRODUCTION**

The oxygen isotope composition of natural waters is a conservative parameter reflecting origin and modifications of a given water sample. During the field course in arctic biology at Arctic Station, Disko (NW Greenland) in the summer of 2002, more than 100 water samples were collected for oxygen isotope analyses. About half of the samples were seawater collected off Disko at depths from 0 to 300 meters. The remaining samples were fresh-water types from lakes, rivers, glacial ice and the well-known homothermic springs at Disko (KRISTENSEN 1988). Marine samples were analysed as a supplementary parameter to the standard identification of salinity and temperature variations in the waters around Disko, and the results have been interpreted in an oceanographic context. Spring-water samples on the other hand were obtained in an attempt to provide new understanding to the origin of the unique homothermic spring systems on Southern Disko. Other fresh-water samples were included to give better insight into the oxygen isotope composition of the meteoric water systems on Disko. No oxygen isotope analyses have been carried out before on Disko area waters, and the total number of isotopic data from Greenland waters is still very limited. An overview of southern Greenland isotope data is given in BUCHARDT et al. (2001).

#### Background

Oxygen has three stable isotopes: <sup>16</sup>O, <sup>17</sup>O and <sup>18</sup>O, differing only in number of neutrons and therefore in mass. Their natural occurrence is roughly 99.8 : 0.04 : 0.2 measured as weight. Oxygen-bearing compounds in nature show small differences in isotopic concentrations as result of isotopic fractionation. Oxygen in carbonate minerals is thus 30 ‰ (permil) enriched in <sup>18</sup>O (we normally exclude <sup>17</sup>O from the calculations owing to its smaller concentration) compared to seawater, which again is enriched 30 to 50 ‰ compared to ice-sheet ice. These fractionations are caused by the tiny differences in kinetic and zero point energy of the oxygen-bearing molecules involved in the exchange processes. In the meteoric-water cycle (meteoric means "in the air") relating seawater, precipitation and major fresh-water reservoirs the most important isotopic fractionation processes are evaporation and condensation. Water molecules carrying an <sup>16</sup>O isotope will evaporate slightly faster than <sup>18</sup>O-carrying molecules, and water vapour will be depleted by about 10 ‰ in <sup>18</sup>O compared to the evaporating water mass. Similarly, <sup>18</sup>O-carrying molecules will condensate slightly faster than the <sup>16</sup>O-carrying

molecules, and precipitation will be enriched in  $^{18}$ O by about 10 % compared to the condensating vapour.

In nature, precipitation falling in the temperate and arctic regions originates as evaporation formed in the subtropical high-pressure cells. As discussed above, this atmospheric water vapour will be about 10 ‰ depleted in <sup>18</sup>O as compared to the seawater. As the air masses and their water vapour move northwards towards the temperate regions and the polar front, temperatures decrease, and increasingly larger amounts of water vapour condensate and leave the air mass as precipitation. The precipitation will be enriched in the heavy oxygen isotope <sup>18</sup>O due to the condensation fractionation, and consequently the remaining water vapour will be depleted in <sup>18</sup>O. As the fractionation between vapour and water remains approximately constant (actually it decreases slightly with lower temperature), precipitation formed from these increasingly more depleted vapour masses will itself be more depleted. In principle the whole system can be viewed as a major distillation column leading to gradually more depleted condensates.

This isotopic depletion is termed the latitude effect (or rain-out effect) as the depletion generally is correlated to increasing latitudes. A similar effect is seen between summer and winter precipitation (seasonal effect) and in precipitation falling at elevated altitudes (altitude effect), both related to the temperature differences. The effects are important in the arctic, as temperature differences between high and low latitudes and between summer and winter are large. On a longer time scale, climatic differences also play an important role in the changing isotopic composition of precipitation. This is the basis for the Greenland ice-core analyses, where precipitation is preserved over long time spans as snow and ice. Generally, the Greenland ice sheet is depleted in <sup>18</sup>O by 25 to 40 ‰ compared to normal seawater.

Isotopic enrichments are normally expresses as differences relative to a standard by the  $\delta$  (delta)-function and expressed as % deviations from a standard composition. In hydrological studies, Standard Mean Ocean Water or SMOW is used as the internationally standard, as the oceans have been found to function as a stable reservoir. Seawater thus has  $\delta^{18}$  values close to 0 % SMOW. Precipitation falling in the temperate regions will typically have  $\delta^{18}$  values around -8 to -10 %, while arctic precipitation may have considerably more negative  $\delta^{18}$  values. Rain sampled at the Arctic Station was thus found to have  $\delta^{18}$  values around -18 %, while ice sheet samples are known to vary between -25 and -45 %.

Where seawater and fresh water mixes, modifications in the seawater isotopic composition will reflect the isotopic composition of the fresh water component. As salinity and isotopic composition both are related to amount of fresh water contribution, these parameters will be linearly related. The slope of the "mixing line" as seen in a salinity- $\delta^{18}$ O diagram will differ according to the oxygen isotope composition of the fresh water component. Brackish water in a temperate area will thus be characterized by a lesser slope than similar water in an arctic area, as fresh water in temperate areas is less depleted in <sup>18</sup>O than fresh water in the Arctic. Saline waters having obtained their dissolved ions from processes other than seawater mixing will keep their original  $\delta^{18}$ O values and thus directly reflect the origin of the water. The basic principles of oxygen isotope hydrology were first treated by EPSTEIN & MAYEDA (1953). A comprehensive summary is given in the textbook by CLARK & FRITZ (1997).

In the following we will treat the Disko water samples with these relations in mind. The  $\delta^{18}$ O values of the marine samples will be compared to water depth, salinity and temperature as a mean of delineating different water masses and identifying differences in fresh water admixture. The isotopic composition of the spring water samples will be related to

temperature and altitude and compared to the local precipitation and run-off water. This may help to identify differences in the sources of the homothermic spring water systems.

## 2. MATERIAL AND METHODS.

A Niskin water sampler was used to collect marine water samples from the research vessel Porsild 2 during several cruises to the sea south and west of Disko Island. The locations of the marine stations are shown on Figure 1. Seawater samples were generally collected at the surface, at 2.5 m and at 20 m depth. At 6 stations, deeper samples were obtained down to the maximum depth of 300 m allowed by the sampling gear at Porsild. The Seabird CTS instrument normally used from Porsild was inoperational, and water temperatures were determined by a handheld digital thermometer immediately after sampling. Precision was better than 0.2°C. This method of measuring salinity at the surface and not in situ probably introduces errors, and the temperature data should be interpreted with some caution. Salinity was estimated by a handheld refractometer and later determined by a Guildline Portasal 8410 salinometer at the Niels Bohr Institute for Astronomy, Geophysics and Physics at University of Copenhagen. Measurements were calibrated to the IAPSO Standard Seawater P 115. Precision was better that 0.05 psu (practical salinity units). Fresh-water samples were collected at spring, river and lake localities, from rain and from the Lyngmark glacier above Godhavn. Temperature was determined directly on the spring water by a digital thermometer similar to the one used for the marine samples. Localization of sampling sites for both marine and fresh-water samples was performed by use of a handheld GPS-instrument reading at least 4 different satellite signals. The GPS data have not been corrected for deviations in Disko area projections. All water samples were stored cold in 250 ml polyethylene bottles and brought to Copenhagen directly after end of the course. Oxygen isotope determinations of water samples were performed at the ice-core laboratory at the Niels Bohr Institute for Astronomy, Geophysics and Physics. Reproducibility is better than  $\pm 0.02$  ‰ on the  $\delta$ -scale.

### **3. RESULTS**

The results of the oxygen isotope determinations are given in Table 1 and 2 together with information on sample localities, salinity and temperature. The marine data are illustrated in Figure 2, which relates depth, salinity and temperature to oxygen isotope composition of the 60 investigated seawater samples. The isotopic compositions of the 41 fresh-water samples are shown in Figure 3.

# 4. DISCUSSION

#### Marine waters

The water masses in Disko Bay can be divided into three layers in the summer period: a relatively warm surface layer down to 20-30 m affected by insulation and fresh-water run-off, a cold intermediate layer between 30 and 100 m generated by vertical downwards convection of cold surface water during the winter, and a warm and saline deep layer below 100 m related to the Atlantic Mode Water by the West Greenland Current (HERMANN 1971, BUCH 1990 and 2000). As seen from Figure 2, this division can also be identified in our Disko Bay data. During the summer of 2002, the surface layer was characterized by salinities below 33.2 ‰ and temperatures between 4 and 10°C. The layer reflects mixing between seawater and fresh-water run-off. Downwards, this layer gradually changed to a cold, intermediate layer with salinities between 33 and 34 ‰ and temperatures as low as 1°C. This layer was best developed between 80 and 100 m depth. Further down, both salinity and temperature increased as we approached the bottom layer. This layer probably originates in the West Greenland Current. The deepest sample at 300 m had a salinity of 34.4 ‰ and a temperature of 4.2°C and seemingly reflects advection of Atlantic Mode water (Buch 2000).

The oxygen isotope composition of seawater samples with salinities above 30 ‰ is linearly related to salinity (Figure 4) with a correlation coefficient  $r^2$  of 0.92. This relationship suggests a two-component mixing system between Atlantic water (salinity 35.2 ‰ and  $\delta^{18}$ O 0.22 ‰, EPSTEIN & MAYEDA 1953, CRAIG & GORDON 1965) carried into Disko Bay by the West Greenland Current and an unknown fresh-water component. The  $\delta^{18}$ O value of this



**Figure 1.** Map of Disko Island showing the locations of sampling sites visited during the course in Arctic Biology 2002. Marine stations were sampled from the research vessel Porsild 2.

Station	Locality	Remarks	Date	Latitude	Longitude	Depth m	Salinity o/oo	Temp. C	d18O o/oo
0	Diaka Duat	fixed CTD station	hub i E	60 11	E2 201	200	22.04		0.72
0	Disko Bugi	fixed CTD-station	July 5	60 11'	53 30	-209	33,04 33,18		-0,73
0	Disko Bugt	fixed CTD station	July 5	60 11'	53 30	-15	22 426		-1,20
1	Disko Bugi	Off Ignik	July 5	09     60 17 317'	53 30 53 13 214'	-2,5 102	32,430		-1,03
1	Disko Bugt	Official	July 5	60 17 217	52 12 214	-192	22 505		-0,33
1	Disko Bugt	Official	July 5	60 17 217	52 12 214	-30	22,000		-0,79
1	Disko Bugi		July 5	69 17.317	53 13.214	-10,5	<i>33,290</i>		-1,00
1	Disko Bugt	Off Ignik	July 5	60 17 217	53 13.214 52 12 214	-2,5	32,20	E 0	-1,70
2	Mollomfiord	Enoks Have Mellomfiord		60.45	53 15.214	70	32,244	5,6	-1,01
2	Mellemfiord	Enoks Havn, Mellemfjord	July 8	69 45	54 51	-70	33,020		-0,09
2	Mellemfiord	Enoks Havn, Mellemfjord	July 8	69 45'	54 51'	-70	32 / 07		-1.42
3	Mellemfiord	Inner Mellemfiord	luly 8	69 45'	54 51'	-70	33 656		-0.75
3	Mellemfiord	Inner Mellemfjord	luly 8	69 45'	54 51'	-20	33 177		-1.02
3	Mellemfiord	Inner Mellemfjord	July 8	69 45'	54 51'	-2.5	32 334		-1.51
4	Mellemfiord	Outer Mellemfiord	July 9	69 45'	54 51'	-70	33 728		-0.62
4	Mellemfiord	Outer Mellemfiord	July 9	69 45'	54 51'	-20	33 343		-0.82
4	Mellemfiord	Outer Mellemfiord	July 9	69 45'	54 51'	-2.5	32,939		-1.13
5	Disko Fiord	Off Diskofiord	July 9	69 32.9'	54 58.5'	-70	33.832		-0.5
5	Disko Fiord	Off Diskofiord	July 9	69 32.9'	54 58.5'	-30	33.611		-0.82
5	Disko Fiord	Off Diskofiord	July 9	69 32 9'	54 58 5'	-2.5	33 118		-1.07
6	Disko Fiord	Diskofiord	July 9	69 28.02'	54 16.01'	-70	33.659		-0.83
6	Disko Fiord	Diskofiord	July 9	69 28.02'	54 16.01'	-30	33,255		-1.06
6	Disko Fiord	Diskofiord	July 9	69 28.02'	54 16.01'	-2.5	29,785		-2.87
7	Disko Buat	West of Blåfield	July 9	69 18.41'	54 13.41'	-70	33.689		-0.73
7	Disko Bugt	West of Blåfjeld	July 9	69 18.41'	54 13.41'	-30	33,463		-0,86
7	Disko Bugt	West of Blåfjeld	July 9	69 18.41'	54 13.41'	-2,5	33,1		-1,13
8	Mudderbugt	East of Flakkerhuk	July 12	69 38.257'	51 46.518	-100	33,736	1,7	-0,79
8	Mudderbugt	East of Flakkerhuk	July 12	69 38.257'	51 46.518	-20	33,205	3,3	-1,05
8	Mudderbugt	East of Flakkerhuk	July 12	69 38.257'	51 46.518	-2,5	31,587	6,6	-2,33
9	Disko Bugt	Off Skansen	July 12	69 25.4'	52 20.9'	-95	33,463	1,4	-0,8
9	Disko Bugt	Off Skansen	July 12	69 25.4'	52 20.9'	-20	33,309	2,3	-0,98
9	Disko Bugt	Off Skansen	July 12	69 25.4'	52 20.9'	-2,5	32,738	7	-1,46
10	Disko Bugt	Off Igpik	July 12	69 17.8'	53 11.9'	-150	34,064	2,6	-0,34
10	Disko Bugt	Off Igpik	July 12	69 17.8'	53 11.9'	-100	33,846	1,8	-0,72
10	Disko Bugt	Off Igpik	July 12	69 17.8'	53 11.9'	-30	33,229	2,4	-1,05
10	Disko Bugt	Off Igpik	July 12	69 17.8'	53 11.9'	-2,5	32,719	7,6	-1,64
10	Disko Bugt	Off Igpik	July 12	69 17.8'	53 11.9'	0	32,769	8,2	-1,49
11	Disko Bugt	Godhavn-Grønne Ejland	July 15	69 08.206'	53 33.299'	-150	34,094	3	-0,46
11	Disko Bugt	Godhavn-Grønne Ejland	July 15	69 08.206'	53 33.299'	-70	33,508	1	-0,75
11	Disko Bugt	Godhavn-Grønne Ejland	July 15	69 08.206'	53 33.299'	-2,5	32,393	9	-1,74
12	Disko Bugt	Godhavn-Grønne Ejland	July 15	69 03.838'	53 30.912'	-20	33,241	4	-1,09
12	Disko Bugt	Godhavn-Grønne Ejland	July 15	69 03.838'	53 30.912'	-2,5	32,511	8,8	-1,67
13	Disko Bugt	Godhavn-Grønne Ejland	July 15	68 58.759'	53 22.684'	-300	34,393	4,2	-0,23
13	Disko Bugt	Godhavn-Grønne Ejland	July 15	68 58.759'	53 22.684'	-150	34,154	3,3	-0,37
13	Disko Bugt	Godhavn-Grønne Ejland	July 15	68 58.759	53 22.684	-70	33,822	2,8	-0,78
13	Disko Bugt	Godhavn-Grønne Ejland	July 15	68 58.759	53 22.684	-20	33,261	4,7	-1,14
13	Disko Bugt	Godhavn-Grønne Ejland	July 15	68 58.759	53 22.684	-2,5	33,026	6	-1,31
100	Disko Fjord		JUIY 17	69 27.502	54 18.503	0	32,553		-1,51
101	Disko Fjord	Between Kana and Hut	July 17	69 26.905	53 50.590	0	16,188		-10,17
102	Disko Fjord	Kuanerssuit, siity red water	July 17	69 31.999	53 34.290	0	0		-18,39
103	Disko Fjord	Kuanerssuit, off radioactive spring	JUIY 17	69 33.346	53 35.104	0	2,08		-17,65
104	Disko Fjord		July 17	09 33.108	53 31.329	0	24,190 24,906		-0,09
105	Muddorbuct	Mudderbusten	July 17	60 24 100	52 20 207	0	24,000	4.6	-0,07
100	Muddorbudt	Mudderbugten	July 19	60 41 274	51 51 054	0	31,810	4,0	-2,00
107	Muddorbugt	Mudderbugten	July 19	60 42 620	51 55 100	0	32,02	4,0 5 7	-1,00
100	Muddorbuct	Mudderbugten	July 19 July 10	60 27 142	52 14 702	0	20,020	5,7	-0,07
109	Mudderbugt	Mudderbugten	July 19	60 32 640	52 14.702	0	24,102	5,7	-0,0 _1 Q
110	Mudderbugt	Flakkerhuk	July 19	69 32 580'	52 00.030	_5	32,121		-1,0 _1 /0
112	Disko Buat	Beach at mouth of Røde Flv	July 22	33 32.309	52 00.000	0	17 856		-8 25
2	Liono Dugi		July 22			5	,000		0,20

**Table 1.** Seawater samples from Disko Bay and Disko Fjord, July 2002

fresh water can be estimated from the Y-axis intercept of the regression line determined from the Disko Bay seawater samples of -25.5 ‰. This value is much more negative than the analysed fresh-water samples from Disko (average -16 ‰, see Figure 3), but corresponds to the two iceberg values measured from grounded ice. The data can be explained from mixing of Atlantic water and glacial melt water from the Greenland Ice Sheet as the major process forming the salinity variations in the Disko Bay water.

The few marine surface samples with salinities below 30 ‰ were collected in areas near to the coast either in the Disko Fjord system or close to major river outlets (Røde Elv, Mudderbugten). These samples illustrate a different salinity-oxygen isotope relationship than

Locality	Spe cification	Water type	Date	La titude	Lon gitud e	Height m	Temp. C	d 180 o/oo
Arktisk Station	rain	precipitation	July 19	69 15.190'	53 31.051'	20		-18,89
Arktisk Station	Drin king water brook	nver water	July 10	69 15.190'	53 31.051	20		-15,38
Arktisk Station	lce from iceberg, coast by A.S. fin e crystallin e	ice	July 16			0		-29,63
Arktisk Station	Ice from iceberg, coast by A.S. coarse crystalline	ice	July 16			0		-25,43
Blæs edal	Spring at east side	spring water	July 21			200		
Blæs edal	Morænesø	lake water	July 21	69 16.171'	53 28.906	100		-13, 75
Blæs edal	Spring at east side, opposite water fall	spring water	July 21			150		-15,72
Engelskmandens Havn	Orchid s pring	spring water	July 14	69 15.588'	53 34.001	0	8,8	-14,54
Engelskm andens Havn	Tardigrad spring	spring water	July 14	69 15.588'	53 34.001	0	13,4	-16,54
Engelskmandens Havn	Spring	spring water	July 14	69 15.883'	53 33.956'	100	4,9	-16,52
Engelskm andens Havn	Thermistor spring, B	spring water	July 14	69 15.588'	53 34.001	0	14,6	-16,66
Engelskmandens Havn	Brook	nver water	July 14	69 15.588'	53 34.001	0	4,8	-16,11
Engelskmandens Havn	Thermistor spring, B	spring water	July 14	69 15.588'	53 34.001'	0	14,6	-16,7
Engelskm andens Havn	Bubble spring, D	spring water	July 14	69 15.588'	53 34.001	0	13,5	-16,34
Engelskmandens Havn	Spring at plate au by basalt wall	spring water	July 14	69 15.883'	53 33.956'	180	3,1	-16,38
Eqalunguit	Tarajungitsok , spring m outh	spring water	July 17	69 33.405	53 36.387'	50	12,1	-17,44
Eqalunguit	Tarajungitsok, spring mouth	spring water	July 17	69 33.405	53 36.387'	50	12,1	-17,44
Eqalunguit	Tarajungitsok , spring m outh	spring water	July 17	69 33.405'	53 36.387'	50	12,1	-17,6
Godhavn	Red River, at bridge	nver water	July 22					-16,04
Isun gua	Spring, 2 nd b og	spring water	July 19	69 43.850'	51 56.437	20		-1,99
Isun gua	Spring, 1 st bog, small basin	spring water	July 19	69 43.763	51 56.437	20		-15,32
lsun gua	Spring, 2 nd b og	spring water	July 19	69 43.850'	51 56.437	20		-15,24
lsun gua	3rd bog	spring water	July 12	69 43.778'	51 56.483'	20		-17,98
Kuan it	Spring, west	spring water	July 9			30		-14,61
Kuan it	Spring, east	spring water	July 9			20		-16,1
Kuan it	Spring, m id	spring water	July 9			20		-15,59
Kvan dale n	Spring m outh, Lym naea Lake	spring water	July 12	454 568	33 143	200		-17,97
Kvan dale n	Lym naea Lake	lake water	July 12	454 568	33 143	30		-15,66
Lyngmark sbræen	Melt water, glacier	glacial	July 21	69 17.318'	53 34.906	840		-15,94
Lyngmark sbræen	Cryochonite holes at glacier	glacial	July 21	69 17.307'	53 35.013'	860		-16,24
Lyngmark sbræen	Melt water, glacier	glacial	July 21	69 17.494'	53 35.533'	920		-16,49
Lyngmark sbræen	Melt water, glacier	glacial	July 21					-15,93
Østerlien	Spring, cave	spring water	July 7	69 15.456'	53 31.990'	100	1,6	-14,71
Østerlien	Spring 1	spring water	July 7	69 15.456'	53 31.990'	100	1,8	-15,34
Østerlien	Spring at drinking water river	spring water	July 7	69 15.456'	53 31.990'	100	2,1	-15,37
Qaru suit	Spring with kv an	spring water	July 17	69 33.100'	53 31.120'	55	5,6	-17,7
Ravn eklø ft	Spring	spring water	July 20	69 15.945'	53 33.775'	160		-15,57
Ravn eklø ft	River, Akuarut	niver water	July 20	69 15.945'	53 33.775'	150		-15,53
Sdr. Strømfjord	River, at bridge	nver water	July 26					-23,7
Sdr. Strømfjord	Lymnaea Lake	lake water	July 26					-15,82
Sdr. Strømfjord	Great Salt Lake	lake water	July 26					-11,47

 Table 2. Fresh water samples, July 2002

the fully marine samples. As seen from Figure 5, a regression line through these samples (salinity <30.0 ‰) intercepts with the Y-axis at an  $\delta^{18}$ O value of -18.5 ‰ (r<sup>2</sup> = 0.99). This value is close to the fresh water  $\delta^{18}$ O values measured from Disko Island (-18 to -15 ‰, see Figure 3) and identifies the lowered salinity of the surface seawater samples to be caused by local fresh-water run-off.

The deeper-water samples fit the same salinity- $\delta^{18}$ O relation as the surface water (see Figure 4 and 5) and obviously belong to the same mixing system. The low temperature of the water mass at 100 m has been explained from vertical convection of surface water during the winter, which does not affect the oxygen isotope composition. The deepest water sample from 300 m has the salinity- $\delta^{18}$ O signature closest to normal Atlantic water.



Figure 2. Results of the marine water analyses. Samples are from Disko Bay, Disko Fjord and Davis Strait west of Disko Island.

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Spring water

The springs at Disko can be divided into three groups, the ephemeral springs, the homothermic low temperature springs (0 to 4°C), and the homothermic warm water springs (8 to 18°C), KRISTENSEN 1988 and 2000. The latter group includes springs with elevated salinity and measurable radioactivity, probably from dissolved radon gas. The warm water springs are geographically related to a Precambrian gneissic basement high underlying the Early Tertiary Disko basalts in a north-south direction, and many of the springs have their outlet at or close to the nonconformity between gneiss and basalt. A remanent volcanic heating from the basalt can probably be excluded as heat source, and no valid explanation of the existence of these warm springs has yet been presented.



Figure 3. Results of the oxygen isotope analyses of fresh-water samples from Disko Island.

We have compared the oxygen isotope composition of spring water from two of the warm water spring localities (Engelskmandens Havn: the Bubble, Orchid, Tardigrad and Thermistor springs and Eqalunguit: Tarajungitsok springs) to a series of homothermic low temperature springs (Figure 6). All springs have  $\delta^{18}$ O values between -18 and -14 ‰ corresponding to the general composition of meteoric water at Disko (Figure 3), and none of the values exclude a meteoric water origin for the spring water. Two of the springs (Cave spring behind Arctic Station and Orchid spring at Engelskmandens Havn) are markedly enriched in <sup>18</sup>O compared to the other springs in the area. Of special interest is the Orchid spring, which flows only a few meters from the other springs at Engelskmandens Havn but differs significantly in both temperature and  $\delta^{18}$ O. The other springs at Engelskmandens Havn have a  $\delta^{18}$ O signature identical to springs at the plateau above the descent and close to the Akuarut brook flowing down from the Devils Canyon. The lower temperature and the enriched oxygen isotope composition of the Orchid spring could possibly be explained from a minor seawater contribution to this spring, but such an explanation would not be valid for the higher located Cave spring.



Figure 4. Plot of oxygen isotope composition of marine water samples versus salinity. Only samples with salinities greater than 30 ‰ have been included. The North Atlantic data point is from GRAIG & GORDON (1965) and has not been used in the calculation of the regression line. Samples have been divided according to their sampling depths.

The remaining springs fall in three  $\delta^{18}$ O groups (Figure 6): the springs from the more northerly localities at Kvandalen and Disko Fjord/ Kuanerssuit including Qarusuit and Tarajungitsok ( $\delta^{18}$ O values between -18 and -17 ‰), the springs at Engelskmandens Havn ( $\delta^{18}$ O between -17 and -16 ‰), and springs east of Godhavn at Lierne behind Arctic Station, Blæsedal and Kuanit ( $\delta^{18}$ O between -16 and -15 ‰). This distribution probably reflects an altitude effect in the <sup>18</sup>O composition of local precipitation. In such a model, springs at Engelskmandens Havn are feed from more <sup>18</sup>O-depleted precipitation falling at higher altitudes (e.g. Lyngmarks Glacier) than the springs at Arctic Station and Kuanit. The more northerly springs at Kvandalen and Tarajungitsok are fed by precipitation depleted in <sup>18</sup>O from the passage over the 1000 m high mountains at south Disko. Fresh surface water from the fjord off Tarajungitsok has an  $\delta^{18}$ O value of about -18 ‰ supporting this interpretation.

Figure 7 is an attempt to evaluate this model. In the figure I have plotted the  $\delta^{18}$ O values for spring and river water from related areas. The atypically enriched samples from the orchid and cave springs are marked separately. No river value exists for the Kvandalen area and a number from the Tarajungitsok area has been used. The figure indicates a direct relationship between isotopic composition of spring and river water in the different local areas and thus supports the hypothesis that springs in most cases are fed from local precipitation.



**Figure 5.** Plot of mixing systems of the Disko Bay marine waters. Fresh-water values are from rivers and melt-water collected at Disko and from icebergs grounded off Godhavn. The mixing line for Disko Bay seawater is from Figure 4

But does this analysis bring any understanding to the origin of the heat in the homothermal warm water springs at Disko? The data clearly exclude warm, deep-seated magmatic or juveline water as a source to the springs. Such water would typically be strongly enriched in <sup>18</sup>O compared to the actual spring waters (Figure 8) and would not follow a meteoric water trend. It is well known that hot volcanic rocks can create meteoric water convection cells where infiltrating surface water is heated by the hot rocks and returned to the surface as hot springs and geysers, but the heat contained in the present system probably is not enough to drive a pure convection system. Further to the north on Nuussuag Peninsula oxidation of pyrite in black shale is known to generate enough heat to ignite the shale, but such rocks are not developed on southern Disko. The coincidence between warm springs and the north-south trending basement high points to these old rocks as the heat source, probably related to decay of radioactive elements as uranium and thorium. No studies have been performed to investigate heat flow from the basement in this area, and no conclusions can be reached based on the existing data. Nevertheless, the present investigation of oxygen isotope composition of the spring water strongly points to a local, meteoric origin of the spring waters, which must have obtained their heat content from contact with the basement rocks along shallow cracks and faults.

# **5. CONCLUSIONS**

During the course in Arctic Biology at the Arctic Station on Disko, North West Greenland, more than 100 water samples were collected for analysis of oxygen isotope composition. Marine waters sampled at varying depths were also analysed for salinity and temperature. Fresh-water samples come from springs, rivers, lakes and glaciers at Disko. The oxygen



**Figure 6**. Spring water samples from Disko Island plotted in a temperature  $\delta^{18}O$  diagram. Names refer to individual springs. Orchid, Bubble, Tardigrad and Thermistor springs all belong to the spring system at Engelskmandens Havn west of Godhavn

isotope data in this report are the first given for the Disko Bay area (see Figure 8 for overview).

- The temperature and salinity data confirm the division of the Disko Bay water column into three layers. The strong correlation between salinity and oxygen isotope data can tentatively be interpreted as reflecting a two-component mixing system between Atlantic water and <sup>18</sup>O-depleted melt water from the Greenland Ice Sheet. Surface waters close to Disko Island are affected by mixing with local fresh-water run-off.
- Spring water, both from warm and cold homothermic springs, have oxygen isotope compositions comparable to local meteoric water, and an origin as deep-seated juveline water can be excluded. Local variations in  $\delta^{18}$ O values can be related to altitude effects as the condensating moisture travels across Disko.
- Oxygen isotope analyses of arctic waters look promising as a hydrological tool at Disko, and further, more systematic sampling programs are recommended.



**Figure 7.** Comparison of spring water and river water  $\delta^{18}O$  values for selected spring systems. The figure illustrates the close relationship between spring water and meteoric water composition. This supports the concept of a local, meteoric origin for the springs.



Oxygen isotope composition of different water types

**Figure 8.** Summary figure of the oxygen isotope composition of different water types at Disko and other Greenland areas. Data on magmatic water are from HOEFS (1987).

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tadpole-shrimp (Lepidurus arcticus). Drawing by S. W. Knudsen

# Diversity and Biomass of Marine Flagellates and Diatoms in the vicinity of Disko Island, West Greenland

Knud Andreas JØNSSON, Lone MUNK & Morten SMITH

Department of Phycology, Botanical Institute, University of Copenhagen

Abstract. Changes in the climate due to e.g. global warming could affect the organisms of the lower trophic levels such as phytoplankton and heterotrophic flagellates and thus change the structure of the food web. Therefore it is important to know more about the composition of these groups. Climate changes are thought to have the highest impact on the polar regions because it would lead to melting of the sea ice and the polar ice caps and thus change the environment. The present study focuses on the biomass and diversity of marine flagellates and diatoms in the area around Disko, Greenland. Both the nanofraction and the netfraction were examined. By counting the cells in an inverted microscope using the Utermöhl method it was possible to fractionate the biomass into ecological and taxonomical groups. Carbon biomasses were calculated on the basis of cell counts with the aid of the computer programme Aquabase. Micrographs were made in both LM and TEM of the encountered organisms in order to document the species diversity. Both biomass and diversity were compared with similar investigations in the Disko area as well as with a similar study in Spitsbergen. The estimated total biomass (heterotrophic and autotrophic) per litre in the present study is within the range 3-82  $\mu$ g C L<sup>-1</sup> (the mean value is 26  $\mu$ g C L<sup>-1</sup>) and this is in accordance with the biomass estimations of similar studies in the area. The diversity of the netplankton is likewise within the range of previous diversity investigations conducted in the area. A total of 81 species distributed over 9 classes was found of which 62 belonged to the netplankton fraction and 19 belonged to the nanoplankton fraction. The nanoplankton however, is less diverse than known from previous studies, which is thought due to less appropriate processing procedures and improvements of methods are therefore suggested.

#### Keywords.

#### **1. INTRODUCTION**

Changes in the climate due to e.g. global warming is expected to have greatest impact in polar regions since even minor temperature changes will dramatically reduce the amount of sea ice and thus alter the structure of the arctic food web. This goes for the endemic sea ice biota as well as the pelagic species, which depend on the duration of the ice cover. Between 1948-1998 the temperatures in the upper 300 metres of the oceans globally have risen 0.3°C (LEVINSEN 2000). There is reason to believe that this rise in temperature will affect the marine phytoplankton quantitatively and qualitatively. Deviations in the constitution of this group could have severe consequences for the delicate balance of the whole arctic ecosystem since marine phytoplankton form the basis of the food web. As fishing constitutes 95% of the total export of Greenland, it would without doubt have dire effects on Greenland's economy and people if the arctic ecosystem was disturbed or changed. This in particular applies to the Disko Bay area which is the most densely populated area in Greenland (LEVINSEN 2000).

Arktisk Station at Qeqertarsuaq is located on the southern tip of Disko Island. It is with its excellent location, well equipped laboratory and the research vessel M/S Porsild a perfect start-out point for profound investigations and analyses of the marine life including the protists. Therefore the area is relatively well studied when it comes to species diversity of netplankton as well as nanoplankton and biomass calculations for the whole community (THOMSEN 1982, CLAUSEN et al. 1994, NIELSEN & HANSEN 1995, TRIER 1998, NIELSEN & HANSEN 1999, LEVINSEN 2000).

The aim of the project was to make a survey of the current biomass composition and diversity of phytoplankton species in the Disko Bay area. We realized that we would hardly be able to

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contribute much to the general species list of the area and decided to focus on the biomass of flagellates. With the use of an inverted microscope (Utermöhl method) as opposed to the much faster and much easier chl. a measurements we wished to fractionate the different flagellate groups (including the nanofraction) and investigate how much each group contributed to the total biomass of protists per litre. Previously the arctic pelagic food web was considered to be rather simple being mostly dominated by diatoms which were then consumed by copepods which were then again consumed by larger animals (NIELSEN & HANSEN 1999). This very simplified view has now been superseded by a greater understanding of the dynamics in an often complicated pelagic ecosystem which includes not only other species rich protist groups like for instance dinoflagellates and nanoflagellates (CLAUSEN et al. 1994, NIELSEN & HANSEN 1999). By fractionating the flagellate groups we would get a much more detailed picture of the ecological importance of the different phytoplankton classes in the Disko area. The emphasis was mainly on the flagellates but of course we looked at the other groups in the water samples as well and compared the findings of the present study with previous findings.

Due to time constraints it is only possible to get a very fragmentary picture of the phytoplankton composition and diversity in the vicinity of Disko Island. However, the present investigation should fill in a gap in the understanding of the biomass distributions in the arctic pelagic food web. And in conjunction with further studies on biomass distributions among different protist classes it should be possible to put together an overall picture of the diversity and biomass distribution in the area around Disko.

The project furthermore gave us knowledge of new methods and techniques, which were often difficult to apprehend and rather time consuming to carry out. Among these were preparations of EM-grids, counting the organisms in an inverted microscope and assembling photo plates. When working in the Arctic conditions change all the time and unpredictable situations inevitably occur. The equipment did not always work as expected and it was not always possible to do things the way we had planned in Denmark. This field project forced us to cope with many unforeseen situations. It made us change plans a lot of times and made us improvise whenever equipment broke down. All in all it taught us what it is like to work far from civilization with limited resources in the Arctic.

# 1.1 Hydrography

Since the distribution of protists in time and space depends on hydrography we have included a brief paragraph addressing this.

The origin of the water masses surrounding Disko is quite complex and changes throughout the year, but the two most important components are the West Greenland Current (WGC) and the melt water from sea ice, icebergs, fjord glaciers and from land.

The WGC coming from the south is relatively warm and saline. Part of the current continues east of Disko, beneath the fresher surface layer, into Disko Bay and out of the Vaigat. The WGC also leads to upwelling along the south coast of Disko. The WGC itself consists of a mixture of the East Greenland Current (relatively cold and fresh) and the Irminger Current (relatively warm and saline). Off Disko the WGC mixes with the Baffin Current creating counterclockwise eddies.

The cold freshwater from the melting of snow, sea ice, icebergs and ice glaciers (most importantly the Ilulissat fjord glacier) passes both west and north around Disko, from Ilulissat and into the open sea.

Throughout the year the contribution of freshwater changes with the shift in temperature. In spring and summer a great quantity of melt water creates a fresher surface layer, which in addition is warmed by the increased solar radiation. This results in a rather stable thermo- and halocline (pycnocline).

In autumn a reduction in the outflow of melt water occurs and this in combination with less solar heating and stronger winds creates a water column with uniform salinity and uniform low temperatures in the upper 80 metres.

Another aspect in the Disko Bay area is the tidal fluctuations. The rising tides can halt outgoing currents, which are either forced back into the bay or towards Kronprinsens Ejlande (ANDERSEN 1981a).



Figure 1. Map indicating the most important currents in the waters around Greenland (modified from HERMANN 1971).

#### **1.2 Introduction to marine phytoplankton**

Since we in this project have identified as many organisms belonging to different groups of protists as possible we will present a brief outline of the major classes. The marine phytoplankton includes all photosynthetic planktonic organisms in the sea. When

investigating phytoplankton other organisms are caught as well. In the division Dinophyta for example there is a considerable number of heterotrophic species (approximately 50%). The heterotrophic dinoflagellates are caught while sampling for photosynthetic dinoflagellates. However, the heterotrophs are considered members of the protozooplankton because of their lack of chloroplasts. The choanoflagellates are a constituent of the kingdom Animalia and therefore considered part of the protozooplankton as well. In fact it is due to tradition more than to taxonomy that this particular group is included in the work of phycologists.

Diatoms on the other hand are extremely important photosynthetic organisms. They are an immensely important group in coastal production as opposed to the nano- and picoplankton that we set out to investigate which are by far the most dominant in the open ocean. This is due to their large surface to volume ratio which is favourable when nutrients are scarce (VAN DEN HOEK et al. 1995).

# **1.2.1 Prasinophyceae (Prasinophytes)**

The prasinophytes are a class in the division Chlorophyta (green algae) and the majority are planktonic flagellates. They are equipped with either 1,2,4,8 or 16 flagella inserted apically or laterally. They also bear several types of scales on the cell body as well as on the flagella. The scales are important for precise identification and are only visible in the electron microscope. Prasinophytes occur in both marine and limnic waters (VAN DEN HOEK et al. 1995).

# 1.2.2 Haptophyceae (Haptophytes)

The haptophytes are predominantly unicellular spherical flagellates with two flagella. They are furthermore equipped with a haptonema which protrudes between the two flagella not unlike a third flagellum. The internal structure is, however, entirely different. The lumen holds a crescent shaped structure of microtubules which is unlike the 9+2 microtubular construction of a flagellum. The cell surface is covered with cellulose scales and some species have calcified scales (coccolithophorids). The haptophytes are mostly marine with only a minute number of the about 500 species living in freshwater (VAN DEN HOEK et al. 1995).

# 1.2.3 Chrysophyceae (Golden algae)

These organisms have yellow to brown coloured chloroplasts and this feature has given them their name. They are members of the division Heterokontophyta. Most species are unicellular or colonial and are often equipped with a long forward pointing flagellum with two rows of tripartite hairs (mastigonemes) and a shorter smooth flagellum pointing backwards. This is common for most heterokonts. The flagella arise apically on the cell. The chrysophytes are most abundant in fresh water and only a few species are found in the sea (VAN DEN HOEK et al. 1995).

# 1.2.4 Choanoflagellida (Choanoflagellates)

There has been much debate over the correct taxonomic position of the choanoflagellates. It has been suggested that they belong to Chrysophyceae because of the superficial similarity with this group. However, the vast majority of people now considers the choanoflagellates as being part of the kingdom Animalia because of the close and very convincing resemblance to choanocyte cells in sponges (THOMSEN 1982). The status of the choanoflagellates as a sister group to the metazoa is also supported by molecular data (CHRISTENSEN 1997). Choanoflagellates are unicellular organisms with one apical flagellum and an anterior ring of tentacles that creates a collar-like structure. The cells are characterized by the lorica composed of silicified ribs (costae) with which they surround themselves. Species identification and systematics of the choanoflagellates rely entirely on the arrangement of the

lorica. Choanoflagellates are very common and often one of the most dominating (number of cells) plankton groups in marine and brackish waters (THOMSEN 1982).



Figure 2: Schematic overview of a choanoflagellate-cell with the most characteristic features for identification (modified from TRONDSEN 1997).

# 1.2.5 Cryptophyceae (Cryptophytes)

The cryptophytes are predominantly bilaterally symmetrical unicellular flagellates. The cell is drop-shaped with a subapical gullet containing numerous trichocysts. The flagella arise from just inside the gullet. The two flagella are of unequal length. The longer flagellum is equipped with two rows of hairs but these are not tripartite like the hairs of the heterokonts. The shorter flagellum bears one row of hairs, which are shorter than the hairs on the long flagellum. This class is also of considerable evolutionary interest because it holds an organelle, the nucleomorph, which is the reduced nucleus of an autotrophic endosymbiont (a red algal cell) in a heterotrophic ancestor of the cryptophytes. This morphological piece of evidence in favour of the endosymbiont theory is also supported by molecular studies of the nucleomorph (VAN DEN HOEK et al. 1995).

# **1.2.6 Bacillariophyceae (Diatoms)**

The diatom cell is surrounded by a box and lid shaped construction called the frustule. This box can assume two general shapes: 1) pennate (bilatterally symmetrical) or 2) centric (radially symmetrical). Both shapes can be unicellular or colonial. As a member of the heterokonts we would expect them to be flagellated which they normally are not. Only the reproductive cells (zoids) of the radially symmetrical order Centrales exhibit this trait. Of course the morphology of the frustule shows great variation with as many as 100,000 diatom species being proposed. Diatoms are not only diverse they are also very important in carbon fixation. One estimate suggests that they are responsible for 20-25% of the dry mass produced on Earth per year. Therefore much marine life depends on these organisms in order to survive (VAN DEN HOEK et al. 1995).



**Figure 3.** Schematic overview of a diatom cell with the characteristic box and lid form (modified from HASLE & SYVERTSEN 1997)



**Figure 4.** Schematic overview of the dinoflagellate cell. A) Desmokont cell. B) Dinokont cell. C) Thecate cell. D) Athecate (naked) cell (modified from FAUST & GULLUDGE 2002).

### **1.2.7 Dinophyceae (Dinoflagellates)**

The dinoflagellates differ from almost all other organisms by having spiralized chromosomes (the euglenoids also present this feature) in the cells' interphase referred to as a dinokaryon.

There are only few exceptions to this e.g. the genus *Noctiluca*. The dinoflagellates are nearly always flagellated cells. The flagella emerge where the cingulum (transverse furrow) and the sulcus (longitudial furrow) intersect. The so-called dinokont dinoflagellates have one flagellum running in the cingulum and one in the sulcus. Other flagella arrangements are present in the dinoflagellates, but since we did not find any of these it will not be further described. The dinoflagellates are divided into two major groups: the thecate and the athecate (naked). The thecate dinoflagellates carry a specific number of cellulose plates in flat vesicles (amphiesma vesicles) just beneath the plasmalemma. The naked dinoflagellates have these vesicles too but no cellulose is found here. 90% of the dinoflagellates are marine and the rest resides in fresh water. Dinoflagellates are not only photosynthetic organisms about 50% are mixotrophic or heterotrophic (VAN DEN HOEK et al. 1995).



**Figure 5.** *Map of Disko Island indicating the sampling locations. Station numbers in bold represent those water samples used for biomass estimation (modified from ANDERSEN 1981b).* 

Station	Date	Position	Locality
0	05.07.2002	N69°11,W53°30	"fixed station"
1	05.07.2002	N69°17,W53°13	lppik
2	08.07.2002	N69°45,W54°51	Enoks Havn, Mellemfjord
3	08.07.2002	N69°45,W54°35	Mellemfjord
4	09.07.2002	N69°47,W54°57	Mellemfjord
5	09.07.2002	N69°11,W54°59	Disko Fjord
6	09.07.2002	N69°28.025,W54°16.009	Disko Fjord
7	09.07.2002	N69°18.408,W54°13.443	Blåfjeld
8	12.07.2002	N69°38,W51°46	Mudderbugten
9	12.07.2002	N69°25,W52°20	Flakkehuk
10	12.07.2002	N69°18,W52°11	Skansen
11	15.07.2002	N69°08.206,W53°33.299	Brændevinsskærene
12	15.07.2002	N69°03.838,W53°30.912	Brændevinsskærene
13	15.07.2002	N68°58.759,W53°22.684	Kronprinsens Ejlande

**Table 1.** Positions of the stations at which samples were collected. Station numbers in bold represent those water samples used for biomass estimation.

# 2. MATERIALS AND METHODS

#### 2.1 Sampling site

Between July 3rd and July 26th 14 stations were sampled in the vicinity of Disko Island. The sampling area covered an area from Mudderbugten in the east to Mellemfjord in the west. The last 3 stations were positioned around Kronprinsens Ejlande and Brændevinsskærene (see figure 5).

#### 2.2 Collection of water samples at sea

At all stations phytoplankton was collected using a 20  $\mu$ m plankton net. The plankton net was moved up and down in the upper ca. 20 metres of the water column until the collected water sample had a yellowish colour. Live samples were kept cool using blue ice and a second net haul was fixed in Lugol iodine.

Initially it was planned to collect water samples with a Niskin bottle at three depths. The first at 2.5 metres, the second at the flourescense peak and the third underneath the pycnocline (thermocline or halocline). In order to find the depths with the flourescense peak and the pycnocline we used a CTD to obtain a complete profile of the water column. Unfortunately the CTD only worked properly at the first station. We were therefore forced to collect samples based on the information we had obtained from the first station in combination with the visible depth of the plankton net, when it was lowered into the water. From all depths we kept live and lugol fixed samples with a volume of 500 mL.

At stations 0, 1 and 2 we took samples according to the plan mentioned above, but soon realized that processing of the samples was extremely time consuming (1-2 hours of centrifugation per sample) and hence only samples from 2.5 metres and at the estimated chl.a maximum in a depth of 20-30 metres were collected from the remaining stations.

#### 2.3 Light microscopy

The laboratory at Arktisk Station where all processing of live samples was done was air-conditioned to  $5^{\circ}$ C in order to keep the organisms alive. In the laboratory we looked at the live netplankton samples and identified as many species as possible in the light microscope. This is particulary necessary when identifying the athecate dinoflagellates as they round up in fixed samples thus becoming virtually impossible to identify. The plankton organisms were photodocumented using a Leitz Dialux 20 microscope, a Sony DXC-390 3ccd colour video camera and a Sony digital video cassette recorder DSR-V10P. The lugol fixed samples were used for estimating the biomass and as a platform for identification of *Chaetoceros* spp. and *Thalassiosira* spp. upon our return to Denmark.

#### 2.4 Transmission electron microscopy of uranyl acetate stained whole mounts

The nanoplankton fraction (2-20  $\mu$ m) was also collected from the different depths and prepared for EM-grids for later species identification using transmission electron microscope. The water samples were coarsely filtered through a 20  $\mu$ m filter to isolate the nanoplankton fraction. This fraction was further more centrifuged for 30 min at 2500 rpm to concentrate the sample. We started to centrifuge 1 L/station, but as one of the centrifuges with large capacity broke down, it was necessary to only centrifuge approximately 0,5 L. Droplets of this concentrate were then placed on a formvar coated copper grid and immediately afterwards they were exposed to the vapour from four "hanging" drops of a 2% solution of OsO<sub>4</sub> for 40 seconds. This fixative kills the organisms instantly and therefore prevents the break down of the cytoskeleton which means that the organisms can be correctly identified and importantly that the flagella and body scales stick to the nanoflagellates. When dry the grids were rinsed very gently in a Petri dish containing distilled water in order to remove the corrosive salt crystals (MOESTRUP & THOMSEN 1980).

Back in Denmark the grids were stained with uranyl acetate. The electron dense uranyl component adheres to phospholipids and proteins giving the organisms a useful contrast in the electron microscope. The grids were put formvar side down on a drop of uranyl acetate and left there in darkness for 20 min. Afterwards the grids were rinsed twice in double distilled water (MOESTRUP & THOMSEN 1980). The electron microscope used was a Jeol 100SX and the film used was Kodak electron microscopy film.

The photo plates were made from the EM and LM micrographs. After having made a rough layout in hand the plates were scanned in a computer and were then "fine tuned" with the computer program Adobe Photoshop ver. 6.0 and 7.0.

#### 2.5 Biomass estimation

50 mL of the Lugol fixed samples were poured into a 50 mL Hydro Bios sedimentation chamber. After 24 hrs. the suspended organisms had sunk to the bottom of the chamber ready to be counted in an inverted microscope by use of the Utermöhl method (UTERMÖHL 1958). We used a Leitz Labovert FS inverted microscope and a Nikon TMS inverted microscope. The idea was to count at least 400 cells of each taxon because this equals a 10% margin of error (OLRIK 1991). Often this was not possible because the chamber did not hold 400 cells of each taxon and furthermore the counting procedure was extremely time consuming (approximately 6 hrs. per chamber). Thus regarding the nanoplankton we agreed on counting four rows (diameter of the chamber) at 40x equalling an area of 0.32 mm<sup>2</sup> as we were working on a deadline. This means that for many taxa the margin of error due to the counting exceeds 10%.

The number of diameters counted along with the results of the counting was put into Aquabase. Aquabase is a computer program that uses cell volume together with genus specific carbon contents to estimate biomass. For correct volume estimation several groups were split in distinct size classes. The computed data was presented using Sigmaplot 8.0.

#### 2.6 The reason to use sedimentation chambers for biomass estimation of phytoplankton

Often total biomass estimations are based on the amount of chl. a in a certain amount of water. However, the amount of chl. a in an organism depends on the amount of sun light which means that a period with little sun light will make the organisms produce much more chl. a than in periods with a lot of light. The amount of chl. a therefore will vary over the season and perhaps even vary on a daily basis. Biomass estimations based on spectrophotometric measurements of chl. a are therefore potentially inaccurate as they are either underestimates or overestimates depending on the season (DAUGBJERG pers. comm.). Using the sedimentation chambers the heterotrophic organisms are not left out of the biomass calculation which would be the case if estimations were based on measurements of chl. a only. It is without doubt a much more exact method but it is also extremely time consuming.

#### **3. RESULTS**

#### **3.1 Diversity**

All cell dimensions given in this chapter are litterature values.

#### 3.1.1 Prasinophyceae

# Pyramimonas Schmarda 1850

# Pyramimonas cirolaneae Pennick 1982 (Plate 1.A-B)

The cell is 4-8  $\mu$ m long and 4  $\mu$ m wide. The cell is pyramid shaped with four equally long flagella. At the anterior end it has a stigma consisting of a single layer of globules. Trichocysts are also present. It is virtually impossible to distinguish between *P. cirolaneae* and *P. grossii* in light microscope. The box scales and crown scales are however, distinguishable in TEM. The box scales of *P. cirolaneae* are divided into four quadrants and the crown scales are spiked. Previously recorded in the Barents Sea and the English Channel. This is the first finding in Greenland (MCFADDEN et al. 1986).

Pyramimonas sp. nov. (Plate 1. C-D)

# Pyramimonas virginica Pennick 1977 (Plate 1.E-H)

The cell is 2-3.5  $\mu$ m long and 2  $\mu$ m wide. It is of pyramidial shape and has four flagella. Trichocysts are found in the lobes of the anterior end. A stigma composed of a single layer of globules is also present anteriorly. It differs from all other trichocyst bearing members of the genus *Pyramimos* by having hexagonal basket scales. Previously found on the east coast of USA and in Greenland (MCFADDEN et al. 1986).

# 3.1.2 Haptophyceae

# Pappomonas Manton & Oates 1975

Pappomonas flabellifera Manton & Oates 1975 var. borealis (Plate 2.A and 2.D)

Small autotrophic cell with two flagella and a short haptonema (HEIMDAL 1997). Heterococcolithophorid; the cell is covered by oval coccoliths and around the flagellar pole the coccoliths have an appendage in the shape of a stalk with two triangular plates attached (resembles the end of an arrow). The species is divided in two variants: var. *flabellifera* and var. *borealis*. Var. *borealis* has more dentate coccolith appendages and the columnar stalk and the plates overlap for a longer distance. Temperate and arctic regions in the northern hemisphere (MANTON et al. 1976a, THOMSEN et al. 1988).

# Pappomonas virgulosa Manton & Sutherland 1975 (Plate 2.B)

Resembles *Pappomonas flabellifera*, but instead of the columnar stalk and the two triangular plates, the stalk ends in a terminal tuft of four finger-like rods. Probably cosmopolitan (MANTON & SUTHERLAND 1975, THOMSEN et al. 1988).

# Phaeocystis Lagerheim 1893

# Phaeocystis pouchetii (Hariot) Lagerheim 1893 (Plate 2.E-G)

This species can be observed in two different life stages. It has a non-motile colony-forming stage and a free-living motile stage. In the colony-forming stage the cells lose their flagella and haptonema and lie in a gelatinous colony. In the motile stage the cells have a short haptonema and two flagella of equal length (approximately 1,5 times the cell length). The cell is 4-8 µm long and contains two chloroplasts. The presence of *P. pouchetii* in samples can



**Plate 1.** *A)* Pyramimonas cirolanae. *B)* Box scale of *P*. cirolanae. *C)* Pyramimonas sp. nov. *D)* Crown, footprint & limuloid scales of Pyramimonas sp. nov. *E) P. virginica. F)* Limuloid scale of *P. virginica. G)* Diamond shaped scale of *P. virginica. H)* Hexagonal basket scale of *P. virginica.* 



Plate 2. A) Pappomonas flabellifera var. borealis. B) P. virgulosa. C) Apedinella radians. D)
P. flabellifera var borealis close up of the cocolithphorids. E) Phaeocystis pouchetii.
F) P. pouchetii and its chitin threads in a five-star pattern. G) P. pouchetii in the colony forming life stage.

also be revealed by the observation of a five-pointed star pattern made of discharged chitin threads. Distributed in cold water in both hemispheres (THRONDSEN 1997).

# 3.1.3 Dictyochophyceae

# Apedinella Throndsen 1971

Apedinella radians (Lohmann) Campbell 1973 (Plate 2.C)

The cells' length are 6-10  $\mu$ m. Has six yellow to brown coloured chloroplasts. The cell is covered with cellulose scales of which 4-9 bear easy recognizable spiny scales. Distributed coastally in the Atlantic, Arctic, Mediterranean and Pacific (TRONDSEN 1997).

# 3.1.4 Chrysophyceae

# **Dinobryon** Ehrenberg 1834

Dinobryon balticum (Schütt) Lemmermann 1900 (Plate 3.A-C)

Forms large tree-like colonies of ochromonadoid cells in loricae. The loricae are 50-66  $\mu$ m long in the basal part of the colony and 32-35  $\mu$ m long in the distal part and 3-5  $\mu$ m wide. Mixotrophic. Distributed in the Baltic Sea, Atlantic Ocean and the Arctic (THRONDSEN 1997).

Unidentified heterotrophic flagellates (Plate 3.D-E)

Unidentified autotrophic flagellate (Plate 3.F)

# 3.1.5 Choanoflagellida

## Bicosta Leadbeater 1978

#### *Bicosta spinifera* (Throndsen) Leadbeater 1978 (Plate 4.A)

The lorica consists of just seven costal strips. The two slightly uneven longitudinal costae cross midway. The posterior spine is terminally S-shaped and the total length of the lorica is 45-80  $\mu$ m long. The number of tentacles is usually around 30. Worldwide distribution in waters below 16 °C (MANTON et al. 1980).

#### Bicosta minor (Reynolds) Leadbeater 1978 (Plate 4.B-D)

The lorica of *B. minor* consists of only seven costal strips. They are arranged as two longitudinal costae which meet posteriorly. The lorica length of this species in Greenland is 17-34 µm. Widely distributed (THOMSEN 1982).

# Crinolina Thomsen 1976

#### Crinolina aperta Leadbeater 1975 (Plate 4.F)

The lorica is shaped like a barrel that is open in both ends. The width of the anterior end is 17-25  $\mu$ m and the width of the posterior end 25-35  $\mu$ m. The length of the lorica is 45-50  $\mu$ m. The lorica is composed of twelve longitudinal costae and two transverse costae. The protoplast (when kept intact during fixation) is centrally located. The type specimen originates from northern Canada (MANTON et al. 1975).

# Pleurasiga Schiller 1925



**Plate 3.** *A)* Colony of Dinobryon balticum. *B)* Single D. balticum with lorica. *C)* Single D. balticum. *D)* Unidentified heterotrophic nanoflagellate (I). *E)* Unidentified heterotrophic nanoflagellate. *F)* Unidentified autotrophic nanoflagellate.


Plate 4. A) Bicosta spinifera. B & D) B. minor. C) B. minor and Parvicorbicula serrulata. E) P. serrulata. F) Crinolina aperta.

Pleurasiga minima Throndsen 1970 (Plate 5.)

The protoplast is enclosed in a tight fitting broadly amphora-shaped lorica. The lorica is composed of seven longitudinal costae and two transverse costae of equal size. The anterior junctions of the longitudinal costae and the transverse costae are T-junctions. The lorica measures approximately 10  $\mu$ m both in width and length. From the protoplast a long flagellum protrudes. Cosmopolitan (THRONDSEN 1970, MANTON et al. 1976b and HANSEN et al. 1989).



Plate 5. Pleurasiga minima.



Plate 6. A) Unidentified choanoflagellate. B) Parvicorbicula quadricosta. C) Unidentified choanoflagellate. D) Acanthoecopsis sp. E) Conion groenlandicum.

# Conion Thomsen 1982

*Conion groenlandicum* Thomsen 1982 (Plate 6.E)

Cone-shaped lorica constructed of approximately eleven longitudinal and three transverse costae. The lorica is 13-14  $\mu$ m long and 9-12  $\mu$ m wide. The longitudinal costae are composed of four or five costal strips. The anterior longitudinal costal strips join the next costal strip two-thirds from the anterior end. At the posterior end of the lorica the longitudinal costae join

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and a pedicel with a length of 7-10  $\mu$ m is formed. Known from arctic and subarctic regions (THOMSEN 1982).

## Parvicorbicula Deflandre 1960

#### Parvicorbicula quadricostata Throndsen 1970 (Plate 6.B)

The lorica consists of four longitudinal costae and two transverse costae. The inner transverse costa creates a square with a longitudinal costa in each corner. The outer transverse costa and the four longitudinal costae connect in T-joints. The species is observed in the northern hemisphere (HANSEN et al. 1989).

## Parvicorbicula serrulata Leadbeater 1975 (Plate 4.C and 4.E)

*P. serrulata* is characterized by the broad and serrated costal strips. The lorica is composed of seven longitudinal and two transverse costae. The lorica ends in a posterior stalk, which in the top consists of two costal strips and further down consists of single costal strips. The species is only observed in the northern hemisphere (HANSEN et al. 1989).

## 3.1.6 Bacillariophyceae

## **Centric species**



Figure 6. Schematic overview of a Chaetoceros-cell showing the most characteristic features for identification (modified from HASLE & SYVERTSEN 1997).

## **Chaetoceros** Ehrenberg 1844

Chaetoceros concavicornis Mangin 1917 (Plate 7.A-B)

Cells are 12-30  $\mu$ m wide (apical axis) and connected to form a straight chain. The valves are unlike; the upper valve is rounded and setae arise almost from the centre, the lower valve is flat and the setae arise almost from the valve corners. All setae bend towards the lower end and become broader and with spines further out. Cosmopolitan, except in the southern cold water region, common in the arctic, subarctic and temperate regions (CUPP 1943, HASLE & SYVERTSEN 1997).

## Chaetoceros convolutus Castracane 1886 (Plate 7.A-B)

Cells are 10-27  $\mu$ m wide and connected in a chain that sometimes twists around the pervalvar axis. The shape of the cells is very similar to *C. concavicornis*, but the setae do not increase in width distally. Both cells and setae contain numerous small chloroplasts (RINES & HARGRAVES 1988). Girdle zone is broad about 1/3 of the cell. Cosmopolitan (HASLE & SYVERTSEN 1997).

## *Chaetoceros debilis* Cleve 1894 (Plate 7.C)

Apical axis is 12-30 µm long. The cells form spirally twisted chains and have slightly rounded corners. The intercalary setae are relatively short and originate within the valve margin The terminal setae are likewise short and appear similar to the intercalary setae. Apertures nearly rectangular. Each cell has one chloroplast. Worldwide distribution, mainly in cold water (HASLE & SYVERTSEN 1997, JENSEN & MOESTRUP 1998).

## Chaetoceros diadema (Ehrenberg) Gran 1897 (Plate 7.D)

Cells are connected to form chains. Apical axis is 10-50  $\mu$ m long. Setae are stiff, originate inside the valve margin and cross at the chain edge. One chloroplast per cell. Resting spores are conspicuous with dissimilar valves. The primary valve is convex and has dichotomously branching spines; the secondary valve is smooth and convex. Cosmopolitan (HASLE & SYVERTSEN 1997).

#### *Chaetoceros furcellatus* Bailey 1856 (Plate 7.E)

Cells are 8-20  $\mu$ m wide (apical axis). The chain is straight or somewhat curved. The intercalary setae arise just inside the valve margin and they are relatively thin. The apertures are rectangular, sometimes compressed in the centre. Each cell contains one chloroplast. This species is easily identified by its resting spores. The resting spores lie in pairs within the mother cell, are smooth and have thick setae, which are fused for a long distance (sometimes the setae also twist). Distributed in the northern cold-water region (HASLE & SYVERTSEN 1997).

#### *Chaetoceros decipiens* Cleve 1873 (Plate 7.F)

Cells are 9-84  $\mu$ m wide (Cleve 1873: 27-34 $\mu$ m wide) and connected by the corners to form straight chains. Sibling setae are fused for a length of two to three setae diameters. Terminal setae are shorter, but thicker than the intercalary ones. The intercalary setae diverge at an acute angle. Apertures between cells are slit-like to elliptic. Cosmopolitan (CUPP 1943).

# *Chaetoceros socialis* Lauder 1864 *forma socialis* Proschkina-Lavrenko 1963 and *forma radians* (Schütt) Proschkina-Lavrenko 1963 (Plate 7.G)

The 2-14  $\mu$ m wide cells form short chains. The corners of adjacent cells do not touch, but the setae cross just at the valve margin. The setae are of unequal length; three setae are short and the fourth is long and entwine with other long setae (both from the original chain and other chains) in the centre of the colony. *Forma socialis* is with its smooth resting spores

distinguished from *forma radians*, which has spiny resting spores. Cosmopolitan (HASLE & SYVERTSEN 1997, JENSEN & MOESTRUP 1998)



**Plate 7.** *A-B)* Chaetoceros concavicornis/convolutus. C) C. debilis. D) C. diadema. E) C. furcellatus with resting spores. F) C. decipiens. G) C. socialis/radians.

# Thalassiosira Cleve 1873 emend. Hasle 1973

## Thalassiosira nordenskioeldii Cleve 1873 (Plate 8.A-B)

Cells are octagonal in girdle view and circular in valve view. The valves are 10-50  $\mu$ m in diameter (HASLE & SYVERTSEN 1997) and slightly concave in the middle. An organic thread from the central strutted process connects the cells. The connecting thread is no longer than the pervalvar axis. From the margin of the valve face a ring of thinner threads can be observed. Distributed in the northern cold water to temperate region (CUPP 1943, HASLE & SYVERTSEN 1997).

*Thalassiosira anguste-lineata* (A. Schmidt) Fryxell & Hasle 1977 (Plate 8.C) Cells are box-shaped with rounded corners in girdle view. The diameter is 14-78 µm (HASLE & SYVERTSEN 1997). The cells are connected by 4-9 threads. Cosmopolitan (CUPP 1943).

## *Bacterosira* Gran 1900

*Bacterosira bathyomphala* (Cleve) Syvertsen & Hasle 1993 (Plate 8.F)

In girdle view the chain consists of tightly connected cells with abutting valve faces. The diameter is  $18-24 \mu m$  and the length of the cell is usually a little longer. The cell wall of this centric diatom is only weakly silicified. Distributed in the northern cold water region (HASLE & SYVERTSEN 1997).

## *Leptocylindrus* Cleve 1889

## *Leptocylindrus danicus* Cleve 1889 (Plate 8.H)

Cylindrical cells with thin cell walls. Cells are 5-16  $\mu$ m wide and 2-10 times as long. Neighbouring cells are connected over the whole valve face, one cell is slightly convex and the other slightly concave. Many small rounded chloroplasts are evenly dispersed in the cell. Intercalary bands are present, but very difficult to see in LM. The species is cosmopolitan, but more common in the arctic to temperate region, than in the antarctic and subantarctic region (HASLE & SYVERTSEN 1997).

#### Pennate diatoms

*Nitzschia longissima* (Brébisson) Ralfs 1861 / *Cylindrotheca closterium* (Ehrenberg) Lewin & Reimann 1964 (Plate 8.E)

It is necessary to study these two species in EM to distinguish them and the taxonomic position is still much debated. In LM the cells are long (linear to lanceolate) and pennate with two centrally placed chloroplasts. The cell is slightly thicker in the middle where the chloroplasts are situated. The apical axis is 30-400  $\mu$ m for *C. closterium* and 125-450  $\mu$ m for *N. longissima*. The transapical axes are 2.5-8  $\mu$ m (*C. closterium*) and 6-7  $\mu$ m wide (*N. longissima*). Cosmopolitan (HASLE & SYVERTSEN 1997).

#### Pseudo-nitzschia H. Peragallo 1897-1908

Pseudo-nitzschia cfr. seriata. (Cleve) H. Peragallo 1897-1908 (Plate 8.I)

Cells are elongate and spindle-shaped in girdle view. The cells are overlapping at the ends to form chains. Two chloroplasts on either side of the median transapical plane. The are 95-115  $\mu$ m long and 6.5-7  $\mu$ m wide (CUPP 1943). It is distributed is temperate to cold water in the northern hemisphere (HASLE & SYVERTSEN 1997).



Plate 8. A-B) Thalassiosira nordenskioeldii. C) T. anguste-lineata. D) T. sp. E) Nitzschia longissima/Cylindrotheca closterium. F) Bacterosira bathyomphala. G) Licmophora sp. H) Leptocylindrus danicus. I) Pseudo-nitzschia cfr. seriata. J-K) Unidentified pennate diatom. L) Unidentified diatom.

Unidentified pennate diatom (Plate 8.J-K)

Unidentified diatom (Plate 8.L)

# **3.1.7 Dinophyceae**

# Thecate dinoflagellates

# **Dinophysis** Ehrenberg 1839

Dinophysis norvegica Claparéde & Lachmann 1859 (Plate 9.A-C)

Cells are rather variable but tend to be large and ovoid 48-80 µm long, 39-70 µm wide (Faust & Gulledge 2002). Hypotheca pointed with antapical protrusions. The widest part of the cell is halfway between the lower cingular list and the antapex just above third rib (R3) of the left sulcal list. Thecal plates are heavily areolated, areolae are large and with pores. Phototrophic. Neritic; widespread in cold and temperate waters in the northern hemisphere (DODGE 1982, HANSEN & LARSEN 1992, FAUST & GULLEDGE 2002).

## Dinophysis rotundata Claparéde & Lachmann 1859 (Plate 9.D)

Medium sized cell 36-56 µm long, 36-43 µm wide (FAUST & GULLEDGE 2002). In lateral view a rounded cell with convex apex and antapex, in ventral view the cell looks more compressed but still with convex sides. Small cap-like epitheca. Left sulcal list is sigmoid and widens posteriorly, its length extends over half the length of the hypotheca. The deepest part of the cell is between second rib (R2) and third rib (R3) of the left sulcal list. Heterotrophic. Cosmopolitan (DODGE 1982, HANSEN & LARSEN 1992, FAUST & GULLEDGE 2002).

## Dinophysis acuminata Claparéde & Lachmann 1859 (Plate 9.E-F)

Small to medium sized cell 38-58 µm long, 30-40 µm wide (FAUST & GULLEDGE 2002). Almost oval or elliptical in shape. The antapex is rounded with more or less well-developed posterior protrusions. Left sulcal list extends beyond the midpoint of the cell. Depending on the age of the cell the thecal plates are covered with more or less prominent areolae each with a pore. Phototrophic. Neritic; cold and temperate waters worldwide (DODGE 1982, HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997, FAUST & GULLEDGE 2002).

## Dinophysis cfr. braarudii Nordli 1951 (Plate 9.G)

The cell is 25-32  $\mu$ m long, 21-29  $\mu$ m wide in side view and 18-20  $\mu$ m wide in ventral view. The cell resembles *D. rotundata* in the side view but it is more arrow shaped when viewed from the ventral side. The wing has three spines. This species is not formally described but only mentioned by Nordli in his work from 1951. Earlier reported from the Barents Sea and West Greenland (NORDLI 1951, CLAUSEN et al. 1994).

## Gonyaulax Diesing 1866

## Gonyaulax cfr. spinifera (Claparéde & Lachmann) Diesing 1866 (Plate 9.H-J)

A medium sized cell 24-50  $\mu$ m long, 30-40  $\mu$ m wide. The epitheca has convex sides leading into a rather short apical horn. The hypotheca is rounded and has a variable number of antapical spines (usually two). The cingulum is descending two or more cingulum widths. The sulcus is sigmoidal and extends from apex to antapex. The theca is prominently reticulated. This species is highly variable and might actually be split into several species on account of six different cyst-types that all turned out to be species recognized as *G. spinifera*.

Phototrophic. Cosmopolitan (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

# Protoceratium Bergh 1881

## *Protoceratium reticulatum* (Claparéde & Lachmann) Bütschli 1885 (Plate 9.K) Synonym: *Gonyaulax grindleyi* Reinecke 1967

A medium sized cell 28-43  $\mu$ m long, 25-35  $\mu$ m wide with a subspheroidal shape. The epitheca is conical while the hypotheca is rounded and longer than the epitheca. Theca is heavily reticulated with a pore at the centre of each reticulation. Cingulum slightly above the middle of the cell and the sulcus straight almost reaching the antapex. Phototrophic. Worldwide distribution in cold to temperate waters (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

# Ceratium Schrank 1793

# Ceratium longipes (Bailey) Gran 1902 (Plate 10.A and 10.C)

A large cell up to 250 µm long, 40-60 µm wide (DODGE 1982, HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997). The hypothecal horns bend anteriorly, the right one being nearly parallel to and occasionally as long as the apical horn, the left one a great deal shorter and curved. Is rather similar to *C. horridum* but tend to be more robust and with the apical horn bent to the right. Phototrophic. Coastal; arctic to cold temperate waters (DODGE 1982, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

## Ceratium arcticum (Ehrenberg) Cleve 1901 (Plate 10.B and 10.D)

A large cell about the size of *C. longipes* with which it is easily confused. The apical horn is directed to the right. The hypothecal horns are only slightly curved compared to *C. longipes* and form an almost straight line. Phototrophic. Cold water in the northern hemisphere (HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997).

## Protoperidinium Bergh 1881

## Protoperidinium depressum (Bailey) Balech 1974

A large cell 116-200  $\mu$ m long, 116-144  $\mu$ m wide. The epitheca is concave towards the horn like apex. The hypotheca is convex near the girdle and ends in two divergent antapical horns. The cytoplasm can be pink. Cosmopolitan (DODGE 1982, THOMAS 1997).

## Protoperidinium bipes (Paulsen) Balech 1974

A small cell 20-35  $\mu$ m long, 17-19  $\mu$ m wide which is quite easily identified from its characteristic shape. The epitheca is triangular ending in a long apical horn. The hypotheca is shorter than the epitheca and has two antapical spines that diverge outwards. The thecal plates are delicate and difficult to analyse but not particularly important for proper identification. Heterotrophic. Is found throughout the Atlantic Ocean, the Mediterranean and the Baltic Sea (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994)

## Protoperidinium brevipes (Paulsen) Balech 1974

A small cell 20-40  $\mu$ m long, 20-40  $\mu$ m wide, which is characterized by its pentagonal shape and the two rather indistinct antapical spines. Cingulum is wide and the sulcus is deep and broadens towards the antapex. Heterotrophic. Coastal cold water (STEIDINGER & TANGEN 1997).



**Plate 9.** *A-C)* Dinophysis norvegica. D) D. rotundata. E-F) D. acuminata. G) D. cfr. braarudii. H-J) Gonyaulax cfr. spinifera. K) Protoceratium reticulatum.

#### Protoperidinium pellucidum Bergh 1881

Medium-sized cell 40-68  $\mu$ m long, 36-70  $\mu$ m wide (Dodge 1982) that is more rounded than *P. pallidum* and only slightly flattened dorsoventrally. A pyriform cell with a short apical horn and two antapical spines. The sulcus widens towards the antapex and the left sulcal list may give the appearance of a third spine. The cingulum is bordered by lists supported by spines. Heterotrophic. Cosmopolitan in temperate to tropical waters (DODGE 1982, STEIDINGER & TANGEN 1997).



Plate 10. A & C) Ceratium longipes. B & D) C. arcticum.

## Protoperidinium pallidum (Ostenfeld) Balech 1973 (Plate 11)

In size a very variable cell 38-107 µm long, 30-85 µm wide (DODGE 1982, HANSEN & LARSEN 1992). An elongate pyriform cell with two divergent antapical spines (the right spine usually longer than the left) and a short apical spine. Dorsoventrally flattened and with reticulated surface. Heterotrophic. Coastal and oceanic worldwide (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et al. 1994, STEIDINGER & TANGEN 1997).

## Protoperidinium steinii (Jørgensen) Balech 1974

A medium-sized cell 39-60  $\mu$ m long, 22-44  $\mu$ m wide which is pyriform with an elongated apical horn and a rounded hypoteca with long (9-14  $\mu$ m) antapical spines. The cingulum is bordered by lists supported by spines and the surface of the cell is prominently reticulated. Heterotrophic. Worldwide distribution (DODGE 1982, HANSEN & LARSEN 1992).



Plate 11. Protoperidinium pallidum.

# Heterocapsa Stein 1883

Heterocapsa triquetra (Ehrenberg) Stein 1883 (Plate 13.K)

Small thecate cell (<20  $\mu$ m), which in LM appears to be athecate. The epitheca is rounded to conical and the hypotheca is attenuated into a horn. Slightly displaced cingulum. Distributed worldwide (STEIDINGER & TANGEN 1997).

## Athecate dinoflagellates

# Gyrodinium Kofoid & Swezy 1921

## Gyrodinium spirale (Bergh) Kofoid & Swezy 1921 (Plate 12.G-M)

Large spindle-shaped asymetric cell, 40-200 (usually 50-103)  $\mu$ m long. The cingulum is narrow and displaced one-third to half the body length. The sulcus is only a shallow depression between the longitudinal stripes. Heterotrophic. The nucleus is situated in the middle of the cell. Cosmopolitan (DODGE 1982, STEIDINGER & TANGEN 1997).

*Gyrodinium* spp. (Plate 12.A-F & L-M) Four different taxa.

## Gymnodinium Stein 1878

*Gymnodinium rubrum* Kofoid & Swezy 1921 (Plate 13.A-C)

The cells are 100-145  $\mu$ m long and 75-90  $\mu$ m wide. This species is quite variable in shape but is normally elipsoid with a circular cross section. Its most recognizable features are the

longitudinal striation and its size. Heterotrophic. The cytoplasm has a pale red colour at the apex which gradually changes into diffuse yellow posteriorly. Formerly reported from California, Greenland and Denmark (KOFOID & SWEZY 1921, CLAUSEN et al. 1994).

## Cochlodinium Schütt 1896

# *Cochlodinium* cfr. *brandtii* Wulff 1916 (Plate 13.D-E)

A middle-sized cell 50-110  $\mu$ m long which is spindle-shaped with rounded ends. It is characterized by the deep cingulum that makes about 3-4 turns around the cell and the sulcus that slightly invades the epitheca. Heterotrophic. Information on distribution is poor but it is known from the Barents Sea and the English Channel and is probably widespread (DODGE 1982, CLAUSEN et al. 1994).

# Cochlodinium helicoides Lebour 1925 (Plate 13.F-G)

A middle-sized cell 29-54  $\mu$ m long, 24-30  $\mu$ m wide which is asymmetrical sub-oval. The cingulum makes 1.5 turns around the cell. Large nucleus in the centre of the cell and many chloroplasts with pyrenoids. Phototrophic (mixotrophic). Is known from the Mediterranean, the Pacific Ocean, the North Sea and West Greenland (DODGE 1982, CLAUSEN et al. 1994).

# Togula Flø Jørgensen & Daugbjerg 2002

# Togula jolla Flø Jørgensen & Daugbjerg 2002 (Plate 13.H-I)

A small cell 25-43  $\mu$ m long and 19-35  $\mu$ m wide, which is ellipsoidal in dorsoventral view and more elongated in lateral view. The cingulum has a sigmoid shape and is displaced 0,3-0,4 cell lengths. Several chloroplast lobes present. Probably worldwide (FLØ JØRGENSEN 2002).

# Katodinium Fott 1957

## Katodinium glaucum (Lebour) Loeblich III 1965 (Plate 13.J)

Medium-sized cell 40-56  $\mu$ m long which is characterized by the spindle-shaped form. The cingulum is displaced 4 or 5 cingulum widths. The epicone has about 20 longitudinal stripes and is much longer than the hypocone with 2-3 longitudinal stripes. Heterotrophic. Cosmopolitan, common in estuarine areas (DODGE 1982).

## Amphidinium Claparéde & Lachmann 1859

## Amphidinium cfr. operculatum Claparède & Lachmann 1859 (Plate 13.L-M)

A medium-sized cell 29-50  $\mu$ m long (normally in the range of 31-39  $\mu$ m), 21-28  $\mu$ m wide. The cell is flattened dorso-ventrally and has a elongate ellipsoidal shape in lateral view. The epicone is small and triangular in outline. The cingulum is deeply incised and originates approximately 0.3 cell lengths from the anterior end of the cell. The hypocone is broadly rounded and the antapex is slightly asymmetrical. The chloroplasts are yellow-brown and the chloroplast lobes radiate in a characteristic way from a central pyrenoid. Cosmopolitan (FLØ JØRGENSEN 2002, KOFOID & SWEZY 1921).

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Plate 12. A-B) Gyrodinium sp. 1. C-D) Gyrodinium sp. 2. E-F) Gyrodinium sp. 3. G-K) Gyrodinium spirale (pic. G-I are in deep focus and therefore appear mirrored). L-M) Gyrodinium sp. 4.



Plate 13. A-C) Gyrodinium rubrum (pic. A are in deep focus and therefore appear mirrored).
D-E) Cochclodinium brandtii. F-G) C. helicoides. H-I) Togula jolla. J) Katodinium glaucum.
K) Heterocapsa triquetra (thecate). L-M) Amphidinium cfr. Operculatum. N-O) Gyrodinium sp.

# 3.1.8 Species lists

Choanoflagellida (choanoflagellates)
Bicosta minor
Bicosta spinifera
Parvicorbicula serrulata
Crinolina aperta
Pleurasiga minima
Parvicorbicula quadricostata
Pleurasiga sp.
Conion groenlandicum
Acanthoecopsis sp.
Prasinophyceae (prasinophytes)
Pyramimonas cirolaneae
Pyramimonas virginica
<i>Pyramimonas</i> sp. nov.
<i>Mantoniella</i> sp.
Haptophyceae (haptophytes)
Phaeocystis pouchetii
Pappomonas flabellifera var. borealis
Pappomonas virgulosa
Chrysophyceae (golden algae)
Dinobryon balticum
Dictyochophyceae (dictyochophytes)
Apedinella radians
Protista insertae sedis
Telonema subtilis

 Table 2. Complete species list of nanoplankton.

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	St. 0	St.1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8	St. 9	St. 10	St. 11	St. 12	St. 13
Gyrosigma/Pleurosigma sp.			х		х		х							
Leptocylindrus danicus					х	х	х					х	х	х
Licmophora sp.			х					х						
Navicula sp.							х							
Pennate diatom				х						х				
Proboscia spp.						х								
Pseudo-nitzschia cfr. seriata				х	х	х	х	х	х	х	х	х	х	х
Rhizosolenia hebetata												х		
Thalassiosira anguste-lineata						х								
Thalassiosira nordenskioeldii					х	х	х	х	х	х	х	х	х	
Thalasiosira cfr. rotula														х
Thalassiosira spp.	х		х		х									
Chrysophyceae														
Dinobryon balticum	х		х	х	х	х	х	х	х	х	х	х	х	х
Prasinophyceae														
Pyramimonas cirolanae	х	х	х	х	х	х	х		х		х			х
Pyramimonas virginica	х	х	х	х	х	х	х	х	х		х			х
Pyramimonas spp.	х													
Mantoniella sp.														
Haptophyceae														
Phaeocystis pouchetii					х	х	х	х		х			х	х
Dictyochophyceae														
Dictyocha speculum						х		х						
Apedinella radians														
Cryptophyceae														
Several unidentified species														
Protista insertae sedis														
Telonema subtilis														

**Table 3.** Complete species list of netplankton.

#### **3.2 Biomass**

The species names mentioned in the following are due to our studies of living material in the light microscopy in Greenland. The suffixes of roman numbers e.g. (I) or (II) refer to different size classes in the same genus or undistinguishable groups.

## 3.2.1 Biomass distribution at 2.5 m (fig. 7)

The total biomass in the 2.5 metre samples varies from approximately 6  $\mu$ g C L<sup>-1</sup> to approximately 82  $\mu$ g C L<sup>-1</sup>. Station 0, 1 and 8 are all dominated by diatoms (>80%) whereas small groups of autotrophic nanoflagellates, heterotrophic nanoflagellates (station 0) and heterotrophic dinoflagellates (stations 1 and 8) contribute the rest of the total biomass. At station 13 half the biomass is contributed by diatoms and half is contributed by heterotrophic dinoflagellates. At stations 2, 3, 5 and 7 the total biomass is much smaller than at stations 0, 1,

8 and 13. These stations are dominated by autotrophic nanoflagellates and heterotrophic dinoflagellates and diatoms only play a minor role (station 3 and 7).

#### 3.2.2 Biomass distribution for the deep samples (fig. 7)

The total biomass in the deep samples varies from approximately 3  $\mu$ g C L<sup>-1</sup> to approximately 37  $\mu$ g C L<sup>-1</sup>. Diatoms dominate (>50 %) at stations 0, 1 and 8 and heterotrophic dinoflagellates dominate at stations 2 and 3 (samples from Mellemfjord). Autotrophic nanoflagellates dominate at stations 5, 7 and 13.



**Figure 7.** Biomass distributions indicating the accumulated biomass of diatoms, nanoflagellates and heterotrophic dinoflagellates at eight selected stations at 2.5 metres and deep samples. Notice the different scales on the y-axis.

#### 3.2.3 Station 0, 2.5 m (fig. 8)

In this sample we were able to distinguish between 19 different taxa. These represent all the taxonomical groups we observed in Greenland. It was a sample dominated by diatoms. Especially the genus *Chaetoceros* was dominant with a biomass per litre in excess of 30  $\mu$ g C and a concentration of approx. 700,000 cells L<sup>-1</sup>. The genus *Thalassiosira* contributed with a biomass exceeding 20  $\mu$ g C L<sup>-1</sup> based on a cell concentration of approx. 80,000 cells L<sup>-1</sup>. The remaining contribution was more or less negligible in comparison to the two dominant diatom genera. Though it is probably worth noticing that the two nanoflagellates *Dinobryon balticum* and *Phaocystis pouchetii* contributed more than the rest of the nanoflagellates in terms of both biomass and cell concentration.

#### 3.2.4 Station 0, 15 m (fig. 8)

This station held 18 taxa. The two diatom genera *Chaetoceros* and *Thalassiosira* were again the top biomass contributors with 4.5 and 14.5  $\mu$ g C L<sup>-1</sup>, respectively. *Chaetoceros* was much more numerous with approximately 160,000 cells L<sup>-1</sup> compared to around 60,000 for

*Thalassiosira*. A concentration more or less the same for *Phaocystis pouchetii* though the biomass was only around 0.5  $\mu$ g C L<sup>-1</sup>.



**Figure 8.** Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 0 at 2.5 and 15 metres.

#### 3.2.5 Station 1, 2.5 m (fig. 9)

Again the diatoms dominate among the 24 taxa. *Thalassiosira* showed a high biomass of around 33  $\mu$ g C L<sup>-1</sup> and a corresponding concentration of approximately 130,000 cells L<sup>-1</sup>. *Chaetoceros* spp. (I) had a high cell count of around 830,000 cells L<sup>-1</sup>.



Figure 9. Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 1 at 2.5 and 18.5 metres.

A group of unidentified autotrophic flagellates was also a major contributor with 15  $\mu$ g C L<sup>-1</sup> and just below 800,000 cells L<sup>-1</sup>. The heterotrophic dinoflagellate genus *Protoperidinium* was represented by almost 3.5  $\mu$ g C L<sup>-1</sup> with only 220 cells L<sup>-1</sup>.

# 3.2.6 Station 1, 18.5 m (fig. 9)

This station held 15 taxa and was characteristic by having the *Chaetoceros* spp. (I) and *Protoperidinium* genera contributing an equal amount of 5  $\mu$ g C L<sup>-1</sup>. Again we saw a difference in concentration of the two groups with *Chaetoceros* spp. I having a much higher concentration. *Thallasiosira*, however, was the main biomass contributor with nearly 9  $\mu$ g C L<sup>-1</sup>.

# 3.2.7 Station 2, 2.5 m (fig. 10)

A total of 15 taxa were encountered on this station. A group of unidentified autotrophic flagellates (I) exceeded 2.5  $\mu$ g C L<sup>-1</sup> and had a concentration of approximately 140,000 cells L<sup>-1</sup>. *Phaocystis pouchetii* was represented with a similar concentration though its biomass was only around 1  $\mu$ g C L<sup>-1</sup>. The prasinophyte *Pyramimonas cirolaneae* was, with regards to biomass, placed in between the two others with barely 80,000 cells L<sup>-1</sup>. Diatoms at this station represent less than 0.5  $\mu$ g C L<sup>-1</sup>.

# 3.2.8 Station 2, 40 m (fig. 10)

At this station we found that *Protoperidinium* was the most dominant of the 20 taxa in terms of biomass with almost 18.5  $\mu$ g C L<sup>-1</sup>. It is, however, among the organisms with the lowest concentration (1,595 cells L<sup>-1</sup>). With the highest concentration of around 325,000 cells L<sup>-1</sup> we found the chrysophyte *Dinobryon balticum* with a biomass contribution of approximately 2.5  $\mu$ g C L<sup>-1</sup>. A group of unidentified autotrophic flagellates (I) also made a prominent contribution to the biomass of approx. 4  $\mu$ g C L<sup>-1</sup> distributed over 190,000 cells L<sup>-1</sup>. *Phaocystis pouchetii* accounted for 160,000 cells L<sup>-1</sup> and approximately 1  $\mu$ g C L<sup>-1</sup>. The diatom biomass for this depth was less than 1  $\mu$ g C L<sup>-1</sup>.

## 3.2.9 Station 3, 2.5 m (fig. 11)

Out of a total of 18 taxa found at this station *Protoperidinium* was the dominant genus with around 9.5  $\mu$ g C/L and a correspondingly low cell concentration of 668 cells L<sup>-1</sup>. The most numerous taxon per litre was *Dinobryon balticum* with almost 325,000 cells L<sup>-1</sup> of which a biomass of 2.3  $\mu$ g C L<sup>-1</sup> was deducted. The prasinophyte *Pyramimonas cirolaneae* also contributed with a similar value (2.1  $\mu$ g C L<sup>-1</sup>). The dominant diatom was *Proboscia alata* with a contribution of approximately 1.5  $\mu$ g C L<sup>-1</sup>.

## 3.2.10 Station 3, 20 m (fig. 11)

Of the 19 taxa *Protoperidinium* was again the dominant organism accounting for around 17  $\mu$ g C L<sup>-1</sup>. It was followed by *Phaocystis pouchetii* which contributed with a little less than 13  $\mu$ g C L<sup>-1</sup> and the corresponding cell concentration of around 170,000 cells L<sup>-1</sup>. The diatoms at this station were responsible for approximately 4  $\mu$ g C L<sup>-1</sup>.

## 3.2.11 Station 5, 2.5 m (fig. 12)

A group of large *Protoperidinium* (I) was the largest biomass contributor of the 11 taxa (around 7  $\mu$ g C L<sup>-1</sup>) while the biomass of *Dinobryon balticum* with just around 1.5  $\mu$ g C L<sup>-1</sup> was responsible for the highest cell concentration. A group of smaller *Protoperidinium* species contributed with a similar amount as *Dinobryon balticum*. No diatoms were recorded at this station.



**Figure 10.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 2 at 2.5 and 40 metres.* 

#### 3.2.12 Station 5, 30 m (fig. 12)

A group of unidentified autotrophic flagellates (II) was the main contributor at this station with a little more than 11  $\mu$ g C L<sup>-1</sup> and a correspondingly high cell count in excess of 1.6 mill. cells L<sup>-1</sup>. *Phaocystis pouchetii* contributed with around half the biomass and concentration i.e. approximately 6  $\mu$ g C L<sup>-1</sup> and 800,000 cell L<sup>-1</sup>. Another group of unidentified autotrophic



**Figure 11.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 3 at 2.5 and 20 metres.* 

flagellates (I) had around the same biomass as *Phaocystis pouchetii* but a considerably lower cell concentration of around 265,000 cells  $L^{-1}$ . Various heterotrophic flagellates also made a notable biomass contribution with around 2 µg C  $L^{-1}$ . The accumulated diatom biomass at this station was less than 1 µg C  $L^{-1}$ .



**Figure 12.** Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 5 at 2.5 and 30 metres.

#### 3.2.13 Station 7, 2.5 m (fig. 13)

Of the 17 taxa recorded *Dinobryon balticum* had the largest biomass contribution of almost 2.5  $\mu$ g C L<sup>-1</sup> as a result of a concentration 325,000 cells L<sup>-1</sup>. As a group the heterotrophic dinoflagellates contributed with a biomass of 4.5  $\mu$ g C L<sup>-1</sup>. The accumulated diatom biomass on this station was less than 1.5  $\mu$ g C L<sup>-1</sup>.

# 3.2.14 Station 7, 30 m (fig. 13)

This station held 14 taxa of which none exceeded  $1.5\mu g \text{ C L}^{-1}$ . The prominent taxa being *Phaocystis pouchetii* with 1.2  $\mu g \text{ C L}^{-1}$  and *Protoperidinium* with around 1  $\mu g \text{ C L}^{-1}$ .

# 3.2.15 Station 8, 2.5 m (fig. 14)

The main contributor to the biomass of the 21 taxa found on this station was *Chaetoceros* spp. (I) with almost 45  $\mu$ g C L<sup>-1</sup> and a concentration of approximately 1.6 mill. cells L<sup>-1</sup>. Another diatom genus *Thalassiosira* spp. contributed with around 4  $\mu$ g C L<sup>-1</sup>. The remaining taxa barely recorded biomasses of more than 3  $\mu$ g C L<sup>-1</sup>, respectively.

## 3.2.16 Station 8, 20 m (fig. 14)

The dominant taxon of the represented 16 taxa was *Dinobryon balticum* with a biomass of almost 3.5  $\mu$ g C L<sup>-1</sup>. *D. balticum* also had the highest concentration with more than 450,000 cells L<sup>-1</sup>. The two diatom genera *Chaetoceros* and *Thalassiosira* both contributed with 2-2.5  $\mu$ g C L<sup>-1</sup> but, of course, with much lower cell concentrations of *Dinobryon balticum*. *Protoperidinium* accounted for a similar contribution with a little less than 2  $\mu$ g C L<sup>-1</sup>.

# 3.2.17 Station 13, 2.5 m (fig. 15)

*Protoperidinium* was the organism that recorded the largest biomass contribution of the 14 taxa with almost 14  $\mu$ g C L<sup>-1</sup> based on a concentration of 1,200 cells L<sup>-1</sup>. Eleven of the taxa at this station were diatoms. The most important of these were *Chaetoceros* spp. (I) and *Thalassiosira* spp. with contributions between 3.5 and 8  $\mu$ g C L<sup>-1</sup>. *Chaetoceros* spp. (I) was much more numerous than *Thalassiosira* spp. with concentrations of 130,000 and 30,000, respectively.

## 3.2.18 Station 13, 20 m (fig. 15)

The group of unidentified autotrophic flagellates (I) and *Thalassiosira* spp. was the two most important groups with a biomass contribution of approximately 5  $\mu$ g C L<sup>-1</sup> and had the corresponding concentration of around 250,000 and 15,000 cells L<sup>-1</sup>, respectively. The two nanoflagellates *Dinobryon balticum* and *Phaocystis Pouchetii* both contributed with around half the biomass of the others i.e. 2-2.5  $\mu$ g C L<sup>-1</sup> and held about 300,000 cells L<sup>-1</sup>.

## 4. DISCUSSION

## 4.1 Nanoplankton diversity

The original idea of the project was to attach particular attention to the nanoplankton fraction. However, surprisingly few nanoplankton species were observed on our EM grids. Our list only includes 19 species distributed over five classes. The list furthermore reveals that not only does it contain fewer taxa but certain genera and even classes are completely missing in our samples compared to studies conducted by Thomsen in 1977 (98 species, 6 classes) (THOMSEN 1982) and Clausen et al. in 1994 (60 species, 6 classes) (CLAUSEN et al. 1994). This cannot be a matter of pure coincidence and there certainly are several things that might explain this comparatively low diversity of nanoplankton.

## 4.1.1 Comparison with Thomsen 1977

Compared to the survey conducted by Thomsen in 1977 dinoflagellates and diatoms (except for a few torn apart ones) were not represented in our nanoplankton fraction at all. These two classes alone accounted for 13 species in 1977. Of the golden algae we found only 14% of the



**Figure 13.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 7 at 2.5 and 30 metres.* 

species found by Thomsen. With three species of haptophytes we recorded 8% of the number of species collected in 1977. In the class Prasinophyceae we found four species including three species of *Pyramimonas* of which *P. cirolaneae* was neither found in 1977 nor in 1994. Furthermore more we found an undescribed species of the genus *Pyramimonas*. In 1977



**Figure 14.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 8 at 2.5 and 20 metres.* 



**Figure 15.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 13 at 2.5 and 20 metres.* 

Thomsen found nine species assigned to five genera belonging to the prasinophytes so finding only two of those genera was again rather disappointing. The pedinellid *Apedinella radians* was found in the samples from 1994 but not in those from 1977. The choanoflagellates was by far the largest group in our samples but only amounted to nine species compared to 28 found by Thomsen and none were new observations (THOMSEN 1982, CLAUSEN et al. 1994).

All together a poor result compared to 1977 and there are several reasons for this. In 1977 the collecting sites were in Disko Fjord and in the immediate vicinity of Qeqertarsuaq, which means that the collecting sites themselves could not be the reason for the differences in diversity. The sampling was done in July and August so a certain amount of seasonal variation compared to our investigations cannot be entirely ruled out. Finally the sampling was done not only at the surface but also at depths as deep as 300 metres and this has indeed had influence on the sampling results. Five species of choanoflagellates were only found at 60 metres or below and only one of these species was represented in our samples, which were mostly collected at 40 metres or above. In order to investigate if there were more heterotrophic flagellates at greater depths, two grids from water samples from 70 metres and two grids from water samples from 300 metres were examined but although this did increase the amount of choanoflagellate species found it was not nearly enough to bring the total species list to match the list from 1977 (THOMSEN 1982).

## 4.1.2 Comparison with Clausen et al. 1994

Compared to the investigations conducted by Clausen et al. in 1994 the overall impression is nearly the same as when compared with Thomsen. They found three groups *Parmales*, *Euglenophyceae* and nine diatom species, which we did not observe in our samples. The golden algae and the haptophytes amounted to 33% and 14% respectively of the species found in 1994 and the haptophytes were represented by ten genera in 1994 compared to two genera found by us. *Pyramimonas cirolaneae* and *Mantoniella* sp. were not found in 1994 but apart from that only 17% of the species we found were identical to the prasinophytes found by Clausen et al. We managed to find 47% of the choanoflagellate species found in 1994. As in 1977 Clausen et al. sampled more extensively at depths below 40 metres (CLAUSEN et al. 1994). This could explain the lack of certain taxa especially for the choanoflagellates since these are bacteria and detritus feeding organisms and thus predominantly found at greater depths (THOMSEN 1982). Six species of choanoflagellates were only found at 50 metres of which only one species was found in our samples. Another three species belonging to the haptophytes are the prasinophytes were also found at a depth of 50 metres only.

In 1994 the collecting site for nanoplankton was at one station just south of Qeqertarsuaq between July 27th and August 3rd. Sampling was carried out from the surface down to 50 metres. Furthermore one water sample was taken at the surface in the harbour basin on August 1st. Seasonal variation and collection sites are thus unlikely to explain differences in species diversity. Clausen et al. intensively studied one station and therefore probably recorded more species as opposed to the present investigation, which covered a large area.

For both studies season and sampling sites did not seem to explain the entire poorness of our species list. However, when processing the samples there were several things, which might have had a negative impact on the amount of species found. All water samples were centrifuged for no less than 30 min in three rounds of 10 min. at a supposedly but no way certain speed of 2500 rounds pr. min. This could have damaged some of the delicate organisms and made them impossible to identify. Also the temperature in the laboratory was most of the time near 10°C (even 15°C one day when the cooling system broke down) and not

5°C which is preferable since temperatures from 10-15°C can deform or even kill the cells. When the cooling system broke down we observed that the netplankton simply died so we must assume that the nanoplankton did too. A minor temperature rise probably has little impact on the organisms as opposed to great temperature fluctuations which might be fatal to the organisms. We expect the rough handling and the temperature changes to be the main reasons for finding less species than similar investigations. Lack of time prevented us from looking through all five grids per depth per station. Instead two grids per depth were investigated and this too might have contributed to the disappointing nanoplankton species list.

## 4.2 Netplankton diversity

The netplankton fraction was not centrifuged and was therefore in a relatively good condition compared to the nanoplankton fraction. Additionally four people spent a lot of time looking through the live samples in Greenland, which is easily seen from the species list. Naturally the organisms have been affected by the high temperatures in the laboratory too but many of the samples were processed within 24 hours of our arrival back at Arktisk Station which meant that many organisms could be readily identified and filmed or photographed for later identification.

## 4.2.1 Netplankton diversity compared with Clausen 1994 and Trier 1996

This does not mean that the species list is identical with the species list from 1994 but the amount of species is nearly the same. Twenty-three species of diatoms were found in both 1994 and 2002. Clausen et al. found nine species, which we did not find, and we found nine species, which were not found in 1994. For the dinoflagellates there were 24 species identical with 1994 and 2002 but Clausen et al. even discovered 17 species, which we did not see in our samples whereas we had 5 species not found in 1994. The differences in diversity could be due to the fact that Clausen et al. also collected samples in Vaigat. Apart from this the difference in species diversity is not easily explained and is hardly due to changed environmental conditions in the area. The nine diatom species found in 2002 which were not present in 1994 are neither particularly tied to cold nor warm water and the same is the case for the nine diatom species found in 1994 which were not present in 2002 (HASLE & SYVERTSEN 1997). There seems to be no pattern for the dinoflagellates either (STEIDINGER & TANGEN 1997) and it is therefore not possible to connect the differences in species composition with changing climatic conditions. The differences are more likely due to the difference in time spent for identification, the people identifying the organisms and again the fact that one will never find the exact same organisms every time one samples from day to day or from year to year due to a patchy distribution.

Trier (1998) investigated the species composition of a transect (10 stations) from Disko and westwards between June 26th and July 5th 1996. As in 1994 there is a considerable variation in species diversity. Trier found 24 species of diatoms that were not found in our samples and we on the other hand found 17 species not found at Trier's transect. For the dinoflagellates Trier's transect and the present investigation had 18 species in common and Trier found another nine species whereas we found another 11. Particularly for the diatoms this is a great deal more variable than compared to Clausen et al. but it must again be emphasized that the species lists very much depend on the people doing the identification. We for instance put a lot of work into identification of *Chaetoceros*-species, which resulted in a total of twelve *Chaetoceros* species on our list compared to only six on Trier's. This does not necessarily mean that the other six species were not present at Trier's transect since Clausen et al. found thirteen species belonging to this genus in 1994.

One species was found by neither Clausen et al. nor Trier. *Togula jolla* a newly described benthic athecate dinoflagellate (FLØ JØRGENSEN 2002) was for the first time ever discovered in Greenland. However, this species has previously been found in USA, Japan and Australia (FLØ JØRGENSEN 2002) and was expected to be discovered in the sediments along the shores on Disko Island, as this is presumably a cosmopolitan species.

The rest of the species are otherwise nearly identical except that Clausen et al. found a euglenophyte that we did not. Clearly there will always be differences in the species diversity when observed at different times even though the sampling sites seem unchanged.

## 4.2.2 Netplankton compared with Spitsbergen

Okolodkov et al. (2000) investigated the phytoplankton diversity at a transect with 16 stations in Kongsfjorden, Spitsbergen (79°N 12°E) between July 10th-16th 1996. This high-latitude locality, which is situated about 1100 km farther north than Disko, is under marked influence of the Gulf Current. The water in Kongsfjorden is therefore relatively warm and free of ice early in the year compared to other localities this far north (NORDENHAUG 1989). Though the sampling by Okolodkov et al. was carried out at the same time of the year, as the present investigation the netplankton diversity was rather different compared to the diversity in the vicinity of Disko. The two places had only nine species of dinoflagellates in common. Spitsbergen had 13 species not found around Disko whereas 18 species found around Disko were not found in the investigation in Spitsbergen. Thus more than half the species found at Disko in 2002 differed from the species found in Spitsbergen in 1996 and the total amount of dinoflagellates summed up to a total of only 22 species in Spitsbergen compared to 27 at Disko.

For the diatom species the situation is even more extreme. Only eight species were found in Spitsbergen compared to 31 at Disko, nearly four times as many. Only two species found in Spitsbergen were not found at Disko whereas Disko proved to have 26 species, which were not found in Spitsbergen. Even for other classes the picture is the same fewer genera and fewer species in Spitsbergen compared to Disko. It is difficult to know how much effort Okolodkov et al. has put into species identification but there seems to be a clear trend showing that the species diversity is poorer in Spitsbergen. This could be due to the locality e.g. a shorter season caused by the longer polar night and higher concentrations of suspended matter, which is found in the fjords of Spitsbergen (OKOLODKOV et al. 2000).

## 4.3 Biomass distribution.

Figures 7 clearly show that most of the deep water samples are not located at the expected fluorescence maximum. It was expected to observe a subsurface fluorescence maximum just above the pycnocline (halocline, thermocline or nutricline), but the sample depth has probably been incorrect. Because of the patchy distribution of phytoplankton the biomass can change a lot over a few vertical meters (NIELSEN & HANSEN 1999).

Stations 0 and 1 are dominated by diatoms with a comparatively high biomass, especially at 2.5 metres but also in the deep samples (15 and 18.5 metres). This means that silicate (SiO<sub>2</sub>) must be present, even though silicate and nitrate is often depleted in the surface layer in July after the spring bloom of diatoms (NIELSEN & HANSEN 1999). But summer blooms do occur in this area, especially along the southeast coast of Disko, because of wind and tidewater driven upwelling with nutrient rich water (ANDERSEN 1981a, ANDERSEN 1999). Phosphate is

not considered a limiting factor since it is almost never depleted in Arctic waters (HARRISON & COTA 1991).

Station 2 and 3 are both situated in Mellemfjord and interestingly this fjord is not markedly influenced by melt water, in fact salinities at these two stations ranged between 32.5 ‰ and 34 %. This means that Mellemfjord is influenced by the tide that also explains that the species found here are the same as at the other two stations west of Disko Island. The stations probably represent a phytoplankton community some time after a diatom bloom. The silicate and most of the nitrate  $(NO_3)$  is depleted and the majority of diatoms have settled out of the photic zone or they could simply just have been grazed by the protozooplankton. The diatom and nanoplankton grazing heterotrophic dinoflagellates are still present with a high biomass. The autotrophic biomass is dominated by nanoflagellates (Phaeocystis pouchetii, Dinobryon balticum, Pyramimonas spp. and several unidentified autotrophic flagellates), which have the advantage of having a large surface to volume ratio that facilitate nutrient uptake. Phaeocystis pouchetii also has an advantage when it comes to grazing. In the colony-forming life stage the cells lie embedded in a mucilaginous gel (THRONDSEN & EIKREM 2001) that has a low nutritional value for grazers (GREEN & LEADBEATER 1994). The relatively low biomass and the dominance of smaller species are typical for a summer situation (NIELSEN & HANSEN 1995, NIELSEN & HANSEN 1999, JENSEN et al. 1999). Another aspect in the fjords of Disko is the melt-water from glaciers and run-off from land that wash small sediment particles into the fjord and diminish the light penetration (ANDERSEN & BORN 1999). If the light penetration is dramaticly reduced the photic zone decreases and this will affect the standing stock negatively. However, it is not very likely to be the case at station 2 (at the time of our sampling) since we find the bulk of autotrophic biomass at a depth of 40 metres and not near the surface. An identical picture is revealed at Station 3 even though the deep sample here is taken at 20 metres.

Station 5 had a very low autotrophic biomass at 2.5 metres and was probably nutrient depleted. But the deep sample (30 metres) had the highest biomass of all stations due to autotrophic nanoflagellates and this might be because the sample is taken at the fluorescence maximum around the pycnocline. We propose that this is due to the fact that this station is the one farthest away from the coast i.e. the most "oceanic" and not affected by the upwelling in Disko Bay. If this is the case it indicates a "normal" oceanic summer situation with a dominating nanofraction in this particular instance by an unidentified autotrophic flagellate (II). Also at this station we see the largest biomass contribution of the heterotrophic nanoflagellates, which is expected to be able to feed on the remains of dead nanoflagellates (POC and DOM) and bacteria (THOMSEN 1982, HANSEN & NIELSEN 1995).

Station 7 has a very low biomass both at the surface and at 30 metres. When netplankton samples were taken the plankton net was visible down to 18 metres, therefore it is possible that a fluorescence maximum exists at a depth below 18 metres or that the biomass is low in the whole water column. If the latter is the case a possible explanation is nutrient depletion in combination with grazing by ciliates and copepods (which the present investigation did not take into account).

Diatoms with a high surface biomass dominate at station 8. This is most likely due to large amounts of nutrients perhaps caused by either upwelling or perhaps a later melting of the sea ice.

Station 13 has an intermediate biomass of diatoms and a relatively high biomass of heterotrophic dinoflagellates in the surface that graze on the diatoms. Station 13 was situated near a small island with a lot of nesting birds and these birds could be enriching the surrounding water with small amounts of nitrate.

The biomass at all stations has been compared with salinity measurements but no trend or connection could be established.

To sum up the general picture; Stations 0, 1, 8 and 13 are directly comparable since they are all situated in Disko Bay and are thus presumably affected by upwelling. This results in a comparatively high diatom biomass at 2.5 metres. Stations 5 and 7 are both located west of Disko Island and stations 2 and 3 are heavily influenced by water with origins west of Disko Island. The influence of nutrient poor oceanic water combined with a comparatively high biomass of heterotrophic dinoflagellates results in a total biomass no greater than 40  $\mu$ g C L<sup>-1</sup> which is more or less half of what has been recorded at any of the Disko Bay stations. So what is observed is a normal summer situation in an area with a complex hydrography. The biomass is low at the surface, except for stations with presumed upwelling. The deeper samples are more difficult to compare since they do not originate from the same depth and the fluorescence maximum is unknown. The information from the biomass distributions only gives a momentary picture and it is not possible to deduct whether the observed biomass values represent the beginning, the peak or the remaining biomass of a population.

## 4.3.1 Biomass comparison with other investigations in the Arctic.

Biomass comparisons are made with 1) an investigation of a transect in Disko Bay and Baffin Bay carried out by Trier in 1996 (TRIER 1998), 2) with an investigation done along part of the coast of West Greenland by Jensen et al. sampled in 1993 (JENSEN et al. 1999), 3) with the study of Nielsen and Hansen from 1994 from a permanent station just off Qeqertarsuaq (NIELSEN & HANSEN 1999) and finally with a study from Kongsfjorden, Svalbard (OKOLODKOV et al. 2000).

Trier has biomass estimations from 5 metres and from the fluorescence maximum, but aside from that we assume that it is suitable for comparison. Trier's biomass is composed of both autotrophic and heterotrophic biomass and is just like ours estimated by the Utermöhl method. In the 5 metre samples Trier estimated a biomass of  $21 \pm 28 \ \mu g \ C \ L^{-1}$  compared to our 35  $\ \mu g \ C \ L^{-1}$ . In the deeper water samples Trier estimated  $42 \pm 32 \ \mu g \ C \ L^{-1}$  and we estimated a biomass of  $20 \ \mu g \ C \ L^{-1}$ . These differences are within the margin of error and therefore agree well with our results.

Jensen et al. covers a very large area and therefore only stations in the vicinity of Disko are presented in this study (stations 431 to 453). In the study by Jensen et al. the biomass is presented as chl. a values and it was therefore necessary to multiply the chl. a values with a carbon conversion factor. This factor is not constant, as explained in section 2.6, but in order to compare our biomass estimates a value of 40 was chosen based on literature values of 31 and 43 estimated by Nielsen and Hansen in 1995 and 1999 respectively (NIELSEN & HANSEN 1995, NIELSEN & HANSEN 1999) and of 40 by Vismann & Hansen in 1998 (VISMANN & HANSEN 1998). The chl. a values are given for the whole of the euphotic zone for each station and for that reason an average of our 2.5 meter and deep water samples are used for comparison. When transforming the chl. a values of Jensen et al, a carbon biomass of 33  $\mu$ g C L<sup>-1</sup> is obtained. Compared to our 21  $\mu$ g C L<sup>-1</sup> their value is a little higher, but not more than the uncertainty of the conversion factor can account for.

Nielsen and Hansen 1999 also use chl. a as a biomass indicator and multiply with a carbon conversion factor of 43. They only sample one station, but monitor the succession through August and September. The station is situated close to our station 0. They estimate an autotrophic average biomass of 37  $\mu$ g C L<sup>-1</sup> in that period which is within the range of our results. The biomass is a little higher than the biomass of the present study, but during August 1994 strong winds occurred and brought nutrients to the euphotic zone (NIELSEN & HANSEN 1999).

In general a great consistency is found between our data and the data from previous biomass studies in the area, even though different methods have been applied. However, the Utermöhl method is probably the most accurate (if 400 cells of each taxon are counted) and facilitates partition of the biomass into the desired groups (taxonomical and ecological).

In order to compare with results from other studies in the arctic region a study from Kongsfjorden, Spitsbergen was chosen. The sampling area is further north (79°N), but the stations are also situated in and around a fjord and the sampling has also taken place in July. Abundance is expressed as cell numbers per litre. The cell counts show that the dinoflagellates are represented with larger numbers in Kongsfjorden (70,300 cells  $L^{-1}$ ) compared with Disko (2,300 cells  $L^{-1}$ ) and that diatoms are represented with lower numbers in Spitsbergen (445 cells  $L^{-1}$ ) compared with Disko (270,000 cells  $L^{-1}$ ). The lower total cell count (204,000 cells  $L^{-1}$ ) in Kongsfjorden compared with (1,136,000 cells  $L^{-1}$ ) in Disko might be due to more sediment in the water which would reduce the light penetration and result in a lower standing stock. The lower diatom cell numbers could be due to less silica in Kongsfjorden or simply due to the presence of large amounts of heterotrophic dinoflagellates. It is rather difficult to compare cell numbers for different investigations since they do not tell anything about the size of the cells and thus the actual biomass.

## 4.4 Improvements of materials and methods

In retrospect we realize that some things could have been done to improve the quality of the present phytoplankton study. As always time is an important factor and we have definitely worked against it while the project lasted. This is meant as a suggestion and recommendation section aimed at those people who in the future might conduct similar investigations.

Samples could be taken at the permanent station (station 0) as often as possible in order to investigate the extent of variation due to other parameters than change of location. The amount of cells being deformed or killed might have been reduced if concentration of the samples had been done by means of filtration instead of centrifugation. A combination of filtration and few minutes of centrifugation would probably be the best solution. Or perhaps do the centrifugation with a centrifuge capable of centrifuging one litre at a time or use a continuous centrifuge.

As for the CTD and other technical equipment it would be useful to bring some spare (less sophisticated) instruments for back up. We lost a lot of potential data on account of a useless CTD. With on location measurements of temperature and salinity in the water column, it would have been possible to detect the pycnocline and thus collect the deep samples at the depth of the theoretical fluorescence maximum.

In order to achieve a greater diversity of nanoplankton it might have been a good idea to study the EM grids at a larger magnification (for example 10,000 times magnification). We did not

observe solitary scales from ruptured cells since the grids were predominantly examined with 2000 times magnification and only with larger magnification in certain interesting areas in the vicinity of newly found cells.

# 4.5 Additional work

If we had had more time available several other methods could have been used in order to improve the species identification.

- SEM (Scanning Electron Microscopy) is useful when identifying for example *Thalassiosira*, *Pseudo-Nitzschia* and *Protoperidinium*.
- Slides with acid cleaned diatom frustules examined in LM at 100x magnification are excellent for identification of a wide range of diatoms.
- Staining cells with calcofluor white and further examination in epifluorescence microscope is a good method for identifying thecate dinoflagellates.
- Five hours of video were recorded in Greenland mainly of athecate dinoflagellates however; time did not permit us to look at them.

For improvement of the biomass estimations, it might have given us a more detailed picture of the biomass distribution if samples from all stations had been counted.

# **5. CONCLUSION**

The nanoplankton diversity (19 species, 5 classes) was quite poor compared to previous studies. The explanation for this is hardly due to changed environmental conditions in the area but should be seen in the light of the many technical difficulties when collecting and processing the samples. The netplankton diversity on the other hand corresponded very well with other studies conducted in the area. Minor differences in species composition between the present study and previous studies are expected to be explained by ordinary monthly and annual fluctuations, the time spent for identification and the different people doing the identification again rather than changed climatic and environmental conditions in the area. Compared to an investigation in the more northerly located Kongsfjorden in Spitsbergen the Disko area clearly displayed a much greater netplankton diversity presumably due to a longer season and a more variable hydrography.

By use of the Utermöhl method we calculated the biomasses of fractionated groups of flagellates and diatoms. By so doing we obtained a very detailed picture of the importance of the different protist groups. At the four stations in Disko Bay which is rather nutrient rich due to the presumed upwelling we observed a high biomass of mainly diatoms. This contrasted markedly with the observations from the four stations west of Disko Island where the water is comparatively nutrient poor. In these "ocean" influenced localities the biomass was much smaller and dominated by heterotrophic flagellates, which with their larger surface to volume ratio are better suited for life in a nutrient poor environment. Our biomass calculations are in fine agreement with previous studies conducted in the area and therefore support the general picture of the biomass distributions in the area. Furthermore our study gives a much more detailed picture of the importance of different protist groups based on biomasses. Compared to Kongsfjorden in Spitsbergen the Disko area displays larger numbers of diatoms and smaller numbers of dinoflagellates. Explanations for these findings are only speculative but might again be related to a longer season and less light attenuation in the Disko area compared to Kongsfjorden.
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# Diversity and Biomass of Marine Flagellates and Diatoms in the vicinity of Disko Island, West Greenland

Knud Andreas JØNSSON, Lone MUNK & Morten SMITH

Department of Phycology, Botanical Institute, University of Copenhagen

Abstract. Changes in the climate due to e.g. global warming could affect the organisms of the lower trophic levels such as phytoplankton and heterotrophic flagellates and thus change the structure of the food web. Therefore it is important to know more about the composition of these groups. Climate changes are thought to have the highest impact on the polar regions because it would lead to melting of the sea ice and the polar ice caps and thus change the environment. The present study focuses on the biomass and diversity of marine flagellates and diatoms in the area around Disko, Greenland. Both the nanofraction and the netfraction were examined. By counting the cells in an inverted microscope using the Utermöhl method it was possible to fractionate the biomass into ecological and taxonomical groups. Carbon biomasses were calculated on the basis of cell counts with the aid of the computer programme Aquabase. Micrographs were made in both LM and TEM of the encountered organisms in order to document the species diversity. Both biomass and diversity were compared with similar investigations in the Disko area as well as with a similar study in Spitsbergen. The estimated total biomass (heterotrophic and autotrophic) per litre in the present study is within the range 3-82  $\mu$ g C L<sup>-1</sup> (the mean value is 26  $\mu$ g C L<sup>-1</sup>) and this is in accordance with the biomass estimations of similar studies in the area. The diversity of the netplankton is likewise within the range of previous diversity investigations conducted in the area. A total of 81 species distributed over 9 classes was found of which 62 belonged to the netplankton fraction and 19 belonged to the nanoplankton fraction. The nanoplankton however, is less diverse than known from previous studies, which is thought due to less appropriate processing procedures and improvements of methods are therefore suggested.

#### Keywords.

### **1. INTRODUCTION**

Changes in the climate due to e.g. global warming is expected to have greatest impact in polar regions since even minor temperature changes will dramatically reduce the amount of sea ice and thus alter the structure of the arctic food web. This goes for the endemic sea ice biota as well as the pelagic species, which depend on the duration of the ice cover. Between 1948-1998 the temperatures in the upper 300 metres of the oceans globally have risen 0.3°C (LEVINSEN 2000). There is reason to believe that this rise in temperature will affect the marine phytoplankton quantitatively and qualitatively. Deviations in the constitution of this group could have severe consequences for the delicate balance of the whole arctic ecosystem since marine phytoplankton form the basis of the food web. As fishing constitutes 95% of the total export of Greenland, it would without doubt have dire effects on Greenland's economy and people if the arctic ecosystem was disturbed or changed. This in particular applies to the Disko Bay area which is the most densely populated area in Greenland (LEVINSEN 2000).

Arktisk Station at Qeqertarsuaq is located on the southern tip of Disko Island. It is with its excellent location, well equipped laboratory and the research vessel M/S Porsild a perfect start-out point for profound investigations and analyses of the marine life including the protists. Therefore the area is relatively well studied when it comes to species diversity of netplankton as well as nanoplankton and biomass calculations for the whole community (THOMSEN 1982, CLAUSEN et al. 1994, NIELSEN & HANSEN 1995, TRIER 1998, NIELSEN & HANSEN 1999, LEVINSEN 2000).

The aim of the project was to make a survey of the current biomass composition and diversity of phytoplankton species in the Disko Bay area. We realized that we would hardly be able to

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contribute much to the general species list of the area and decided to focus on the biomass of flagellates. With the use of an inverted microscope (Utermöhl method) as opposed to the much faster and much easier chl. a measurements we wished to fractionate the different flagellate groups (including the nanofraction) and investigate how much each group contributed to the total biomass of protists per litre. Previously the arctic pelagic food web was considered to be rather simple being mostly dominated by diatoms which were then consumed by copepods which were then again consumed by larger animals (NIELSEN & HANSEN 1999). This very simplified view has now been superseded by a greater understanding of the dynamics in an often complicated pelagic ecosystem which includes not only other species rich protist groups like for instance dinoflagellates and nanoflagellates (CLAUSEN et al. 1994, NIELSEN & HANSEN 1999). By fractionating the flagellate groups we would get a much more detailed picture of the ecological importance of the different phytoplankton classes in the Disko area. The emphasis was mainly on the flagellates but of course we looked at the other groups in the water samples as well and compared the findings of the present study with previous findings.

Due to time constraints it is only possible to get a very fragmentary picture of the phytoplankton composition and diversity in the vicinity of Disko Island. However, the present investigation should fill in a gap in the understanding of the biomass distributions in the arctic pelagic food web. And in conjunction with further studies on biomass distributions among different protist classes it should be possible to put together an overall picture of the diversity and biomass distribution in the area around Disko.

The project furthermore gave us knowledge of new methods and techniques, which were often difficult to apprehend and rather time consuming to carry out. Among these were preparations of EM-grids, counting the organisms in an inverted microscope and assembling photo plates. When working in the Arctic conditions change all the time and unpredictable situations inevitably occur. The equipment did not always work as expected and it was not always possible to do things the way we had planned in Denmark. This field project forced us to cope with many unforeseen situations. It made us change plans a lot of times and made us improvise whenever equipment broke down. All in all it taught us what it is like to work far from civilization with limited resources in the Arctic.

### 1.1 Hydrography

Since the distribution of protists in time and space depends on hydrography we have included a brief paragraph addressing this.

The origin of the water masses surrounding Disko is quite complex and changes throughout the year, but the two most important components are the West Greenland Current (WGC) and the melt water from sea ice, icebergs, fjord glaciers and from land.

The WGC coming from the south is relatively warm and saline. Part of the current continues east of Disko, beneath the fresher surface layer, into Disko Bay and out of the Vaigat. The WGC also leads to upwelling along the south coast of Disko. The WGC itself consists of a mixture of the East Greenland Current (relatively cold and fresh) and the Irminger Current (relatively warm and saline). Off Disko the WGC mixes with the Baffin Current creating counterclockwise eddies.

The cold freshwater from the melting of snow, sea ice, icebergs and ice glaciers (most importantly the Ilulissat fjord glacier) passes both west and north around Disko, from Ilulissat and into the open sea.

Throughout the year the contribution of freshwater changes with the shift in temperature. In spring and summer a great quantity of melt water creates a fresher surface layer, which in addition is warmed by the increased solar radiation. This results in a rather stable thermo- and halocline (pycnocline).

In autumn a reduction in the outflow of melt water occurs and this in combination with less solar heating and stronger winds creates a water column with uniform salinity and uniform low temperatures in the upper 80 metres.

Another aspect in the Disko Bay area is the tidal fluctuations. The rising tides can halt outgoing currents, which are either forced back into the bay or towards Kronprinsens Ejlande (ANDERSEN 1981a).



Figure 1. Map indicating the most important currents in the waters around Greenland (modified from HERMANN 1971).

### **1.2 Introduction to marine phytoplankton**

Since we in this project have identified as many organisms belonging to different groups of protists as possible we will present a brief outline of the major classes. The marine phytoplankton includes all photosynthetic planktonic organisms in the sea. When

investigating phytoplankton other organisms are caught as well. In the division Dinophyta for example there is a considerable number of heterotrophic species (approximately 50%). The heterotrophic dinoflagellates are caught while sampling for photosynthetic dinoflagellates. However, the heterotrophs are considered members of the protozooplankton because of their lack of chloroplasts. The choanoflagellates are a constituent of the kingdom Animalia and therefore considered part of the protozooplankton as well. In fact it is due to tradition more than to taxonomy that this particular group is included in the work of phycologists.

Diatoms on the other hand are extremely important photosynthetic organisms. They are an immensely important group in coastal production as opposed to the nano- and picoplankton that we set out to investigate which are by far the most dominant in the open ocean. This is due to their large surface to volume ratio which is favourable when nutrients are scarce (VAN DEN HOEK et al. 1995).

# **1.2.1** Prasinophyceae (Prasinophytes)

The prasinophytes are a class in the division Chlorophyta (green algae) and the majority are planktonic flagellates. They are equipped with either 1,2,4,8 or 16 flagella inserted apically or laterally. They also bear several types of scales on the cell body as well as on the flagella. The scales are important for precise identification and are only visible in the electron microscope. Prasinophytes occur in both marine and limnic waters (VAN DEN HOEK et al. 1995).

# 1.2.2 Haptophyceae (Haptophytes)

The haptophytes are predominantly unicellular spherical flagellates with two flagella. They are furthermore equipped with a haptonema which protrudes between the two flagella not unlike a third flagellum. The internal structure is, however, entirely different. The lumen holds a crescent shaped structure of microtubules which is unlike the 9+2 microtubular construction of a flagellum. The cell surface is covered with cellulose scales and some species have calcified scales (coccolithophorids). The haptophytes are mostly marine with only a minute number of the about 500 species living in freshwater (VAN DEN HOEK et al. 1995).

# 1.2.3 Chrysophyceae (Golden algae)

These organisms have yellow to brown coloured chloroplasts and this feature has given them their name. They are members of the division Heterokontophyta. Most species are unicellular or colonial and are often equipped with a long forward pointing flagellum with two rows of tripartite hairs (mastigonemes) and a shorter smooth flagellum pointing backwards. This is common for most heterokonts. The flagella arise apically on the cell. The chrysophytes are most abundant in fresh water and only a few species are found in the sea (VAN DEN HOEK et al. 1995).

# 1.2.4 Choanoflagellida (Choanoflagellates)

There has been much debate over the correct taxonomic position of the choanoflagellates. It has been suggested that they belong to Chrysophyceae because of the superficial similarity with this group. However, the vast majority of people now considers the choanoflagellates as being part of the kingdom Animalia because of the close and very convincing resemblance to choanocyte cells in sponges (THOMSEN 1982). The status of the choanoflagellates as a sister group to the metazoa is also supported by molecular data (CHRISTENSEN 1997). Choanoflagellates are unicellular organisms with one apical flagellum and an anterior ring of tentacles that creates a collar-like structure. The cells are characterized by the lorica composed of silicified ribs (costae) with which they surround themselves. Species identification and systematics of the choanoflagellates rely entirely on the arrangement of the

lorica. Choanoflagellates are very common and often one of the most dominating (number of cells) plankton groups in marine and brackish waters (THOMSEN 1982).



**Figure 2:** Schematic overview of a choanoflagellate-cell with the most characteristic features for identification (modified from TRONDSEN 1997).

# 1.2.5 Cryptophyceae (Cryptophytes)

The cryptophytes are predominantly bilaterally symmetrical unicellular flagellates. The cell is drop-shaped with a subapical gullet containing numerous trichocysts. The flagella arise from just inside the gullet. The two flagella are of unequal length. The longer flagellum is equipped with two rows of hairs but these are not tripartite like the hairs of the heterokonts. The shorter flagellum bears one row of hairs, which are shorter than the hairs on the long flagellum. This class is also of considerable evolutionary interest because it holds an organelle, the nucleomorph, which is the reduced nucleus of an autotrophic endosymbiont (a red algal cell) in a heterotrophic ancestor of the cryptophytes. This morphological piece of evidence in favour of the endosymbiont theory is also supported by molecular studies of the nucleomorph (VAN DEN HOEK et al. 1995).

### **1.2.6 Bacillariophyceae (Diatoms)**

The diatom cell is surrounded by a box and lid shaped construction called the frustule. This box can assume two general shapes: 1) pennate (bilatterally symmetrical) or 2) centric (radially symmetrical). Both shapes can be unicellular or colonial. As a member of the heterokonts we would expect them to be flagellated which they normally are not. Only the reproductive cells (zoids) of the radially symmetrical order Centrales exhibit this trait. Of course the morphology of the frustule shows great variation with as many as 100,000 diatom species being proposed. Diatoms are not only diverse they are also very important in carbon fixation. One estimate suggests that they are responsible for 20-25% of the dry mass produced on Earth per year. Therefore much marine life depends on these organisms in order to survive (VAN DEN HOEK et al. 1995).



**Figure 3.** Schematic overview of a diatom cell with the characteristic box and lid form (modified from HASLE & SYVERTSEN 1997)



**Figure 4.** Schematic overview of the dinoflagellate cell. A) Desmokont cell. B) Dinokont cell. C) Thecate cell. D) Athecate (naked) cell (modified from FAUST & GULLUDGE 2002).

### **1.2.7 Dinophyceae (Dinoflagellates)**

The dinoflagellates differ from almost all other organisms by having spiralized chromosomes (the euglenoids also present this feature) in the cells' interphase referred to as a dinokaryon.

There are only few exceptions to this e.g. the genus *Noctiluca*. The dinoflagellates are nearly always flagellated cells. The flagella emerge where the cingulum (transverse furrow) and the sulcus (longitudial furrow) intersect. The so-called dinokont dinoflagellates have one flagellum running in the cingulum and one in the sulcus. Other flagella arrangements are present in the dinoflagellates, but since we did not find any of these it will not be further described. The dinoflagellates are divided into two major groups: the thecate and the athecate (naked). The thecate dinoflagellates carry a specific number of cellulose plates in flat vesicles (amphiesma vesicles) just beneath the plasmalemma. The naked dinoflagellates have these vesicles too but no cellulose is found here. 90% of the dinoflagellates are marine and the rest resides in fresh water. Dinoflagellates are not only photosynthetic organisms about 50% are mixotrophic or heterotrophic (VAN DEN HOEK et al. 1995).



**Figure 5.** *Map of Disko Island indicating the sampling locations. Station numbers in bold represent those water samples used for biomass estimation (modified from ANDERSEN 1981b).* 

Station	Date	Position	Locality				
0	05.07.2002	N69°11,W53°30	"fixed station"				
1	05.07.2002	N69°17,W53°13	lppik				
2	08.07.2002	N69°45,W54°51	Enoks Havn, Mellemfjord				
3	08.07.2002	N69°45,W54°35	Mellemfjord				
4	09.07.2002	N69°47,W54°57	Mellemfjord				
5	09.07.2002	N69°11,W54°59	Disko Fjord				
6	09.07.2002	N69°28.025,W54°16.009	Disko Fjord				
7	09.07.2002	N69°18.408,W54°13.443	Blåfjeld				
8	12.07.2002	N69°38,W51°46	Mudderbugten				
9	12.07.2002	N69°25,W52°20	Flakkehuk				
10	12.07.2002	N69°18,W52°11	Skansen				
11	15.07.2002	N69°08.206,W53°33.299	Brændevinsskærene				
12	15.07.2002	N69°03.838,W53°30.912	Brændevinsskærene				
13	15.07.2002	N68°58.759,W53°22.684	Kronprinsens Ejlande				

**Table 1.** Positions of the stations at which samples were collected. Station numbers in bold represent those water samples used for biomass estimation.

### 2. MATERIALS AND METHODS

### 2.1 Sampling site

Between July 3rd and July 26th 14 stations were sampled in the vicinity of Disko Island. The sampling area covered an area from Mudderbugten in the east to Mellemfjord in the west. The last 3 stations were positioned around Kronprinsens Ejlande and Brændevinsskærene (see figure 5).

### 2.2 Collection of water samples at sea

At all stations phytoplankton was collected using a 20  $\mu$ m plankton net. The plankton net was moved up and down in the upper ca. 20 metres of the water column until the collected water sample had a yellowish colour. Live samples were kept cool using blue ice and a second net haul was fixed in Lugol iodine.

Initially it was planned to collect water samples with a Niskin bottle at three depths. The first at 2.5 metres, the second at the flourescense peak and the third underneath the pycnocline (thermocline or halocline). In order to find the depths with the flourescense peak and the pycnocline we used a CTD to obtain a complete profile of the water column. Unfortunately the CTD only worked properly at the first station. We were therefore forced to collect samples based on the information we had obtained from the first station in combination with the visible depth of the plankton net, when it was lowered into the water. From all depths we kept live and lugol fixed samples with a volume of 500 mL.

At stations 0, 1 and 2 we took samples according to the plan mentioned above, but soon realized that processing of the samples was extremely time consuming (1-2 hours of centrifugation per sample) and hence only samples from 2.5 metres and at the estimated chl.a maximum in a depth of 20-30 metres were collected from the remaining stations.

### 2.3 Light microscopy

The laboratory at Arktisk Station where all processing of live samples was done was air-conditioned to  $5^{\circ}$ C in order to keep the organisms alive. In the laboratory we looked at the live netplankton samples and identified as many species as possible in the light microscope. This is particulary necessary when identifying the athecate dinoflagellates as they round up in fixed samples thus becoming virtually impossible to identify. The plankton organisms were photodocumented using a Leitz Dialux 20 microscope, a Sony DXC-390 3ccd colour video camera and a Sony digital video cassette recorder DSR-V10P. The lugol fixed samples were used for estimating the biomass and as a platform for identification of *Chaetoceros* spp. and *Thalassiosira* spp. upon our return to Denmark.

### 2.4 Transmission electron microscopy of uranyl acetate stained whole mounts

The nanoplankton fraction (2-20  $\mu$ m) was also collected from the different depths and prepared for EM-grids for later species identification using transmission electron microscope. The water samples were coarsely filtered through a 20  $\mu$ m filter to isolate the nanoplankton fraction. This fraction was further more centrifuged for 30 min at 2500 rpm to concentrate the sample. We started to centrifuge 1 L/station, but as one of the centrifuges with large capacity broke down, it was necessary to only centrifuge approximately 0,5 L. Droplets of this concentrate were then placed on a formvar coated copper grid and immediately afterwards they were exposed to the vapour from four "hanging" drops of a 2% solution of OsO<sub>4</sub> for 40 seconds. This fixative kills the organisms instantly and therefore prevents the break down of the cytoskeleton which means that the organisms can be correctly identified and importantly that the flagella and body scales stick to the nanoflagellates. When dry the grids were rinsed very gently in a Petri dish containing distilled water in order to remove the corrosive salt crystals (MOESTRUP & THOMSEN 1980).

Back in Denmark the grids were stained with uranyl acetate. The electron dense uranyl component adheres to phospholipids and proteins giving the organisms a useful contrast in the electron microscope. The grids were put formvar side down on a drop of uranyl acetate and left there in darkness for 20 min. Afterwards the grids were rinsed twice in double distilled water (MOESTRUP & THOMSEN 1980). The electron microscope used was a Jeol 100SX and the film used was Kodak electron microscopy film.

The photo plates were made from the EM and LM micrographs. After having made a rough layout in hand the plates were scanned in a computer and were then "fine tuned" with the computer program Adobe Photoshop ver. 6.0 and 7.0.

### 2.5 Biomass estimation

50 mL of the Lugol fixed samples were poured into a 50 mL Hydro Bios sedimentation chamber. After 24 hrs. the suspended organisms had sunk to the bottom of the chamber ready to be counted in an inverted microscope by use of the Utermöhl method (UTERMÖHL 1958). We used a Leitz Labovert FS inverted microscope and a Nikon TMS inverted microscope. The idea was to count at least 400 cells of each taxon because this equals a 10% margin of error (OLRIK 1991). Often this was not possible because the chamber did not hold 400 cells of each taxon and furthermore the counting procedure was extremely time consuming (approximately 6 hrs. per chamber). Thus regarding the nanoplankton we agreed on counting four rows (diameter of the chamber) at 40x equalling an area of 0.32 mm<sup>2</sup> as we were working on a deadline. This means that for many taxa the margin of error due to the counting exceeds 10%.

The number of diameters counted along with the results of the counting was put into Aquabase. Aquabase is a computer program that uses cell volume together with genus specific carbon contents to estimate biomass. For correct volume estimation several groups were split in distinct size classes. The computed data was presented using Sigmaplot 8.0.

### 2.6 The reason to use sedimentation chambers for biomass estimation of phytoplankton

Often total biomass estimations are based on the amount of chl. a in a certain amount of water. However, the amount of chl. a in an organism depends on the amount of sun light which means that a period with little sun light will make the organisms produce much more chl. a than in periods with a lot of light. The amount of chl. a therefore will vary over the season and perhaps even vary on a daily basis. Biomass estimations based on spectrophotometric measurements of chl. a are therefore potentially inaccurate as they are either underestimates or overestimates depending on the season (DAUGBJERG pers. comm.). Using the sedimentation chambers the heterotrophic organisms are not left out of the biomass calculation which would be the case if estimations were based on measurements of chl. a only. It is without doubt a much more exact method but it is also extremely time consuming.

### **3. RESULTS**

### **3.1 Diversity**

All cell dimensions given in this chapter are litterature values.

### 3.1.1 Prasinophyceae

# Pyramimonas Schmarda 1850

# Pyramimonas cirolaneae Pennick 1982 (Plate 1.A-B)

The cell is 4-8  $\mu$ m long and 4  $\mu$ m wide. The cell is pyramid shaped with four equally long flagella. At the anterior end it has a stigma consisting of a single layer of globules. Trichocysts are also present. It is virtually impossible to distinguish between *P. cirolaneae* and *P. grossii* in light microscope. The box scales and crown scales are however, distinguishable in TEM. The box scales of *P. cirolaneae* are divided into four quadrants and the crown scales are spiked. Previously recorded in the Barents Sea and the English Channel. This is the first finding in Greenland (MCFADDEN et al. 1986).

Pyramimonas sp. nov. (Plate 1. C-D)

# Pyramimonas virginica Pennick 1977 (Plate 1.E-H)

The cell is 2-3.5  $\mu$ m long and 2  $\mu$ m wide. It is of pyramidial shape and has four flagella. Trichocysts are found in the lobes of the anterior end. A stigma composed of a single layer of globules is also present anteriorly. It differs from all other trichocyst bearing members of the genus *Pyramimos* by having hexagonal basket scales. Previously found on the east coast of USA and in Greenland (MCFADDEN et al. 1986).

# 3.1.2 Haptophyceae

### Pappomonas Manton & Oates 1975

Pappomonas flabellifera Manton & Oates 1975 var. borealis (Plate 2.A and 2.D)

Small autotrophic cell with two flagella and a short haptonema (HEIMDAL 1997). Heterococcolithophorid; the cell is covered by oval coccoliths and around the flagellar pole the coccoliths have an appendage in the shape of a stalk with two triangular plates attached (resembles the end of an arrow). The species is divided in two variants: var. *flabellifera* and var. *borealis*. Var. *borealis* has more dentate coccolith appendages and the columnar stalk and the plates overlap for a longer distance. Temperate and arctic regions in the northern hemisphere (MANTON et al. 1976a, THOMSEN et al. 1988).

### Pappomonas virgulosa Manton & Sutherland 1975 (Plate 2.B)

Resembles *Pappomonas flabellifera*, but instead of the columnar stalk and the two triangular plates, the stalk ends in a terminal tuft of four finger-like rods. Probably cosmopolitan (MANTON & SUTHERLAND 1975, THOMSEN et al. 1988).

### Phaeocystis Lagerheim 1893

### Phaeocystis pouchetii (Hariot) Lagerheim 1893 (Plate 2.E-G)

This species can be observed in two different life stages. It has a non-motile colony-forming stage and a free-living motile stage. In the colony-forming stage the cells lose their flagella and haptonema and lie in a gelatinous colony. In the motile stage the cells have a short haptonema and two flagella of equal length (approximately 1,5 times the cell length). The cell is 4-8 µm long and contains two chloroplasts. The presence of *P. pouchetii* in samples can



**Plate 1.** *A)* Pyramimonas cirolanae. *B)* Box scale of *P*. cirolanae. *C)* Pyramimonas sp. nov. *D)* Crown, footprint & limuloid scales of Pyramimonas sp. nov. *E) P. virginica. F)* Limuloid scale of *P. virginica. G)* Diamond shaped scale of *P. virginica. H)* Hexagonal basket scale of *P. virginica.* 



Plate 2. A) Pappomonas flabellifera var. borealis. B) P. virgulosa. C) Apedinella radians. D)
P. flabellifera var borealis close up of the cocolithphorids. E) Phaeocystis pouchetii.
F) P. pouchetii and its chitin threads in a five-star pattern. G) P. pouchetii in the colony forming life stage.

also be revealed by the observation of a five-pointed star pattern made of discharged chitin threads. Distributed in cold water in both hemispheres (THRONDSEN 1997).

# 3.1.3 Dictyochophyceae

### Apedinella Throndsen 1971

Apedinella radians (Lohmann) Campbell 1973 (Plate 2.C)

The cells' length are 6-10  $\mu$ m. Has six yellow to brown coloured chloroplasts. The cell is covered with cellulose scales of which 4-9 bear easy recognizable spiny scales. Distributed coastally in the Atlantic, Arctic, Mediterranean and Pacific (TRONDSEN 1997).

### 3.1.4 Chrysophyceae

### **Dinobryon** Ehrenberg 1834

Dinobryon balticum (Schütt) Lemmermann 1900 (Plate 3.A-C)

Forms large tree-like colonies of ochromonadoid cells in loricae. The loricae are 50-66  $\mu$ m long in the basal part of the colony and 32-35  $\mu$ m long in the distal part and 3-5  $\mu$ m wide. Mixotrophic. Distributed in the Baltic Sea, Atlantic Ocean and the Arctic (THRONDSEN 1997).

Unidentified heterotrophic flagellates (Plate 3.D-E)

Unidentified autotrophic flagellate (Plate 3.F)

### 3.1.5 Choanoflagellida

### Bicosta Leadbeater 1978

### *Bicosta spinifera* (Throndsen) Leadbeater 1978 (Plate 4.A)

The lorica consists of just seven costal strips. The two slightly uneven longitudinal costae cross midway. The posterior spine is terminally S-shaped and the total length of the lorica is 45-80  $\mu$ m long. The number of tentacles is usually around 30. Worldwide distribution in waters below 16 °C (MANTON et al. 1980).

### Bicosta minor (Reynolds) Leadbeater 1978 (Plate 4.B-D)

The lorica of *B. minor* consists of only seven costal strips. They are arranged as two longitudinal costae which meet posteriorly. The lorica length of this species in Greenland is 17-34 µm. Widely distributed (THOMSEN 1982).

### Crinolina Thomsen 1976

### Crinolina aperta Leadbeater 1975 (Plate 4.F)

The lorica is shaped like a barrel that is open in both ends. The width of the anterior end is 17-25  $\mu$ m and the width of the posterior end 25-35  $\mu$ m. The length of the lorica is 45-50  $\mu$ m. The lorica is composed of twelve longitudinal costae and two transverse costae. The protoplast (when kept intact during fixation) is centrally located. The type specimen originates from northern Canada (MANTON et al. 1975).

### Pleurasiga Schiller 1925



**Plate 3.** *A)* Colony of Dinobryon balticum. *B)* Single D. balticum with lorica. *C)* Single D. balticum. *D)* Unidentified heterotrophic nanoflagellate (I). *E)* Unidentified heterotrophic nanoflagellate. *F)* Unidentified autotrophic nanoflagellate.



Plate 4. A) Bicosta spinifera. B & D) B. minor. C) B. minor and Parvicorbicula serrulata. E) P. serrulata. F) Crinolina aperta.

Pleurasiga minima Throndsen 1970 (Plate 5.)

The protoplast is enclosed in a tight fitting broadly amphora-shaped lorica. The lorica is composed of seven longitudinal costae and two transverse costae of equal size. The anterior junctions of the longitudinal costae and the transverse costae are T-junctions. The lorica measures approximately 10  $\mu$ m both in width and length. From the protoplast a long flagellum protrudes. Cosmopolitan (THRONDSEN 1970, MANTON et al. 1976b and HANSEN et al. 1989).



Plate 5. Pleurasiga minima.



Plate 6. A) Unidentified choanoflagellate. B) Parvicorbicula quadricosta. C) Unidentified choanoflagellate. D) Acanthoecopsis sp. E) Conion groenlandicum.

# Conion Thomsen 1982

*Conion groenlandicum* Thomsen 1982 (Plate 6.E)

Cone-shaped lorica constructed of approximately eleven longitudinal and three transverse costae. The lorica is 13-14  $\mu$ m long and 9-12  $\mu$ m wide. The longitudinal costae are composed of four or five costal strips. The anterior longitudinal costal strips join the next costal strip two-thirds from the anterior end. At the posterior end of the lorica the longitudinal costae join

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and a pedicel with a length of 7-10  $\mu$ m is formed. Known from arctic and subarctic regions (THOMSEN 1982).

### Parvicorbicula Deflandre 1960

### Parvicorbicula quadricostata Throndsen 1970 (Plate 6.B)

The lorica consists of four longitudinal costae and two transverse costae. The inner transverse costa creates a square with a longitudinal costa in each corner. The outer transverse costa and the four longitudinal costae connect in T-joints. The species is observed in the northern hemisphere (HANSEN et al. 1989).

### Parvicorbicula serrulata Leadbeater 1975 (Plate 4.C and 4.E)

*P. serrulata* is characterized by the broad and serrated costal strips. The lorica is composed of seven longitudinal and two transverse costae. The lorica ends in a posterior stalk, which in the top consists of two costal strips and further down consists of single costal strips. The species is only observed in the northern hemisphere (HANSEN et al. 1989).

### 3.1.6 Bacillariophyceae

### **Centric species**



Figure 6. Schematic overview of a Chaetoceros-cell showing the most characteristic features for identification (modified from HASLE & SYVERTSEN 1997).

### **Chaetoceros** Ehrenberg 1844

Chaetoceros concavicornis Mangin 1917 (Plate 7.A-B)

Cells are 12-30  $\mu$ m wide (apical axis) and connected to form a straight chain. The valves are unlike; the upper valve is rounded and setae arise almost from the centre, the lower valve is flat and the setae arise almost from the valve corners. All setae bend towards the lower end and become broader and with spines further out. Cosmopolitan, except in the southern cold water region, common in the arctic, subarctic and temperate regions (CUPP 1943, HASLE & SYVERTSEN 1997).

### Chaetoceros convolutus Castracane 1886 (Plate 7.A-B)

Cells are 10-27  $\mu$ m wide and connected in a chain that sometimes twists around the pervalvar axis. The shape of the cells is very similar to *C. concavicornis*, but the setae do not increase in width distally. Both cells and setae contain numerous small chloroplasts (RINES & HARGRAVES 1988). Girdle zone is broad about 1/3 of the cell. Cosmopolitan (HASLE & SYVERTSEN 1997).

### *Chaetoceros debilis* Cleve 1894 (Plate 7.C)

Apical axis is 12-30 µm long. The cells form spirally twisted chains and have slightly rounded corners. The intercalary setae are relatively short and originate within the valve margin The terminal setae are likewise short and appear similar to the intercalary setae. Apertures nearly rectangular. Each cell has one chloroplast. Worldwide distribution, mainly in cold water (HASLE & SYVERTSEN 1997, JENSEN & MOESTRUP 1998).

### Chaetoceros diadema (Ehrenberg) Gran 1897 (Plate 7.D)

Cells are connected to form chains. Apical axis is 10-50  $\mu$ m long. Setae are stiff, originate inside the valve margin and cross at the chain edge. One chloroplast per cell. Resting spores are conspicuous with dissimilar valves. The primary valve is convex and has dichotomously branching spines; the secondary valve is smooth and convex. Cosmopolitan (HASLE & SYVERTSEN 1997).

### *Chaetoceros furcellatus* Bailey 1856 (Plate 7.E)

Cells are 8-20  $\mu$ m wide (apical axis). The chain is straight or somewhat curved. The intercalary setae arise just inside the valve margin and they are relatively thin. The apertures are rectangular, sometimes compressed in the centre. Each cell contains one chloroplast. This species is easily identified by its resting spores. The resting spores lie in pairs within the mother cell, are smooth and have thick setae, which are fused for a long distance (sometimes the setae also twist). Distributed in the northern cold-water region (HASLE & SYVERTSEN 1997).

### *Chaetoceros decipiens* Cleve 1873 (Plate 7.F)

Cells are 9-84  $\mu$ m wide (Cleve 1873: 27-34 $\mu$ m wide) and connected by the corners to form straight chains. Sibling setae are fused for a length of two to three setae diameters. Terminal setae are shorter, but thicker than the intercalary ones. The intercalary setae diverge at an acute angle. Apertures between cells are slit-like to elliptic. Cosmopolitan (CUPP 1943).

# *Chaetoceros socialis* Lauder 1864 *forma socialis* Proschkina-Lavrenko 1963 and *forma radians* (Schütt) Proschkina-Lavrenko 1963 (Plate 7.G)

The 2-14  $\mu$ m wide cells form short chains. The corners of adjacent cells do not touch, but the setae cross just at the valve margin. The setae are of unequal length; three setae are short and the fourth is long and entwine with other long setae (both from the original chain and other chains) in the centre of the colony. *Forma socialis* is with its smooth resting spores

distinguished from *forma radians*, which has spiny resting spores. Cosmopolitan (HASLE & SYVERTSEN 1997, JENSEN & MOESTRUP 1998)



**Plate 7.** *A-B)* Chaetoceros concavicornis/convolutus. C) C. debilis. D) C. diadema. E) C. furcellatus with resting spores. F) C. decipiens. G) C. socialis/radians.

# Thalassiosira Cleve 1873 emend. Hasle 1973

### Thalassiosira nordenskioeldii Cleve 1873 (Plate 8.A-B)

Cells are octagonal in girdle view and circular in valve view. The valves are 10-50  $\mu$ m in diameter (HASLE & SYVERTSEN 1997) and slightly concave in the middle. An organic thread from the central strutted process connects the cells. The connecting thread is no longer than the pervalvar axis. From the margin of the valve face a ring of thinner threads can be observed. Distributed in the northern cold water to temperate region (CUPP 1943, HASLE & SYVERTSEN 1997).

*Thalassiosira anguste-lineata* (A. Schmidt) Fryxell & Hasle 1977 (Plate 8.C) Cells are box-shaped with rounded corners in girdle view. The diameter is 14-78 µm (HASLE & SYVERTSEN 1997). The cells are connected by 4-9 threads. Cosmopolitan (CUPP 1943).

### *Bacterosira* Gran 1900

*Bacterosira bathyomphala* (Cleve) Syvertsen & Hasle 1993 (Plate 8.F)

In girdle view the chain consists of tightly connected cells with abutting valve faces. The diameter is  $18-24 \mu m$  and the length of the cell is usually a little longer. The cell wall of this centric diatom is only weakly silicified. Distributed in the northern cold water region (HASLE & SYVERTSEN 1997).

### *Leptocylindrus* Cleve 1889

### *Leptocylindrus danicus* Cleve 1889 (Plate 8.H)

Cylindrical cells with thin cell walls. Cells are 5-16  $\mu$ m wide and 2-10 times as long. Neighbouring cells are connected over the whole valve face, one cell is slightly convex and the other slightly concave. Many small rounded chloroplasts are evenly dispersed in the cell. Intercalary bands are present, but very difficult to see in LM. The species is cosmopolitan, but more common in the arctic to temperate region, than in the antarctic and subantarctic region (HASLE & SYVERTSEN 1997).

### Pennate diatoms

*Nitzschia longissima* (Brébisson) Ralfs 1861 / *Cylindrotheca closterium* (Ehrenberg) Lewin & Reimann 1964 (Plate 8.E)

It is necessary to study these two species in EM to distinguish them and the taxonomic position is still much debated. In LM the cells are long (linear to lanceolate) and pennate with two centrally placed chloroplasts. The cell is slightly thicker in the middle where the chloroplasts are situated. The apical axis is 30-400  $\mu$ m for *C. closterium* and 125-450  $\mu$ m for *N. longissima*. The transapical axes are 2.5-8  $\mu$ m (*C. closterium*) and 6-7  $\mu$ m wide (*N. longissima*). Cosmopolitan (HASLE & SYVERTSEN 1997).

### Pseudo-nitzschia H. Peragallo 1897-1908

Pseudo-nitzschia cfr. seriata. (Cleve) H. Peragallo 1897-1908 (Plate 8.I)

Cells are elongate and spindle-shaped in girdle view. The cells are overlapping at the ends to form chains. Two chloroplasts on either side of the median transapical plane. The are 95-115  $\mu$ m long and 6.5-7  $\mu$ m wide (CUPP 1943). It is distributed is temperate to cold water in the northern hemisphere (HASLE & SYVERTSEN 1997).



Plate 8. A-B) Thalassiosira nordenskioeldii. C) T. anguste-lineata. D) T. sp. E) Nitzschia longissima/Cylindrotheca closterium. F) Bacterosira bathyomphala. G) Licmophora sp. H) Leptocylindrus danicus. I) Pseudo-nitzschia cfr. seriata. J-K) Unidentified pennate diatom. L) Unidentified diatom.

Unidentified pennate diatom (Plate 8.J-K)

Unidentified diatom (Plate 8.L)

# 3.1.7 Dinophyceae

# Thecate dinoflagellates

# **Dinophysis** Ehrenberg 1839

Dinophysis norvegica Claparéde & Lachmann 1859 (Plate 9.A-C)

Cells are rather variable but tend to be large and ovoid 48-80 µm long, 39-70 µm wide (Faust & Gulledge 2002). Hypotheca pointed with antapical protrusions. The widest part of the cell is halfway between the lower cingular list and the antapex just above third rib (R3) of the left sulcal list. Thecal plates are heavily areolated, areolae are large and with pores. Phototrophic. Neritic; widespread in cold and temperate waters in the northern hemisphere (DODGE 1982, HANSEN & LARSEN 1992, FAUST & GULLEDGE 2002).

### Dinophysis rotundata Claparéde & Lachmann 1859 (Plate 9.D)

Medium sized cell 36-56 µm long, 36-43 µm wide (FAUST & GULLEDGE 2002). In lateral view a rounded cell with convex apex and antapex, in ventral view the cell looks more compressed but still with convex sides. Small cap-like epitheca. Left sulcal list is sigmoid and widens posteriorly, its length extends over half the length of the hypotheca. The deepest part of the cell is between second rib (R2) and third rib (R3) of the left sulcal list. Heterotrophic. Cosmopolitan (DODGE 1982, HANSEN & LARSEN 1992, FAUST & GULLEDGE 2002).

### Dinophysis acuminata Claparéde & Lachmann 1859 (Plate 9.E-F)

Small to medium sized cell 38-58 µm long, 30-40 µm wide (FAUST & GULLEDGE 2002). Almost oval or elliptical in shape. The antapex is rounded with more or less well-developed posterior protrusions. Left sulcal list extends beyond the midpoint of the cell. Depending on the age of the cell the thecal plates are covered with more or less prominent areolae each with a pore. Phototrophic. Neritic; cold and temperate waters worldwide (DODGE 1982, HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997, FAUST & GULLEDGE 2002).

### Dinophysis cfr. braarudii Nordli 1951 (Plate 9.G)

The cell is 25-32  $\mu$ m long, 21-29  $\mu$ m wide in side view and 18-20  $\mu$ m wide in ventral view. The cell resembles *D. rotundata* in the side view but it is more arrow shaped when viewed from the ventral side. The wing has three spines. This species is not formally described but only mentioned by Nordli in his work from 1951. Earlier reported from the Barents Sea and West Greenland (NORDLI 1951, CLAUSEN et al. 1994).

### Gonyaulax Diesing 1866

### Gonyaulax cfr. spinifera (Claparéde & Lachmann) Diesing 1866 (Plate 9.H-J)

A medium sized cell 24-50  $\mu$ m long, 30-40  $\mu$ m wide. The epitheca has convex sides leading into a rather short apical horn. The hypotheca is rounded and has a variable number of antapical spines (usually two). The cingulum is descending two or more cingulum widths. The sulcus is sigmoidal and extends from apex to antapex. The theca is prominently reticulated. This species is highly variable and might actually be split into several species on account of six different cyst-types that all turned out to be species recognized as *G. spinifera*.

Phototrophic. Cosmopolitan (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

# Protoceratium Bergh 1881

### *Protoceratium reticulatum* (Claparéde & Lachmann) Bütschli 1885 (Plate 9.K) Synonym: *Gonyaulax grindleyi* Reinecke 1967

A medium sized cell 28-43  $\mu$ m long, 25-35  $\mu$ m wide with a subspheroidal shape. The epitheca is conical while the hypotheca is rounded and longer than the epitheca. Theca is heavily reticulated with a pore at the centre of each reticulation. Cingulum slightly above the middle of the cell and the sulcus straight almost reaching the antapex. Phototrophic. Worldwide distribution in cold to temperate waters (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

# Ceratium Schrank 1793

# Ceratium longipes (Bailey) Gran 1902 (Plate 10.A and 10.C)

A large cell up to 250 µm long, 40-60 µm wide (DODGE 1982, HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997). The hypothecal horns bend anteriorly, the right one being nearly parallel to and occasionally as long as the apical horn, the left one a great deal shorter and curved. Is rather similar to *C. horridum* but tend to be more robust and with the apical horn bent to the right. Phototrophic. Coastal; arctic to cold temperate waters (DODGE 1982, CLAUSEN et. al. 1994, STEIDINGER & TANGEN 1997).

### Ceratium arcticum (Ehrenberg) Cleve 1901 (Plate 10.B and 10.D)

A large cell about the size of *C. longipes* with which it is easily confused. The apical horn is directed to the right. The hypothecal horns are only slightly curved compared to *C. longipes* and form an almost straight line. Phototrophic. Cold water in the northern hemisphere (HANSEN & LARSEN 1992, STEIDINGER & TANGEN 1997).

### Protoperidinium Bergh 1881

### Protoperidinium depressum (Bailey) Balech 1974

A large cell 116-200  $\mu$ m long, 116-144  $\mu$ m wide. The epitheca is concave towards the horn like apex. The hypotheca is convex near the girdle and ends in two divergent antapical horns. The cytoplasm can be pink. Cosmopolitan (DODGE 1982, THOMAS 1997).

### Protoperidinium bipes (Paulsen) Balech 1974

A small cell 20-35  $\mu$ m long, 17-19  $\mu$ m wide which is quite easily identified from its characteristic shape. The epitheca is triangular ending in a long apical horn. The hypotheca is shorter than the epitheca and has two antapical spines that diverge outwards. The thecal plates are delicate and difficult to analyse but not particularly important for proper identification. Heterotrophic. Is found throughout the Atlantic Ocean, the Mediterranean and the Baltic Sea (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et. al. 1994)

### Protoperidinium brevipes (Paulsen) Balech 1974

A small cell 20-40  $\mu$ m long, 20-40  $\mu$ m wide, which is characterized by its pentagonal shape and the two rather indistinct antapical spines. Cingulum is wide and the sulcus is deep and broadens towards the antapex. Heterotrophic. Coastal cold water (STEIDINGER & TANGEN 1997).



**Plate 9.** *A-C)* Dinophysis norvegica. D) D. rotundata. E-F) D. acuminata. G) D. cfr. braarudii. H-J) Gonyaulax cfr. spinifera. K) Protoceratium reticulatum.

### Protoperidinium pellucidum Bergh 1881

Medium-sized cell 40-68  $\mu$ m long, 36-70  $\mu$ m wide (Dodge 1982) that is more rounded than *P. pallidum* and only slightly flattened dorsoventrally. A pyriform cell with a short apical horn and two antapical spines. The sulcus widens towards the antapex and the left sulcal list may give the appearance of a third spine. The cingulum is bordered by lists supported by spines. Heterotrophic. Cosmopolitan in temperate to tropical waters (DODGE 1982, STEIDINGER & TANGEN 1997).



Plate 10. A & C) Ceratium longipes. B & D) C. arcticum.

### Protoperidinium pallidum (Ostenfeld) Balech 1973 (Plate 11)

In size a very variable cell 38-107 µm long, 30-85 µm wide (DODGE 1982, HANSEN & LARSEN 1992). An elongate pyriform cell with two divergent antapical spines (the right spine usually longer than the left) and a short apical spine. Dorsoventrally flattened and with reticulated surface. Heterotrophic. Coastal and oceanic worldwide (DODGE 1982, HANSEN & LARSEN 1992, CLAUSEN et al. 1994, STEIDINGER & TANGEN 1997).

### Protoperidinium steinii (Jørgensen) Balech 1974

A medium-sized cell 39-60  $\mu$ m long, 22-44  $\mu$ m wide which is pyriform with an elongated apical horn and a rounded hypoteca with long (9-14  $\mu$ m) antapical spines. The cingulum is bordered by lists supported by spines and the surface of the cell is prominently reticulated. Heterotrophic. Worldwide distribution (DODGE 1982, HANSEN & LARSEN 1992).



Plate 11. Protoperidinium pallidum.

# Heterocapsa Stein 1883

Heterocapsa triquetra (Ehrenberg) Stein 1883 (Plate 13.K)

Small thecate cell (<20  $\mu$ m), which in LM appears to be athecate. The epitheca is rounded to conical and the hypotheca is attenuated into a horn. Slightly displaced cingulum. Distributed worldwide (STEIDINGER & TANGEN 1997).

### Athecate dinoflagellates

# Gyrodinium Kofoid & Swezy 1921

### Gyrodinium spirale (Bergh) Kofoid & Swezy 1921 (Plate 12.G-M)

Large spindle-shaped asymetric cell, 40-200 (usually 50-103)  $\mu$ m long. The cingulum is narrow and displaced one-third to half the body length. The sulcus is only a shallow depression between the longitudinal stripes. Heterotrophic. The nucleus is situated in the middle of the cell. Cosmopolitan (DODGE 1982, STEIDINGER & TANGEN 1997).

*Gyrodinium* spp. (Plate 12.A-F & L-M) Four different taxa.

### Gymnodinium Stein 1878

*Gymnodinium rubrum* Kofoid & Swezy 1921 (Plate 13.A-C)

The cells are 100-145  $\mu$ m long and 75-90  $\mu$ m wide. This species is quite variable in shape but is normally elipsoid with a circular cross section. Its most recognizable features are the

longitudinal striation and its size. Heterotrophic. The cytoplasm has a pale red colour at the apex which gradually changes into diffuse yellow posteriorly. Formerly reported from California, Greenland and Denmark (KOFOID & SWEZY 1921, CLAUSEN et al. 1994).

### Cochlodinium Schütt 1896

# *Cochlodinium* cfr. *brandtii* Wulff 1916 (Plate 13.D-E)

A middle-sized cell 50-110  $\mu$ m long which is spindle-shaped with rounded ends. It is characterized by the deep cingulum that makes about 3-4 turns around the cell and the sulcus that slightly invades the epitheca. Heterotrophic. Information on distribution is poor but it is known from the Barents Sea and the English Channel and is probably widespread (DODGE 1982, CLAUSEN et al. 1994).

# Cochlodinium helicoides Lebour 1925 (Plate 13.F-G)

A middle-sized cell 29-54  $\mu$ m long, 24-30  $\mu$ m wide which is asymmetrical sub-oval. The cingulum makes 1.5 turns around the cell. Large nucleus in the centre of the cell and many chloroplasts with pyrenoids. Phototrophic (mixotrophic). Is known from the Mediterranean, the Pacific Ocean, the North Sea and West Greenland (DODGE 1982, CLAUSEN et al. 1994).

# Togula Flø Jørgensen & Daugbjerg 2002

# Togula jolla Flø Jørgensen & Daugbjerg 2002 (Plate 13.H-I)

A small cell 25-43  $\mu$ m long and 19-35  $\mu$ m wide, which is ellipsoidal in dorsoventral view and more elongated in lateral view. The cingulum has a sigmoid shape and is displaced 0,3-0,4 cell lengths. Several chloroplast lobes present. Probably worldwide (FLØ JØRGENSEN 2002).

# Katodinium Fott 1957

### Katodinium glaucum (Lebour) Loeblich III 1965 (Plate 13.J)

Medium-sized cell 40-56  $\mu$ m long which is characterized by the spindle-shaped form. The cingulum is displaced 4 or 5 cingulum widths. The epicone has about 20 longitudinal stripes and is much longer than the hypocone with 2-3 longitudinal stripes. Heterotrophic. Cosmopolitan, common in estuarine areas (DODGE 1982).

### Amphidinium Claparéde & Lachmann 1859

### Amphidinium cfr. operculatum Claparède & Lachmann 1859 (Plate 13.L-M)

A medium-sized cell 29-50  $\mu$ m long (normally in the range of 31-39  $\mu$ m), 21-28  $\mu$ m wide. The cell is flattened dorso-ventrally and has a elongate ellipsoidal shape in lateral view. The epicone is small and triangular in outline. The cingulum is deeply incised and originates approximately 0.3 cell lengths from the anterior end of the cell. The hypocone is broadly rounded and the antapex is slightly asymmetrical. The chloroplasts are yellow-brown and the chloroplast lobes radiate in a characteristic way from a central pyrenoid. Cosmopolitan (FLØ JØRGENSEN 2002, KOFOID & SWEZY 1921).

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Plate 12. A-B) Gyrodinium sp. 1. C-D) Gyrodinium sp. 2. E-F) Gyrodinium sp. 3. G-K) Gyrodinium spirale (pic. G-I are in deep focus and therefore appear mirrored). L-M) Gyrodinium sp. 4.



Plate 13. A-C) Gyrodinium rubrum (pic. A are in deep focus and therefore appear mirrored).
D-E) Cochclodinium brandtii. F-G) C. helicoides. H-I) Togula jolla. J) Katodinium glaucum.
K) Heterocapsa triquetra (thecate). L-M) Amphidinium cfr. Operculatum. N-O) Gyrodinium sp.

# 3.1.8 Species lists

Choanoflagellida (choanoflagellates)
Bicosta minor
Bicosta spinifera
Parvicorbicula serrulata
Crinolina aperta
Pleurasiga minima
Parvicorbicula quadricostata
Pleurasiga sp.
Conion groenlandicum
Acanthoecopsis sp.
Prasinophyceae (prasinophytes)
Pyramimonas cirolaneae
Pyramimonas virginica
<i>Pyramimonas</i> sp. nov.
<i>Mantoniella</i> sp.
Haptophyceae (haptophytes)
Phaeocystis pouchetii
Pappomonas flabellifera var. borealis
Pappomonas virgulosa
Chrysophyceae (golden algae)
Dinobryon balticum
Dictyochophyceae (dictyochophytes)
Apedinella radians
Protista insertae sedis
Telonema subtilis

 Table 2. Complete species list of nanoplankton.

### 52 K. A. Jønsson, L. Munk & M. Smith

	St. 0	St.1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8	St. 9	St. 10	St. 11	St. 12	St. 13
<i>Gyrosigma/Pleurosigma</i> sp.			х		х		х							
Leptocylindrus danicus					х	х	х					х	х	х
Licmophora sp.			х					х						
Navicula sp.							х							
Pennate diatom				х						х				
Proboscia spp.						х								
Pseudo-nitzschia cfr. seriata				х	х	х	х	х	х	х	х	х	х	х
Rhizosolenia hebetata												х		
Thalassiosira anguste-lineata						х								
Thalassiosira nordenskioeldii					х	х	х	х	х	х	х	х	х	
Thalasiosira cfr. rotula														х
Thalassiosira spp.	х		х		х									
Chrysophyceae														
Dinobryon balticum	х		х	х	х	х	х	х	х	х	х	х	х	х
Prasinophyceae														
Pyramimonas cirolanae	х	х	х	х	х	х	х		х		х			х
Pyramimonas virginica	х	х	х	х	х	х	х	х	х		х			х
Pyramimonas spp.	х													
<i>Mantoniella</i> sp.														
Haptophyceae														
Phaeocystis pouchetii					х	х	х	х		х			х	х
Dictyochophyceae														
Dictyocha speculum						х		х						
Apedinella radians														
Cryptophyceae														
Several unidentified species														
Protista insertae sedis														
Telonema subtilis														

**Table 3.** Complete species list of netplankton.

### **3.2 Biomass**

The species names mentioned in the following are due to our studies of living material in the light microscopy in Greenland. The suffixes of roman numbers e.g. (I) or (II) refer to different size classes in the same genus or undistinguishable groups.

### 3.2.1 Biomass distribution at 2.5 m (fig. 7)

The total biomass in the 2.5 metre samples varies from approximately 6  $\mu$ g C L<sup>-1</sup> to approximately 82  $\mu$ g C L<sup>-1</sup>. Station 0, 1 and 8 are all dominated by diatoms (>80%) whereas small groups of autotrophic nanoflagellates, heterotrophic nanoflagellates (station 0) and heterotrophic dinoflagellates (stations 1 and 8) contribute the rest of the total biomass. At station 13 half the biomass is contributed by diatoms and half is contributed by heterotrophic dinoflagellates. At stations 2, 3, 5 and 7 the total biomass is much smaller than at stations 0, 1,
8 and 13. These stations are dominated by autotrophic nanoflagellates and heterotrophic dinoflagellates and diatoms only play a minor role (station 3 and 7).

### 3.2.2 Biomass distribution for the deep samples (fig. 7)

The total biomass in the deep samples varies from approximately 3  $\mu$ g C L<sup>-1</sup> to approximately 37  $\mu$ g C L<sup>-1</sup>. Diatoms dominate (>50 %) at stations 0, 1 and 8 and heterotrophic dinoflagellates dominate at stations 2 and 3 (samples from Mellemfjord). Autotrophic nanoflagellates dominate at stations 5, 7 and 13.



**Figure 7.** Biomass distributions indicating the accumulated biomass of diatoms, nanoflagellates and heterotrophic dinoflagellates at eight selected stations at 2.5 metres and deep samples. Notice the different scales on the y-axis.

### 3.2.3 Station 0, 2.5 m (fig. 8)

In this sample we were able to distinguish between 19 different taxa. These represent all the taxonomical groups we observed in Greenland. It was a sample dominated by diatoms. Especially the genus *Chaetoceros* was dominant with a biomass per litre in excess of 30  $\mu$ g C and a concentration of approx. 700,000 cells L<sup>-1</sup>. The genus *Thalassiosira* contributed with a biomass exceeding 20  $\mu$ g C L<sup>-1</sup> based on a cell concentration of approx. 80,000 cells L<sup>-1</sup>. The remaining contribution was more or less negligible in comparison to the two dominant diatom genera. Though it is probably worth noticing that the two nanoflagellates *Dinobryon balticum* and *Phaocystis pouchetii* contributed more than the rest of the nanoflagellates in terms of both biomass and cell concentration.

### 3.2.4 Station 0, 15 m (fig. 8)

This station held 18 taxa. The two diatom genera *Chaetoceros* and *Thalassiosira* were again the top biomass contributors with 4.5 and 14.5  $\mu$ g C L<sup>-1</sup>, respectively. *Chaetoceros* was much more numerous with approximately 160,000 cells L<sup>-1</sup> compared to around 60,000 for

*Thalassiosira*. A concentration more or less the same for *Phaocystis pouchetii* though the biomass was only around 0.5  $\mu$ g C L<sup>-1</sup>.



**Figure 8.** Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 0 at 2.5 and 15 metres.

#### 3.2.5 Station 1, 2.5 m (fig. 9)

Again the diatoms dominate among the 24 taxa. *Thalassiosira* showed a high biomass of around 33  $\mu$ g C L<sup>-1</sup> and a corresponding concentration of approximately 130,000 cells L<sup>-1</sup>. *Chaetoceros* spp. (I) had a high cell count of around 830,000 cells L<sup>-1</sup>.



Figure 9. Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 1 at 2.5 and 18.5 metres.

A group of unidentified autotrophic flagellates was also a major contributor with 15  $\mu$ g C L<sup>-1</sup> and just below 800,000 cells L<sup>-1</sup>. The heterotrophic dinoflagellate genus *Protoperidinium* was represented by almost 3.5  $\mu$ g C L<sup>-1</sup> with only 220 cells L<sup>-1</sup>.

## 3.2.6 Station 1, 18.5 m (fig. 9)

This station held 15 taxa and was characteristic by having the *Chaetoceros* spp. (I) and *Protoperidinium* genera contributing an equal amount of 5  $\mu$ g C L<sup>-1</sup>. Again we saw a difference in concentration of the two groups with *Chaetoceros* spp. I having a much higher concentration. *Thallasiosira*, however, was the main biomass contributor with nearly 9  $\mu$ g C L<sup>-1</sup>.

## 3.2.7 Station 2, 2.5 m (fig. 10)

A total of 15 taxa were encountered on this station. A group of unidentified autotrophic flagellates (I) exceeded 2.5  $\mu$ g C L<sup>-1</sup> and had a concentration of approximately 140,000 cells L<sup>-1</sup>. *Phaocystis pouchetii* was represented with a similar concentration though its biomass was only around 1  $\mu$ g C L<sup>-1</sup>. The prasinophyte *Pyramimonas cirolaneae* was, with regards to biomass, placed in between the two others with barely 80,000 cells L<sup>-1</sup>. Diatoms at this station represent less than 0.5  $\mu$ g C L<sup>-1</sup>.

## 3.2.8 Station 2, 40 m (fig. 10)

At this station we found that *Protoperidinium* was the most dominant of the 20 taxa in terms of biomass with almost 18.5  $\mu$ g C L<sup>-1</sup>. It is, however, among the organisms with the lowest concentration (1,595 cells L<sup>-1</sup>). With the highest concentration of around 325,000 cells L<sup>-1</sup> we found the chrysophyte *Dinobryon balticum* with a biomass contribution of approximately 2.5  $\mu$ g C L<sup>-1</sup>. A group of unidentified autotrophic flagellates (I) also made a prominent contribution to the biomass of approx. 4  $\mu$ g C L<sup>-1</sup> distributed over 190,000 cells L<sup>-1</sup>. *Phaocystis pouchetii* accounted for 160,000 cells L<sup>-1</sup> and approximately 1  $\mu$ g C L<sup>-1</sup>. The diatom biomass for this depth was less than 1  $\mu$ g C L<sup>-1</sup>.

### 3.2.9 Station 3, 2.5 m (fig. 11)

Out of a total of 18 taxa found at this station *Protoperidinium* was the dominant genus with around 9.5  $\mu$ g C/L and a correspondingly low cell concentration of 668 cells L<sup>-1</sup>. The most numerous taxon per litre was *Dinobryon balticum* with almost 325,000 cells L<sup>-1</sup> of which a biomass of 2.3  $\mu$ g C L<sup>-1</sup> was deducted. The prasinophyte *Pyramimonas cirolaneae* also contributed with a similar value (2.1  $\mu$ g C L<sup>-1</sup>). The dominant diatom was *Proboscia alata* with a contribution of approximately 1.5  $\mu$ g C L<sup>-1</sup>.

### 3.2.10 Station 3, 20 m (fig. 11)

Of the 19 taxa *Protoperidinium* was again the dominant organism accounting for around 17  $\mu$ g C L<sup>-1</sup>. It was followed by *Phaocystis pouchetii* which contributed with a little less than 13  $\mu$ g C L<sup>-1</sup> and the corresponding cell concentration of around 170,000 cells L<sup>-1</sup>. The diatoms at this station were responsible for approximately 4  $\mu$ g C L<sup>-1</sup>.

### 3.2.11 Station 5, 2.5 m (fig. 12)

A group of large *Protoperidinium* (I) was the largest biomass contributor of the 11 taxa (around 7  $\mu$ g C L<sup>-1</sup>) while the biomass of *Dinobryon balticum* with just around 1.5  $\mu$ g C L<sup>-1</sup> was responsible for the highest cell concentration. A group of smaller *Protoperidinium* species contributed with a similar amount as *Dinobryon balticum*. No diatoms were recorded at this station.



**Figure 10.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 2 at 2.5 and 40 metres.* 

### 3.2.12 Station 5, 30 m (fig. 12)

A group of unidentified autotrophic flagellates (II) was the main contributor at this station with a little more than 11  $\mu$ g C L<sup>-1</sup> and a correspondingly high cell count in excess of 1.6 mill. cells L<sup>-1</sup>. *Phaocystis pouchetii* contributed with around half the biomass and concentration i.e. approximately 6  $\mu$ g C L<sup>-1</sup> and 800,000 cell L<sup>-1</sup>. Another group of unidentified autotrophic



**Figure 11.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 3 at 2.5 and 20 metres.* 

flagellates (I) had around the same biomass as *Phaocystis pouchetii* but a considerably lower cell concentration of around 265,000 cells  $L^{-1}$ . Various heterotrophic flagellates also made a notable biomass contribution with around 2 µg C  $L^{-1}$ . The accumulated diatom biomass at this station was less than 1 µg C  $L^{-1}$ .



**Figure 12.** Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 5 at 2.5 and 30 metres.

### 3.2.13 Station 7, 2.5 m (fig. 13)

Of the 17 taxa recorded *Dinobryon balticum* had the largest biomass contribution of almost 2.5  $\mu$ g C L<sup>-1</sup> as a result of a concentration 325,000 cells L<sup>-1</sup>. As a group the heterotrophic dinoflagellates contributed with a biomass of 4.5  $\mu$ g C L<sup>-1</sup>. The accumulated diatom biomass on this station was less than 1.5  $\mu$ g C L<sup>-1</sup>.

## 3.2.14 Station 7, 30 m (fig. 13)

This station held 14 taxa of which none exceeded  $1.5\mu g \text{ C L}^{-1}$ . The prominent taxa being *Phaocystis pouchetii* with 1.2  $\mu g \text{ C L}^{-1}$  and *Protoperidinium* with around 1  $\mu g \text{ C L}^{-1}$ .

### 3.2.15 Station 8, 2.5 m (fig. 14)

The main contributor to the biomass of the 21 taxa found on this station was *Chaetoceros* spp. (I) with almost 45  $\mu$ g C L<sup>-1</sup> and a concentration of approximately 1.6 mill. cells L<sup>-1</sup>. Another diatom genus *Thalassiosira* spp. contributed with around 4  $\mu$ g C L<sup>-1</sup>. The remaining taxa barely recorded biomasses of more than 3  $\mu$ g C L<sup>-1</sup>, respectively.

### 3.2.16 Station 8, 20 m (fig. 14)

The dominant taxon of the represented 16 taxa was *Dinobryon balticum* with a biomass of almost 3.5  $\mu$ g C L<sup>-1</sup>. *D. balticum* also had the highest concentration with more than 450,000 cells L<sup>-1</sup>. The two diatom genera *Chaetoceros* and *Thalassiosira* both contributed with 2-2.5  $\mu$ g C L<sup>-1</sup> but, of course, with much lower cell concentrations of *Dinobryon balticum*. *Protoperidinium* accounted for a similar contribution with a little less than 2  $\mu$ g C L<sup>-1</sup>.

## 3.2.17 Station 13, 2.5 m (fig. 15)

*Protoperidinium* was the organism that recorded the largest biomass contribution of the 14 taxa with almost 14  $\mu$ g C L<sup>-1</sup> based on a concentration of 1,200 cells L<sup>-1</sup>. Eleven of the taxa at this station were diatoms. The most important of these were *Chaetoceros* spp. (I) and *Thalassiosira* spp. with contributions between 3.5 and 8  $\mu$ g C L<sup>-1</sup>. *Chaetoceros* spp. (I) was much more numerous than *Thalassiosira* spp. with concentrations of 130,000 and 30,000, respectively.

### 3.2.18 Station 13, 20 m (fig. 15)

The group of unidentified autotrophic flagellates (I) and *Thalassiosira* spp. was the two most important groups with a biomass contribution of approximately 5  $\mu$ g C L<sup>-1</sup> and had the corresponding concentration of around 250,000 and 15,000 cells L<sup>-1</sup>, respectively. The two nanoflagellates *Dinobryon balticum* and *Phaocystis Pouchetii* both contributed with around half the biomass of the others i.e. 2-2.5  $\mu$ g C L<sup>-1</sup> and held about 300,000 cells L<sup>-1</sup>.

### 4. DISCUSSION

### 4.1 Nanoplankton diversity

The original idea of the project was to attach particular attention to the nanoplankton fraction. However, surprisingly few nanoplankton species were observed on our EM grids. Our list only includes 19 species distributed over five classes. The list furthermore reveals that not only does it contain fewer taxa but certain genera and even classes are completely missing in our samples compared to studies conducted by Thomsen in 1977 (98 species, 6 classes) (THOMSEN 1982) and Clausen et al. in 1994 (60 species, 6 classes) (CLAUSEN et al. 1994). This cannot be a matter of pure coincidence and there certainly are several things that might explain this comparatively low diversity of nanoplankton.

### 4.1.1 Comparison with Thomsen 1977

Compared to the survey conducted by Thomsen in 1977 dinoflagellates and diatoms (except for a few torn apart ones) were not represented in our nanoplankton fraction at all. These two classes alone accounted for 13 species in 1977. Of the golden algae we found only 14% of the



**Figure 13.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 7 at 2.5 and 30 metres.* 

species found by Thomsen. With three species of haptophytes we recorded 8% of the number of species collected in 1977. In the class Prasinophyceae we found four species including three species of *Pyramimonas* of which *P. cirolaneae* was neither found in 1977 nor in 1994. Furthermore more we found an undescribed species of the genus *Pyramimonas*. In 1977



**Figure 14.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 8 at 2.5 and 20 metres.* 



**Figure 15.** *Biomass (columns) and cell numbers (cross-hairs) for the protist taxa identified during the present investigation at station 13 at 2.5 and 20 metres.* 

Thomsen found nine species assigned to five genera belonging to the prasinophytes so finding only two of those genera was again rather disappointing. The pedinellid *Apedinella radians* was found in the samples from 1994 but not in those from 1977. The choanoflagellates was by far the largest group in our samples but only amounted to nine species compared to 28 found by Thomsen and none were new observations (THOMSEN 1982, CLAUSEN et al. 1994).

All together a poor result compared to 1977 and there are several reasons for this. In 1977 the collecting sites were in Disko Fjord and in the immediate vicinity of Qeqertarsuaq, which means that the collecting sites themselves could not be the reason for the differences in diversity. The sampling was done in July and August so a certain amount of seasonal variation compared to our investigations cannot be entirely ruled out. Finally the sampling was done not only at the surface but also at depths as deep as 300 metres and this has indeed had influence on the sampling results. Five species of choanoflagellates were only found at 60 metres or below and only one of these species was represented in our samples, which were mostly collected at 40 metres or above. In order to investigate if there were more heterotrophic flagellates at greater depths, two grids from water samples from 70 metres and two grids from water samples from 300 metres were examined but although this did increase the amount of choanoflagellate species found it was not nearly enough to bring the total species list to match the list from 1977 (THOMSEN 1982).

### 4.1.2 Comparison with Clausen et al. 1994

Compared to the investigations conducted by Clausen et al. in 1994 the overall impression is nearly the same as when compared with Thomsen. They found three groups *Parmales*, *Euglenophyceae* and nine diatom species, which we did not observe in our samples. The golden algae and the haptophytes amounted to 33% and 14% respectively of the species found in 1994 and the haptophytes were represented by ten genera in 1994 compared to two genera found by us. *Pyramimonas cirolaneae* and *Mantoniella* sp. were not found in 1994 but apart from that only 17% of the species we found were identical to the prasinophytes found by Clausen et al. We managed to find 47% of the choanoflagellate species found in 1994. As in 1977 Clausen et al. sampled more extensively at depths below 40 metres (CLAUSEN et al. 1994). This could explain the lack of certain taxa especially for the choanoflagellates since these are bacteria and detritus feeding organisms and thus predominantly found at greater depths (THOMSEN 1982). Six species of choanoflagellates were only found at 50 metres of which only one species was found in our samples. Another three species belonging to the haptophytes are the prasinophytes were also found at a depth of 50 metres only.

In 1994 the collecting site for nanoplankton was at one station just south of Qeqertarsuaq between July 27th and August 3rd. Sampling was carried out from the surface down to 50 metres. Furthermore one water sample was taken at the surface in the harbour basin on August 1st. Seasonal variation and collection sites are thus unlikely to explain differences in species diversity. Clausen et al. intensively studied one station and therefore probably recorded more species as opposed to the present investigation, which covered a large area.

For both studies season and sampling sites did not seem to explain the entire poorness of our species list. However, when processing the samples there were several things, which might have had a negative impact on the amount of species found. All water samples were centrifuged for no less than 30 min in three rounds of 10 min. at a supposedly but no way certain speed of 2500 rounds pr. min. This could have damaged some of the delicate organisms and made them impossible to identify. Also the temperature in the laboratory was most of the time near 10°C (even 15°C one day when the cooling system broke down) and not

5°C which is preferable since temperatures from 10-15°C can deform or even kill the cells. When the cooling system broke down we observed that the netplankton simply died so we must assume that the nanoplankton did too. A minor temperature rise probably has little impact on the organisms as opposed to great temperature fluctuations which might be fatal to the organisms. We expect the rough handling and the temperature changes to be the main reasons for finding less species than similar investigations. Lack of time prevented us from looking through all five grids per depth per station. Instead two grids per depth were investigated and this too might have contributed to the disappointing nanoplankton species list.

### 4.2 Netplankton diversity

The netplankton fraction was not centrifuged and was therefore in a relatively good condition compared to the nanoplankton fraction. Additionally four people spent a lot of time looking through the live samples in Greenland, which is easily seen from the species list. Naturally the organisms have been affected by the high temperatures in the laboratory too but many of the samples were processed within 24 hours of our arrival back at Arktisk Station which meant that many organisms could be readily identified and filmed or photographed for later identification.

### 4.2.1 Netplankton diversity compared with Clausen 1994 and Trier 1996

This does not mean that the species list is identical with the species list from 1994 but the amount of species is nearly the same. Twenty-three species of diatoms were found in both 1994 and 2002. Clausen et al. found nine species, which we did not find, and we found nine species, which were not found in 1994. For the dinoflagellates there were 24 species identical with 1994 and 2002 but Clausen et al. even discovered 17 species, which we did not see in our samples whereas we had 5 species not found in 1994. The differences in diversity could be due to the fact that Clausen et al. also collected samples in Vaigat. Apart from this the difference in species diversity is not easily explained and is hardly due to changed environmental conditions in the area. The nine diatom species found in 2002 which were not present in 1994 are neither particularly tied to cold nor warm water and the same is the case for the nine diatom species found in 1994 which were not present in 2002 (HASLE & SYVERTSEN 1997). There seems to be no pattern for the dinoflagellates either (STEIDINGER & TANGEN 1997) and it is therefore not possible to connect the differences in species composition with changing climatic conditions. The differences are more likely due to the difference in time spent for identification, the people identifying the organisms and again the fact that one will never find the exact same organisms every time one samples from day to day or from year to year due to a patchy distribution.

Trier (1998) investigated the species composition of a transect (10 stations) from Disko and westwards between June 26th and July 5th 1996. As in 1994 there is a considerable variation in species diversity. Trier found 24 species of diatoms that were not found in our samples and we on the other hand found 17 species not found at Trier's transect. For the dinoflagellates Trier's transect and the present investigation had 18 species in common and Trier found another nine species whereas we found another 11. Particularly for the diatoms this is a great deal more variable than compared to Clausen et al. but it must again be emphasized that the species lists very much depend on the people doing the identification. We for instance put a lot of work into identification of *Chaetoceros*-species, which resulted in a total of twelve *Chaetoceros* species on our list compared to only six on Trier's. This does not necessarily mean that the other six species were not present at Trier's transect since Clausen et al. found thirteen species belonging to this genus in 1994.

One species was found by neither Clausen et al. nor Trier. *Togula jolla* a newly described benthic athecate dinoflagellate (FLØ JØRGENSEN 2002) was for the first time ever discovered in Greenland. However, this species has previously been found in USA, Japan and Australia (FLØ JØRGENSEN 2002) and was expected to be discovered in the sediments along the shores on Disko Island, as this is presumably a cosmopolitan species.

The rest of the species are otherwise nearly identical except that Clausen et al. found a euglenophyte that we did not. Clearly there will always be differences in the species diversity when observed at different times even though the sampling sites seem unchanged.

### 4.2.2 Netplankton compared with Spitsbergen

Okolodkov et al. (2000) investigated the phytoplankton diversity at a transect with 16 stations in Kongsfjorden, Spitsbergen (79°N 12°E) between July 10th-16th 1996. This high-latitude locality, which is situated about 1100 km farther north than Disko, is under marked influence of the Gulf Current. The water in Kongsfjorden is therefore relatively warm and free of ice early in the year compared to other localities this far north (NORDENHAUG 1989). Though the sampling by Okolodkov et al. was carried out at the same time of the year, as the present investigation the netplankton diversity was rather different compared to the diversity in the vicinity of Disko. The two places had only nine species of dinoflagellates in common. Spitsbergen had 13 species not found around Disko whereas 18 species found around Disko were not found in the investigation in Spitsbergen. Thus more than half the species found at Disko in 2002 differed from the species found in Spitsbergen in 1996 and the total amount of dinoflagellates summed up to a total of only 22 species in Spitsbergen compared to 27 at Disko.

For the diatom species the situation is even more extreme. Only eight species were found in Spitsbergen compared to 31 at Disko, nearly four times as many. Only two species found in Spitsbergen were not found at Disko whereas Disko proved to have 26 species, which were not found in Spitsbergen. Even for other classes the picture is the same fewer genera and fewer species in Spitsbergen compared to Disko. It is difficult to know how much effort Okolodkov et al. has put into species identification but there seems to be a clear trend showing that the species diversity is poorer in Spitsbergen. This could be due to the locality e.g. a shorter season caused by the longer polar night and higher concentrations of suspended matter, which is found in the fjords of Spitsbergen (OKOLODKOV et al. 2000).

### 4.3 Biomass distribution.

Figures 7 clearly show that most of the deep water samples are not located at the expected fluorescence maximum. It was expected to observe a subsurface fluorescence maximum just above the pycnocline (halocline, thermocline or nutricline), but the sample depth has probably been incorrect. Because of the patchy distribution of phytoplankton the biomass can change a lot over a few vertical meters (NIELSEN & HANSEN 1999).

Stations 0 and 1 are dominated by diatoms with a comparatively high biomass, especially at 2.5 metres but also in the deep samples (15 and 18.5 metres). This means that silicate (SiO<sub>2</sub>) must be present, even though silicate and nitrate is often depleted in the surface layer in July after the spring bloom of diatoms (NIELSEN & HANSEN 1999). But summer blooms do occur in this area, especially along the southeast coast of Disko, because of wind and tidewater driven upwelling with nutrient rich water (ANDERSEN 1981a, ANDERSEN 1999). Phosphate is

not considered a limiting factor since it is almost never depleted in Arctic waters (HARRISON & COTA 1991).

Station 2 and 3 are both situated in Mellemfjord and interestingly this fjord is not markedly influenced by melt water, in fact salinities at these two stations ranged between 32.5 ‰ and 34 %. This means that Mellemfjord is influenced by the tide that also explains that the species found here are the same as at the other two stations west of Disko Island. The stations probably represent a phytoplankton community some time after a diatom bloom. The silicate and most of the nitrate  $(NO_3)$  is depleted and the majority of diatoms have settled out of the photic zone or they could simply just have been grazed by the protozooplankton. The diatom and nanoplankton grazing heterotrophic dinoflagellates are still present with a high biomass. The autotrophic biomass is dominated by nanoflagellates (Phaeocystis pouchetii, Dinobryon balticum, Pyramimonas spp. and several unidentified autotrophic flagellates), which have the advantage of having a large surface to volume ratio that facilitate nutrient uptake. Phaeocystis pouchetii also has an advantage when it comes to grazing. In the colony-forming life stage the cells lie embedded in a mucilaginous gel (THRONDSEN & EIKREM 2001) that has a low nutritional value for grazers (GREEN & LEADBEATER 1994). The relatively low biomass and the dominance of smaller species are typical for a summer situation (NIELSEN & HANSEN 1995, NIELSEN & HANSEN 1999, JENSEN et al. 1999). Another aspect in the fjords of Disko is the melt-water from glaciers and run-off from land that wash small sediment particles into the fjord and diminish the light penetration (ANDERSEN & BORN 1999). If the light penetration is dramaticly reduced the photic zone decreases and this will affect the standing stock negatively. However, it is not very likely to be the case at station 2 (at the time of our sampling) since we find the bulk of autotrophic biomass at a depth of 40 metres and not near the surface. An identical picture is revealed at Station 3 even though the deep sample here is taken at 20 metres.

Station 5 had a very low autotrophic biomass at 2.5 metres and was probably nutrient depleted. But the deep sample (30 metres) had the highest biomass of all stations due to autotrophic nanoflagellates and this might be because the sample is taken at the fluorescence maximum around the pycnocline. We propose that this is due to the fact that this station is the one farthest away from the coast i.e. the most "oceanic" and not affected by the upwelling in Disko Bay. If this is the case it indicates a "normal" oceanic summer situation with a dominating nanofraction in this particular instance by an unidentified autotrophic flagellate (II). Also at this station we see the largest biomass contribution of the heterotrophic nanoflagellates, which is expected to be able to feed on the remains of dead nanoflagellates (POC and DOM) and bacteria (THOMSEN 1982, HANSEN & NIELSEN 1995).

Station 7 has a very low biomass both at the surface and at 30 metres. When netplankton samples were taken the plankton net was visible down to 18 metres, therefore it is possible that a fluorescence maximum exists at a depth below 18 metres or that the biomass is low in the whole water column. If the latter is the case a possible explanation is nutrient depletion in combination with grazing by ciliates and copepods (which the present investigation did not take into account).

Diatoms with a high surface biomass dominate at station 8. This is most likely due to large amounts of nutrients perhaps caused by either upwelling or perhaps a later melting of the sea ice.

Station 13 has an intermediate biomass of diatoms and a relatively high biomass of heterotrophic dinoflagellates in the surface that graze on the diatoms. Station 13 was situated near a small island with a lot of nesting birds and these birds could be enriching the surrounding water with small amounts of nitrate.

The biomass at all stations has been compared with salinity measurements but no trend or connection could be established.

To sum up the general picture; Stations 0, 1, 8 and 13 are directly comparable since they are all situated in Disko Bay and are thus presumably affected by upwelling. This results in a comparatively high diatom biomass at 2.5 metres. Stations 5 and 7 are both located west of Disko Island and stations 2 and 3 are heavily influenced by water with origins west of Disko Island. The influence of nutrient poor oceanic water combined with a comparatively high biomass of heterotrophic dinoflagellates results in a total biomass no greater than 40  $\mu$ g C L<sup>-1</sup> which is more or less half of what has been recorded at any of the Disko Bay stations. So what is observed is a normal summer situation in an area with a complex hydrography. The biomass is low at the surface, except for stations with presumed upwelling. The deeper samples are more difficult to compare since they do not originate from the same depth and the fluorescence maximum is unknown. The information from the biomass distributions only gives a momentary picture and it is not possible to deduct whether the observed biomass values represent the beginning, the peak or the remaining biomass of a population.

### 4.3.1 Biomass comparison with other investigations in the Arctic.

Biomass comparisons are made with 1) an investigation of a transect in Disko Bay and Baffin Bay carried out by Trier in 1996 (TRIER 1998), 2) with an investigation done along part of the coast of West Greenland by Jensen et al. sampled in 1993 (JENSEN et al. 1999), 3) with the study of Nielsen and Hansen from 1994 from a permanent station just off Qeqertarsuaq (NIELSEN & HANSEN 1999) and finally with a study from Kongsfjorden, Svalbard (OKOLODKOV et al. 2000).

Trier has biomass estimations from 5 metres and from the fluorescence maximum, but aside from that we assume that it is suitable for comparison. Trier's biomass is composed of both autotrophic and heterotrophic biomass and is just like ours estimated by the Utermöhl method. In the 5 metre samples Trier estimated a biomass of  $21 \pm 28 \ \mu g \ C \ L^{-1}$  compared to our 35  $\ \mu g \ C \ L^{-1}$ . In the deeper water samples Trier estimated  $42 \pm 32 \ \mu g \ C \ L^{-1}$  and we estimated a biomass of  $20 \ \mu g \ C \ L^{-1}$ . These differences are within the margin of error and therefore agree well with our results.

Jensen et al. covers a very large area and therefore only stations in the vicinity of Disko are presented in this study (stations 431 to 453). In the study by Jensen et al. the biomass is presented as chl. a values and it was therefore necessary to multiply the chl. a values with a carbon conversion factor. This factor is not constant, as explained in section 2.6, but in order to compare our biomass estimates a value of 40 was chosen based on literature values of 31 and 43 estimated by Nielsen and Hansen in 1995 and 1999 respectively (NIELSEN & HANSEN 1995, NIELSEN & HANSEN 1999) and of 40 by Vismann & Hansen in 1998 (VISMANN & HANSEN 1998). The chl. a values are given for the whole of the euphotic zone for each station and for that reason an average of our 2.5 meter and deep water samples are used for comparison. When transforming the chl. a values of Jensen et al, a carbon biomass of 33  $\mu$ g C L<sup>-1</sup> is obtained. Compared to our 21  $\mu$ g C L<sup>-1</sup> their value is a little higher, but not more than the uncertainty of the conversion factor can account for.

Nielsen and Hansen 1999 also use chl. a as a biomass indicator and multiply with a carbon conversion factor of 43. They only sample one station, but monitor the succession through August and September. The station is situated close to our station 0. They estimate an autotrophic average biomass of 37  $\mu$ g C L<sup>-1</sup> in that period which is within the range of our results. The biomass is a little higher than the biomass of the present study, but during August 1994 strong winds occurred and brought nutrients to the euphotic zone (NIELSEN & HANSEN 1999).

In general a great consistency is found between our data and the data from previous biomass studies in the area, even though different methods have been applied. However, the Utermöhl method is probably the most accurate (if 400 cells of each taxon are counted) and facilitates partition of the biomass into the desired groups (taxonomical and ecological).

In order to compare with results from other studies in the arctic region a study from Kongsfjorden, Spitsbergen was chosen. The sampling area is further north (79°N), but the stations are also situated in and around a fjord and the sampling has also taken place in July. Abundance is expressed as cell numbers per litre. The cell counts show that the dinoflagellates are represented with larger numbers in Kongsfjorden (70,300 cells  $L^{-1}$ ) compared with Disko (2,300 cells  $L^{-1}$ ) and that diatoms are represented with lower numbers in Spitsbergen (445 cells  $L^{-1}$ ) compared with Disko (270,000 cells  $L^{-1}$ ). The lower total cell count (204,000 cells  $L^{-1}$ ) in Kongsfjorden compared with (1,136,000 cells  $L^{-1}$ ) in Disko might be due to more sediment in the water which would reduce the light penetration and result in a lower standing stock. The lower diatom cell numbers could be due to less silica in Kongsfjorden or simply due to the presence of large amounts of heterotrophic dinoflagellates. It is rather difficult to compare cell numbers for different investigations since they do not tell anything about the size of the cells and thus the actual biomass.

### 4.4 Improvements of materials and methods

In retrospect we realize that some things could have been done to improve the quality of the present phytoplankton study. As always time is an important factor and we have definitely worked against it while the project lasted. This is meant as a suggestion and recommendation section aimed at those people who in the future might conduct similar investigations.

Samples could be taken at the permanent station (station 0) as often as possible in order to investigate the extent of variation due to other parameters than change of location. The amount of cells being deformed or killed might have been reduced if concentration of the samples had been done by means of filtration instead of centrifugation. A combination of filtration and few minutes of centrifugation would probably be the best solution. Or perhaps do the centrifugation with a centrifuge capable of centrifuging one litre at a time or use a continuous centrifuge.

As for the CTD and other technical equipment it would be useful to bring some spare (less sophisticated) instruments for back up. We lost a lot of potential data on account of a useless CTD. With on location measurements of temperature and salinity in the water column, it would have been possible to detect the pycnocline and thus collect the deep samples at the depth of the theoretical fluorescence maximum.

In order to achieve a greater diversity of nanoplankton it might have been a good idea to study the EM grids at a larger magnification (for example 10,000 times magnification). We did not

observe solitary scales from ruptured cells since the grids were predominantly examined with 2000 times magnification and only with larger magnification in certain interesting areas in the vicinity of newly found cells.

## 4.5 Additional work

If we had had more time available several other methods could have been used in order to improve the species identification.

- SEM (Scanning Electron Microscopy) is useful when identifying for example *Thalassiosira*, *Pseudo-Nitzschia* and *Protoperidinium*.
- Slides with acid cleaned diatom frustules examined in LM at 100x magnification are excellent for identification of a wide range of diatoms.
- Staining cells with calcofluor white and further examination in epifluorescence microscope is a good method for identifying thecate dinoflagellates.
- Five hours of video were recorded in Greenland mainly of athecate dinoflagellates however; time did not permit us to look at them.

For improvement of the biomass estimations, it might have given us a more detailed picture of the biomass distribution if samples from all stations had been counted.

## **5. CONCLUSION**

The nanoplankton diversity (19 species, 5 classes) was quite poor compared to previous studies. The explanation for this is hardly due to changed environmental conditions in the area but should be seen in the light of the many technical difficulties when collecting and processing the samples. The netplankton diversity on the other hand corresponded very well with other studies conducted in the area. Minor differences in species composition between the present study and previous studies are expected to be explained by ordinary monthly and annual fluctuations, the time spent for identification and the different people doing the identification again rather than changed climatic and environmental conditions in the area. Compared to an investigation in the more northerly located Kongsfjorden in Spitsbergen the Disko area clearly displayed a much greater netplankton diversity presumably due to a longer season and a more variable hydrography.

By use of the Utermöhl method we calculated the biomasses of fractionated groups of flagellates and diatoms. By so doing we obtained a very detailed picture of the importance of the different protist groups. At the four stations in Disko Bay which is rather nutrient rich due to the presumed upwelling we observed a high biomass of mainly diatoms. This contrasted markedly with the observations from the four stations west of Disko Island where the water is comparatively nutrient poor. In these "ocean" influenced localities the biomass was much smaller and dominated by heterotrophic flagellates, which with their larger surface to volume ratio are better suited for life in a nutrient poor environment. Our biomass calculations are in fine agreement with previous studies conducted in the area and therefore support the general picture of the biomass distributions in the area. Furthermore our study gives a much more detailed picture of the importance of different protist groups based on biomasses. Compared to Kongsfjorden in Spitsbergen the Disko area displays larger numbers of diatoms and smaller numbers of dinoflagellates. Explanations for these findings are only speculative but might again be related to a longer season and less light attenuation in the Disko area compared to Kongsfjorden.

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# **Biodiversity and Taxonomical Revision of selected Species of Macroalgae collected at Disko Island, West Greenland.**

Berit CHRISTENSEN<sup>1</sup>, Anja GEISLER<sup>1</sup> & Majken Them JENSEN<sup>2</sup>

<sup>1</sup> Department of Phycology, Botanical Institute, University of Copenhagen.

<sup>2</sup> Department of Invertebrate Zoology, Zoological Museum, University of Copenhagen.

#### Keywords.

**Abstract.** Macroalgae were sampled at differently exposed coasts at Disko Island, West Greenland. 69 species were identified in total. Earlier investigations in other parts of Greenland have resulted in theoretic distribution patterns of algae at differently exposed coasts. When comparing these distribution patterns to the observed algal composition at Disko, it was found that the observed distribution and composition of algae are comparable to patterns found elsewhere in Greenland. To see if two species of *Elachista* are present at Disko Island (*E. lubrica* and *E. fucicola*), morphometry on sporangia, assimilating filaments and paraphyses of field collected *Elachista* from various host algae was undertaken. In regard to all examined structures, it was found, that there exists a statistical difference between the specimens of *Elachista* living on *Fucus* as compared to specimens growing on other algae species and other types of substrate. If future DNA-analysis shows that these marked morphological size differences are consistent with the two species concept, then it will be much easier to separate the two species in future. Our investigation suggests that *E. fucicola* grows on *Fucus* whereas *E. lubrica* grows on a great variety of other substrates. Oogonia and antheridia from *Fucus vesiculosus* were measured from two localities; Disko Island, West Greenland and Hvidøre, Denmark. The aim was to investigate if the size of these structures is difference between the two populations.

#### **1. INTRODUCTION**

#### **1.1** Algae in the Arctic.

The Arctic is in many ways an extreme environment. To cope with often harsh conditions, the organisms living here have evolved various adaptations such as alternative reproduction strategies, overwintering, decreased metabolic rates in darkness etc. (BORN & BÖCHER, 1999; PEDERSEN, 1994). The main problem an alga faces at such high latitudes, is a reduced growth period as a consequence of an extended winter period. The growth can be further reduced by very low temperatures occurring during winter. At  $\div$ 1,7°C, ice is formed, and since the temperature decreases toward north, the period in which plants are frozen into ice foot formations is prolonged, the further north one goes. Most algae are able to survive this freezing - sometimes it actually protects the plant from extreme temperatures and further damage from ice scouring. Nevertheless, the growth period is reduced and a short life cycle becomes necessary to the algae. The low temperatures also affect the algae in another way by lowering the growth rate which can prevent reproduction or, if severe enough, have a lethal effect on the algae. Consequently, the total number of species primarily takes place in the littoral zone where ice foot formation takes place (WILCE, 1964).

#### **1.2** Vertical distributions.

Many factors determine the vertical distribution of organisms at rocky shores. These factors include; temperature, degree of wave exposure, tidal levels, ice scouring, light intensity, chemical and physical composition of the water as well as the structure and composition of the bottom. Also biotic factors such as competition and grazing can influence distribution. When dealing with algal zonation, two zones are particularly important; the littoral zone and the sublittoral zone. The littoral zone lies between the lowest and the highest tide. In this

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zone, the organisms must be able to tolerate regular shifting periods of desiccation and wetting (BORN & BÖCHER, 1999). In the sublittoral zone, the organisms are always covered by water and do not experience the weather extremes characteristic of the littoral zone. The heights of the zones are highly variable and dependent upon the interaction of the above mentioned factors (LITTLE & KITCHING, 1996). Usually in Greenland, the littoral zone is about 1,4 m high while the sublittoral zone extend from the littoral zone down to 20-25m or to the light compensation point (PEDERSEN, 1994). Generally, patterns of organisms are remarkably similar on rocky shores, which experience the same degree of wave action. Here, plants and animals are not randomly distributed, but found in these distinct zones (LITTLE & KITCHING, 1996) as depicted in fig. 1.



After Pedersen 1987

**Figure 1:** General figure of algae distribution at differently influenced coasts; 1a: Sheltered coast with no ice scouring. 1b: Wave exposed coast. 1c: Exposed coast with ice scouring – the littoral zone. 1d: The same type of coast – illustrating algae distribution in both the littoral and the sublittoral zone.

Some of the main determinators of algal zonation in the Arctic are the degree of wave exposure and ice scouring. At low to moderately exposed shores without ice scouring, large perennial brown algae (Phaeophyta) often dominate the littoral and the upper sublittoral zones. Representatives are *Fucus vesiculosus* and *Ascophyllum nodosum* (PEDERSEN, 1994; ROSENVINGE, 1899). They all posses floating devices, which help them staying afloat at high tide (BORN & BÖCHER, 1999; LITTLE & KITCHING, 1996) (fig. 1a). At less sheltered coasts,

these algae cope in varying degrees. Ascophyllum nodosum is more sensitive to the larger acceleration forces found in such places. Contrary, F. vesiculosus is able to grow there, but the species does not develop airbladders in such locations. In these environments, different species of green algae take over, such as Urospora penicilliformis and species of Ulothrix (PEDERSEN, 1994) (fig. 1b). Ice scouring can have a devastating effect on algae growth. At coasts with massive ice scouring, the ice wears off the algae so that the littoral zone can appear almost without vegetation. The growth of the few remaining algae is modest and restricted to rock crevices and permanent tidal pools (JENSEN, 1999; PEDERSEN, 1994). In the sublittoral, where the effect of the ice is only sporadic, species of Pylaiella, Chordaria flagelliformis and Scytosiphon lomentaria appear (fig. 1c). Where ice scouring is less pronounced, these annual brown algae also moves into the littoral zone. Also different species of red algae are found in the sublittoral zone; Porphyra miniata, Rhodomela lycopodioides and *Palmaria palmata*. In the deeper sublittoral, these algae are replaced by perennial brown algae such as Agarum clathratum, Alaria esculenta, Laminaria saccharina and L. longicruris which often form large continuos covers of vegetation. Further down, this community is replaced by different species of red algae, the most common being Membranoptera denticulatum, Phycodrys rubens and Ptilota serrata (PEDERSEN, 1994). At these depths (15-20m), the brown algae are limited by the diminishing light intensities whereas red algae can cope because of their pigmentation, phycoerythrin, which enables them to absorb light at low light intensities (BORN & BÖCHER, 1999; PEDERSEN, 1994) (fig. 1d). Except for ice bergs, hardly any ice is present at Disko in July, and it is difficult to determine the degree of ice scouring at the different localities. The degree of exposure is easier assessed by pure observation. Considering the factors influencing algal distribution, we wish to examine if the observed algal composition and distribution at Disko Island, West Greenland is comparable to the theoretic generalisations described above.

### 1.3. Elachista fucicola/lubrica.

*Elachista* is a brown algal genus, which is a very common epiphyte on different algae and other substrates. In the northern Atlantic, it is represented by two taxa; *Elachista fucicola* (Vell.) Areschoug and *Elachista lubrica* Ruprecht. The morphological differences between the two species are minute, thereby allowing the following summery of the general structure of these algae (fig. 2 and pl. 1, App.2).



Figure 2: General structure of Elachista (lubrica). The figure is not drawn to scale.

Both species are composed of a prostrate microthallus of uniseriate, branched filaments. From this basal system, erect assimilating filaments are formed. These filaments branch extensively under the basal meristem, forming a characteristic medulla, and additional erect filaments and paraphyses develop from the hypomeristematic laterals. The paraphyses can be mistaken for young assimilating filaments but can be distinguished by their globular cells. The hypomeristematic laterals are also responsible for the formation of pyriform unilocular sporangia, from which swarmers are released. These swarmers develop directly into new prostrate systems. Experiments with unialgal cultures (JAASUND, 1960; PEDERSEN, 1979) have revealed the presence of uniseriate plurilocular sporangia on the microthalli – these are impossible to observe in field-collected material because of the dense medulla. As in the case with the unilocular sporangia, the plurilocular sporangia also release swarmers, which develop directly into new prostrate systems. Gametes have never been observed.

Because of the morphologically similarity of the two species, the North Atlantic species of *Elachista* have posed a taxonomical problem for investigators for many years. In most recent literature, it has been widely accepted, that *Elachista* was represented by only one species in the North Atlantic, being *E. fucicola* (PEDERSEN, 1979). This view changed when ZACHO (2001) found developmental, morphological and especially molecular evidence for dividing this species into two: *E. fucicola* and *E. lubrica*.

Presupposing that two species occur in the North Atlantic, we wish to examine if both species are present at Disko (using morphometry and DNA-sequencing), and if they are – which species of host algae, they colonise. The choice of host algae could prove to be yet another way to separate the two species, as investigations have shown, that *E. fucicola* grows almost exclusively on species of *Fucus* whereas *E. lubrica* colonises a number of other species – both perennials and annuals (PEDERSEN, 1979; RUENESS, 1977).

### 1.4. Fucus vesiculosus.

*Fucus vesiculosus* is a very common and conspicuous alga, which occur in the littoral zone throughout the northern hemisphere. The parenchymatic thallus is flat and dichotomously branched and has a midrib (NIELSEN, 1981). Paired floating devices are usually present, but can be absent in plants from brackish water and exposed environments (LARSEN & HANSEN, 1986). The species is dioeciosus and the reproductive organs are situated in conceptacles in the terminal parts of the thallus (MOSS, 1950). The oogonium contains 8 haploid eggs when ripe. These are expelled from the oogonium by mucous secretion as a cluster, which breaks open, releasing the eggs into the water. The antherida are elongate and contain numerous spermatozoids when mature. Similar to the eggs, the spermatozoids are released in mucous packets, which dissolve in the water. The reproductive products are released during low tide, and fertilization takes place during the next high tide (HOEK ET AL., 1998) (fig. 3a+b). The zygote develops into new diploid plants (NIELSEN, 1981).



Figure 3a: Oogonium from F. vesiculosus showing division into 8 eggs. From: Nielsen, 1981



Figure 3b: Antheridia from F. vesiculosus. From: Nielsen, 1981

The reproductive biology of fucoids has been extensively investigated. The topics for these studies have been receptacle initiation, period of receptacle growth and oogonial maturation (BÄCK *et al.*, 1991), anatomical studies and chemical composition of receptacles (MOSS, 1950) and general reproduction (KNIGHT & PARKE, 1950). All of these studies have worked with *Fucus* from contrasting environments and arrived at the conclusion, that geography and exposure greatly affect the parameters, that they have investigated.

Our aim is to compare *Fucus vesicolusus* from two geographically different areas, which experience similar wave exposure. We wish to see, if Arctic populations of *F. vesiculosus* differ from boreal populations in respect to the size of oogonia and antheridia.

### 2. MATERIALS AND METHODS

### 2.1. Field sampling

Algae were collected at various localities in the surrounding waters and fiords of Disko Island, West Greenland in July 2002. The island is situated about 200 km north of the Arctic Circle (between 69° to 70,5°N and 51° to 54°W) and 100 km from the west coast of Greenland (fig. 4). The waters around Disko are a mixture of cold, low saline water from the Baffin Current and warmer, more saline water with North Atlantic origin (BUCH, 2002; SCHMID & PIEPENBURG, 1993). From October until May, the sea is usually covered by ice which, when it thaws, causes a reduction in the salinity of the surface layers, together with melt water from land. This melt-off reaches a maximum in September, after which the turbulent winter climate creates more uniform salinities (ANDERSEN, 1981b). The sampling was done by means of a triangle and a rectangular dredge – either dragged from aboard the research vessel "Porsild" or from a dinghy. Nine of the localities were in the sublittoral zone: Engelskmandens Havn, Enoks Havn, Mellemfjord, Jernpynten, Satut, Brændevinsskærene, Kronprinsens Ejland, Igpik, and Nunguaq. At these localities, the water was around 5-10 meters deep. Furthermore, algae were handpicked from the littoral zone at one locality, Udkiggen. The material was stored in seawater. In laboratory, it was sorted out and determined to species level (if possible) using the key of RUENESS (1977) plus additional literature (PEDERSEN, 1976; ROSENVINGE, 1893). Stereoscopes (SZX12) and light microscopes (Olympus BH2) were used for identification. In addition, pictures were taken with a digital camera (Olympus 30-30 zoom) mounted on a light microscope (Olympus BX51).

### 2.2. Elachista.

If possible, the length and width of 10 mature unilocular sporangia were measured for each specimen by means of a light microscope (Olympus BH2) - maturity was defined by the presence of red eyespots. Also the width of the basis of 20 assimilating filaments and the distal part of 20 paraphyses were measured. Measurements were done on *Elachista* found on various host algae; 20 specimens living on *Fucus vesiculosus*, 20 specimens found on *Agarum clathratum*, 4 specimens on *Rhodomela lycopodioides*, 3 specimens on *Dictyosiphon foeniculaceus* and 1 specimen growing on *Ptilota pectinata*. The data was statistically tested for differences in variance using an ANOVA test in the software program SAS version 8.2. Germlings for DNA analysis were isolated from Greenlandic material and cultivated in MV30 (CHRISTENSEN, 1988), a modification of Provasoli's ES with addition of vitamins.

#### 2.3. Fucus.

The material from Disko was handpicked in the littoral zone at Udkiggen in July, 2002. In Denmark, material was collected north of Copenhagen, at Hvidøre in November, 2002. Both locations were moderately exposed and the water temperature at the time of sampling was very similar (5-7 °C). From each location, 10 male and 10

female plants were chosen and small portions of the conceptacles were cut for microscope preparation. For each reproductive organ, 10 antheridia or oogonia were measured. Only mature structures were measured, - determined as a division of the oogonia and by red eyespots in the spermatozoids. Also these results were statistically tested with an ANOVA test in SAS 8.2.



Figure 4: Map of the different localities at Disko where macroalgae were collected: 1) Udkiggen, 2) Engelskmandens Havn, 3) Enoks Havn, 4) Mellemfjord bund, 5) Jernpynten, 6) Satut, 7) Brændevinsskærene, 8) Kronprinsens Ejland, 9) Igpik, 10) Nunguaq. After Andersen, 1981b

## 3. RESULTS

### 3.1. Distribution

A total of 69 species of algae were identified. 23 species were found in the littoral zone compared to 60 species in the sublittoral. 14 of the observed species occur in both zones. In App. 1, all the observed species are compiled into a species list together with the respective localities, at which they were found. In addition, selected pictures of these species are presented in plates 1-2 (App.2). In table 1, substrate and number of different species within the present orders are reported for the different localities.

From App. 1 and table 1, it is seen that Udkiggen, Kronprinsens Ejland and Igpik show by far the highest species diversity, each locality numbering almost 1/3 to 1/2 of all species identified. In contrast, only a few species were observed in Mellemfjord and at Satut. Phaeophyceae is the dominant order and observed for all localities. Chlorophyceae and Rhodophyceae are present in lower numbers at most of the localities, except from Mellemfjord and Satut, where they are entirely absent. At Jernpynten only a few species of Chlorophyceae occur apart from the Phaeophyceae.

Locality	Substrate	Phaeophyceae	Chlorophyceae	Rhodophyceae	Chryrophyceae	Total
Udkiggen	Rocky coast, algae sampled from tidal pools and rocky shelves	12	9	2	0	23
Engelskmands Havn	Small rocks and mud	9	1	3	0	13
Enoks Havn	Shell gravel and big rocks	12	5	4	1	22
Mellemfjord (inner part)	Fine mud and silt	5	0	0	0	5
Jernpynten	Mixture; open sandy areas with gravel and others with rocks	18	2	0	0	20
Satut	Rocky bottom with sandy areas in between	2	0	0	0	2
Brændevins- skærene	Rocks	3	0	6	0	9
Kronprinsens Ejland	Rocks	19	6	3	1	29
Igpik	Mixture of mud and gravel	17	2	6	1	26
Nunguaq	Mud	12	0	3	0	15

**Table 1:** Number of identified species within each order present together with the substrate at each locality.

### 3.2. Elachista

The mean values of measures of the different structures of *Elachista* are shown in table 2 and fig. 5. In table 2 and in fig. 5, the results of the measurements of sporangia, paraphyses and assimilating filaments have been compiled for comparison between the different host algae. The values from Nunguaq and Igpik are very similar and about twice as large as the values from the *Elachista* from Udkiggen. The one specimen from Brændevinsskærene has been left out as it was very poorly developed and the maturity of the sporangia was questionable. There were significant differences between the measured structures on *Elachista* from different host algae – with a p value of less than 0.0001, the F values for each structure are; paraphyses: 365.13, assimilating filaments: 485.11, sporangia width: 233.63, sporangia length: 336.53.

	Udkiggen	Nunarsuaq	lgpik	lgpik	Brændevinsskærene
	(on Fucus	(on Agarum	(on Rhodomela	(on Dictyosiphon	(on Ptilota pectinata)
	vesiculosus)	clathratum)	lycopodioides)	foeniculaceus )	
	20 Spec.	20 Spec.	4 Spec.	3 Spec.	1 Spec.
Sporangia length µm	65,8	144,15	136,08	130,54	÷
Sporangia width µm	29,49	66,92	67,02	63,91	÷
Paraphyses µm	9,43	17,89	16,52	14,75	10
Ass. filaments µm	12,55	23,16	22	19,75	13,33

**Table 2:** The mean values obtained from measurements of three different morphological structures of Elachista.

### **3.2.** *Fucus*

Table 3 and fig. 6 exhibit the compiled data of measurements performed on the reproductive structures of *Fucus vesiculosus*. It is evident from table 3 and fig. 6 that the sizes of the reproductive organs are very similar at both locations. This is supported statistically by the ANOVA test giving F values of; antheridia width: 59.63, antheridia length: 19.85, oogonia width: 24.03, oogonia length: 28,17 with p values less than 0.0001.



**Figure 5:** *Mean values with standard deviations of the different morphological structures measured on Elachista. The single specimen found on Ptilota pectinata has been left out.* 



Figure 6: Mean values with standard deviations of the reproductive organs measured on *Fucus vesiculosus.* 

	Udkiggen	Hvidøre
Oogonia length µm	140,9	153,3
Oogonia width µm	130,5	132
Antheridia length µm	42	45,6
Antheridia width µm	15,5	18,7

 Table 3: Mean values obtained from measurements of reproductive structures in Fucus vesiculosus

### 4. DISCUSSION

### 4.1. Distribution.

When looking at the observed composition of algal species compared to the theoretical distribution patterns at differently exposed coasts (fig.1), it becomes clear, that observed distribution patterns are much more complex than pictured.

At Engelskmandens Havn, we did not sample in the littoral zone, but a belt of algae was observed visually in this zone. At Udkiggen, where we did sample in the littoral zone, we found a lot of *Fucus vesiculosus*, *F. evanescens* and a single specimen of *Ascophyllum nodosum*. Besides being typical inhabitants of the littoral zone, these species are also characteristic of somewhat sheltered coasts together with *Devaleraea ramentacea* and *Monostroma fuscum* which were found at both localities. These findings indicate that these localities are somewhat sheltered and do not experience ice scouring – as in fig. 1a. This is in agreement with other algae found there such as *Ulothrix* and *Urospora* species. But the picture is not that simple; even though the *F. vesiculosus* plants at Udkiggen were well developed, airbladders were absent. Besides, a few specimens of species characteristic of the distributions depicted in fig. 1c+d - Chordaria flagelliformis and species of *Pylaiella*, were also found. Based on fig. 1, we believe that these two localities share features from both fig. 1a and fig. 1c+d – being somewhat sheltered coasts with occasionally ice scouring.

Even though *Chordaria flagelliformis* and *Pylaiella* appear at Engelskmandens Havn and Udkiggen, they occur in much higher numbers at Jernpynten, Enoks Havn, Brændevinsskærene, Kronprinsens Ejland, Igpik and Nunguaq. All of these localities had bare rocky coastlines and the degree of wave exposure was varying from moderately to high. We saw no or very little algal growth in the littoral zone, which could indicate that, in this zone, these localities display the distribution pattern shown in fig. 1b.

In the sublittoral zone, we observed large submerged forests of *Laminaria sp., Alaria* esculenta and Agarum clathratum at Nunguaq, Igpik, Kronprinsens Ejland and Jernpynten. Many of the red algal species found at Brændevinskærene were growing on the hapteres of *Laminaria nigripes*. This locality was the only place where red algae outnumbered brown algae. It has been reported for the Disko area, that grazing by green sea urchin (*Strongylocentrotus droebachiensis*) has a great impact on the distribution of larger brown algae (PEDERSEN, 1994), which can be why we observed this phenomenon. At Jernpynten, red algae were absent but this may be caused by inadequate sampling or by brown algae forcing the red algae out on deeper waters and out of sampling area.

Apart from Mellemfjord and Satut, the observed algal composition at all localities seems to be very similar as they display many of the same species. The distribution is in general agreement with the patterns depicted in fig. 1c and 1d, which suggest that they are all influenced by ice scouring to a varying degree.

The above mentioned parameters do not explain, why Mellemfjord and Satut show such a low species diversity. It is clear, that the observed distribution pattern cannot be explained by exposure and ice scouring alone. Beside these two parameters, structure of the shore and bottom texture greatly influence algae distribution. Especially settling algae and larger benthic algae are depending upon a stable substrate, which is probably the reason why we only observed very few specimens of large brown algae, such as Laminaria species at Igpik, Enoks Havn and Engelskmandens Havn. At these localities, the main components of the substrate were mud or gravel, which exclude these species from living there. In contrast, the vegetation was dominated by ephemeral algae. In Mellemfjord, this phenomenon was very obvious. Here, we observed the second lowest number of species, which was clearly influenced by the fact, that this locality was situated in the bottom of a fiord close to a river outlet. A distinct boundary existed between the water of the fiord and the water from the river, which carried a lot of silt. This great sediment supply is especially pronounced in spring, when melting water from fiords and rivers transport silt and mud into the sea. It reduces the transparency of the seawater, which causes a reduction in photosynthesis by lowering light penetration and/or by sedimentation directly on the thalli. If the sedimentation is in great excess, the sea bottom can be covered with so much sediment that the algae can no longer adhere (BORN & BÖCHER, 1999; LITTLE & KITCHING, 1996).

Furthermore, the melt-of in spring sometimes results in a several meters thick layer of nearly freshwater. This can have a lethal effect on the algae, if they cannot submerge to higher salinity (LITTLE & KITCHING, 1996; PEDERSEN, 1994). At Nunguaq we saw a pronounced boundary between the saline seawater and the fresh water from river outlets. The sampling was done on the saline side of the boundary and apparently the salinity and light intensities were sufficient for algal growth, since we found several different species of brown algae and a few red algae.

In reality, these general trends in distribution patterns are an oversimplification; the vertical distribution of algae is controlled by the complex interaction between a lot of different factors. Despite differences in the parameters that influence algal distribution at different coasts of Greenland, the distribution and composition of algae at Disko appear to be comparable with distribution patterns found in other parts of Greenland.

### 4.2. Elachista

Over the years, there has been much controversy as to whether *E. lubrica* is merely a form of *E. fucicola*. ROSENVINGE (1893) and LUND (1959) both agree that *E. lubrica* and *E. fucicola* are taxonomical synonyms, whereas JAASUND (1960) not only separates the two species, but goes as far as to place *E. lubrica* in another genus; *Myriactula* Kuntze. His argument for this placing is partly his observation of phaeophycean hair in connection with the assimilating filaments and the presence of plurilocular sporangia on the prostrate system of *E. lubrica*. These structures are absent in *E. fucicola*. We did not observe hair or hair-like structures in our material, which is in agreement with observations on cultures and living material of *E. lubrica*, where the presence of true hair has never been confirmed (EDELSTEIN ET AL., 1971; LUND, 1959; PEDERSEN, 1979; ZACHO, 2001). In culture, however, hair-like structures have been found. These are in fact elongated, long-celled vegetative filaments as they contain chloroplast. Such do occur in crowded cultures and could be a response to nutritional conditions (PEDERSEN, 1979). With regard to the sporangia, the uniseriate plurilocular

sporangia observed by JAASUND, 1960, have also been observed by PEDERSEN (1976, 1979). KORNMANN (1962) AND KOEMAN & CORTEL-BREEMAN, (1976) observed similar structures on the microthallus of Dutch material of E. fucicola. These structures seem to be different from the ones observed by JAASUND and PEDERSEN, since they are described as parenchymatous plurilocular sporangia and are probably induced by experimental temperatures. Another character of E. lubrica, observed by JAASUND, is the observation of intercalary transverse and longitudinal divisions in the assimilating filaments. These divisions have also been reported by ROSENVINGE (1893) and KUCKUCK (1929), which interpret them as plurilocular sporangia. Such divisions have never been observed in culture and seem to serve no reproductive function, which led LUND (1959) to call them abortive sporangia. PEDERSEN (1979) proposes that they are somehow induced by epiphytic algae or bacteria, as seen in other cases. PEDERSEN does agree to the fact that there is a morphological difference between plants from different regions, concerning the fertile basal system. This difference makes him suggest a genetic variation, but not one justifying a separation at species level. As in most recent literature, he lists only one species of Elachista from Greenland; Elachista fucicola. ZACHO (2001) reassessed in a recent investigation, the taxonomic status of E. fucicola/lubrica. Four strains of *Elachista* from different regions were investigated concerning morphology, temperature response, reproduction and phylogeny. The author found, that germlings from the NW Atlantic strains develop differently from the NE Atlantic and NE Pacific strains and only the NW Atlantic strains developed uniseriate plurilocular sporangia. Furthermore, the number of these sporangia was most excessive at low temperature, which corresponds to the local conditions of the origin of these strains. Based on those findings and on phylogenetic analysis, she proposed to resurrect E. lubrica. It is likely, that E. lubrica gains a reproductive advantage because of the formation of uniseriate plurilocular sporangia on the microthallus. Since the microthallus is capable of overwintering, the onset for swarmer release is earlier and reproduction is independent of development of macrothalli. This enhances the colonisation efficiency, which enables this species to grow on host algae with shorter life spans. It is most likely a reproductive adaptation to the short growth periods in the Arctic which is in agreement with ZACHO (2001), as she conclude, that E. lubrica has an arctic to cold-temperate distribution as compared to E. fucicola's arctic to temperate distribution. Our measurements show that there exist a distinct difference between Elachista growing on Fucus and Elachista growing on other species of host algae. Since the specimens growing on Fucus vesiculosus were collected in the littoral zone whereas all other *Elachista* were found in the sublittoral zone, one could suspect, that this result is caused by different growth conditions at the various localities, resulting in individual size differences. This seems unlikely though, because Udkiggen shares quite a lot of traits with the other localities, even though the composition of algae found here was different when compared to the other localities. Also the salinity and temperature in the upper waters around Disko are quite uniform, which does not seem to justify such large and consistent size differences. Another parameter supporting the presence of two species in Greenland is the great difference in number of algae used as substratum; in Greenland, Elachista is an epiphyte on approximately 25 species of host algae (both perennials and annuals) whereas they are restricted to only 5 perennial species in Denmark mostly fucoids (PEDERSEN, 1979). Bases on that fact and our morphological data, we believe, that we have indeed found both species of Elachista at Disko; E. fucicola living on Fucus vesiculosus and E. lubrica growing on a variety of other substrates. We would liked to have tested this assumption by using DNA analysis on the material, but the unialgal cultures did not develop in time for this procedure.

### 4.3. Fucus vesiculosus

The receptacle development of *Fucus vesiculosus* proceeds over a period of several months. First, receptacle initials are developed as small swellings on the tip of the fronds. After the initiation period, which lasts for several months, conceptacles form on the receptacles followed by the formation of gametes. The onset and length of initiation differ for different locations (BÄCK ET AL., 1991; KNIGHT & PARKE, 1950). BÄCK et al. (1991) observed, that initiation occurred during short day conditions whereas low temperatures (< 5°C) seem to control the development of the conceptacles. At both our localities, the sampling was done in sea water with temperatures between 5-7°C. At Hvidøre, sampling was performed over a period of four weeks in November. Apparently the temperature was to high at the beginning of November because no fertile Fucus plants was found during the first two weeks. Previous investigations of *Fucus vesiculosus* have shown, that different populations differ markedly in the vegetative thallus morphology (BÄCK et al., 1991; Moss, 1950). Our observations support this view since the plants from Disko Island were smaller and more compact compared to the plants from Hvidøre. Furthermore airbladders were absent in the material from Greenland but usually found in the Danish population. The degree of wave exposure and sea temperature were compatible at both locations at the time of sampling, so the differences are probably caused by the occasional ice scouring at Udkiggen. Among other things, BÄCK et al. (1991) and MOSS (1950) looked at the receptacles of F. vesiculosus and found differences in regard to both morphology and sizes of these structures at differently exposed localities. We observed the same phenomenon, as the conceptacles of specimens from Greenland were markedly larger and more inflated than those from Denmark. This result is probably influenced by the fact that plants from Hvidøre were collected in November, when they had just reached maturity, whereas plants from Greenland were collected in July, which is near the end of their fertility period. Moss (1950) reported that the intercellular mucilage in the conceptacles increased with maturation, which explains the larger amount of mucilage and size observed in the Greenlandic population. For differently exposed localities, BÄCK et al. (1991) found no differences in oogonia size in contrast to Moss (1950) who reports size differences for both oogonia and antheridia. Our results shows that no ecotypic variation exist between Fucus vesiculosus plants from Denmark and plants from Disko Island with regard to oogonia and antheridia sizes. Although unlikely, it cannot be excluded that the oogonia and antheridia from Fucus collected at Hvidøre attain larger sizes later in the fertile period. Already they appear a bit larger as compared to the plants from Greenland.

### **5. CONCLUSION**

Three projects concerning macroalgae were carried out during the Arctic Field Course, 2002; The observed distribution of macroalgae at Disko Island were compared to theoretic distribution patterns which are based upon observations from other parts of Greenland. Despite local differences regarding biotic and abiotic factors influencing algal distribution, it was found that both the observed algal distribution as well as the species composition at Disko are comparable to distribution patterns in other regions of Greenland.

Morphometry on selected structures of *Elachista* were measured to see if there was a difference between *Elachista* growing on different host algae. Our morphological data revealed a statistically significant difference between *Elachista* growing on *Fucus* and *Elachista* growing on other substrates, suggesting that two species of *Elachista* are present at Disko; *E. fucicola* and *E.lubrica*.

Antheridia and oogonia of *Fucus vesiculosus* were measured for two different geographically locations (Disko Island, West Greenland and Hvidøre, Denmark). These populations were

examined concerning ecotypic variation and despite differences in growth period and time of fertility, no differences were found in regard to the examined structures.

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# Appendix 1

#### Phaeophyceae

Lokalitet	Udkiggen	Engelskmandens	Enoks	Mellemfjord	Jernpynten	Satut	Brændevins-	Kronprinsens	Igpik	Nunguaq
Art		Havn	Havn	bund			skærene	Ejland		
Agarum clathratum		Х			Х			Х	Х	
Alaria esculenta					Х	Х		Х		Х
Ascophyllum nodosum	Х									
Chorda filum	Х				Х				Х	
Chordaria flagelliformis	Х		Х	Х	Х		Х	Х	Х	Х
Coelocladia arctica			Х		Х					
Delamarea attenuata	Х		Х		Х					
Desmarestia aculeata		Х	Х					Х		
Desmarestia viridis		Х	Х							
Dictyosiphon foeniculaceus	Х		Х	Х	Х			Х	Х	Х
Ectocarpus fasciculatus			Х		Х			Х		
Ectocarpus pycnocarpus		Х								
Ectocarpus siliculosus										Х
Elachista fucicola	Х									
Elachista lubrica		Х						Х	Х	(X)
Eudesme virescens					Х			Х	Х	Х
Fucus evanescens	Х				Х					
Fucus vesiculosus	Х									
Halosiphon tomentosus		Х							Х	
Haplospora globosa									Х	
Hincksia ovata								Х		
Laminaria longicruris					Х	Х		Х		Х
Laminaria nigripes					Х		Х			
Laminaria saccharina			Х		Х				Х	Х
Laminaria sp. (juvenil)									Х	
Leptonematella fasciculata								Х		
Petalonia fascia									Х	
Phaeostroma pustulosum								Х		
Pylaiella littoralis	Х		Х	Х			Х		Х	
Pylaiella varia		Х	Х	Х	Х				Х	Х
Porterinema fluviatile								Х		
Protectocarpus speciosus								Х		
Punctaria glacialis								Х		
Punctaria plantaginea		Х			Х			Х	Х	
Scytosiphon complanatus			Х		Х			Х	Х	Х
Scytosiphon lomentaria					X			X	Х	Х
Sphacelaria arctica	Х	Х								
Sphacelaria plumosa			Х		Х			Х	Х	Х
Stictyosiphon tortilis	Х			Х						
Symphyocarpus strangulans								Х		

#### Chlorophyceae

Lokalitet	Udkiggen	Engelskmandens	Enoks	Mellemfjord	Jernpynten	Satut	Brændevins-	Kronprinsens	Igpik	Nunguaq
Art		Havn	Havn	bund			skærene	Ejland		
Acrosiphonia arcta					Х					
Acrosiphonia centralis	Х		Х		Х			Х		
Acrosiphonia sonderi			Х					Х		
Chaetomorpha capillaris	Х									
Chaetomorpha melagonium			Х							
Chlorochytrium dermatocolax									Х	
Cladophora sp.	Х									
Enteromorpha intestinalis	Х									
Enteromorpha prolifera	Х									
Kornmannia leptoderma		Х						Х		
Monostroma fuscum	Х		Х							
Ulothrix flacca	Х		Х					Х		
Ulothrix speciosa								Х		
Ulva lactuca		Х								
Ulvopsis grevillei	Х									
Urospora penicilliformis	X									
Urospora wormskioldii								Х	Х	

#### Rhodophyceae

Lokalitet	Udkiggen	Engelskmandens	Enoks	Mellemfjord	Jernpynten	Satut	Brændevins-	Kronprinsens	Igpik	Nunguaq
Art		Havn	Havn	bund			skærene	Ejland		
Acrochaetium microscopium									Х	
Callophyllis cristrata			Х							
Devaleraea ramentaceum	Х	Х							Х	
Membranoptera denticulata							X			



# Appendix 2

Plate 1: 1) Elachista lubrica - with mature sporangia, 2) Elachista fucicola – also with mature sporangia, 3) Punctaria glacialis – base with marginal rhizoids, 4) Haplospora globosa – with both oogonia (horisontal arrow) and antheridia (vertical arrow), 5) Hincksia ovata – opposite branches and sporangia (arrow), 6) Eudesme virescens – with sporangium.



Plate 2: 1) Symphyocarpus strangulans – some of the distal cells branche subdichotomously,
2) Ectocarpus fasciculatus – nucleus suspended in cytoplasm (arrow) and bandshaped choroplast with several pyrenoids, 3) Ralfsia bornetii – squash preparation showing the long distal cells, paraphyses and several empty unilocular sporangia (arrow), 4) Coelocladia arctica – surface covered with filaments which are transformed into plurilocular sporangia, 5) Urospora wormskioldii – the vegetative cells are transformed into sporangia, from which zoospores are released,
6) Urospora wormskioldii – zoospores, 7) Urospora penicilliformis – reticulate chloroplast. Some of the cells are transformed into sporangia, 8) Ulothrix speciosa (left) and U. flacca (right) – Both species contain girdle-shaped chloroplast. The width of the cells is the distinguishing character, 9) Ulothrix speciosa – when mature, the cells are transformed into sporangia and the alga curls up, 10) Scagelia pylaisaei – tetrasporangium producing four haploid tetraspores from which male and female gametophytes develop.

# Some Vascular Plants in the Vicinity of Godhavn, Disko Island – July 2002

#### Niels DAUGBJERG

Botanical Institute, Øster Farimagsgade 2D, DK-1353 Copenhagen K, Denmark

**Abstract.** Vascular plants were identified during hiking trips mostly in the vicinity of Godhavn in July 2002. A total of 73 species from 30 families were identified and of these 32 are illustrated in 5 color plates. No attempt was made to identify grasses and species in the sedge family. During our 3-week long stay we recorded 34% of the vascular plants at Disko and 14% of the total number of vascular plants in Greenland.

#### **1. INTRODUCTION**

This chapter provides a list of the vascular plants observed during our field course at Arktisk Station in July 2002. Since the taxonomy and distribution of higher plants was not studied thoroughly the species list should not be considered as a detailed survey of the Godhavn flora. Rather it is included here to reveal some of the most common plants in addition to a few 'exotic' orchids. The flora was studied mostly during hiking trips to places like Skarvefjeld, Engelskmandshavn, Kuannit, Lyngmarksbræen, Blæsedalen and the Lymnaea lake in Kvandalen. These observations formed the basis of an understanding of the plants typically occurring in the arctic terrestrial ecosystem.

For identification of vascular plants the following books were used

- 'Grønlands flora' by BÖCHER et al. (1966)
- 'Grønlands blomster' by FEILBERG et al. (1984)
- 'Den store nordiske flora' by MOSSBERG et al. (1999)

#### **2. PHOTODOCUMENTATION**

The photographs shown in plate 1-5 were taken with a Canon EOS E50 camera equipped with a Canon compact macro lens EF 50 mm 1:2.5. The film used was kodachrome 64. The slides were scanned using a Nikon coolscanIII slide scanner and individual photographs were assembled using Adobe Photoshop ver. 7.01.

#### **3. RESULTS AND DISCUSSION**

A total of 73 species of vascular plants from 30 families were determined (Table 1). Thirtytwo of these are illustrated in five photographic plates. It should be noted that no serious attempt was made to identify grasses and plants belonging in the sedge family. Bryophytes (though not vascular plants) also were not identified albeit numerous taxa were observed, particularly in connection with the many hot springs present on Disko Island. A total of 513 higher plants have been recorded from Greenland (http://www.nat.ku.dk/as/ASuk-nat.htm) and we observed  $\approx 14\%$  of these. The total number of higher plants at Disko Island is 213 and we saw  $\approx 34\%$  of these. Five orchid species are known from Greenland and we saw three of these (Table 1). Though *Corallorhiza trifida* (koralrod in Danish) has its northern distribution on Disko Island we did not encounter it. *Amerorchis rotundiflora* (rhizomgøgeurt in Danish) is not present on Disko.

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**Table 1.** List of higher plants observed in the vicinity of Godhavn, Disko Island, July 2002.Family names are given in Danish and in Latin. Latin names in boldface indicateplants shown on photographic plates (Plate 1-5).

Family (Danish / Latin)	Latin name	Danish name
Padderokkefam. /	Equisetum arvense	Ager-padderok
Equisetaceae	Equisetum variegatum	Liden padderok
Ulvefodfam. /	Huperzia selago	Otteradet ulvefod
Lycopodiaceae	Lycopodium annotium	Femradet ulvefod
- 1	Diphasiastrum alpinum	Bjerg-ulvefod
Slangetungefam. /	Botrychium lunaria	Alm. Månerude
Ophioglossaceae	Botrvchium lanceolatum	Fliget månerude
Mangeløvfam. /	Polystichum lonchitis	Krumfinnet skjoldbregne
Dryopteridaceae		5 8
5 F	Gymnocarpium dryopteris	Tredelt egebregne
Ranunkelfam. /	Ranunculus lapponicus	Laplands-ranunkel
Ranunculaceae	Ranunculus pygmaeus	Dværg ranunkel
	Anemone richardsonii	Sne-anemone
Rosenfam. / Rosaceae	Drvas integrifolia	Grønlandsk fieldsimmer
	Potentilla vahliana	Vahls potentil
	Alchemilla glomerulans	Kilde-løvefod
Stenbrækfam. /	Saxifraga caesnitosa	Tue-stenbræk
Saxifragaceae		
	Saxifraga tricuspidata	Tornet stenbræk
Natlysfam / Onagraceae	Chamaenerion latifolium	Storblomstret gederams
	Epilobium hornemannii	Mos-dueurt
Nellikefam /	Honckenva peploides	Arktisk strandarve
Carvophyllaceae	Stellaria longines	Stilk fladstierne
	Stellaria calvcantha	Field fladstierne
	Cerastium arcticum	Arktisk hønsetarm
	Viscaria alpina	Alpe-tiærenellike
	Silene acaulis	Tue-limurt
Valmuefam. /	Papaver radicatum	Alm. field-valmue
Papaveraceae		
Korsblomstfam, /	Draha sp.	
Brassicaceae		
Skærmblomstfam. /	Angelica archangelica	Field-kvan
Aniaceae		<b>J</b>
Pilefam. / Salicaceae	Salix arctophila	Tundra-pil
	Salix herbacea	Dværg-pil
	Salix arctica	Arktisk pil
	Salix glauca	Blågrå pil
Birkefam. / Betulaceae	Betula nana	Dværg-birk
Syrefam. / Polygonaceae	Oxvria digvna	Fjeldsvre
,	Polygonum viviparum	Topspirende pileurt
Rubladfam. / Boraginaceae	Mertensia maritime	Hestetunge
Lyngfam. / Ericaceae	Cassiope tetragona	Kantlyng
, <u> </u>	Harrimanella hvnnoides	Moslyng
	Ledum palustre	Mose-post
	Phyllodoce coerulea	Blålvng
	Rhododendron lannonicum	Alperose
	Vaccinium uliginosum	Mosebølle
	Loiseleuria procumbens	Kryblyng
Fieldprydfam. /	Diapensia lapponica	Fieldprvd
Diapensiaceae	z mponsta tappontoa	- Jerupi J u
Revlingefam /	Empetrum hermanhroditum	Field-revling
Empetraceae		- Jera re rang
Hindebægerfam. /	Armeria scahra	Field-engelskgræs
Plubaginaceae		- Jera engeröngræb
Maskeblomstfam. /	Veronica alpina	Alpe-ærenpris

Scrophulariaceae	Veronica fruticans	Klippe-ærenpris
-	Pedicularis flammea	Brand-troldurt
	Pedicularis hirsuta	Lådden troldurt
	Pedicularis lanata	Uldhåret troldurt
	Pedicularis lapponica	Laplands-troldurt
	Euphrasia frigida var. frigida	Arktisk øjentrøst
	Bartsia alpina	Sorttop
Vintergrønfam. /	Pyrola grandiflora	Storblomstret
Pyrolaceae		sommerkonval
Blærerodfam. /	Pinguicola vulgaris	Alm. vibefedt
Lentibulariaceae	0 0	
Klokkefam. /	Campanula uniflora	Enblomstret klokke
Campanulaceae	1 0	
Kurvblomstfam. /	Erigeron humilia	Sort bakkestjerne
Asteraceae	Antennaria alpina var. canescens	Grå kattefod
	Gnaphalium norvegicum	Sæter-evighedsblomst
	Arnica angustifolia	Arktisk guldblomme
	Taraxacum lacerum	Grønlandsk mælkebøtte
Liljefam. / Lilaceae	Tofieldia pusilla	Fjeld-bjørnebrod
Gøgeurtfam. /	Leucorchis albida	Satyrblomst
Orchidaceae	Platanthera hyperborea	Grønlandsk gøgelilje
	Listera cordata	Hjerte-fliglæbe
Sivfam. / Juncaceae	Juncus arcticus	Arktisk siv
	Juncus trifidus	Treblad-siv
Halvgræsfam. / Cyperaceae	Carex misandra	Bue-star
	Carex bigelowii spp. nardeticola	Rank star
	Eriophorum scheuchzeri	Polar-kæruld
	Eriophorum angustifolium	Smalbladet kæruld
Græsfam. / Poaceae	Elymus mollis	Dunet marehalm

A comparison of the number of plants recorded in 1982 and 2002 shows that during the 1982 field course they recorded nearly 1.8 times as many plants as we did. The total number of plants identified in 1992 and 2002 was identical (table 2).

**Table 2.** Total number of higher plants recorded in the vicinity of Godhavn during field courses in 1982, 1992 and 2002.

Year of observation	1982	1992	2002
Total number of	131	75	74
higher plants			

Going through the species lists in more detail we observed 9 species not observed in 1982 and 21 species not observed in 1992. On the other hand 27 species were recorded in 1992 but not in 2002. In the 1982 survey they observed 68 species not recorded in 2002. Of these approx. 34% belong to species of *Carax*, *Draba*, *Festuca*, *Poa*, *Luzula*, *Ahelum* and *Agrostis*; taxa that were not given high priority among the vascular plants recorded in 2002. Still the flora list from 1982 is the most complete.

Acknowledgmets. Thanks to Reinhardt Møbjerg Kristensen and Marianne Philip for showing us the many beautiful places in the vicinity of Godhavn to 'look' for vascular plants.

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Plate 1. A. *Pedicularis lapponica* (laplands-troldurt). B. *Pedicularis hirsuta* (låd-den troldurt). C. *Pedicularis flammea* (brand-troldurt). D. *Pedicularis lanata* (uldhåret troldurt). E. *Bartsia alpina* (sorttop). F. *Veronica alpina* (alpe-ærenpris). G. *Veronica fruticans* (klippe-ærenpris).



Plate 2. A. Leucorchis albida (satyrblomst). B-C. Platanthera hyperborean (grøn-landsk gøgelilje). D. Listera cordata (hjerte-fliglæbe). E. Botrychium lanceolatum (fliget månerude).



Plate 3. A. Saxifraga tricuspidata (tornet stenbræk). B. Saxifraga caespitosa (tue-stenbræk). C. Polygonum viviparum (topspirende pileurt). D. Viscaria alpina (alpetjærenellike). E. Silene acaulis (tue-limurt). F. Chamaenerion latifolium (storblomstret gederams).



Plate 4. A. Ledum palustre (mose-post). B. Phyllodoce coerulea (blålyng). C. Harrimanella hypnoides (moslyng). D. Loiseleuria procumbens (kryblyng). E. Cassiope tetragona (kantlyng). F. Papaver radicatum (alm. fjeld-valmue). G. Pyrola grandiflora (storblomstret sommerkonval).



Plate 5. A. *Betula nana* (dværg-birk). B. *Salix arctica* (arktisk pil). C. *Campanula uniflora* (enblomstret klokke). D. *Erigeron humilia* (sort bakkestjerne). E. Erio- phorum scheuchzeri (polar-kæruld). F. *Arnica angustifolia* (arktisk guldblomme). G. *Angelica archangelica* (fjeld-kvan).

# Marine, Benthic Algae – Annotated List

Poul Møller PEDERSEN

Department of Phycology, Botanical Institute, University of Copenhagen.

**Abstract.** During the course in arctic biology, July 2002, on the island of Disko and with base at Arctic Station, Qeqertarsuaq, 69 species of marine macroalgae were identified. Most of the species were collected in the sublittoral zone at depth from 3-10 m. The aim of this annotated list is to give a detailed description of some interesting reproductive and distributional patterns in some selected species, and to be an inspiration for future studies.

#### Coelocladia arctica Rosenvinge

The type locality for this species is Ujarasugssuk, Vaigattet, Disko Island. Since ROSENVINGE's (1893) description this species has not been found in nature in Greenland with reproductive structures, but it occurs quite often in culture by chance (PEDERSEN 1976, PEDERSEN et al. 2000), and therefore it is probably quite abundant in West Greenland. However, the occurrence of the characteristic sporangial filaments from the surface cells is very important for a safe identification as sterile thalli can easily be taken for a species of *Stictyosiphon*.

Specimens with plurilocular sporangia have been collected at Enoks Havn, Mellemfjord and at Jernpynten at the entrance to Mellemfjord. This is the first report on such structures in nature in Greenland since they were described by ROSENVINGE (1893).

#### Hincksia ovata (Kjellman) P.C. Silva

Plants with opposite plurilocular sporangia, a sporangium opposite a lateral or opposite laterals have been found at Kronprinsens Island.

#### Haplospora globosa Kjellman

Plant with antheridia and oogonia have been found.

*Trachynema groenlandicum* (Lund) Pedersen Syn. *Litosiphon groenlandicus* Lund

This species was formally described by LUND (1959), but it was already mentioned in an earlier paper (LUND 1951). The distribution was at that time restricted to East Greenland (Jørgen Brønlund Fjord, Kejser Franz Josephs Fjord and Scoresby Sund). Only very few additional reports exist in the literature since it was described. KAWAI & KUROGI (1983) found similar but more robust plants in Japan and erected var. *japonicus*. PEDERSEN (1985) discussed the generic placing and studied additional material with plurilocular sporangia from the Cape Farewell area, South Greenland. He concluded that *L. groenlandicus* could not be accommodated in the genus *Litosiphon* due to the presence of basally sheated true hairs and the hecatonematoid microthallus observed by KAWAI AND KUROGI (1983). Therefore, the new genus *Trachynema* was erected and the new combinations *T. groenlandicum* and *T. mortensenii* were formally introduced (PEDERSEN 1985).

The distribution of *Trachynema groenlandicum* is now extended to the west coast of Greenland. We have found found several specimens of this species with unilocular sporangia slightly protruding from the surface of the parenchyma being solitary or formed by transformation of adjacent cells. This genus is probably rather common, but a careful inspection of the material is required to disentangle this species from other species in the community of filamentous and parenchymatous algae with few longitudinal walls.

# Trachynema mortensenii (S. Lund) Pedersen

The differences between the two species of *Trachynema* are only minor and refer to the overall dimension of the plants and the plurilocular sporangia, and the fact that unilocular sporangia are unknown in *T. mortensenii*. Size and the development of reproductive structures on juvenile plants are under strong environmental control, for example by light intensity as in *Myriotrichia clavaeformis*.

*Trachynema groenlandicum* is now in unialgal culture and the validity of the differences between the two species will be tested, and the life history can be studied and compared with the Japanese plants.

# Porterinema fluviatile (Porter) Wærn

A careful examination of the older part of the laminae of members of the Laminariales will often reveal this species. It grows together with the green alga *Bolbocoleon piliferum* and other epi-/endopytes. In fertile condition *Porterinema* can be recognised by the subdivision of vegetative cells into sporangia with few loculi.

The synonymy with *Sorapion kjellmanii* (Wille) Rosenvinge has never been fully supported (see PEDERSEN 1981). *Porterinema*-like plants develop from unilocular sporangia of *S. kjellmanii*, but the process is seemingly not resilient. Therefore, *P. fluviatile* is maintained as a distinct species.

# Pylaiella varia Kjellman

All samples in the sublittoral zone contain rather large amounts of filamentous browns. One of the most conspicuous components in this filamentous association is *Pylaiella varia*, which can be rather easily recognized by its branching pattern being often unilateral, and the laterals issue from 2-3 adjacent cells in a downward succession.

# Ectocarpus fasciculatus Harv.

This species is another important component among the filamentous brown algae in the sublittoral zone. It occurs with unilocular sporangia.

Ectocarpus pycnocarpus Rosenvinge is probably a phenotype of E. fasciculatus.

# Ulothrix scutata Jónsson

This species was described by JÓNSSON (1904), but it has rarely been mentioned in the literature and its separation from *Ulothrix flacca* is questionable being based on the discoid holdfast and regular constrictions in the erect filaments. It has been argued that *U. flacca* also shows increased differentiation between the basal system and the erect filaments under more exposed conditions, and most authors consider *U. scutata* as a synonym of *U. flacca*.

A unialgal culture of *U. scutata* isolated from a crude culture of *Elachista fucicola* growing on *Fucus* collected at Udkiggen near Qeqertarssuaq/Godhavn in the littoral zone develops constantly a discoid holdfast in a stagnant culture medium. Further investigations are needed on the possible genetic basis for the development of a discoid holdfast together with further details on its life-history and developmental patterns for an evaluation of its taxonomic status.

Scytosiphon complanatus (Rosenvinge) Doty

The genus *Scytosiphon* in Greenland comprises at least two species, *S. lomentaria* and *S. complanatus*. In addition, some cylindrical plants occur, and they develop discs composed of bilateral symmetric elements, never radiary growing discs similar to normal *Ralfsia* (PEDERSEN 1980).

*S. complanatus* is characterized by the absence of paraphyses, the hollow thallus, and the presence of true hairs. These characters separate it from *Petalonia zosterifolia* (Reinke) Kuntze (PEDERSEN et al. 1987).

In Enoks Havn, Mellemfjord, we have found another complanate *Scytosiphon* growing on small stones and fragments of bivalves at c. 3 m depth. The plants have plenty paraphyses among the plurilocular sporangia, the true hairs often occur in fascicles, and the macrothallus is hollow. The identity of these plants is uncertain and further studies on plants in unialgal culture are needed. The possibility exists that some Pacific taxa (see for example KOGAME 1996) may have passed through the Canadian arctic archipelago and are distributed along the northern part of the Greenland west coast.

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# New rotifer recordings from Disko Island, with a complete list of the Greenlandic rotifer fauna

Martin Vinther SØRENSEN

Zoological Museum, University of Copenhagen, Denmark. E-mail: mvsorensen@zmuc.ku.dk

Abstract. The rotifer fauna in Moraine Lake on Disko Island was investigated. Twenty-five taxa were found, 22 of which were identified to species level; 21 of the recorded taxa are new for Disko Island and three are new for Greenland. The new findings are presented in a species list that summarizes all records of rotifers from Greenland, inclusive several records that not earlier have been published. Furthermore, notes are given on the morphology of some selected species, inclusive *Eothinia elongata*, *Microcodides chlaena* and *Resticula nyssa*.

Key words. Arctic, Rotifera, trophi morphology, SEM, species list.

#### **1. INTRODUCTION**

Our knowledge on the Greenlandic rotifer fauna is scarce, and most of the collections have been concentrated to the Disko Bay area and around Kangerlussuaq and Ammassalik. The first study of the Greenlandic rotifer fauna was done by LEVINSEN (1881) who reported that he had observed almost 30 different species in samples from Ilulissat. However, he was only able to identifying three of them, and only to genus level. Ten years later BERGENDAL (1892) made a much more comprehensive sampling from freshwater and brackish water localities near Ilulissat and Aasiat, and recorded 82 species of which 53 are valid today. The following hundred years the rotifer samplings on Greenland were extremely sporadic, until DE SMET et al. (1993) could present a list that included 67 identified species, based on material from Kangerlussuaq and Ammassalik. Since 1993, observations on the Greenlandic rotifer fauna have been restricted to the Disko Island area.

In the summer of 2002 I visited the Danish Arctic Station on Disko Island during the biannual course in Arctic biology held by the University of Copenhagen, and during my stay I had the opportunity to sample rotifers from different freshwater localities near Godhavn. The present contribution deals with the rotifers found in the periphyton of a stagnant freshwater lake in Blæsedalen, approximately 3 km from Godhavn. Comments are given on the morphology of some selected species and a complete list that summarizes all rotifer recordings from Greenland through time is presented.

# 2. MATERIALS AND METHODS

#### 2.1 Locality description

The southern part of Disko Island is traversed by three valleys that connect the south coast with Disko Fjord. One of these, Blæsedalen, is approximately 20 km long and opens to the south right next to Godhavn and the Danish Arctic Station. The sampling locality, Moraine Lake (69°16'09"N 053°28'19"'W), is located at the foot of the prominent moraine hill, Pjeturssons Moraine, 2 km from the southern entry point of Blæsedalen. The lake covers an area of 0.75 km<sup>2</sup> and receives water from two tributaries in its eastern end. In its western end there is an outlet to the river Rødeelv (RØEN, 1962). Most of the bottom is covered with stones or dark mud, but near the eastern tributaries there is dense vegetation of the moss *Drepanocladus exannulatus*. The lake is ice free from the beginning of July to late September.

#### 2.2 Sampling and sorting

Periphyton samples from Moraine Lake were taken on 27 July 2002, with a conical plankton net with a diameter of 30 cm and a mesh size of 40  $\mu$ m that was dragged through the *D. exannulatus* vegetation. Subsequently, *D. exannulatus* was collected and squeezed into a 30  $\mu$ m mesh sieve. At the sampling time temperature was 7.4°C and pH 6.8.

The samples were sorted alive under an Olympus SZX12 dissecting microscope. Identification and observation of the specimens were done with an Olympus BX51 Nomarski microscope. Some specimens were photographed with a Camedia C-3030 digital camera or filmed with a JVC TK-C1381 video camera. Specimens for whole mounts were fixed in 3% formaldehyde, dehydrated through a series of glycerin, and mounted on glass slides. Trophi were prepared for LM and SEM following the procedure given by DE SMET (1998). Trophi for SEM were examined and photographed with a JEOL JSM-6335F field emission SEM.

Species
Bdelloidea indet.
C. gibba (Ehrenberg, 1832)
C. ventripes Dixon-Nuttall, 1901
Collotheca sp.
C. obtusa (Gosse, 1886)
Dicranophorus luetkeni Bergendal, 1892
Eosphora cf. najas Ehrenberg, 1830
Eothinia elongata (Ehrenberg, 1832)
Euchlanis dilatata Ehrenberg, 1832
<i>Floscularia</i> sp.
K. quadrata (Müller, 1786)
L. hamata (Stokes, 1896)
L. lunaris (Ehrenberg, 1832)
L. patella (Müller, 1786)
Lindia torulosa Dujardin, 1841
Microcodides chlaena (Gosse, 1886)
Mytilina mucronata (Müller, 1773)
N. foliacea (Ehrenberg, 1838)
Proales fallaciosa Wulfert, 1937
Proalinopsis caudatus (Collins, 1872)
Resticula nyssa Harring & Myers, 1924
Scaridium longicaudum (Müller, 1786)
Stephanoceros fimbriatus (Goldfuss, 1820)
Trichocerca rattus (Müller, 1776)
Trichotria tetractis (Ehrenberg, 1830)

**Table 1.** Rotifers recorded from the Moraine Lake in July 2002.

# **3. RESULTS AND DISCUSSION**

#### **3.1 Recorded species**

The rotifer species recorded from the Moraine Lake are listed in Table 1. The samples yielded a total of 25 rotifer taxa (24 species + Bdelloidea indet.). Three species, *Eothinia elongata, Resticula nyssa* and *Stephanoceros fimbriatus* are new to Greenland. Some selected species are illustrated on fig. 1.

The new recordings are furthermore included in Table 2 that presents a complete list of the Greenlandic rotifer fauna. The list is based on earlier published records (LEVINSEN, 1881; DE GUERNE & RICHARD, 1889; BERGENDAL, 1892, VANHÖFFEN, 1897; RØEN, 1966; ANDERSEN, 1990; DE SMET et al., 1993; SØRENSEN, 1998; SØRENSEN & KRISTENSEN, 2000; KRISTENSEN & FUNCH, 2000; FUNCH & SØRENSEN, 2001), personal unpublished collections, and an unpublished species list by Dr. Ruttner-Kolisko (RUTTNER-KOLISKO, in lit.).

## **3.2 Species account**

## Eothinia elongata (Ehrenberg, 1832)

Several fairly large specimens were found in the sample (Fig. 1A). All investigated specimens had trophi from bdelloid rotifers in their stomachs. The species has not earlier been recorded from Greenland, but has been found in Europe (GLASCOTT, 1893; BĒRZIŅŠ, 1949; WULFERT, 1960), Russia, the Asian parts of the former Soviet Union (KUTIKOVA, 1970), South America (SEGERS & DUMONT, 1995) and the United States and Canada (HARRING & MYERS, 1922; MYERS, 1942; CHENGALATH & KOSTE, 1989). It is considered a rare cosmopolite.

The species is distinguished by its trophi morphology combined with the presence of one large salivary gland, and one cerebral and two frontal eyes. The recorded specimens were generally typical but deviated in some details in the trophi. Trophi from two specimens were prepared for SEM. The rami are large and slightly asymmetrical, with long, pointed alulae (Fig. 2A-D). Most posteriorly on the ventral side, two extensions from each ramus form a small hinge (Fig. 2E). Basifenestrae and subbasifenestrae are present. The apical rami parts bend dorsally and have a dense row of fine teeth (Fig. 2C). Each ramus has approximately 20 teeth. A pair of small, twisted, fan-shaped oral plates is attached on the ventral side of rami (Fig. 2B). The fulcrum is clearly divided into a dorsal and ventral part (Fig. 2A, C-D). The dorsal part is broadened laterally and composed of relatively thick sclerofibrillae that are arranged both side-by-side and on top of each other. The ventral fulcrum part is much more narrow laterally, composed of one horizontal row of thick sclerofibrillae. Each uncus has one tooth with a basal lamella (Fig. 2F). The manubria are composed of a long distal cauda and a small proximal head. The head is formed by the walls of the medial and posterior manubrium chambers. Apertures from both chambers are located 1/3 from the proximal end of the manubrium (Fig. 2A, D). A paired epipharynx composed of two large, fan-shaped plates is present (Fig. 2A). The shape of the epipharynx is most easily seen with LM. It differs from the more rod-shaped epipharyngeal elements with spatulate terminals, which were described from the specimens investigated by HARRING & MYERS (1922) and WULFERT (1960).

Measurements: Body: 504 µm; toes 37 µm; trophi 64 µm; rami 38 µm; fulcrum 37 µm; manubria 47 µm; unci 17 µm; epipharynx 15 µm; oral plates 6 µm.

Table 2. Rotifers recorded from Greenland. Auth. Ref. numbers referrer to following publications: 1 = LEVINSEN (1881), 2 = DE GUERNE & RICHARD (1889), 3 = BERGENDAL (1892), 4 = VANHÖFFEN (1897), 5 = RØEN (1966), 6 = ANDERSEN (1990), 7 = DE SMET et al. (1993), 8 = SØRENSEN (1998), 9 = SØRENSEN & KRISTENSEN (2000), 10 = KRISTENSEN & FUNCH (2000), 11 = FUNCH & SØRENSEN (2001), 12 = RUTTNER-KOLISKO, in lit., 13 = SØRENSEN, unpubl. obs., 14 = present study.

Species	Auth Ref	Region
Aspelta chydona H&M 1928	10	Degertarsuag
Asplanchna sp	4	Pearyland
A priodonta Gosse 1850	3 4 7 12	Ilulissat Uummannaa Ammassalik Oegertarsuaa
A priodonta helvetica (Imhof 1884)	2	Oagortog Aasiat
Brachionus angularis Gosse 1851	7	Kangerlussuag
B hidentata Anderson 1889	3	Aggiat
B. calveiflorus Pallas 1766	7	Kangerlussuag
B. levdigi Cohn 1862	7	Kangerlussuag
<i>B. urceolaris rubens</i> (Ehrenberg 1838)	7	Kangerlussuag
<i>B. variabilis</i> (Hempel 1896)	7	Kangerlussuag
Cenhalodella sp. 1	7	Kangerlussuag
Canhalodalla sp. 2	7	Kangerlussuag
C cf. auriculata (Müller, 1773)	7	Kangerlussuag
C. cl. duffculata (Wuffert, 1775)	7	Kangerhussung
C. oungulata Wuller, 1957	2 7 12	Aggiet Ilulisset Kangerlussung Ammerselik
C. calellina (Mullel, 1776)	5, 7, 12	Adsiat, hunssat, Kangenussuaq, Animassank,
C. doligata Wulfort 1027	7	Ammagaalik
C. deficula (Ebranharg, 1937)	7	Annassank
C. gibba (Ebrophorg, 1932)	2 7 14	Rangeriussuag
C. globa (Enterlocig, 1852)	3, 7, 14	Kangerluggung
C. cl. globala (Gosse, 1887)	7 2 7 11	Aggiet Kangerlussung Obgertersung
C. gracuis (Entenderg, 1852)	3, 7, 11	Kangerluggung
C. mula Myers, 1924	/	Cagarteriusg
C. plicala Myers, 1924	15	Vegenarsuag
C. stered (Gosse, 1887)	/	Kangeriussuaq, Ammassank
C. tantilloides Hauer, 1935	13	Veqertarsuaq
C. ventripes angustior Donner, 1950	/	Kangeriussuaq, Ammassalik
C. ventripes Dixon-Nuttall, 1901	14	Qegertarsuaq
Collotneca sp.	14	Qegertarsuag
C. campanulata (Doble, 1849)	3	Northeast Greenland
C. mutabilis (Hudson, 1885)	4	
C. coronetta (Cubitt, 1869)	3	
C. ornata (Enrenberg, 1832)	2	Northeast Greenland
C. ornata cornuta (Doble, 1849)	2, /	Kangerlussuaq
Colurella adriatica Ehrenberg, 1831	/	Kangerlussuaq, Ammassalik
C. colurus (Ehrenberg, 1830)	3, 9, 11	Aasiat, Ilulissat, Ritenbenk, Ikka Fjord, Qegertarsuag
C. obtusa (Gosse, 1886)	7, 10, 13, 14	Kangerlussuaq, Ammassalik, Qeqertarsuaq
C. uncinata (Muller, 1773)	3, 7, 11	Aasiat, Ilulissat, Ritenbenk, Kangerlussuaq,
	6.0.11	Qegertarsuag
C. unicauda Godske Eriksen, 1968	6, 9, 11	Ikka Fjord, Qegertarsuag
Conochilus hippocrepis (Schrank, 1830)	2, 3, 4	Oummannaq, Aasiat
C. unicornis Rousselet, 1892	12	Qegertarsuaq
Dicranophorus jorcipatus (Muller, 1/86)	3, /	Aasiat, Kangeriussuaq
D. luetkeni Bergendal, 1892	3, 14	Ilulissat, Qeqertarsuaq
Dissotrocha sp.	1	Ilulissat
D. aculeata (Ehrenberg, 1832)	3	Aasiat, Ilulissat, Ritenbenk
Encentrum sp.	7	Ammassalik
<i>E. algente</i> Harring, 1921	11	Qeqertarsuaq
E. ct. diglandula (Zavadowski, 1926)	/	Kangerlussuaq
E. graingeri Chengalath, 1985	8	Qeqertarsuaq
E. limicola Otto, 1936	9,11	Ikka Fjord, Qeqertarsuaq
<i>E. marinum</i> (Dujardin, 1841)	3, 8, 11	Aasıat, Qeqertarsuaq
<i>E. mustela</i> (Milne, 1885)	7	Kangerlussuaq
E. porsildi Sørensen, 1998	8	Qeqertarsuaq

## Microcodides chlaena (Gosse, 1886)

This species was extremely common in the samples (Fig. 1C). It has earlier been recorded in Scandinavia, Central Europe, the former Soviet Union (WULFERT, 1940, KUTIKOVA, 1970), the United States (HARRING & MYERS, 1922), and Papua New Guinea (SEGERS & DE MEESTER, 1994). On Greenland it has been recorded in the Ilulissat region (BERGENDAL, 1892).

The species is easily distinguished by the presence of toes with unequal length and lateral antennae in depressions located posterior to a pair of characteristic integumental elevations (Fig. 1C).

The adult animals are gibbous in lateral view and conical in dorso-ventral view (Fig. 1D); immature animals are considerably smaller, with S-shaped body (Fig. 1B). The body is divided into a head, a trunk and a foot region. The head is large, especially in the immature specimens. The dorsal part of the trunk has distinct longitudinal folds in the integument. The foot is composed of two pseudosegments and both toes are attached to the distal pseudosegment. This observation is in agreement with that of KOSTE (1978), but contradicts WULFERT (1940) who describes *M. chlaena* as single-toed with a dorsal spur on the basal foot segment. The corona is complicated, consisting of a continuous circumapical band that encircles the buccal field. A pseudotroch is formed by four rows of long, stiff cirri. All cirri curve away from the mouth opening. Two other paired rows of cirri are located closer to the mouth opening. These cirri are shorter and curve towards the mouth opening. The buccal field is small and comprises a group of motile cilia around the mouth. The brain is large and saccate. Cerebral glands were not identified. A large eyespot is located on the ventral side of the brain. A pair of dorsal antennae is located dorsally on the head. Lateral antennae are located laterally, slightly behind the mid length of the trunk. Each antenna attaches inside a small depression in a prominent wing-shaped elevation formed by the integument (Fig. 1C).

A short ciliated esophagus connects the pharynx and stomach. The stomach is large and contains often a yellowish material. The gastric glands are granulated and unstalked.

Trophi from six specimens were prepared for SEM. The trophi are modified malleate. The rami are trapezoid, but with elongated, dorsally curved apical tips. The inner margins of the rami have six small teeth in the basal part; apical rami parts have paired combs with numerous fine denticles (Fig. 3B). A pair of small, caudally pointed alulae are located on the lateral sides of the rami (Fig. 3A). A pair of large basifenestrae are visible on the dorsal side (Fig. 3B). The ventral side furthermore has a pair of basal apophyses bearing a band of sarciopili (Fig. 3B). A pair of delicate epipharyngeal plates is associated with the apical rami parts (Fig. 3). Fulcrum is short, broadest in distal half. Unci are large with 8 teeth. Principal tooth is bipartite (Fig. 3B). Manubria are composed of a well-developed proximal head and a caudal rod. Proximal head contains three chambers, which all have large apertures.

Measurements: Body (adults): 190-240  $\mu$ m; body (immatures): 90-128  $\mu$ m; right toe 27  $\mu$ m; left toe 24  $\mu$ m; trophi 27  $\mu$ m; rami 20  $\mu$ m; fulcrum 6  $\mu$ m; manubria 16  $\mu$ m; unci 14  $\mu$ m.



Figure 1. LM-photos of selected rotifers. A: Eothinia elongata. B: Dicranophorus luetkeni. C. Microcodides chlaena. D. Scaridium longicaudum. E. Lepadella patella. F. Stephanoceros fimbriatus. G. Keratella quadrata. H. Notholca foliacea.



**Figure 2.** Eothinia elongata. SEM-photos of trophi. A: lateral view. B: rami, ventral view. C: frontal view. D: ventrocaudal view. E: Detail showing basal hinge on ventral side of rami. F: unci. Abbreviations: bf – basifenestra; ep – epipharynx; mm – aperture from medial manubrium chamber; op – oral plates; pm – aperture from posterior manubrium chamber; sf – subbasifenestra; ul – uncus lamellae; ut – principal uncus tooth.

# Resticula nyssa Harring & Myers, 1924

A few specimens were found in the samples. The species is a rare cosmopolite and has been recorded from Europe (WULFERT, 1940; KOCH-ALTHAUS, 1962, 1963), the United States (HARRING & MYERS, 1924), arctic Canada (NOGRADY & SMOL, 1989; DE SMET & BAFORT,

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**Figure 3.** Microcodides chlaena. SEM-photos of trophi. A: dorsal view. B: ventral view. Abbreviations: al - alula; bf - basifenestra; ep - epipharynx; sf - subbasifenestra.



**Figure 4.** *Resticula nyssa. SEM-photos of trophi. A: dorsal view. B: frontal view. Abbreviations:* pr - pleural rod; mm - aperture from medial manubrium chamber; ra - ramus.

1990; DE SMET & BEYENS, 1995) and Svalbard (DE SMET, 1988, 1993). The species is new to Greenland.

The species is distinguished by its trophi morphology and absence of a true eyespot. The recorded specimens were generally typical. Trophi from one specimen were prepared for SEM. The rami are large and triangular. The apical rami parts are lamellate, forming an apical basket (Fig. 4). A pair of pleural rods with fanned ends are attached to the dorsal side of the rami (Fig. 4A). The fulcrum is long with a dorsally curved terminal tip. Unci have two teeth, furthermore, left uncus has five accessory teeth forming a fan. Manubria are composed of a well-developed proximal head and a caudal rod. The proximal head contains three chambers. Anterior and posterior chambers have large apertures; the medial chamber has a smaller aperture in the distal part of the manubrium head (Fig. 4A).

Measurements: Trophi 50 µm; rami 28 µm; fulcrum 29 µm; manubria 30 µm; unci 13 µm.

# 4. CONCLUDING REMARKS

Twenty-five rotifer taxa were reported from the Moraine Lake. Twenty-one of these are new for Disko Island and three are new for Greenland. Rotifer species new for Greenland are *Eothinia elongata, Resticula nyssa* and *Stephanoceros fimbriatus*. Furthermore, six of the species listed in Table 1 are new for Disko Island. These species were not recorded during this study, but were found in connection with earlier samplings. *Euchlanis incisa* and *Notommata glyphura* were recorded from a freshwater pond on the southwestern part of Disko Island (SØRENSEN, pers. obs.). *Conochilus unicornis, Synchaeta grandis, S. kitina* and *Taphrocampa* cf. *selenura* were recorded by Dr. Ruttner-Kolisko, who sampled different brackish and freshwater localities in August 1977. All four species are new to Greenland. These recordings have not been published earlier and the data are only available as an unpublished species list. Besides these four species, RUTTNER-KOLISKO (in lit.) also reports an undetermined *Polyarthra* from a small pond in the Disko Fjord area. According to her notes, the species may either be identical with *P. euryptera* or *P. major*.

Including the species reported in this study, a total of 57 rotifer species have been recorded from Disko Island (Table 1). The total number of species for Greenland is now approximately 133 valid species (Table 2).

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# Functional Morphology of two Mud-dwelling Meiofauna Polychaetes of the Family Nerillidae (Annelida)

Katrine WORSAAE

Zoological Museum, University of Copenhagen, Denmark. E-mail: kworsaae@zmuc.ku.dk

**Abstract.** Two meiofauna polychaete species were collected from mud bottom at ca. 200 meters depth off Iqpik, Disko, Greenland. *Paranerilla* cf. *limicola* is an obligate mud-dweller whereas *Meganerilla* cf. *swedmarki* is a facultative mud-dweller. They both belong to the family Nerillidae, generally known to be interstitial. Their motility was studied and video recorded in compound microscope and their morphology was studied in light microscope and scanning electron microscope. The functional morphology of the two species was compared and the possible evolution of the "interstitial" family in mud is discussed.

Key words. *Meganerilla* cf. *swedmarki*, *Paranerilla* cf. *limicola*, mud, interstitial, evolution, video recordings, SEM.

#### **1. INTRODUCTION**

Meiofauna polychaetes are mainly known from interstitial habitats in shallow water. Nerillidae is the largest meiofauna family in Polychaeta with 48 species in 17 genera. They generally have a length of 300 µm to 2 mm and are nearly all marine and distributed worldwide from the intertidal to abyssal depths (3660 m - see WORSAAE & KRISTENSEN 2003). Nerillids have generally been characterised as an interstitial polychaete family (e.g., WESTHEIDE 1990). However, only three monotypic genera of the seventeen existing genera have been reported solely from the interstitial sandy habitat (WORSAAE & KRISTENSEN, in review). Many species have been found in gravel with or without shells. This may in fact not represent a true interstitial environment because of the very large interstices, which often contain deposits of silt. Taking this into consideration, most nerillid genera have actually been found outside the interstitial environment (sometimes exclusively) (WORSAAE & KRISTENSEN, in review). Three species, Meganerilla swedmarki Boaden, 1961, Paranerilla cilioscutata Worsaae & Kristensen, 2003 and P. limicola Jouin & Swedmark, 1965, have been found in mud bottoms (Jouin & Swedmark 1965; Saphonov & Tzetlin 1997; Worsaae & KRISTENSEN 2003). However, only the two species of Paranerilla have solely been reported from mud. The two described species of this genus are apparently the only obligate muddwellers among meiofauna polychaetes. However, mud fauna is not very well sampled in regards of meiofauna polychaetes.

Nerillids as well as most other interstitial polychaetes generally move by gliding with the help of the midventral ciliary band. In addition, most nerillids observed alive are also capable of making a quick escape reaction by rapid undulation of the body (e.g., JOUIN & SWEDMARK 1965). *Paranerilla cilioscutata* and *P. limicola* have moreover been observed to enter the upper layers of the sediment by moving particles across its dorsal surface (JOUIN & SWEDMARK 1965; WORSAAE & KRISTENSEN 2003). This transport of particles is enabled by the dense covering of cilia on the prostomium and first segment, which is unique for this genus (WORSAAE & KRISTENSEN 2003). The facultative mud-dwelling nerillid, *Meganerilla swedmarki*, has a very different external morphology, which has never been studied in details. The purpose of this study is to video record the motility (in compound and light microscope (LM)) and compare the morphology (in scanning electron microscope (SEM)) of the obligate mud-dwelling *Paranerilla* cf. *limicola* and the facultative mud-dwelling *Meganerilla* cf. *swedmarki* living sympatrically off Disko, Greenland.

#### 2. MATERIALS AND METHODS

Mud samples were taken with a triangle-dredge in June 2003 at 195-220 m near Iqpik, off Disko (between 69°17.25'N, 53°13.08'W and 69°17.34'N, 53°13.24'W). The station, Iqpik was established within the area locally known as "shrimp field", an area of high benthic productivity characterised by well-oxygenated, stable, brown, muddy sediment (see HIGGINS & KRISTENSEN 1988). Animals were extracted from the sediment by whirling up the top layer of the mud in a bucket with plenty of water and thereafter screen the surface and upper layers with a 200  $\mu$ m mesh net. The content of this net was washed in a finer conical mesh (60 $\mu$ m) before sorting out the animals alive under compound microscope.

Live animals were studied, photographed in an Olympus BX51 microscope mounted with a digital camera (Olympus c-3030), and video recorded in a Olympus SZX12 mounted with a JVC TK-C1381 colour video camera (see Fig. 1). Video recordings were made in a small petri dish (kept cold), with a small amount of unsieved mud. Edited recordings of *Paranerilla* cf. *limicola* and *Meganerilla* cf. *swedmarki* can be viewed at http://www.zmuc.dk/InverWeb/staff/kworsaae.htm. Before fixation the animals were anaesthetized in an isotonic solution of MgCl<sub>2</sub>. Four specimens were used for whole mounts with the same method as described above after fixation in a 4% formaldehyde solution buffered with borax. Specimens for SEM were either fixed in a 2% formaldehyde solution and postfixed in 1% osmium tetroxide or fixed directly for one hour in 1% osmium tetroxide, transferred to distilled water, dehydrated through an ethanol series, and transferred to 100% acetone. Further handling of fixed material was done in Denmark at the Zoological Museum, University of Copenhagen. The material for SEM was critical point dried, mounted on stubs, sputter coated with palladium, and examined with a JEOL JSM-840 or a JEOL JSM-6335F Field Emission scanning electron microscope. It was not possible to make a successful fixation of *Paranerilla* cf. *limicola* from Iqpik and even though this material could be studied in SEM, I have chosen to instead show scanning electron micrographs of old material of *P. limicola* previously collected in Kristineberg, Sweden (see WORSAAE & KRISTENSEN 2003 for details).



**Figure 1.** Microscopes with mounted AV- equipment on Arctic Station, Godhavn used for the observations on live animals. The scientific leader of Arctic Station, Bente Jessen Graae, is posing.

#### **3. RESULTS**

#### 3.1. Observations on ciliation

*Meganerilla* cf. *swedmarki* from Iqpik: The animals differ from *M. swedmarki* from Sweden by the leaf shaped anal cirri (versus filiform) and longer chaetae. The prostomium possesses an anterior and a posterior group of sensory cilia as well as two lateral short bands of motile cilia in between. The two ventro-lateral palps possess a ciliary band as well as ciliary tufts. Small longitudinal bands of motile cilia are the only ciliation present on the dorsal surface of segment 2-8, apart from a very few randomly spaced single cilia (Fig. 3B). The longitudinal bands are located laterally, extending from near the base of each parapodium to the border of the following segment (mid between the parapodia of the respective segments). Segment 9 possesses a single dorsal transverse band of cilia.

A dense ciliation is present on the ventral surface of the prostomium and segment 1 surrounding the mouth. A midventral ciliary band extends from the pharyngeal region to the pygidium, and further on to the anus on the dorsal side. Transverse ventral ciliary bands are found on segments 1-9 from the parapodia to very near the midventral band. The bands of segment 3-9 are not strictly transverse as they make a 90° bend, extending anteriorly, before bending back 90° a second time towards the midventral band, and ends mid between the lateral side and the midventral band somewhat anterior to the parapodia. An additional ventral transverse band is found on the prostomium posterior to the palps.

*Paranerilla* cf. *limicola* from Iqpik: The material from Iqpik seems of unknown reasons to be especially difficult to fix, as it has not been possible throughout several testing to get a proper fixation of the ciliation for SEM studies. Although not very illustrative, the ciliation could be described from observations of the SEM specimens as well as from the observations of live animals. The morphology generally resembles that of *P. limicola* from Sweden (type locality) except for a few details in the ciliation. Micrographs of material previously collected in Sweden (see WORSAAE & KRISTENSEN 2003), which were better fixated, are therefore shown in Figure 2 and 3, and will better illustrate many of the characters mentioned below for the studied material of *P. cf. limicola* from Iqpik.

Most of the dorsal surface of the prostomium and segment 1 are covered with motile cilia (see Figs. 2A, 3C). Exceptions are a minor non-ciliated area on the frontal surface of each lateral horn, a small non-ciliated pit frontally on the prostomium extending dorsally and posteriorly and broadening out towards a narrow non-ciliated gap on the borderline between the prostomium and segment 1. In the "non-ciliated" areas of the prostomium two groups of non-motile sensory cilia were found as described for *P. limicola* (see Fig. 3C). The anterior group is located in the frontal nonciliated area and posterior group in the nonciliated area in front of segment 1. The ciliation on segment 2 is seen as a ciliary plate with motile cilia covering about one third of segment 2 (versus a double ciliary band in *P. limicola*), whereas the segments 3-7 possess either a double or single ciliary band of motile cilia (see Fig. 2A). The ventral surface of the prostomium and segment 1 are fully covered by cilia (see Fig. 2B-C). A midventral ciliary band extends from the pharyngeal region to the pygidium, and further on to the anus on the dorsal side (see Fig. 2B). The band broadens on segment 7 and the pygidium. Transverse ventral ciliary bands are found on segments 2-7 from the parapodia to very near the midventral band (see Fig. 2B). An additional ventral transverse band is found

between segments 1 and 2 (see Fig. 2B-C).



Figure 2. Scanning electron micrographs of Paranerilla limicola (Gullmar Fjord). Note that the pygidial cirri are missing in A and B, and only one cirrus is present in C. A. Dorsal view of the whole animal. B. Ventral view of whole animal. C. Lateral view of the whole animal. Abbreviations: an, anus; db<sub>1-7</sub>, dorsal ciliary bands (or plates) on segments 1 to 7; lh, lateral horn; mb, midventral ciliary band; mc, ciliary field around mouth; mo, mouth; pb, prostomial ciliary plate; pc<sub>1</sub>, parapodial cirrus on segment 1; vb<sub>1-7</sub>, ventral ciliary bands on segments 1 to 7; xb, extra ventral ciliary band.



Figure 3. Scanning electron micrographs of Paranerilla cilioscutata, P. limicola and Meganerilla cf. swedmarki. A. Dorsal view of Paranerilla cilioscutata from North East Greenland, showing dorsal ciliary plates on segment 1-7 (db<sub>1-7</sub>). C. Close-up of the prostomium of Paranerilla limicola from Kristineberg, Sweden, showing densely ciliated prostomium and first segment with prostomial ciliary plate (pdb) and dorsal ciliary plate on segment 1 (db<sub>1</sub>). Parapodia of segment 1 (par<sub>1</sub>) and two groups of sensory cilia are shown: anterior sensory cilia (as) and posterior sensory cilia (ps). B. Dorsal view of Meganerilla cf. swedmarki from Disko, West Greenland, showing sparsely ciliated dorsal surface only with parapodial ciliary tufts (cit). Abbreviations: pr, prostomium; pa, palp; pc, parapodial cirrus.



Figure 4. Hypothetical evolutionary pathways in muddy habitats (following the schemes presented by Westheide (1987)). A. Regressive evolution, by gradual decrease in size from endopsammic macrofaunal form to meiofaunal form in the mud interface. B. Example of progenetic origin of present meiofauna in the mud interface from a temporary meiofaunal juvenile stage of an epibenthic macrofauna organism with a pelago-benthic life-cycle.

# **3.2.** Observations on motility

*Meganerilla* cf. *swedmarki* from Iqpik: These animals only have a sparse dorsal ciliation and were never observed to burrow. They are capable of gliding on the sediment surface by help of the ventral ciliation. When doing so, it generates a thick mucus-string from the midventral

band, which is detached from the body at the posterior end of the midventral ciliary band. The mucus string following the animal is attaching it to the uppermost flocculent layer. This may prove helpful during strong current as well as when the animal makes a quick escape reaction. The escape reaction is performed by fast undulations of the body and makes the animal enter the water column for several seconds. When the animal relaxes it slowly drops to the sediment surface near where it left, since it is still attached by the mucus string to the surface.

*Paranerilla* cf. *limicola* from Iqpik: The animals studied alive were observed to glide over the sediment, enter the upper layers of the sediments as well as make quick escape reactions by undulation of the body. They enter the sediment by moving particles over the prostomium and dorsal body surface with their distinct dorsal ciliation. The dense motile ciliation on the prostomium, first and second segment carries particles from beneath the prostomium, across to the dorsal surface. On the middle of the dorsal trunk surface the particles are removed to one of the sides. The animals are also able to simply glide across the sediment by help of the ventral ciliation. Moreover, they may leave the substratum to swim into the water column by means of the dorsal ciliary plates and the midventral ciliary band. When they enter the sediment and while swimming gently through the water, parapodia and chaetae are compressed along the body.

## 4. DISCUSSION

All species of the genus Paranerilla are obligate mud-dwellers. Only minor differences in the dorsal ciliation exist between P. limicola from Sweden and P. cf. limicola from Greenland. Although the differences between P. limicola (Fig. 2A) and P. cilioscutata (Fig. 3A) are more substantial no difference in the motility patterns was observed. The unique dorsal ciliation of Paranerilla makes it capable of entering the mud, and thereby gain more protection against predation. Meganerilla cf. swedmarki is a facultative mud-dweller, with no dense dorsal ciliation on prostomium and body. It can therefore not burrow, and does not seem as well adapted morphologically to the muddy habitat as the obligate mud-dwelling species of Paranerilla. Most nerillids and other interstitial polychaetes have a rather sparse dorsal ciliation. Meganerilla cf. swedmarki is never the less found in mud bottom and it seems very possible that more nerillids may be facultative mud-dwellers. However, other factors may influence the choice of habitat. Although Paranerilla and Meganerilla differ in morphology, they are both relatively large and robust compared to many interstitial nerillids. This is in accordance with the generally larger size of mud-dwelling meiofauna (COULL & BELL 1979). It is therefore not expected that some of the very tiny fragile interstitial nerillids living in clean sand are capable of living in mud. Moreover, many nerillids have direct development, sometimes including brooding. Species with direct development would mainly be able to spread from one locality to another and from the interstitial environment to other habitats by migration, dispersal of the sediment or continental drift. However, the two mud-dwelling nerillids Paranerilla limicola and Meganerilla swedmarki are found to have indirect development with a pelagic trochophore larva, which are more easily spread by currents over larger distances to different habitats (BOADEN 1961; JOUIN & SWEDMARK 1965). The advantage of pelagic larvae is only gained when living in a non-interstitial environment where it is possible to disperse the larvae. The development of many nerillids is still unknown.

According to COULL & BELL (1979) the sand fauna tends to be long and slender, whereas the mud fauna is not restricted to a particular morphology. Compared to most other interstitial polychaetes, the nerillids possess many appendages and long chaetae and are not particularly slender. They are as most meiofauna polychaetes generally referred to as interstitial. Nerillids

may have evolved in the interstitial habitat and secondarily spread to non-interstitial habitats. However, the opposite evolutionary history cannot be rejected based on current knowledge of their variable morphology, distribution and inconsistent development.

WESTHEIDE (1987) considered different evolutionary pathways of meiofauna in the interstitial environment and the selective forces supporting them. The induction of progenetic development was linked closely to the interstitial habitat, which possessed so extraordinary adaptational demands, that a one-step adaptation in size would be necessary to enter this habitat (WESTHEIDE 1987). However, some of these pathways, including progenesis, may also be applicable to the muddy environment. If an evolutionary pathway in mud should be considered for the nerillids, some questions need to be raised: Does a size-specific niche in mud exist, as in the interstitial environment? Does an evolution of meiofauna in mud require a progenetic origin or could it take place by gradual transition in size?

The highest concentration of meiofauna in mud has generally been found in the upper one centimetre of the bottom (COULL & BELL 1979). The sediment-water interface, in which several nerillids have been found, may be a size-specific niche for meiofauna organisms. This flocculent layer contains a higher concentration of small sized food particles compared to the underlying layers of the mud due to suspension and resuspension of less heavy particles. Furthermore, the layer is very oxygenated, thereby facilitating a higher production of bacteria and algae (diatom-enriched). The meiofauna has therefore access to higher concentrations of appropriate food items in this layer. In the interface, only small organisms would be able to gain some protection from larger epibenthic and pelagic selective predators, whereas large motile organisms foraging in this layer would be more exposed to selective predation. In some areas, the lower part of the sediment may be anoxic thereby limiting the meiofauna to the upper aerated layers. The uppermost part of the sediment-water interface may also be so loose that only small organisms could move around by ciliary motion.

A size-specific niche, comparable to what is found in the interstitial environment, may therefore exist in the sediment-water interface of muddy habitats. The specific size limitations of the environment are dependent on the depth and density of the sediment-water interface. These factors are defined by local current conditions and the composition of sediment particles, which are the same abiotic parameters that define the space available in the interstitial environment (SWEDMARK 1964).

In lower parts of the mud bottom with less organic material and oxygen, the expenditure/use analysis for motile deposit-feeding macrofauna may be negative. The same may apply to existence above the bottom, where the current is higher. Energetically expensive burrowing or swimming is then only worthwhile when a great deal of food is available and small distances have to be covered. Thus, a selection pressure may exist in or above mud bottoms for a decrease in size down to dimensions that allow an exploration of the sediment-water interface. If the sediment-water interface is very thin or loose a progenetic development would be advantageous in providing a one-step speciation to the size-specific niche (WESTHEIDE 1987). If the interface is more extensive or particle dense, middle-sized animals may be able to explore some of the same benefits of this environment, and a development by gradual decrease in size is possible.

Reduction in size would be the main adaptational demand for entering the niche of the interface environment. Adhesive organs, as found on many interstitial forms would not be useful. It is more important to have the ability to move on or in the transparent flocculent
layer as well as sticking to it during current and other turbulent influence to avoid dispersal to the water column. Species of *Paranerilla* possess a distinct dense dorsal ciliation (Fig. 2A) making them capable of burrowing in mud and *Meganerilla swedmarki* can generate a thick mucus-string from the posterior end of the midventral ciliary band, attaching it to the uppermost flocculent layer. Whereas indirect development is unfavourable in the interstitial environment it is an advantage in mud, where it creates a possible way of spreading and of decreasing intraspecific competition for food. It is striking that this developmental pattern is in fact found among the mud-dwelling nerillids (see above).

Based on the characters on the mud-dwelling nerillids as well as the general variable distribution and morphology of nerillids, it is at present not possible to reject the idea that the nerillids could have evolved in mud – either by a gradual transition in size (Fig. 4A) or by progenesis (Fig. 4B). However, more sampling in muddy habitats as well phylogenetic analyses are needed to clarify their evolution.

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# A Study of the Genus *Amphibolus* from Disko Island with Special Attention on the Life Cycle of *Amphibolus nebulosus* (Eutardigrada: Eohypsibiidae)

Jesper Guldberg HANSEN<sup>1</sup> & Agnete Krabbe KATHOLM<sup>2</sup>

<sup>1</sup>Zoological Museum, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup> Department of Life Science and Chemistry, Roskilde University, Roskilde, Denmark

Abstract. Samples of mosses from four habitats were collected on Disko Island, Greenland, in July 2002, in order to study the life cycle of *Amphibolus nebulosus*. Additional material collected in the period 1976-2001 was examined for the occurrence of *Amphibolus* species. The three species *A. nebulosus*, *A. weglarskae* and *Amphibolus* nov. sp., are unequally distributed at 24 locations, and their preferences for specific environments are indicated. Supplementary, the zoogeography of the family Eohypsibilidae is discussed. The study of *Amphibolus nebulosus* signifies that the life cycle involves two types of cysts and two types of eggs. It seems that both kinds of cysts are related to reproduction as well as to environmental changes. New information on the sclerified structures, claws and the characteristics of the egg-shell within the genus are presented, and a modification of terminology is suggested.

Key words. Eutardigrada, Eohypsibiidae, Amphibolus, zoogeography, distribution, life cycle, encystment.

#### **1. INTRODUCTION**

In the last twenty-six years numerous investigations of the Disko Island tardigrade fauna has been carried out from the Danish Arctic Station, Qegertarsuag, Disko Island, West Greenland. The results have partly been obtained by scientific leaders of the Arctic Station and partly from several field courses in Arctic Biology. At present, more than a hundred different species of limnic/terrestrial tardigrades have been collected on the Disko Island (HEIDE-JØRGENSEN & KRISTENSEN, 1998, 1999). The tardigrade fauna of the thousands of springs on the Island has a high diversity of species, thus more than 50 species have been recorded in or near the springs (KRISTENSEN 1987). The genus Amphibolus Bertolani, 1981 (KRISTENSEN, 1987) is a relatively common element of the spring fauna, and have been collected in both heterothermic (GRØNGAARD et al., 1990; KRISTENSEN & FUNCH, 2000; WESTH & KRISTENSEN, 1992) and homothermic springs (HANSEN et al., 1988; HEIDE-JØRGENSEN & KRISTENSEN, 1998, 1999; KRISTENSEN, 1987; STARK & KRISTENSEN, 1999; WESTH & KRISTENSEN, 1992). According to these investigations, three species of Amphibolus are present in the springs: Amphibolus weglarskae Dastych, 1972, Amphibolus nebulosus Dastych, 1983, both showing the ability of periodical cyst formation, and a third species of Amphibolus new to science. Encystment has been recorded in other limnic and soil tardigrades, but is induced by unknown ecological factors (for further information on this topic, consult WEGLARSKA, 1957). The species Amphibolus nebulosus seems to use different strategies in response to changes in environmental conditions. These strategies include the formation of two types of cysts (WESTH & KRISTENSEN, 1992) and the production of two different kinds of eggs (GRØNGAARD et al., 1990), which indicates that the species Amphibolus nebulosus has a highly complicated life cycle. REBECCHI & BERTOLANI (1994) noted that "in Amphibolus volubilis the cyst is part of a more complex cyclomorphosis, also present in Amphibolus weglarskae", but they apparently only found one type of cyst. In spite of these facts, the life cycle of Amphibolus nebulosus has not been investigated until now, neither has the role of encystment in reproduction. This situation prompted us to perform an investigation of the species Amphibolus nebulosus from Disko Island, with special attention to the formation of cysts and the production of eggs.

HISTORICAL REVIEW. - The genus Amphibolus was established by BERTOLANI (1981) to which he attributed the three species Isohypsibius smreczynskii Weglarska, 1970, I. weglarskae Dastych, 1972 and I. volubilis Durante Pasa & Maucci, 1975 based on similarities in the sclerified structures, characteristics of the egg-shell and the structure of claws. At the same time, he instituted a new family Amphibolidae for the new genus, unaware of the fact, that a family of prosobranch Mollusca already occupied this name. A year later, KRISTENSEN (1982a) created the genus Eohypsibius, with a description of a new species, Eohypsibius nadjae Kristensen, 1982 and attributed this new genus to the family Calohypsibiidae Pilato, 1969, mainly because of the asymmetrical structure of claws. In 1983, DASTYCH described a new species, A. nebulosus and made the same observation as BERTOLANI (1982) that the species of Amphibolus are extremely similar, and can be distinguished only by small differences, if one does not consider the characteristics of the egg-shell. Later, BERTOLANI & KRISTENSEN (1987) attributed Eohypsibius and Amphibolus to the family Eohypsibiidae, the new name of the former Amphibolidae. Simultaneously, they transferred the former Macrobiotus mahunkai IHAROS, 1971 to the genus Amphibolus in recognition of the close relationship to A. weglarskae. ITO (1988) described the new species Eohypsibius terrestris based on small differences to E. nadjae, but in this survey, no eggs were found. In 1991, the same author erected the genus Fujiscon, with a description of a new species Fujiscon *diphasconiellum* Ito, 1991 and attributed this new genus to the family Eohypsibildae. MAUCCI (1996) did not accept his conduct and pointed out that the claws of the genus Fujiscon were of the Hypsibius type and therefore should be placed in the Family Hypsibiidae Pilato, 1969. Lastly, BISEROV (1992) described the species, A. markevichi, as the first Amphibolus species in the genus without eyes.

#### 2. MATERIALS AND METHODS

#### 2.1 Sampling and preparation

This study is based on material from Disko Island partly collected during a field course in Arctic Biology in July 2002, and partly collected by professor Reinhardt Møbjerg Kristensen (Copenhagen, Denmark) from 1976 to 2001. From his personal collection, which also includes the material collected by various field course students, we have examined more than 400 specimens, cysts and eggs belonging to the genus *Amphibolus*. The sampling in 2002 was carried out between the 5th and the 23rd of July 2002. At four different locations, dry and wet samples of aquatic mosses were collected on the banks and in the water, respectively. Several samples were taken at each location to ensure sampling of the ecological variety of the habitats.

For measuring abiotic factors, we used a thermometer for water-, bank- and air temperature in situ. Geographical positions were measured with a Magellan GPS 2000. In the laboratory, each sample was dispersed in water and was squeezed to sort out the tardigrades. The detritus was transferred to petri dishes and the tardigrades were collected under a stereomicroscope. Live animals, eggs, exuvia and cysts were examined mounted on microscopic slides using water or dilute acetic acid. For permanent preservation, we used glycerol or polyvinyllactophenol. For examination and determinations of live and preserved animals, we used an Olympus BX 51 interference-contrast microscope (Normaski-tecnique). In addition, pictures and videos were taken by Olympus digital camera C-3030 zoom and a JVC TK-C 1381 color video camera. Three days before leaving Disko Island we collected new samples from the two sites at Østerlien, where many specimens of *A. nebulosus* and *Amphibolus* nov. sp. had been found. The samples were frozen immediately and stored for later study. From this material 4 adult specimens and 6 eggs of *A. nebulosus*, 4 adults of *Amphibolus* nov. sp. and 3 eggs, were prepared for scanning electron microscopy. Frozen material were thawed and fixed in 1 % osmium tetroxide, buffered with 0.1 M sodium cacodylate adjusted to pH 7.4, for 1 hour at 20 °C. The animals were dehydrated in a graded series of ethanol and acetone prior to critical point drying. The dehydrated specimens were then mounted on aluminium stubs, coated with gold and observed in a JEOL JSM-840 scanning electron microscope.

#### 2.2. Locations

Location 1 and 2 are situated in Østerlien near Røen Sø. Where most of Disko Island consists of basalt, the ground of Østerlien consists of gneiss bedrock. The vegetation in this area is extremely rich as the snow, covering the numerous homothermic springs in wintertime, creates a "greenhouse effect" (KRISTENSEN, 1987).



Figure 1. Map showing Disko Island with indication of the location sites of A. nebulosus, A. weglarskae and Amphibolus nov. sp.: 1. Blæsedalen, 2. Moræne Søen, 3. Østerlien, 4. Vandelven, 5. Røen Sø, 6. Engelskmandens Havn, 7. Lyngmarkselven, 8. Fortune Bay, 9. Nipisat, 10. Uunartoq, 11. Field laboratory, 12. Anguujaatuutit, 13. Kuanarsuit, 14. Orpit, 15. Tarajornitsoq, 16. Eqalunguit, 17. Langsø, 18. Kuannit, 19. Puilasoq, 20. Qullissat, 21. Qullissaaqqat, 22. Isuungua, 23. Kvandalen, 24. Kronprinsens Ejland.

The two locations were chosen in light of the presence of two well-established populations of *Amphibolus nebulosus* (See WESTH & KRISTENSEN, 1992).

LOCATION 1 (Fig. 1: nr. 3). At a stagnant heterothermic pond north east of Arctic Station (GPS: N 69° 15.394' / W 53° 31.350' / 60 m.a.s.l.), samples were taken on the 5th and the 7th of July 2002. The pond had a rather high temperature of 10.2°C. Bank temperature was 9.7°C and air temperature was 10.8°C. In wintertime, the pond is frozen for 4-5 months (WESTH & KRISTENSEN, 1992).

LOCATION 2 (Fig. 1: nr. 3). Southeast of Arctic Station (GPS: N 69° 15.356' / W 53° 31.657' / 60 m.a.s.l.) an outflow from a homothermic spring created a small pond. From this pond, samples were collected the 7th of July. The water of the spring had a temperature of  $5.1^{\circ}$ C. The air temperature was  $10.8^{\circ}$ C.

LOCATION 3 (Fig. 1: nr. 22). For variations of sites of which we searched for species of *Amphibolus*, we visited the heterothermic springs of Isuungua (Fig. 1) where KRISTENSEN & FUNCH (2000) found specimens of *A. weglarskae* and *A. nebulosus* in the summer of 1994 and 1995. The springs of Isuungua (GPS: N 69° 43.778' / W 51° 56.483') are located approximately 90 km in a northeast direction of Qeqertarsuaq. Rivers transporting melt water from the glacier to the sea traverse the old moraine landscape and makes the area quite wet. The temperature of the springs varies throughout the year and is far below 0°C during the long winter (KRISTENSEN & FUNCH, 2000). Samples were collected on the 12th of July 2002. On this day the water level was very low, and the water temperature remarkably high (11,7°C). Another group attending the field course visit the springs on the 19th of July. It had been raining the previous 24 hours and the water level was very high. The temperature varied over a distance of 200 m from 4,5°C to 7,5°C downstream.

LOCATION 4 (Fig. 1: nr. 15). Tarajornitsoq (Fig. 1) is situated in Disko Fjord at Eqalunguit (GPS: N 69° 33.448'/ W 53° 36.839') and is warmer (11.0-12.5°C) than most homothermic springs on Disko Island. What make the spring extraordinary, is its high level of radioactivity, as well as the records of both of *A. nebulosus* and *A. weglarskae*, cysts and tardigrades of the peculiar "*Apodibius* form" (See DASTYCH, 1983b) by students from the Arctic Biology Course (HANSEN et al., 1988). The spring has its outflow 30-40 m.a.s.l. on a slightly rising plateau, running towards the fjord. The vegetation along the spring differentiates from other homothermic springs on Disko Island by limited prevalence of the moss *Mniobryum wahlenbergii*, and its stunted growth. Samples were collected on the 17th of July 2002.

#### **3. RESULTS AND DISCUSSION**

#### 3.1. Distribution of Amphibolus on Disko Island

Three species of *Amphibolus* are unequally distributed all over Disko Island. These are, in order of frequency, *A. nebulosus, A. weglarskae* and *Amphibolus* nov. sp. (Tab. 1). *A. nebulosus* occurs at 15 locations, with a total of 76 specimens, 38 cysts and 77 eggs. *A. weglarskae* occurs at 9 locations, with 39 specimens, 10 cysts and 11 eggs, and *Amphibolus* nov. sp. occurs at 7 locations, and a total of 34 specimens, 6 cysts and 9 eggs. All are included in this study.

To provide an impression of the pattern of habitats for the three species we have conferred the literature regarding the tardigrade fauna on Disko Island as well as the material we have had at our disposal. Tardigrades from the genus Amphibolus are considered limnoterrestrial or semi terrestrial, and are typically living in reservoirs not drying out. There are, however, characteristically differences between the habitat preferences of the species within the genus. A. nebulosus is commonly found in moist habitats and is in literature referred to as "probably hydrophilous" (DASTYCH, 1985). We found specimens of A. nebulosus in substrates of limnic mosses and algae in homothermic and heterothermic springs as well as in soil from the springs. This supports the idea about them being hydrophilous. They are found, however, in mud (HEIDE-JØRGENSEN & KRISTENSEN, 1999), in moist soil (HANSEN et al., 1988; STARK & KRISTENSEN, 1999), in lichens, liverworts and mosses (STARK & KRISTENSEN, 1999). Hence, the preferences of A. nebulosus are apparently for wet habitats, as running or calm water, for mud, soil and mosses, and for more dry environments as lichens. On basis of these data, it seems probable to consider A. nebulosus being hygrophilous and not a true hydrophilous species. The environments preferred by A. weglarskae are drier than those preferred by A. nebulosus. The two species are hardly ever found in the same substrate (i.e. HANSEN et al., 1988). Altogether, we only found one specimen of A. weglarskae at the four locations visited this summer. Nevertheless, A. weglarskae is very common in dry mosses, soil and turf on Disko Island (HANSEN et al., 1988; HEIDE-JØRGENSEN & KRISTENSEN, 1999; KRISTENSEN & FUNCH, 2000). This is supported by descriptions of A. weglarskae from habitats of beech-, chestnut- and wood litter in Italy (BERTOLANI, 1981; BERTOLANI & MANICARDI, 1986).

Living in a range of dry to moist habitats, it seems that *A. weglarskae* is a eurytopic species. *Amphibolus* nov. sp. is probably a real hydrophilous tardigrade. It is found in permanent freshwater habitats like springs, lakes and rivers. We found *Amphibolus* nov. sp. in aquatic mosses and algae in a heterothermic pool where we also found *A. nebulosus*.

#### 3.2 Additional tardigrade fauna

All known members of the tardigrade fauna found on the four locations, visited the summer of 2002, are common in freshwater or in wet soil at Disko Island. The samples included some new species. The following additional species was found: LOCATION 1: Dactylobiotus ambigus, Dactylobiotus dispar, Dactylobiotus nov. sp., Doryphorus macrodon, Hybsibius zetlandicus, Hybsibius nov. sp., Isohybsibius sp., Macrobiotus sp., Murrayon pullari. (LOCATION 2: Only Amphibolus) LOCATION 3: Dactylobiotus nov. sp., Eohybsibius nadjae, Hybsibius dujardini, Macrobiotus richtersi, Macrobiotus echinogenitus, Microhybsibius minimus. LOCATION 4: Isohybsibius elegans.

#### 3.3 The encystment of Amphibolus nebulosus

As previously mentioned, the life cycle of *Amphibolus nebulosus* includes two types of cysts. WESTH & KRISTENSEN (1992) observed white (type 1) and red (type 2) cysts, depending on the habitat in which they were found. In the present study, it was discovered that the species *A. weglarskae* also is capable of producing two types of cysts. The type 1 has a white-yellow to light brown appearance in an interference-contrast microscope (Fig. 2A, 2C and 2E) whereas the type 2 has a darker brown appearance (Fig. 2B, 2D and 2F). When examining the cysts by stereomicroscopy, the type 1 appears completely white and non transparent and is easily seen with the naked eye, whereas the type 2 appears dark brown, almost black. REBECCHI & BERTOLANI (1994) observed brown cysts in the species *A. volubilis* and *A. weglarskae*, corresponding to the red cysts of *A. nebulosus*. As many types of cysts may have a brownish appearance in a light microscope, we suggest using the terms type 1 and type 2, in order to prevent any confusion of properties between cysts of different colours.

The type 1 encystment begins with the discharging of the sclerified parts of the buccalpharyngeal apparatus. Often the gut is emptied by defecation (normal simplex stage), but gut contents have been observed in some cysts. The animal moults with normal ecdysis and starts synthesizing a new buccal-pharyngeal apparatus, staying inside the old single layer cuticle (stage 1). At the same time, the old cuticle crumples up, diminishing the protuberance of the legs, giving it an elliptical appearance. The old cuticle now constitutes the cyst cuticle (Fig. 2A, 2C and 2E). At this stage, the animal is normally immobile, but handled under a microscope at room temperature the animal starts moving inside the cvst cuticle. When the buccal-pharyngeal apparatus is fully developed (stage 2), the animal is ready to perforate the cyst-wall with the stylets and leave the cyst (Figs. 2A, 2C, 2E and 3A-F). The type 2 encystment begins with a modified simplex stage. The sclerified parts of the buccalpharyngeal apparatus is discharged and the gut is emptied by defecation, but eventually the cuticle grows thicker and becomes tanned red or dark brown. At this stage, the digestive system, stylets and claws regress into embryonic cell bodies by histolysis (KRISTENSEN, pers. comm.). The animal then synthesizes a new, thinner and less pigmented cuticle (mummy cuticle) without any protuberances for legs or claws (stage 1). Simultaneously, the old cuticle (cyst cuticle) crumples up, giving the cyst the characteristic elliptical appearance. The next stage almost has the character of a *pseudosimplex* stage (see KRISTENSEN, 1982b), indicating that cyclomorphosis may be involved in this process. A new but highly modified and apparently non-functional buccal-pharyngeal apparatus is synthesized. The buccal tube is



Figure 2. Cysts of some Amphibolus species. Interference phase contrast micrographs (Nomarski-technique). A. Type 1 cyst of A. nebulosus. B. Type 2 cyst of A. nebulosus. C. Type 1 cyst of A. weglarskae. D. Type 2 cyst of A. weglarskae. E. Type 1 cyst of A. nov. sp. F. Type 1 cyst of A. smreczynskii.



Figure 3. A-F. Hatching type 1 cyst of Amphibolus nebulosus. Interference phase contrast micrographs (Nomarski-technique).

narrow and flexible with only traces of stylets and their supports (Fig. 4B-C). REBECCHI & BERTOLANI (1994) observed a similar phenomenon in *A. volubilis* and *A. weglarskae*, but a citation shows that they might have made the wrong interpretation: "at the end of the encystment period, these animals may not uncommonly appear without at thick cuticle, with a

modified buccal-pharyngeal apparatus, and without claws on the legs". What actually happens is, when the cyst is exposed to "unnatural" environmental conditions i.e. radioactivity or high temperature (HANSEN et al., 1988), the cyst cuticle cracks, and the encystment is interrupted. This animal, not fully developed and still encapsulated in the mummy cuticle (Fig. 4B) is able to move around, but eventually it dies within a few hours. If encystment is not interrupted, a third cuticle with legs and claws is synthesized and a normal buccal-pharyngeal apparatus with stylets is regenerated (stage 2). The animal is now ready to exit the cyst by means of the stylets (Figs. 2B, 2D, 2F and 4A).

#### 3.4 The eggs of Amphibolus nebulosus

DASTYCH (1983a) described the egg of *A. nebulosus* and illustrated the variability of the eggs processes. However, in addition to the type of egg described by Dastych, another type has been encountered at several locations on Disko Island. This egg is sculptured with long conical processes for which shape and number cannot be accounted by variability of the normal egg. The egg surface has the same structure as the normal egg, with the characteristic areolation orderly arranged around each process (Fig. 13) and the surface of the processes has a pattern resembling a very delicate net with irregular meshes. This pattern is similar to the other eggs in the genus (Figs. 12F, 14F and 15F), except for the tips of the processes where the meshes are greatly enlarged (Fig. 13F). The diameter of the egg, excluding the processes, is approximately 90-130  $\mu$ m and the length of the processes varies between 40-50  $\mu$ m. For reasons, which will be revealed in the following, the term *winter egg* will be used for this type of egg.

### 3.5 The life cycle of Amphibolus nebulosus

The large number of different spring types on Disko Island, constitutes a unique basis studying the life strategies of *Amphibolus nebulosus*. Unremitting exploration of these springs has revealed the different stages in the life cycle of A. nebulosus, but it was only by accident that the necessary clue was given, to understand the course of events. In 1978, on the 14th of August, samples were taken from a homothermic spring at Qullissat (Fig. 1), in the Northeastern part of Disko. The samples were processed in the laboratory of the Arctic Station and contained a large number of adults, eggs and type 1 cysts of the species A. nebulosus. Subsequently, the samples were placed outside in an open bucket, for later sorting. However, the bucket was forgotten for a while, and the samples began to decay. Forty days later, the samples were re-examined and were found to contain a large number of winter eggs and type 2 cysts. Some of the encysted animals were observed carrying a single, almost fully developed, egg. One of these eggs was successfully removed from the encysted animal, and proved to be a winter egg. This indicates that the type 2 encystment is triggered by environmental stress factors as low pH, lack of oxygen or desiccation, and that the thick walled cyst serves as a defensive mechanism to endure unfavourable environmental conditions, while a true resting egg is produced. When the egg matures, the encysted animal leaves the cyst, lays the egg and then eventually dies. Thus, the egg is believed to stay dormant until favourable environmental conditions are re-established. At present time it is not known how long the egg is able to stay dormant, but winter eggs with developing embryos have been found from early May until the middle of July. In spite of the ability to produce dormant eggs, this is not the primary over wintering strategy of A. nebulosus. Samples collected from heterothermic springs in winter shows that 80-90 % of the A. nebulosus population over winters as type 1 cysts, the remainder comprises eggs and large adults (WESTH & KRISTENSEN, 1992). From this, it could easily be hypothesised, that the type 1 encystment is the true hibernating stage in the life cycle of A. nebulosus. However, in the same paper, the survival of active A. nebulosus, as well as type 1 cysts, was found to be 70-80 %, when exposed to freezing. Thus, the active *A. nebulosus* uses crucial energy on encystment, even though being freeze-tolerant. This indicates that the type 1 encystment serves other purposes than freeze-tolerance only. Furthermore, populations of *A. nebulosus* living in homothermic springs, and thus not exposed to freezing, most commonly comprise active animals, eggs and type 1 cysts all the year round (WESTH & KRISTENSEN, 1992). Of all the collected active specimens of *A. nebulosus* examined in this study, only two specimens were found to contain eggs. In strong contrasts, more than 60 % of the type 1 cysts contain eggs, always 3-6 in number (Fig. 5). Spermatids/spermatozoans were only observed in two cysts (Fig. 5). From this, it seems that type 1 encystment is obligatory in the production of eggs. Summarizing the different events (Fig. 6), it seems most obvious to divide the life cycle of *A. nebulosus* into two parts; a normal life cycle and an alternative life cycle, determined by the type of cysts involved.

The normal life cycle. The life in arctic and alpine environments is characterised by long periods of winter freezing and short intervals of active life, and the need of a rapid reproduction cycle is crucial. To meet these demands, the normal life cycle of *A. nebulosus* is divided into phases in which the utilization of resources is optimised. The active *A. nebulosus* uses all its time on foraging and an excessive intake of food (phase 1), accumulating the energy needed for reproduction. When enough energy is stored, the animal encysts (phase 2). Sheltered inside the type 1 cyst, the accumulated energy is utilized in a rapid maturation of 3-6 quick-developing summer eggs. When the eggs have maturated, the animal leaves the cyst, lays the eggs and repeats the life cycle. When temperature drops, prior to winter freezing, the animals continue to form cysts, but the maturation of eggs is paused and the animals stay encysted. At the time of winter freezing, 80-90 % of the population have encysted and consequently, the population over winter as type 1 cysts. Besides being obligatory in summer egg maturation, the type 1 encystment is the true hibernating stage in the life cycle of *A. nebulosus*.

**The alternative life cycle.** If active specimens of *A. nebulosus* is exposed to a gradually deterioration of the environmental conditions, they form thick-walled type 2 cysts. The cyst seems to provide a temporary protection against unfavourable environmental conditions while a true resting egg is produced, but it also seems that the process of encystment provides the energy needed for a rapid maturation of a single, highly resistant winter egg. Besides serving as a temporary protection, the type 2 encystment is obligatory in winter egg maturation.

#### 3.6 Indication of a similar life cycle, in congeneric species.

The ability to form cysts has been recorded in three other species of *Amphibolus*. These are *A. smreczynskii* (see WEGLARSKA, 1970), *A. volubilis* and *A. weglarskae* (see REBECCHI & BERTOLANI, 1994). In addition, MARLEY & WRIGHT (1996) observed cysts formation in *A. weglarskae*. We have examined two cysts of *A. smreczynskii* from the personal collection of Dr. Barbara Weglarska and they proved to be type 2 cysts (Fig. 2F). According to REBECCHI & BERTOLANI (1994), the cysts observed in *A. volubilis* and *A. weglarskae* corresponds to the red cysts (type 2) found by WESTH & KRISTENSEN (1992). Currently, the type 1 encystment in *Amphibolus* species has therefore only been recorded in *A. nebulosus*. However, in the present study it was discovered that the species *A. weglarskae* and *Amphibolus* nov. sp. also shows the ability to form type 1 cysts (Fig. 2C and 2E). Regarding *A. weglarskae*, only one type of egg has been found. It is possible that a potential winter egg, being too aberrant, has been rejected as an egg of *A. weglarskae*, but it seems more likely that the two types of egg are only slightly different and that the winter egg has been overlooked. All the collected type 2 cysts of *A. weglarskae* were either in stage 1 or in the *pseudosimplex* stage and consequently



Figure 4. Details of the type 2 cyst. A. Newly hatched type 2 cyst. Arrows indicate the mummy cuticle. B. The pseudosimplex stage of the type 2 cysts of Amphibolus nebulosus with eyes and modified buccal-pharyngeal apparatus. Arrows indicate the mummy cuticle C.-D. Close up on the modified buccal-pharyngeal apparatus.



Figure 5. Reproductive features of type 1 cysts. A. Eggs in the ovary of A. nebulosus. B. Egg in the ovary of Amphibolus nov. sp..C.-D. Spertmatides/spermatozoans of A. nebulosus indicated by arrows.

no eggs were observed inside the cysts. Although the occurrences of both types of cysts indicate a life cycle similar to *A. nebulosus*, further research is needed to elucidate the life cycle of *A. weglarskae*. The third species, in which type 1 encystment has been observed, is

*Amphibolus* nov. sp. This species have exclusively been found in true aquatic habitats. As in *A. nebulosus*, eggs were only observed in encysted animals (Fig. 5), indicating that encystment also is obligatory in egg maturation in *Amphibolus* nov. sp.. On the other hand, no type 2 cysts have been found. Living in permanent freshwater habitats, the need to form type 2 cysts is diminished, since the environmental conditions are more stable, and it is likely that the life cycle of this species does not include type 2 encystment.



**Figure 6.** Illustration of the life cycle of Amphibolus nebulosus showing the correlation between the two types of cysts and the summer- and winter egg. Drawing by Jesper Guldberg Hansen.

#### 3.7 Notes on some morphological features

Establishing the genus *Amphibolus*, BERTOLANI (1981) performed a detailed analysis of the sclerified structures in the three species *A. smreczynskii*, *A. weglarskae* and *A. volubilis*, giving a key to the identification of each species in the genus. With the descriptions of further two species (*A. nebulosus* and *A. markevichi*) and the inclusion of *A. mahunkai*, further information on the genus *Amphibolus* has been obtained. In order to get a better understanding of the variability of morphological characters within the genus, we have examined adult specimens of *A. smreczynskii* and *A. mahunkai* from the personal collection of professor Reinhardt Møbjerg Kristensen, in addition to the species distributed on Disko Island. The observation of some new prominent features encouraged us to perform a preliminary analysis of the sclerified structures, claws and the characteristics of the eggs-hell. Here we present new information on the species are given. The terminology used in this presentation follows the ideas used by BERTOLANI (1981) and BERTOLANI & KRISTENSEN (1987).

The new species of *Amphibolus* (Figs. 9, 11E, 11F, 15, 19, 20 and 21). Like its congeners, the new species has a smooth cuticle, a rather wide mouth which is flanked by 14 peribuccal lamellae, three macroplacoids, and two-branched claws. Each claw has a lunula at their base (Fig. 11E and 11F). *Amphibolus* nov. sp. is very similar to *A. markevichi* in lacking eyes, having claws with wide branches, having two medio-dorsal ridges and two medio-ventral ridges (infrabuccal baffles), and in having smooth lunules. *Amphibolus* nov. sp. differs conspicuously from all other members of the genus by a well developed anterior band (1st band) of teeth (Figs. 9 and 20) and by the characteristic of the egg-shell. The egg (Figs. 15 and 21) is sculptured with mammiform processes having hexagonal ground plans, thereby arranging the processes in a hexagonal pattern. The surface of the processes has a pattern typical of *Amphibolus* species, resembling a very delicate net with irregular meshes. The diameter of the egg, excluding the processes, is approximately 80-100  $\mu$ m and the length of the processes varies between 17-30  $\mu$ m.

**The buccal cavity** (Figs. 7, 8, 9, 10, 17 and 20). In observations on the buccal cavity, BERTOLANI (1981) recognized the presence of two bands of infrabuccal teeth and a series of three sturdy transverse ridges (infrabuccal baffles). Studying these structures in *A. nebulosus* and *Amphibolus* nov. sp. by scanning electron microscopy (Figs. 17 and 20), we discovered that the transverse ridges or infrabuccal baffles are in fact very large teeth (this can not be observed in an interference-contrast microscope). It is possible they may have a secondary supportive function, but their shape suggests that they mainly function as regular teeth. A more appropriate terminology is therefore suggested. The band of teeth situated immediately behind the peribuccal lamellae (anterior band) is the first band of teeth in the buccal cavity, the posterior band of teeth is the second band, and the infrabuccal baffles constitute the third band. In this way the interpretation and the resulting terminology are more consistent with the one used by BERTOLANI & KRISTENSEN (1987) for the genus *Eohypsibius*. In the following description and comparison of morphological characters, the original descriptions as well as the observations of BERTOLANI (1981) are used for the species *A. volubilis* and *A. markevichi*, as preparations of these species were not at our disposal at the time of our investigation.

1ST BAND OF INFRABUCCAL TEETH. - In *Amphibolus* nov. sp the 1st band consists of obvious teeth irregularly arranged in 2-3 rows. In *A. smreczynskii, A. volubilis* and *A. nebulosus*, these teeth are much smaller, but always evident. In *A. markevichi* and *A. mahunkai* they are sometimes hardly visible or even missing, whereas in *A. weglarskae* they are usually missing.



Figure 7. The buccal cavity of Amphibolus nebulosus. Interference phase contrast micrographs (Nomarski-technique). A-B. Ventral view. C-D. Dorsal view. Abbreviations: pe.l, peribuccal lamella; tl, lateral tooth of the 3<sup>rd</sup> band of infra buccal teeth; tm, middle tooth of the 3<sup>rd</sup> band of infra buccal teeth; 1<sup>st</sup> band, 1<sup>st</sup> band of infra buccal teeth; 3<sup>rd</sup> band, 3<sup>rd</sup> band of infra buccal teeth; 3<sup>rd</sup> band, 3<sup>rd</sup> band of the infra buccal teeth.



Figure 8. The buccal cavity of Amphibolus weglarskae. Interference phase contrast micrographs (Nomarski-technique). A-B. Ventral view. C-D. Dorsal view. Abbreviations: pe.l, peribuccal lamella; tl, lateral tooth of the 3<sup>rd</sup> band of infra buccal teeth; tm, middle tooth of the 3<sup>rd</sup> band of infra buccal teeth; 1<sup>st</sup> band, 1<sup>st</sup> band of infra buccal teeth; 3<sup>rd</sup> band, 3<sup>rd</sup> band of infra buccal teeth; 3<sup>rd</sup> band, 3<sup>rd</sup> band of the infra buccal teeth.



Figure 9. The buccal cavity of Amphibolus nov. sp. Interference phase contrast micrographs (Nomarski-technique). A-B. Ventral view. C-D. Dorsal view. Abbreviations: pe.l, peribuccal lamella; tl, lateral tooth of the 3<sup>rd</sup> band of infra buccal teeth; tm, middle tooth of the 3<sup>rd</sup> band of infra buccal teeth; 1<sup>st</sup> band, 1<sup>st</sup> band of infra buccal teeth; 3<sup>rd</sup> band of the infra buccal teeth.



Figure 10. The buccal cavity of Amphibolus mahunkai. Interference phase contrast micrographs (Nomarski-technique). A-B and D Ventral view. C. Dorsal view. Abbreviations: an.r, anterior row of the 2<sup>nd</sup> band of infra buccal teeth; pe.l, peribuccal lamella; tl, lateral tooth of the 3<sup>rd</sup> band of infra buccal teeth; tm, middle tooth of the 3<sup>rd</sup> band of infra buccal teeth; 2<sup>nd</sup> band, 2<sup>nd</sup> band of infra buccal teeth; 3<sup>rd</sup> band, 3<sup>rd</sup> band of infra buccal teeth.

2ND BAND OF INFRABUCCAL TEETH. - In all the species of *Amphibolus*, this band is always present and the teeth are always larger than in the first band. In *A. smreczynskii*, *A. volubilis*, *A. nebulosus*, *A. weglarskae* and *Amphibolus* nov. sp. the posterior teeth are bigger than the middle ones in this band. Especially *A. nebulosus* are capable of evolving a few very large teeth (Fig. 17). In *A. volubilis*, *A. mahunkai* and *A. markevichi* the teeth, which make up the anterior row of the 2nd band, are in line, oblong and larger than all other teeth in this band (Fig. 10).

3RD BAND OF INFRABUCCAL TEETH. - We did not observe any noticeable interspecific variations studying this band in an interference-contrast microscope, except from the middle tooth always being split into two parts in *Amphibolus* nov. sp. (Fig. 9) and *A. markevichi* (observed by BISEROV, 1992). This band always consists of two series (one lateral and one dorsal) of three teeth: A short middle tooth (sometimes divided in two) and two larger teeth extending laterally on each side of the middle tooth. Observations by scanning electron microscopy were only made for *A. nebulosus* and *Amphibolus* nov. sp.. In both species it is seen (Figs. 17 and 20) that the two series of teeth are not perfectly separated, constituting an almost continuous circular band. The middle teeth are the longest, but also the most narrow. The teeth on each side of the middle ones are the broadest, extending from the medio-ventral or medio-dorsal plane (where they are longest) to the medio-lateral plane (where they are shortest). In *Amphibolus* nov. sp. (Fig. 20) all teeth in the 3rd band are strongly serrated at the edges, whereas in *A. nebulosus* the serration of the teeth are more random and not quite as pronounced.

The claws (Fig. 11). Examining the species distributed on Disko Island, we experienced that the claws are similar in appearance in all three species, and it sometimes is difficult to recognize the differences in size and proportions of the claws of the different species, due to a certain intraspecific variability. For the same reason, any investigator should be cautious in the interpretation of claw properties, when solely relying on the diagnosis from the literature. Consequently, we have merely chosen to include A. nebulosus, A. weglarskae and Amphibolus nov. sp. in the present analysis of the claws. In figure 11, the differences in size and proportions of the claws of the three species are obvious. The characteristics of the claws of each species are always most conspicuous on the 4th pair of legs. A. nebulosus has the largest claws in proportion to body size (Figs. 11A and 11B). The external claw is always evidently longer than the internal claw. This is best observed on the 4th pair of legs (sometimes almost twice as long), less obvious on the 3rd and 2nd pair of legs, and still less in the 1st. Although not tested statistically, the proportional size of the claws seems to vary in response to season, with the summer form of A. nebulosus having longer claws. It also seems to vary in response to the habitat. Specimens of A. nebulosus from true aquatic habitats, apparently develops longer claws compared to specimens living in other habitats. The lunules of A. nebulosus are obviously dentated (Figs. 11A, 11B, 16B and 16C). This character was not mentioned in the original description by DASTYCH (1983a), but we consider it to be a conservative and thus reliable character, useful in the identification of this species. The claws of Amphibolus nov. sp. are among the smallest (perhaps the claws of A. markevichi are smaller) in proportion to body size (figs. 11E and 11F). The internal claw is about the same length as the external claw. The branches are proportionally broader than the branches in A. nebulosus and A. weglarskae, and the lunules are smooth (Figs. 19B and 19C). The claws of A. weglarskae are proportionally smaller than the claws of A. nebulosus, and the external claw is slightly longer than the internal claw (Fig. 11C). The lunules appear rough (Fig. 11D), but are not dentated like in A. nebulosus.



Figure 11. Double claws of the 4<sup>th</sup> pair of legs. Interference phase contrast micrographs (Nomarski-technique) A-B. Amphibolus nebulosus. C-D. Amphibolus weglarskae. E-F. Amphibolus nov. sp.. Abbreviations: as.p, accessory point; ex.c, external claw; in.c, internal claw; lu, lunule; pr.b, primary branch; se.b, secondary branch.



Figure 12. Summer eggs of Amphibolus nebulosus. Interference phase contrast micrographs (Nomarski-technique). A.-C. Overview. A. Surface of egg. B. Embryo inside the egg with bucco-pharyngeal apparatus and claws. D.-E. Close up on the areolation around the processes characteristic of the Amphibolus nebulosus eggs. F. Close up, showing the fine structure of the processes.



Figure 13. Winter eggs of Amphibolus nebulosus. Interference phase contrast micrographs (Nomarski-technique). A.-C. Overview. A. Surface of egg. B. Embryo inside the egg with bucco-pharyngeal apparatus and claws. D.-E. Close up on processes and areolation. F. Close up on process showing enlargement of the meshes.



Figure 14. Eggs of Amphibolus weglarskae. Interference phase contrast micrographs (Nomarski-technique). A-C. Overview. D-E. Close up on processes. F. Close up showing the fine structure of processes.



 Figure 15. Eggs of Amphibolus nov. sp. Interference phase contrast micrographs (Nomarskitechnique). A-D. Overview of four different eggs. E. Close up on the hexagonal pattern between the processes. F. Close up on the processes.



Figure 16. SEM of Amphibolus nebulosus. A. Overview of adult specimen. B. Claws of the right 4<sup>th</sup> leg. C. Claws of the left 4<sup>th</sup> leg. Note the dentated lunules at the basis of the claws. Abbreviations: ex.c, external claw; in.c, internal claw; lu, lunule

**Eggs** (Figs. 12, 13, 14, 15, 18, 21 and 22). A short description has already been given for the winter egg of *A. nebulosus* and for the egg of *A. mebulosus* nov. sp. and will not be further elaborated in this study. As for the summer egg of *A. nebulosus* and the egg of *A. weglarskae*, the examination did not reveal any important new characters. Before the description of *A. markevichi*, the eggs of the different species in the genus could be easily distinguished throughout marked differences in the egg processes. On this assumption, the validity of *A. mahunkai* has been questioned due to a strong resemblance between the egg of *A. mahunkai* and the egg of *A. weglarskae*. With the description of *A. markevichi*, the finding of the winter egg of *A. nebulosus* and the discovery of a new species, the distinction between the eggs of some of the species is no longer obvious. The eggs of *A. weglarskae*, *A. volubilis* and *A. nebulosus*, are very diverse and is easily distinguished from one another. Interestingly, these eggs all have areolation (Figs. 22). The eggs of *A. smreczynskii*, *A. markevichi* and *Amphibolus* nov. sp. are much alike in appearance, and can be distinguished from one another only by diminutive differences, with the egg of *Amphibolus* nov. sp. being the most aberrant. The status of *A. mahunkai* within the genus is still uncertain and cannot be clarified without a



**Figure 17.** SEM of mouth opening of Amphibolus nebulosus. *A.* Arrow indicates the 2<sup>nd</sup> band of infra buccal teeth. *B.* White arrow indicates the 3<sup>rd</sup> band of the infra buccal teeth, black arrow indicates the 1<sup>st</sup> band of the infra buccal teeth. *D.* White arrow indicates the serration of the 3<sup>rd</sup> band of infra buccal teeth. *Abbreviations: pe.l*, peribuccal lamella; ss, stylet sheath. The position of the abbreviation pe.l indicates the mid-ventral lamella to specify the dorsal/ventral orientation of the animal.

re-examination of the type material. Considering the characteristics of the egg-shells of the different species, it seems there are two well defined groups. In the first group (*A. weglarskae, A. volubilis* and *A. nebulosus*), the differences of the eggs are distinct, but they all have areolation. In the second group (*A. smreczynskii, A. markevichi* and *Amphibolus* nov. sp.), the eggs are much alike and without areolation.



Figure 18. SEM of Amphibolus nebulosus summer egg. A. Overview. B.-F. Close up on the processes and the areolation.



Figure 19. SEM of Amphibolus nov. sp.. A. Overview of adult specimen. B. Claws of the left 4<sup>th</sup> leg. C. Claws of the left 4<sup>th</sup> leg. Note the smooth lunules at the basis of the claws. Abbreviations: ex.c, external claw; in.c, internal claw; lu, lunule.

#### 3.8 Zoogeography of the family Eohypsibiidae

Many species of tardigrades have broad ecological requirements and they are in consequence considered cosmopolitans. Others, such as the species of the genera *Amphibolus* and *Eohypsibius*, have a more restricted distribution. Extensive faunal studies on terrestrial/freshwater tardigrades have been made, however, only a few publications have summed up the current literature to make a record of the worldwide distribution of terrestrial/freshwater tardigrades (MCINNES, 1994; RAMAZZOTTI & MAUCCI, 1983).

Dealing with the Eohypsibiidae, we have made a distributional record including all known literature of this family. The record contains the altitudinal as well as the geographical distribution (Tab. 2). There is not sufficient data available on the abiotic factors of the tardigrade habitats, to picture the more complex preferences of habitats for some tardigrades. Therefore, we cannot obviously claim to treat the subject of the distribution of the Eohypsibiidae family fully. However, it is an attempt to indicate the impact of physiographic factors on the distribution of the family. Nevertheless, a few factors are considerable. It is



**Figure 20.** SEM of mouth opening of Amphibolus nov. sp. A. Arrow indicates the 1<sup>st</sup> band of the infra buccal teeth. B. Arrows indicate the three bands of infra buccal teeth. C. Arrows indicate the 3<sup>rd</sup> band of the infra buccal teeth. D. White arrow indicates the 1<sup>st</sup> band of the infra buccal teeth, black arrow indicates the 3<sup>rd</sup> band of the infra buccal teeth. The position of the abbreviations: pe.l, peribuccal lamella; ss, stylet sheath. The position of the abbreviation pe.l indicates the mid-ventral lamella to specify the dorsal/ventral orientation of the animal. All the pictures are of the same specimen.

tempting to believe that distribution of different species in different habitats is related to the particular substrate, however, using parametric statistical techniques and with collection of quantitative replicate samples, KATHMAN & CROSS (1991) found no direct correlation between tardigrade distribution and specific species of mosses. DASTYCH (1987) found an increasing number of tardigrade species with increasing altitude and defined A. weglarskae as a mountainous sub alpine species occurring from 1000 m a.s.l. In former studies though, he found the number of tardigrades decreasing with an increase in altitude (DASTYCH, 1985). KATHMAN & CROSS (1991), on the other hand, found no statistical evidence for altitude having any significance. However, it seems that at least some species has preferences for certain altitudes. Confronting the collected literature, all members of the Eohypsibiidae family are found in the Arctic or in mountainous areas between 550-2400 m a.s.l. To explain the disjunctive distribution of the Eohypsibiidae family, BERTOLANI & KRISTENSEN (1987) considered all members of the family as being "glacial relicts". Although more data is required before definitive geographical distributions can be determined, our work supports the idea that distribution of the Eohypsibiidae family is enclosed by their requirements for special ecological features present at high altitude or high latitude. Furthermore, based on the geographical data we can say that all members of the Eohypsibiidae are present exclusively in the Holarctic.

	Species			
	Amphibolus	Amphibolus	Amphibolus	
Location	nebulosus	nov. sp.	weglarskae	Date
Anguujaartuutit (Diskofjord)			x	02.12.1977
Blæsedalen	x <sup>s</sup>			Jul./Aug.1998
Eqalunnguit	x	x		11.08.1978
Field laboratory (Diskofjord)			x	14.07.1977, 17.11.1978,
Fortune Bay		x <sup>h</sup>		26.08.1976, 08.09.1976
Isunngua	x <sup>h</sup>			25.05.1994, 20.05.1995, 06.08.2001, 12.07.2002
Kronprinsens Ejland		xw		
Kuanersuit	x			04.03.1977
Kuannit (Diskofjord)			x	17.05.1977
Kvandalen	x			08.06.1979
Langsø (Diskofjord)		x		11.08.1978
Lyngmarkselven	x			26.08.1977
Moræne Søen		x		26.09.1976
Nipisat (Diskofjord)	x	x	x	20.09.1977, Aug.1988
Orpit (Diskofjord)			x	02.12.1977
Puilasoq (Mellemfjord)	x		x	Aug.1988
Qullissaaqqat	x			14.08.1978, 05.07.1979
Qullissat	x			14.08.1978, 05.07.1979
Røen Sø	x <sup>ho</sup> x <sup>o</sup>	x' x°		03.09.1976, 15.09.1977, 07.07.2002
Tarajornitsoq (Diskofjord)	x		x	05.08.1988, 17.07.2002
Termistorstenen (Engelskmandens Havn)	x		x	Aug.1988
Uunartoq (Engelsmandens Havn)			x	02.12.1977
Vandelven	x			30.07.1976, 21.04.1978
Østerlien	x			29.01.1979

No mark: homothermic spring; <sup>s</sup>: snow field; <sup>h</sup>: heterothermic spring; <sup>w</sup>: well; <sup>ho</sup>: homothermic outflow; <sup>o</sup>: outflow; <sup>l</sup>: lake;

**Table 1.** Location sites of A. nebulosus, A. weglarskae and Amphibolus nov. sp. on DiskoIsland.

## Geographical distribution of the family Eohypsibiidae

#### Genus Amphibolus

Species	Location		Altitude	Date	References
Amphibulus markevechi	Russia	Lake Baikal (Sibiria)		1992	BISEROV, 1992*
Amphibolus nebulosus	Canada	Igloolik (N.W.T)		1989	JØRGENSEN & KRISTENSEN, 1989
	Greenland	Blæsedalen (Disko)	90	July-August 1998	STARK & KRISTENSEN, 1999
		Narsaq			MAUCCI, 1996
		Puilassoq (Mellemfjord, Disko)	25	Summer 1998	HEIDE-JØRGENSEN & KRISTENSEN, 1999
		Issungua (Disko)			KRISTENSEN & FUNCH, 2000
		Østerlien ( Disko)	55	1976-1979	WEST & KRISTENSEN, 1992
	Italy	Italy			BERTOLANI & REBECCHI, 1996
	Norway	Grössfiell Mt. (Atomfiella, West Spitsbergen, Svalbard)	100-200	July 1977	DASTYCH, 1983A
		Spitsbergen, Svalbard)	60-100	August 1977	DASTYCH, 1983A*
		Hammerfest (Svalbard)			Durante Pasa & Maucci, 1979; Maucci, 1996
	Russia	Taimyr, (Dikson Island, Siberia)		July 1991	BISEROV, 1996
Amphibolus	Canada	Axel Heiberg Island (Crusoe Lake, Gypsum Hill)		1970	WEGLARSKA, 1970*
smreczynskii		Axel Heiberg Island (Ermine Ridge, Gypsum Hill)		1970	Weglarska, 1970
		Igloolik (N.W.T)		1989	JØRGENSEN & KRISTENSEN, 1989
	Norway	Edgeøya (Svalbard)		Aug. 1984	DE SMET ET AL., 1988
	Russia	Taimyr (Dikson Island, Siberia)		July 1991	BISEROV, 1996
Amphibolus weglarskae	Canada	Devon Island (N.W.T.)			VAN KOMPU & DE SMET, 1991 IN LITT.
	Currentered	Vancouver Island (British Columbia)			KATHMAN, 1990
	Greenland	Isuungua (Disko)	FO	July August 1009	KRISTENSEN & FUNCH, 2000
		Rudifille (DISKO)	25	Summer 1009	STARK & KRISTENSEN, 1999
	Italy	Pullasoq (Mellelilijord, Disko)	1520	Summer 1996	REDE-JØRGENSEN & KRISTENSEN, 1999
	Italy	Camoldoli (Arezzo)	1200	April 1985	BEDTOLANI & MANICARDI, 1995
		Civago (Pistoia)	1000-1200	April 1905	BERTOLANI 1975: 1981: BERTOLANI &
			1000 1200		MANICARDI, 1986; BERTOLANI & REBECCHI, 1996
		Fonni (Nuoro)			BINDA & GUGLIELMINO, 1982
		Genna Sarbene (Nuoro)			BINDA & GUGLIELMINO, 1982
		Lago Baccio (Modena)	1600	Nov. 1984	BERTOLANI & MANICARDI, 1986
		Lucania			BINDA, 1980
		Pavullo (Modena)	700	Fall-winter 1995-96	REBECCHI ET AL., 2000
		Piane di Mocogno (Modena)	1200	July 1997-May 1999	GUIDETTI & BERTOLANI, 2001
		Reggio Emilia and Modena	1050		Bertolani, 1981
		Rio Aratu (Nuoro)			BINDA & GUGLIELMINO, 1982
		Serra di Buda (Acri, Cosenza)	920		Bertolani, 1981
		Serra di Buda (Rende, Cosenza)	500		BERTOLANI, 1981
		Serra di Buda (S. Fili, Cosenza)	700		BERTOLANI, 1981
	Iceland	Olastjördur	1200	1975	MARLEY & WRIGHT, 1996
	Japan	Kamikuisniki-mura (Mt. Fuji)	1300	1985-86	ITO, 1991
	Newyou	Narusawa-mura (Mt. Full)	1430	1985-1986	110, 1991
	Norway	Parantegua (Svalbard)		1902	VAN ROMPU & DE SMET, 1991
		Bigrogya (Svalbard)			VAN ROMPU & DE SMET, 1991 IN LITT
	Poland	Pysznianski Potok (Tatra Mt.)	1100	Aug. 1967	DASTVCH 1972*
	rolana	Sucha Woda (Tatra Mt.)	1350	Aug. 1967	DASTYCH 1972*
Amphibolus volubilis	Greece	Xilocsalo (Crete)	1000	1979	MAUCCI & DURANTE PASA, 1982
Ampinbolus volubilis	Italy	Baccio Lake (Tuscan-Emilian Appenines)	1520		REBECCHI & GUIDI, 1995
	,	Belmonte (Valsessera)	1500		BERTOLANI, 1981
		Cervatto (Vercelli)	990		BERTOLANI, 1981
		Emilia (Modena)	800		BERTOLANI, 1981
		Mount Rondinaio (Mondena)	1520	April-Nov. 1991	REBECCHI & BERTOLANI, 1994
		Nuoro (Sardinia)	550		Bertolani, 1981
		Sassari (Sardinia)	1600		Bertolani, 1981
		Rio Aratu (Nuero)			BINDA & GUGLIELMINO, 1982
		Sorgenti su Cologone (Nuoro)			BINDA GUGLIELMINO, 1982
	Norway	Norwegian coast		July 1974	DURANTE PASA & MAUCCI, 1979*
Amphibolus mahunkai	Korea	Kum Gan-san (Guriong-Fall)		1970	IHAROS, 1971*
1 -	Russia	Taimyr (Dudinka, Siberia)	1	July 1991	BISEROV, 1996

#### **Genus Eohypsibius**

Species	Location		Altitude	Date	References
			m a.s.l.		
Eohypsibius nadjae	Faroe Island	Vid air (Streymoy Island)	50		BERTOLANI & KRISTENSEN, 1987
	Greenland	Kuugssuaq (Disko)	50	Aug. 1978	KRISTENSEN, 1982A*
		Puilasoq (Mellemfjord, Disko)	25	Summer 1998	HEIDE-JØRGENSEN & KRISTENSEN, 1999
		Uunartoq (Disko)	5-10	Aug. 1978	KRISTENSEN, 1982A
		Isuungua (Disko)			KRISTENSEN & FUNCH, 2000
	Italy	Corno dei Tre Signori (Trentino)	2400		Bertolani & Kristensen, 1987;
					MANICARDI & BERTOLANI, 1987
		Monte Baldo (Veneto)	2000		Bertolani & Kristensen, 1987
		Monte Vettore (Appenine Mts., Marche)	2350		BERTOLANI & KRISTENSEN, 1987
		Passo del Tonale (Trentino)	1900		Bertolani & Kristensen, 1987
		Passo San Pelegrino (Trentino)	2000		BERTOLANI & KRISTENSEN, 1987
		Piane di Mocogno (Nothern Appennine, Modena)	1200	July 1997-May 1999	GUIDETTI & BERTOLANI, 2001
		Rifugio Denza (Trentino)	2200		BERTOLANI & KRISTENSEN, 1987
	Russia	Taimyr (Dikson Island, Siberia)		July 1991	BISEROV, 1996
Eohypsibius terrestris	Japan	Narusawa-mura (Mt. Fuji)	985	1985-1986	Ito, 1988*; Ito, 1991
		Kawaguchiko-machi (Mt. Fuji)	970	1985-1986	Іто, 1988; Іто, 1991
		Kamikuishiki-mura (Mt. Fuji)	1300	1985-1986	Ito, 1988; Ito, 1991

# **Tabel 2.** Distribution of the Eohypsibiidae family on basis of sampling locations registered inliterature. \* indicates the type-locality



Figure 21. SEM of Amphibolus nov. sp. egg. A. Overview. B. –F. Close up on the processes.



Figure 22. SEM of the eggs of some Amphibolus species. A. Close up on the areolation of the egg of A. volubilis. B. Egg of A. weglarskae. C. Close up on the areolation of the summer egg of A. nebulosus. D. Winter egg of A. nebulosus. The SEM-pictures of figure A & B, was kindley provided by Dr. Roberto Guidetti, Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy. Unfortunately, the picture of the egg of A. volubilis was sent to us without scale bar.

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# First Report on a New Tantulocaridan (Crustacea: Maxillopoda) Parasitic on Harpacticoid Copepods Found off the Coast of West Greenland

Maja KIRKEGAARD & Steen Wilhelm KNUDSEN

Department of Invertebrate Zoology, Zoological Museum of Copenhagen, Copenhagen, Denmark

**Abstract.** This study presents the description of a new species of tantulocaridan found off the coast of Disko, West Greenland. At present a total of 29 species of Tantulocarida belonging to 5 families have been found. The new species is described on the basis of the tantulus larva and several stages of developing males and females, by the use of LM and SEM. It is placed in the Deoterthridae on account of the male trunk sac formation, the ornamentation on the tergites and cephalic shield, and the hairs on the thoracopods, the number of setae and the caudal rami. Tubular structures were identified inside the head of the tantulus larvae. This new species was exclusively found on yet unidentified harpacticoid copepod hosts caught at depths of 200 m off the coast in mud sediment. A total of 36 tantulocaridans were found in different life cycle stages: The tantulus larvae, the developing male, the parthenogenetic female and what might be an early developing sexual female. It is the first record of Tantulocarida from West Greenland.

Key words: Tantulocarida, parasite, Harpacticoida, West Greenland, parthenogenetic female, new species.

## **1. INTRODUCTION**

The unusual class of Tantulocarida are tiny marine ectoparasitic crustaceans that are found on other minute crustaceans. Tantulocaridans in general exhibit a peculiar and abbreviated lifecycle with an infecting tantulus larva, which passes through a short benthic phase before infecting a suitable epibenthic host. In the short epibenthic phase, they are regarded as temporary meiobenthos. A male or female is released from the trunk sac of the mature larva, or the tantulus larva develops into a parthenogenetic female, by sloughing the thorax and abdomen and then releasing eggs from which tantulus larva hatch (HUYS 1991). In the Arctic the tantulocaridans are known to parasitize harpacticoid copepods (HUYS et al. 1997). The range of hosts seems to be wide in several cases, with different species of Tantulocarida found on Copepoda, Ostracoda, Isopoda, Cumacea and Tanaida (HUYS 1991). Their life cycle includes an infective tantulus larva, a large free-swimming male and a swollen sac-like female (BOXSHALL & LINCOLN 1987). The distribution of tantulocaridans appears to be worldwide, and new genera and species are continuously discovered. Sampling in coral reefs, shallow subtidal localities, alkaline lava pools (BOXSHALL & HUYS 1989) and hydrothermal vents (HUYS & CONROY-DALTON 1997) have shown that tantulocaridans are not only found in cold water habitats, such as the deep-sea, or at high latitudes, as originally thought in 1983 when the class was recognized by BOXSHALL & LINCOLN. Apparently their diversity has been greatly underestimated due to the fact that the class is often overlooked because of the minute size of the animals, and because of the apparent similarity between the tantulocaridan females and the egg sacs on the copepods. Tantulocaridans are the smallest crustaceans ever found, the larvae varies in size from 82 µm (see HUYS et al. 1994) to 200 µm (see BOXSHALL & LINCOLN1991). The taxonomy of the Tantulocarida is somewhat difficult because of their abbreviated life cycle. Cephalic appendages such as first and second antennae are absent among tantulocaridans in every stage of their life cycle and also parts of the mouth are degenerated. This makes it difficult to assess crustacean relationship according to the ordinary

Family	Genus	Species	Host	Reference	Family characters and notes
Basipodellidae	Stygotantulus Polynyapodella Nipponotantulus Hypertantulus Rimitantulus Basipodella	stocki ambrosei heteroxenus hirsutus atlantica	Harpacticoida (Copepoda) Harpacticoida (Copepoda) Copepods (exclusively) Harpacticoida (Copepoda) Harpacticoida (Copepoda) Harpacticoida (Copepoda)	BOXSHALL & HUYS 1989 HUYS et al. 1997 HUYS et al. 1994 OHTSUKA & BOXSHALL in press in 1997 HUYS & CONROY-DALTON 1997 BECKER 1975 in HUYS 1991 BOXSHALL & LINCOLN 1983	Rostrum present. Cephalic shield with 1 pair of pores anteriorly and 2 pairs posteriorly. Subdorsal pores absent. First Protopod with 2 setae on exopod and 2 setae on endopod. Second to fifth protopod with 3-4 setae on exopod and 2 setae on endopod. Urosme multisegmented. Trunc sac with male develops behind sixth tergite, with additional swelling between cephalon and first tergite.
Deoterthridae	Boreotantulus Itoitantulus Campioxiphos Dicrotrichura Coralliotantulus Aphotocentor Tantulucus Cumoniscus Amphiantulus Deoterthron	kunzi misophiricola dineti tricincta coomansi styx hoegi kruppi harpiniarcheres dentatum lincolni	Harpacticoida (Copepoda) Misophrioida Harpacticoida (Copepoda) Unknown Harpacticoida (Copepoda) Unknown Harpacticoida (Copepoda) Cumaceans Amphipoda Myodocopida (Ostracoda) Harpacticoida (Copepoda)	HUYS & BOXSHALL 1988 HUYS et al. 1992b HUYS 1990 HUYS 1990 HUYS 1980 HUYS 1991 HUYS el at. 1992a BONUER 1903 in HUYS et al. 1993b BONUER 1903 in HUYS et al. 1993b BOXSHALL & VADER 1993 BRADFORD & HEWTT 1980 in HUYS 1991 BOXSHALL 1988; HUYS 1990	Rostrum absent. Cephalic shield with 4 pair of pores anteriorly and 6 pairs posteriorly. 1 pair of subdorsalpores. First Protopod with 2-3 setae on exopod and 0-1 setae on endopod. Second to fifth protopod with 2-5 setae on exopod and 2 setae on endopod. Urosome two-segmented. Trunk sac with male develops behind sixth tergite. Only sexual female found by Huys et al. 1993 within <i>I.</i> <i>misophricola</i> .
Microdajidae	.Xenalytus Microdajus	scotophillus langi gaelicus aporosus pectinatus	Unknown Tanaidacea Tanaidacea Tanaidacea Tanaidacea	HUYS 1991 Greve 1965 in HUYS 1991 Boxshall & Lincoln 1987 Grygier & Sieg 1988 Boxshall et al. 1989	All limbs strongly reduced, with very few setae on exo- and endopods. No endits on thoracopods. Trunk sac with male develops behind sixth tergite. Two-segmented urosome.
Onceroxenus	Onceroxenus	birdi curtus	Tanaidacea Tanaidacea	BOXSHALL & LINCOLN 1987 BOXSHALL & LINCOLN 1987	Rostrum minimal and incorporated in the cephalic shield. Cephalic shield with 3 set of 2 pair of pores dorsally. No subdorsalpores. First Protopod with 2 setae on exopod and 0 setae on 1-segmented endopod. Second to fifth protopod with 4 setae on exopod and 2 setae on endopod. Three- segmented urosome.
Doryphallophoridae	Doryphallophora	aselloticola megacephala harrisoni	Isopoda Isopoda	BOXSHALL & LINCOLN 1983 LINCOLN & BOXSHALL 1983 in HUYS 1991 BOXSHALL & LINCOLN 1987; HUYS 1990	Rostrum distinct. Cephalic shield with 8 pair of pores, of which 1 is slitshaped. No subdorsalpores. First Protopod with 4 setae on exopod and 0-1 setae on endopod. Second to fifth protopod with 4 setae on exopod and 2 setae on endopod. Urosome two-segmented. Trunk sac with male develops behind sixth tergite, with additional swelling between fifth and sixth tergite. All known species have tantulus larvae >100 µm.
Incertia sedis	Tantulocarida	sp. indet		Hansen 1913 in Huys 1991	

Table 1 Overview of known tantulocaridans and their respective hosts, partly composed from HUYS (1991)

crustacean taxonomical approach, which is primarily based on cephalic appendages. Instead the tantulocarid taxonomy is based upon body tagmosis in the tantulus larva, because of the sloughing of somites in the adults. Their phylogenetic affinities based on morphology appear to be near Ostracoda, Branchiura and Thecostraca, all within Maxillopoda (BOXSHALL & HUYS 1989). Tantulocarida compromises 5 families, 20 genera, and 28 species, excluding the Incertia sedis and the nov. gen. in this report, see table 1.

This project was originally set out to provide an estimate of the prevalence of parasites and the number of parasites per infected host in heavily fished shrimp fishing fields, Kuanit, versus undisturbed sediments, Iqpik, off the coast of West Greenland. In heavily fished shrimp fishing fields the continuous disturbance of the tantulus larva in the sediment could cause the absence of Tantulocarida. Therefore, this survey would provide a good estimate of the degree of exploitation in the shrimp fishing fields. It turned out that such a survey would require too huge an effort collecting the data and unfortunately we did not find Tantulocarida at Kuanit. Instead the discovery of the presence of a new species at the Iqpik field made the aim of this report to describe this new tantulocaridan. The prevalence of Tantulocarida found is included for not leaving anything unmentioned.

# 2. MATERIAL AND METHODS

Samples were taken off the rocky coast of West Greenland near Qeqertarsuaq (Disko) on the two shrimp fishing fields Iqpik (Disko Bay) and Kuanit (Disko Fjord) both near shore and in relatively shallow waters (100-200 m). A triangular dregde was hauled behind the research vessel Porsild for a few minutes, dragging the dredge uphill and scraping the muddy bottom by the speed of one or two knots. A total of seven hauls were made: Two hauls on the Iqpik-field on 5. July, two hauls on the Iqpik-field on 12. July, two hauls on the Kuanit-field on 17. July and one haul on the Iqpik-field on 19. July, all samples taken in 2002. The two fields are shown in fig. 1.



**Figure 1.** Localities where mud samples were collected. Iqpik and Kuanit. x marks the sampling spot.

Mud samples of living material were kept cold ( $<4^{\circ}$ C) and analysed within a couple of days, but most were sorted and fixed in buffered formalin or alcohol immediately upon retrieval on board the vessel. This was because of a significant lack of Tantulocarida in the live samples after more than 24 hours. We were not able to explain what caused the tantulocaridans to disappear from the sediment samples. Some samples were stained with Rose Bengal in order to facilitate the sorting.

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Drawings of selected specimens of the parasite were made using camera lucida and by interpreting micrographs obtained by a JEOL JSM-6335F scanning electron microscope (SEM) and pictures were taken with an Olympus light microscope (LM), equipped with Nomarski light interference contrast. SEM techniques involved critical point drying and subsequent coating with platinium. Few samples with living material provided the first opportunity for examining and obtaining the first pictures of living Tantulocarida. The type material is deposited in the Zoological Museum of Copenhagen (ZMUC), Denmark.

6 paratypes were sent to Dr. Rony Huys, British Museum, London, for examination and all six were preserved in 96% ethanol for molecular investigations.



**Figure 2.** Harpacticoid with the new tantulocaridan larva. Place of attachment is typical, but can occur everywhere on the host.

Station and date of sampling	Iqpik, 5. July 2002	Iqpik, 12. July 2002	Kuanit, 17. July 2002	Iqpik, 19. July 2002
Harpacticoid copepods examined	330	754	72	97
Tantulocaridans found	5	25	0	6
Prevalance (%)	1.5	3.3	-	6.2
Mean intensity	1.3	1.3	-	1.0
Depth (m)	195-214	166-170	105-130	198-220
Temp. (°C)	5.8 in surface	2.3	2.8	5.6 in
				surface
Location	69°17.3N,	69°18.0N,	69°33.3N,	69°17.3N,
	53°13.1W	53°11.5W	54°15.0W	53°13.2W

**Table 2.** Sampling localities and depth. Prevalence and mean intensity of harpacticoid hosts infected with tantulocaridans. Mean intensity is determined as the total number of parasites pr. infected hosts.

# **3. RESULTS**

All Tantulocarida were found as ectoparasites on harpacticoid copepods (Fig. 2 and 7B). Apparently two different forms of harpacticoid copepods served as potential hosts for the new species of Tantulocarida, a thin and a thick one. A total of 36 Tantulocarida were found. 16 males inside trunk sacs and 5 tantulus larvae were found. 9 of the Tantulocarida collected at Iqpik were females, and only one of all the females examined carried an egg sac with developing eggs. 6 were not examined, but sent to Dr. Rony Huys, British Museum, London. Both fields contained a variety of meiofauna. The Iqpik-field had Polychaeta, Kinorhyncha, Nematoda, Tanaida and a very rich Harpacticoid fauna. The Kuanit-field had Polychaeta, larvae of Bivalvia, larvae of Ophiura, Kinorhyncha, Nematoda and Tanaida, but fewer Harpacticoid species. The sediment in the Iqpik-field was composed of brown mud and fine organic particles whereas the Kuanit-field was composed of sand and black mud.

No Tantulocarida were found at Kuanit. Table 2 shows the frequency of Tantulocarida found in correspondence with the number of Copepoda examined.

# Description

# Tantalus larva

The body consists of a prosome and an urosome (Fig. 3, 8D, 9E and 9F). The prosome consists of a cephalon and 6 anterior pedigerous thoracic somites (Fig. 3A, t1-t-6). The urosome is two-segmented consisting of a seventh limbless thoracic somite (Fig. 3A, t7) and a free abdominal somite. Total body length varies from 147 µm to 192 µm, measured from anterior margin of the cephalic shield to the posterior end of the caudal rami. The Cephalic shield is 1.5 to 1.3 times longer than wide varying in length from 43 µm to 48 µm, and in width from 29 µm to 37 µm. The Cephalic shield is ornamented with high longitudinal lamellae (approximately 1 µm high) tapering off towards the anteriorly located oral disc, some of these lamellae are damaged considerably due to the preparation technique preceding SEM. Some lamellae were partially torn off and others were totally detached from the cuticula. A rostrum is absent. The Cephalon bears 7 pairs of pores, of which 3 have emergent sensilla. The pore formula is A<sub>I-II</sub>, D<sub>I-IV</sub> and L<sub>I</sub>, with 2 pairs of pores anteriorly on the cephalon, 4 pairs of pores dorsally on the cephalon and 1 pais of pores laterally. Eventually more pores could exist laterally and ventrally. The terminology for the body segmentation of the tantulus larvae and pore terminology for cephalic pores follows BOXSHALL & VADER (1993). The oral disc (Fig. 3A, 3B and 7E) is approximately 10 - 12 µm in diameter. A protruding organ (Fig. 4C, po) through the oral disc into the host is barely visible. The protruding organ is shaped either like a bulbous sac as described by BOXSHALL & LINCOLN (1983) or it is the first part of a network of rootlet system penetrating the hosts' interior (BOXSHALL 1991; HUYS 1991; HØEG pers. com. 2002).

Internal structures in the head are barely discernible. Cephalic stylet (Fig. 4A, cs) protracted and slightly curved, with a hollow base. The tubular structures spread throughout the inside of the head without an apparent symmetry (Fig. 4). No surface openings connected to the tubular structure discernable. Globular bodies seem to be randomly distributed near the posterior rim of the head (Fig. 4B).

Tergites on first to sixth thoracic somites have lamellae in distinct polygonal ornamentation (Fig. 3, 7A, 7D and 7F). The first tergite is largely concealed beneath posterior rim of cephalic shield. Thoracopods (Fig. 5, 7D and 8C) are presumably used for swimming. Thoracopod 1-5 (Fig. 5A-E) consists of an unsegmented protopod with small superficial spines or small hairs, a medial endit with 1 spine, an exopod and an endopod. Thoracopod 1 (Fig. 5A) bears a two-segmented endopod and a two-segmented exopod. The exopod bears 2 long haired setae and 1



Figure 3. A. Larva of the new tantulocaridan. External structures, dorsal view. See text for pore labelelling. Perhaps more pores exist ventrally on the cephalon. B. Larva, lateral view. C. Urosome and setae enlarged, dorsal view. Abbreviations: Oral disc (od), transverse lamellae (tl), first to seventh tergite (t1-t7), high lamellae (hl), fringed edges (fe), caudal rami (cr), small seta (ss) and long seta (ls). Neither in A, B or C are setae on caudal rami shown in full length, but cut off as indicated by the small bars.



Figure 4. A. Larva, internal structures, lateral view. B. Cephalon, internal structures, dorsal view. C. Cephalon of A, enlarged, internal structures, lateral view. Abbreviations: Oral disc (od), cephalic stylet (cs) and caudal rami (cr), tubular structures (ts), globular bodies (gb), high lamellae (hl), protruding organ (po) and swellings on tubular structures (sts).

(smaller) thin seta without hair and 1 short spine on the second segment of the endopod. The posterior, long slender segment of the endopod bears one haired seta and a spatulate process (Fig. 5A, sp). Thoracopod 2 (Fig. 5B) has a two- (or three-?) segmented exopod (Fig. 5B, ex), of which the segment closest to the setae bears 2 small spines (Fig. 5B, sex). The second segment of the exopod bears 2 longhaired setae and 2 unhaired thinner setae. The endopod (Fig. 5B, en) is similar to the endopod on thoracopod 1. Thoracopod 3-4 (Fig. 5C and D) bears a two-segmented exopod with 1 small spine on the distal segment (Fig. 5C, sex and 5D, sex). The exopod has 2 longhaired setae, and 2 thinner unhaired setae. The endopod (Fig. 5C, en and 5D, en) is two-segmented, the first segment thick and the proceeding segment slender and bearing 2 haired setae and 1 spatulate process (Fig. 5C, sp and 5D, sp). Thoracopod 5 (Fig. 5E) has a two-segmented exopod, of which the distal segment bears 2 small spines (Fig. 5E, sex), 2 longhaired setae and 2 thinner unhaired setae. The endopod 5 (Fig. 5E) has a two-segmented exopod, of which the distal segment bears 2 small spines (Fig. 5E, sex), 2 longhaired setae and 2 thinner unhaired setae. The endopod is similar to the endopod

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Figure 5. Thoracopods of tantulus larvae shown seperately in pairs. A. Thoracopod pair 1.
B. Thoracopod pair 2. C. Thorapod pair 3. D. Thorapod pair 4. E. Thorapod pair 5. F. Thorapod pair 6. Abbreviations: Protopod (p), medial endit (me), spine on protopod (ps), endopod (en), exopod (ex), spatulate proces (sp), haired seta (hs), unhaired seta (us), spine on exopod (sex) and spine on medial endit (sme). Notice that the hairs on the setae, the spines on the exopods and the spines on the protopods were not seen in light microscope, but only observed on pictures obtained by SEM. These three characters are included on this drawing, in order to give a full idea of the thoracopods.



Figure 6. Attached tantulocaridan, internal structures A. Parthenogenetic female with eggs, lateral view. B. Male developing inside trunk sac of tantulus larvae, lateral view. Abbreviations: Eggs (egg), aesthestascs (as), cephalic stylet (cs), holes in head shield (hh), protruding organ (po), tubular structures (ts) and a long stretched umbilical cord-like organ (uc)

on thoracopod 3 and 4. Thoracopod 6 (Fig. 5F) consists of a protopod with small spines or hairs but without medial endit. The protopod has an one-segmented exopod with 2 longhaired setae.

Although not apparent on any of the six thoracopods, coupling spines could very well exist on the medial endites of the protopods near the single spine.

4 lamellae transverse the seventh tergite (Fig. 3C, tl). Posterior to the seventh tergite, the abdomen contains 3 fringed edges (Fig. 3C, fe), with the most distal one covering the caudal rami originating from the abdomen. 2 articulated caudal rami originate from the abdomen, each armed with 2 long thick haired setae and 1 thin short haired seta (Fig. 3C, 4A, 7A, 7B and 7F).

# Male

Enclosed inside the larval trunk sac the male develops until released by rupture of the sac. The trunk sac develops behind the sixth tergite of the attached larvae and there is no swelling between the cephalic shield and the first tergite. The larval somites are pressed together dorsally, posterior to the larval head, because of the developing trunk sac (Fig. 6B and 7C). The larva metamorphoses after successful attachment to the host and initiates formation of a male in the trunk sac by allometric growth (BOXSHALL & LINCOLN, 1983).

The mature male inside the trunk sac (Fig. 6B and 8E) has six pairs of thoracopods with setae and fine honeycomb-like ornamentation on the head shield as well as on the six tergites and the urosome. On the head shield of the developing male, holes were visible in LM, but apparently no internal structures connected these superficial holes. A long stretched umbilical cord-like organ is visible through the trunk sac, which connects the male with the head of the attached larvae. BOXSHALL (1988) has referred to this organ as the umbilical cord.

No penis could be discerned in LM, but is usually found behind the sixth thoracopod as reported from *Stygotantulus stocki* (see BOXSHALL & HUYS 1989), *Itotantulus misophricola* (see HUYS et al. 1992b) and *Microdajus* sp. (see BOXSHALL et al. 1989; BOXSHALL 1991). All though not recognized, a penis could very well be present. 3 pairs of aesthetascs appear on the head of the male (Fig. 6B, as and 8E, as).

# Parthenogenetic female

A trunk sac develops behind the head shield after all thoracic somites and the urosome are sloughed. This results in a very early female, with a trunk sac smaller than the head (Fig. 9C). The parthenogenetic female (Fig. 6A and 9D) measured 405  $\mu$ m in length from the neck to the posterior end of the egg sac. The egg sac inside measured 345  $\mu$ m in length.

No gonopore or abscission scar was discernible, but could very easily have been overlooked. The gonopore is usually found on the fifth female somite (BOXSHALL & LINCOLN, 1983). No slender long neck was seen on any specimen, as reported from other females with egg sacs (see BOXSHALL 1991; BOXSHALL & VADER 1993; GRYGIER & SIEG 1988).

Two females (Fig. 9A and 9B) with their larval body sloughed and therefore only composed of head and trunk sac, apparently had an indiscernible mass of cells inside the trunk sac.

The small females (Fig 8B and 9C) range in size from 82  $\mu$ m to 67  $\mu$ m respectively, measured from the anterior part of the oral disc to the posterior part of the trunk sac. One early female (Fig. 8A) has a branching rootlet system originating from the head of the larvae, spreading out through the oral disc and inside the host.



Figure 7. Scanning Electron Micrographs. A. Tantulus larvae, lateral view. A<sub>II</sub>, D<sub>II</sub> and L<sub>I</sub> denotes pores, see text for further explanation. B. Harpacticoid host with larvae (la), dorsal view. C. Harpacticoid host with both a shrunken parthenogenetic female (f) and a smaller tantulus larvae with trunk sac containing a developing male (m). D. Thoracopods on lateral side of tantulus larvae. E. Oral disc of parthenogenetic female, ventral view. F. Tantulus larvae, dorsal view. One pore (L<sub>I</sub>) is visible. Second to sixth thoracic somites (t2-t6).

# **4. DISCUSSION**

## Relationship of the new species

The discovery of a new species of Tantulocarida in the coastal waters of West Greenland broadens the geographic distribution of Tantulocarida, since reports of the closest Tantulocarida is from North East Greenland (HUYS et al. 1997). The new tantulus larvae were exclusively found as ectoparasitic on harpacticoid hosts. This indicates that the new species might belong to the family Basipodellidae, because this family has only been found to parasitize harpacticoid hosts (see HUYS 1991; HUYS & CONROY-DALTON, 1997). The swelling on the larval trunk sac, though, does not separate the larval tergites from the cephalon (Fig. 6B, 7C and 8E), which HUYS (1990) defines as being characteristic of the family Basipodellidae. The two-segmented urosome does not correspond with the multisegmented urosome that is encountered in Basipodellidae, either. BOXSHALL & VADER (1993) describes the urosome as consisting of the abdomen and the caudal rami. The body terminology used before BOXSHALL & VADER (1993) includes the seventh thoracic somit, the abdomen and the caudal rami in the urosome. The body terminology used before BOXSHALL & VADER (1993) is often inconsistent and uses the term urosome and abdomen interchangeably, and therefore we follow the body terminology of BOXSHALL & VADER (1993) and thereby including the seventh limbless somite in the urosome. This lowers the number of abdominal somites in some genera, but the genera Basipodellidae is still regarded as having a multi-segmented abdomen according to the body terminology of BOXSHALL & VADER (1993).

The number of setae on the thoracopods of the tantulus larvae excludes any relationship with the families Microdajidae and Onceroxenidae. The family Doryphallophoridae only compromise tantulus larvae larger than 100  $\mu$ m, and they have only been found in deep-sea as ectoparasitic on Isopoda.

The honeycomb-like ornamentation on the tergites is only known from *Aphotocentor styx* (see HUYS 1991) and *Campyloxiphos dineti* (see HUYS 1990), which both belong to the Deoterthridae. In both cases the ornamentation does not include the head of the tantulus larvae or the male as seen on this new species. The 7 pairs of pores do not correspond with the 4 pairs anteriorly and 6 pairs posteriorly recorded for Deoterthridae, but pores could easily have been overlooked. The setae on the endopods seem to be placed in a groove on the endopod, which also occurs in other genera in the Deoterthridae. The exopod on the first thoracopod bears at least 3 setae, and this is only seen within the Deoterthridae (see HUYS 1990). None of the ten genera in the Deoterthridae posses all of the characters reported on this new species. Only one or two of the characters are recognized in a any single genus of Deoterthridae, e.g. *Amphitantulus harpiniacheres* (see BOXSHALL & VADER 1993) which has haired protopods, haired setae and a relative equal number of setae as in this new species, or *Cuminoscius kruppi* (see HUYS et al. 1993a) which has a fringed distal end of the abdomen, or *Tantulucus hoegi* (see HUYS et al. 1992a) which has distinct epicuticullar lamellae on the cephalon.

It thus seems most reasonable to presume that this new species should be placed within the Deoterthridae near the genera: *Aphotocentor*, *Campyloxiphos*, *Amphitantulus*, *Cuminiscus* and *Tantulucus*.

A brief overview of some known genera is given in appendix 1-5.

## Life cycle

The tantulocaridans hatch as tantulus larvae. The tantulus is probably infective immediately after hatching (BOXHALL & LINCOLN 1987), which implies that the newly hatched tantulus larvae are fully developed with thoracopods and cephalic stylet.

Infection of the host could happen upon a release of a swarm of infective larvae from the parthenogenetic female (BOXSHALL & VADER 1993), but this might be a rare coincidence.

Soon after successful attachment to a suitable host, the larval thorax and abdomen is sloughed and swelling begins posterior to the cephalic shield. The tantulocaridan then becomes a parthenogenetic female with eggs in the trunk sac. Perhaps in some cases a sexual female develops inside the trunk sac. If the larval thorax and abdomen is preserved and the swelling of a trunk sac appears behind the sixth somite, then a male develops inside the trunk sac.

Tantulocarida are not equipped with mouthparts at any stage of life, indicating that all feeding takes place through a host.

Suppression of moulting in the host, also known as "parasitic anecdysis" or parasite-mediated growth arrest, has been proposed by HUYS (1991). Suppression of moulting in the host is not regarded as likely in the cold arctic waters near Disko. The cold waters lowers the metabolic rate of the parasite, as well as the metabolic rate of host. This leaves the lifecycle of the arctic harpacticoid long enough for the tantulus to go through its own life cycle. Warm waters affect the tropical harpacticoid host lifecycle, making it shorter compared to the lifecycle of the arctic host. Unless the hosts' moulting is halted in such warm waters, the metamorphosis of a mature male or female would have to be completed within two successive moults i.e. approximately 2 days or less (HUYS 1991). If the host were to moult before the Tantulocarida has completed it's metamorphosis into a mature male or female, the tantulocaridan would remain attached to the sloughed integument of the host and be prevented in further reproduction. Suppression of moulting in the host has been assumed to be due to hormones injected from the tantulocaridan into the host. These hormones, which not only suppress host moulting, also affect the fertility of the host making it behave as if it were carrying an egg sac. It then starts nursing the ectoparasite as if it were its' own (KRISTENSEN pers. com. 2002).

# Sexual female versus sexual male

Sexual reproduction is the most common way of reproduction in Crustacea, and parthenogenetic reproduction is only encountered in a few cases. The presence of a penis in the mature male tantulocaridan does not correspond to the minute size of the larvae (the penis being almost as large as the larvae), with which it was thought to copulate. In 1993, a sexual female was discovered by HUYS et al. (1993b) in the trunk sac of *Itoitantalus misphricola*. Unfortunately this is still the only discovery of a sexual female, and perhaps not all tantulocaridans produce a sexual female.

The two females in Fig. 9A and B have no visible eggs inside. The indiscernible mass of cells inside the trunk sac of the specimen in Fig. 9A might be a developing sexual female, due to the resemblance of an early stage of a developing adult male.

If these growing individuals indeed are sexual females, their stage of development is clearly not as advanced as in the developing males, see Fig. 8E.

The female in Fig. 9A represents an earlier stage of development, whereas the males in Fig. 8E and F represent a later stage of development. At the time of sampling, there must have been larger and more mature sexual females present in the sediments or in the sea, if males and females are to reach maturity contemporary. The size of the possible developing sexual female inside the trunk sac (Fig. 9A) equals the size of the fully mature male (Fig. 8E), which makes it evident that the adult sexual female will be considerably larger than the adult male. This inequality might be because the female is to carry eggs inside the head, as seen in HUYS et al. (1993b).

The females in Fig. 9B and Fig. 8B also have an indiscernible mass of cells inside the trunk sac.

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Figure 8. Light microscope photographs with Nomarski interference. A. Cephalon of attached larvae, lateral view. Notice protruding organ (po) which differentiates into what might be a bulbous sac or rootlet system. B. Immature female, lateral view. C. Tantulus larvae detached from host, ventral view. D. Tantulus larvae attached on host, lateral view. E. Male developing inside trunk sac of tantulus larvae, lateral view. Notice aesthetascs (as). Photo of living specimen. F. Harpactocoid host infested with three tantulocaridans. A tantulus larvae (la) in early phase of trunk sac formation, a developing male inside trunk sac of tantulus larvae (m) and a larvae with ruptured trunk sac (rl).



Figure 9. Light microscope photographs with Nomarski interference. A + B. Female with indiscernible cell mass inside trunk sac. Possibly a developing individual. C. Very early female with newly formed trunk sac. Photo of living specimen. D. Parthenogenetic female. Photo of living specimen. E. Detached tantulus larvae, lateral view. F. Attached tantulus larvae, ventral view.

Internal structures

The tubular structures inside the head of the tantulus larvae apparently do not connect with any superficial openings. The developing male, though, has superficial openings on the head

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shield, but does not have any internal connecting structure. Perhaps connecting holes on the larval head and tubular structures inside the head of the male exist, but this can probably only be verified by Transmission Electron Microscopy (TEM). The interpretation of tubular structures within the head could be a misinterpretation of glandular structures, but to confirm this would also require TEM. Glandular structures associated with the stylet have previously been recognized by BOXSHALL & HUYS (1989) and HUYS et al. (1994). The purpose of these glandular or tubular structures remains uncertain.

It is presumed that muscles associated with the cephalic stylet degenerate after the stylet has served its purpose by piercing the host when the tantulocaridan attaches itself (BOXSHALL 1991). This could explain the asymmetrical placement of the stylet in the head.

The purpose of the umbilical cord-like structure connecting the male's head with the head of the attached larvae is uncertain. BOXSHALL & LINCOLN (1987) suggests that it provides the male inside with nourishment extracted from the host.

HUYS (1991) suggests that a branching network of cells spreads out from the tantulocaridan and inside the host. This network is established via undifferentiated cells carried from the parasite through the host, via the host's circulatory system. As the cells multiply and spread out in all directions, a rootlet system is established. Perhaps hemolymph from the copepod provides the male tantulocaridan with nourishment as well as oxygen.

# External structures

The presence of a penis in the male possibly derived from the seventh trunk limb and the process of larval body tagmosis, indicates that the tantulocaridan class may have strong phylogenetic affinities within the Cirripedia. The presence of a penis is an indication of phylogenetic position within Thecostraca (BOXSHALL & HUYS 1989). We did not observe a penis, probably because we were unable to distinguish it.

Apparently no one has made records of distinct ornamentation on the head shield of the male, which could make this observation the first ever.

Coupling spines, all though not clearly visible on the collected specimens, serves the purpose of aiding swimming capabilities by coupling the swimming strokes of limbs (HUYS 1991; KRISTENSEN pers. com. 2002).

Chemosensorical aesthetascs on the head of the male are regarded as being essential for the location of sexual females (HUYS et al. 1993b).

## DNA sequencing

To establish the phylogenetic relationships of this animal, 6 specimens in 96 % ethanol were sent to Dr. Rony Huys, British Museum, London. The following total DNA sequencing using whole animals showed that the parasite was a copepod, consequently because it is a parasite on a copepod. If DNA sequencing is to be used for tantulocarids, only tissue not involved with feeding can be used: Preferably the egg sac of a decapitated parthenogenetic female or a free swimming larva.

# **5. CONCLUSION**

This new species is placed in the Deoterthridae on account of the male trunk sac formation behind the sixth tergite, the numbers of setae on the thoracopods of the tantulus larvae and the hairs on the protopods and setae. Also the distinct honeycomb-like ornamentations on the tergites have been observed in other species within Deoterthridae. It should be placed within its own genus because none of the ten known genera in Deoterthridae have more than one or two characters in common with it.

We propose that this new species is given the name *Diskotantulus reinhardti* nov. sp. The generic name *Diskotantulus* derived from the island Disko in West Greenland where the

samples were collected and the Latin word for Tantulocarida, and *reinhardti* in honour of professor Reinhardt Møbjerg Kristensen, who was the first to find this new species and who has precipitated the investigations preceding the discovery of it.

Presumably one early sexual females was discovered inside the trunk sac of a female larvae (Fig. 9A). Characteristic of this female is the missing larval body which has been sloughed, and an indiscernible mass of cells inside the developing trunk sac containing what might be a growing individual.

Tubular structures are spreading through out the interior of the larval head, but apparently without any connection to the surface of the head. It is possible that these tubular structures are important in either the piercing of the host integument and the following degeneration of the internal musculature associated with the cephalic stylet, or they could be important in the retrieval of nourishment from the host.

The holes on the cephalic shield of the male were apparently not connected with anything inside the head, as no internal structures in the head of the male were found.

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## BASIPODELLIDAE

Five out of seven genera are depicted on the right.

Descriptive characteristics according to HUYS (1990):

+ Rostrum.

Cephalic shield with 3 pairs of pores (1 anteriorly, 2 posteriorly).

No subdorsal pores.

Thoracopods with well developed rami.

Leg 1 with 2 setae on exopod. 2 setae on endopod.

Leg 2-5 with 3-4 setae on exopod. 2 setae on endopod.

Urosome six- or sevensegmentet.

Trunk sac with male developed behind sixth tergite.

Additional swelling between cephalon and first tergite.

Male abdomen 2-segmentet.

Caudal rami well developed, cylindrical with 3 setae.

Copulatoric stylet straight.

Ectoparasitic on copepoda.

The major part have been recorded from the Atlantic and the Southern Pacific according to Huys et al (1994).

#### Other species:

Basipodella atlantica Hypertantulus sp.



#### DEOTERTHRIDAE

Nine of the ten known genera are depicted on the right.

Descriptive characteristics according to HUYS (1990):

Rostrum absent.

Cephalic shield with 10 pairs of pores 4 anteriorly, 6 posterioly. Some with 1 pair of subdorsal pores.

2-segmented urosome sometimes with surface lamellae.

Thoracopods with endits, and well developed rami.

Leg 1 with 2-3 setae on exopod. 0-1 setae on endopod.

Leg 2-5 with 2-5 setae on exopod. 2 setae on endopod.

Trunk sac with male develops behind sixth tergit.

Male abdomen 2-segmented

Caudal rami on male well developed, cylindrical, with 3 setae.

Copulatoric stylet firm.

Ectoparasitic on Harpacticoida and Ostracoda

According to HUYS et al. (1994) the majority of the known species have been found in the Atlantic and South Pacific



# DEOTERTHRIDAE

#### Other species:

Deoterthron lincolni Coralliotantulus coomansi Cumoniscus kruppi

Host:Cumacea



Redrawn from HUYS et al. (1993b) Found in the North Sea, 307 m's depth.

#### Amphitantulus harpiniarcheres

Host: Amphipoda



BOXSHALL & VADER (1993) Found in the North Sea, 80 m's depth.

#### Deoterthron dentatum

Host: Myodocopida (Ostracoda)

136 µm

3 1 6 安

Redrawn from HUYS (1990) Found near New Zealand, 384 m's depth.

#### MICRODAJIDAE

Two species of the two known genera are depicted on the right.

Descriptive characteristics according to BOXSHALL & LINCOLN (1985):

2-segmented urosome.

Thoracopods without endits

Trunk sac with male developed behind sixth tergite.

Male has 2-segmented abdomen with caudal rami.

Descriptive characteristics according to HUYS (1993b):

All limbs have strongly reduced rami.

#### Other species:

Microdajus gaelicus Microdajus aprosus Microdajus pectinatus Microdajus langi

Host:Tanaidacea





Redrawn from BOXSHALL et al. (1989) Found near Norway and Scotland, 130 and 22 m's depth respectively.

Xenalytus scotophilus

Host: Unknown, found free in sediment



Redrawn from HUYS (1991)

# ONCEROXENIDAE

Two species within the only known genus are depicted on the right.

Descriptive characters according to HUYS (1990):

+ rostrum developed only weakly.

Cephalic shield with 3 sets of 2 pairs of pores.

No subdorsal pores.

Leg 1 with 2 setae on exopod. 0 setae on endopod.

Leg 2-5 with 4 setae on exopod. 2 setae on endopod.

Trunk sac with male developed posteriorly to sixth tergite.

No additional swelling between tergites.

Abdomen 3-segmented.

Male abdomen two-segmented and with well developed caudal rami.

Copulatoric stylet unconfirmed.

Ectoparasitic on deep-sea Tanaids.

Onceroxenidae and Deotherthridae can be difficult to distinguish, but can be done by looking at the segmentation of the abdomen.

## Onceroxenus birdi



Onceroxenus cortus

Host: Tanaida



Redrawn from BOXSHALL & LINCOLN (1987) Found in Europe at 3000 m's depth

## DORYPHALLOPHORIDAE

Two out of three species within the only genus known are depicted on the right.

Descriptive characters according to HUYS (1990):

+ rostrum strongly developed.

Cephalic shield with 8 pairs of pores, of which one is slit-like.

No subdorsal pores.

Leg 1 with 4 setae on exopod. 0 - 1 setae on endopod.

Leg 2-5 with 4 setae on exopod. 2 setae on endopod.

Trunk sac with male developed between fifth and sixth tergite.

Additional swelling between fourth and fifth tergite.

Abdomen 2-segmentet.

Male abdomen unsegmented and with paired caudal setae. Caudal setae on male are obsolete.

Copulatoric stylet straight, slim and spear shaped.

Ectoparasitic on deep sea Asellote isopods.

All species <100 µm only parasitize Harpacticoid Copepods according to Huys (1989).

## Other species:

Doryphallophora Megacephala

#### Dorypallophora aselloticola



Doryphallophora Harrisoni

Host: Isopoda



# Individual and Microgeographical Variation in the Song of the Snow Bunting (*Plectrophenax nivalis*) on Disko Island, West Greenland.

Linda Solveig ANDERSEN & Anne Marie Mandrup NIELSEN

Department of Animal Behaviour, Zoological Institute, Tagensvej 16, 2200 Copenhagen N, Denmark

Abstract. The intra- and inter-individual variation in song of the snow bunting (*Plectrophenax nivalis*) was studied on Disko Island, West Greenland. The songs of six males were recorded in the vicinity of the Arctic Station, Qeqertarsuaq, at the end of their breeding cycle. The intra-individual variation was studied in a qualitative classification analysis of figure types and it was found, that the males had only one song type each. Furthermore, the songs were characterised to be very stereotypic, with a small degree of intra-individual variation. The inter-individual variation was examined by analysing the four parameters: song duration, song output, minimum frequency and maximum frequency. The four parameters all showed an inter-individual variation. The results of the qualitative classification analysis indicate a possible microgeographical variation.

Key words: Snow bunting, Disko Island, song, intra- and inter-individual variation, microgeographical variation.

## **1. INTRODUCTION**

The snow bunting (*Plectrophenax nivalis*) belongs to the order Passeriformes and the family Emberizidae (THORPE & LADE, 1961). It breeds in the high arctic (LYON et al., 1987), and has a circumpolar distribution. See Plate I, A-E, for illustration of male and female snow buntings.

The snow buntings arrive in Greenland in the first weeks of April, and the breeding season begins in the first half of June and ends in the middle of July. When they depart in August to September, the populations of the south-eastern and south-western coasts of Greenland migrate to pass the winter in Canada and the northern part of the USA, whereas the populations of the north-eastern part of Greenland migrate to Russia (MUUS et al. 1981). Nevertheless, some birds have been observed to pass the winter in Greenland, all the way up to Disko Bugt (SALOMONSEN, 1974). The territory of the snow bunting is as large as 600 m in diameter in the beginning of April and decreases in size in the course of May to a final size of 50-100 m (TINBERGEN, 1939). These territories serve for courtship, nesting and feeding (CRAMP & PERRINS, 1994). Many species of the buntings are very territorial and it is characteristic that their songs are primarily for territorial proclamation (THORPE & LADE, 1961).

The song of the male snow bunting is a brief musical warble, which is quite variable in quality (CRAMP & PERRINS, 1994). The song is about 2 sec in length and has a frequency range from about 2 kHz to 6 kHz. It is composed of varying elements (figures types), which are usually constructed into motifs, i.e. fixed sequences of figures (HOFSTAD et al.,2002). The function of the song seems to be to attract potential mates and to warn off rivals (TINBERGEN, 1939). The songs of the buntings are said to be relatively stereotypic and with little variation, compared to the songs of other passerine species (THORPE & LADE, 1961), which is in agreement with ESPMARK'S (1995) conclusion, that the snow bunting has a stereotypic song. Furthermore, it is more common for the males to have individual characteristic songs than to share songs. The variation in the songs of an individual male consists of either adding figures in the beginning or the end of a song, or leaving out expected figures. Quantitative parameters



Plate I. A-C: Male snow bunting. D-E: Female snow bunting. F: Typical snow bunting habitat. All photos by Morten Smith, 2002.

such as the frequencies and the song duration can also vary between individuals (ESPMARK, 1995). Because of the stereotypy in song pattern, ESPMARK (1995) finds that it is only necessary to analyse 10 songs of the snow bunting to include most of the individual variation. This is in contrast to the repertoire size of many other passeriform species, for example the blackbird (*Turdus merula*), where an analysis of more than 200 songs is necessary to estimate the repertoire size of a male (RASMUSSEN & DABELSTEEN, 2002).

Variation in song can also be seen as a microgeographical variation, where neighbouring groups show distinct differences. Boundaries between these populations can divide them into dialect areas, although there is potential interbreeding. Often dialects are found in species, which only have one song type and where song sharing occurs between neighbours (CATCHPOLE & SLATER, 1995). Local dialects like these have been reported for many passeriform species, for example the redwing, *Turdus iliacus* (ESPMARK, 1982). A study by ESPMARK (1995) can not support that this occurs in the snow bunting on Spitsbergen, Svalbard. It is common that birds learn song features from their neighbours, among other

things, to be able to discriminate between neighbours and strangers (CACHPOLE & SLATER, 1995). In microgeographical variation, the distance between the males can have an influence on the degree of similarities in song features (DABELSTEEN Pers. Comm.).

Most studies of passerine birds at the Arctic Field Courses on Disko Island have primarily concentrated on examining population distributions (FINKE & BRANDT, 1999) and not until this study, has the song of the snow bunting been analysed. In this paper, we studied interindividual and microgeographical variation in songs of male snow buntings on Disko Island. This will be illustrated by examining both temporal and frequency parameters. Furthermore, a qualitative classification of figure types is used to study intra- and inter-individual song variation.

# 2. MATERIALS AND METHODS

## 2.1. Samplings

The recordings and observations were made in Østerlien in the vicinity of the Arctic Station, in an area of Lyngmarken and at the waste disposal site south of Qeqertarsuaq (Godhavn).

The locations were typical snow bunting habitats, consisting of a rocky terrain and sparse vegetation (CRAMP & PERRINS, 1994), see also Plate I, F.

Twelve male snow buntings were recorded in a period of ten days from the 7<sup>th</sup> until the 16<sup>th</sup> of July 2002 on 13 different locations (see Appendices 1 and 2). The recordings were made from 7.30 a.m. to 9.30 p.m. and the time of recording varied depending on weather conditions and other circumstances. There was not a large diurnal variation in song activity because of the midnight sun. Therefore the time of recording was not so important. Males were both observed singing alone and simultaneously. We estimated the size of the territories to be at least 50 meters. Therefore the locations had to be more than 50 meters apart, in order to minimise the risk of recording the same male at two different locations. This estimation of territory size is based on an observation of the three males (1, 2 and 3), who had their territories closest to each other. Here it was found, that two of them had their territories within about 50 meters.

## **2.2. Observation methods**

The snow buntings were not secretive, which made the observations and recordings relatively simple, primarily depending on our ability to get close enough to the birds, i.e. varying from 10 meters to 60 meters. We did not record and observe the first five minutes of an observation period to allow the birds to habituate to our presence. One person was observing and registering their visual behaviour, GPS positions, location, temperature, weather conditions, distance and direction of the observed male, while another observer recorded and registered the time of singing. We decided to observe one male at a time (Focal Sampling) and as far as possible by Continuous Recording (MARTIN & BATESON, 1993). This was the optimal method to allow one person to gather an adequate amount of continuous behavioural observations. Due to the limited time available to complete this study, it was not possible to include these behavioural observations in our analysis.

## 2.3. Equipment

The recordings were made with a Sony cassette recorder (model: WM-D6C) equipped with a microphone (model: Audio Technica Condenser Lo-Z), headphones and 90 minutes tapes (TDK Type II). Behavioural observations were made using binoculars (7x50) and the geographical positions of the territories were found using a Silva compass, a hand-held GPS (model: Trimble Navigation, Ensign GPS) and a "Thommen" altimeter. The GPS has an uncertainty of approximately 50 meters in the x- and y-coordinates.

## 2.4. Song analyses

The computer program Avisoft-SASLab Pro was used to produce spectrograms of the recorded songs. The settings of The Spectrogram Parameters and the Realtime Spectrogram Parameters were always set as follows: FFT-length: 256, Temporal resolution overlap: 75%, Frame size: 50%, a Hamming window function and Peak Freq. Interpol: auto. This gave a frequency and time resolution of 86 Hz and 2.9 ms, respectively. These settings are a compromise between, what is recommended in the manual (AVISOFT) and our assessment of, which settings gave the best illustration of the spectrograms. Some of the background noise was removed by band pass filtering (High Pass filter: 1,7 kHz and Low Pass filter: 8 kHz).In total about 900 songs were recorded, ranging from 3 to 216 songs pr. male.

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By listening through the four tapes, we found, that of the 12 males originally recorded, only eight males had recordings with a high enough signal-to-noise ratio, and therefore these were used for further analyses.

To identify the songs of the different males in later analyses, we noted the time and identity of each of the singing males that were recorded. On most locations there was only one male present, but it was difficult to identify the three males (1, 2 and 3) on locations 1, 1a and 1b, because they had adjacent territories. However, their territories seemed well defined and this was used to distinguish between the males. It would have been better to identify them by their colour patterns but this was only possible with male 2. For each of the eight males, the first 20 songs recorded were selected for the analysis. This number of songs was chosen, because it was the largest possible number of songs that could be achieved from each individual male. Furthermore, ESPMARK (1995) argues that at least ten songs should be included to obtain a reasonable repertoire size and a reliable measure of variation in the different song parameters. Only perched songs are used in this analysis, although calls and songs from flight displaying were recorded as well. It would have been a more objective method to select the first 20 consecutive songs for each individual, compared to the method we used. However, this was not always possible. We did not find it reliable to include songs of individuals, who were out of sight, since it was not acceptable. Therefore, the first 20 usable songs were chosen. Defining a song was not complicated, because each song was followed by a long pause.

We have defined the different units in a song, following the literature (ESPMARK, 1995; THOMPSON et al., 1994; MUNDIGER, 1975). A figure is a well-defined unit in a song, which is sometimes divided into separate parts by short pauses. We found that certain parts of a figure always appeared together, as for example in the marked figure (see Figure 1). Therefore, a figure was defined as one figure, as long as the pause between the parts of the figure did not exceed 0.2 cm measured on the printed spectrograms. This equals 0.022 sec. The unreliability of the spectrogram measurements was 0.05 cm, which is 0.0055 sec. MUNDIGER (1975) and HOFSTAD et al. (2002) also use the duration of a pause (0.02 sec) in defining a figure. To examine the objectivity of defining a figure, an inter-observer reliability test was performed. Each observer defined the figures in the 160 songs. Afterwards, the agreement in the definitions was compared and the reliability was more than 90%. A motif is defined as a sequence of figures recurring in the same order in each song (see Appendices 10-17).

To measure song duration, song output, minimum and maximum frequencies, Avisoft-SASLab Pro was used with the following settings in the "Automatic Parameter Measurements": "Element separation": Threshold: –25 dB and Hold time: 0.022 sec. "Temporal parameters": Duration of elements, Interval between elements, Absolute time of start and end. "Spectrum based parameters": Min. frequency and Max. frequency. The "Hold time" value is 0.022 sec, because this is the maximum duration of a pause in a figure that is accepted, see above for further explanation. The three parameters, minimum frequency, maximum frequency and song duration were measured directly in Avisoft. The latter was measured with a changed Hold time of 0.35 sec, but sometimes varying from 0.2 sec to 0.6 sec. Thus the function, "Duration of elements", measured the duration of the entire song. The song output is a derived measure calculated from the song duration and the "Duration of elements". Several thresholds were tested on the spectrograms and the most suitable value was –25 dB. This setting was used as often as possible to standardise the measurements, but when the settings were not adequate in respect to the quality of a spectrogram, the threshold was changed and/or the volume was increased. An inter-observer reliability test was performed to make sure, that there was an agreement on how to adjust these settings. The reliability was approximately 60%. Nevertheless, there was always an agreement on, when the threshold should be changed and the disagreement concerning the degree of this change was only about 2-5 dB.

Several measures are used for analysing variation in song between individuals. The following measures were used in our analysis: *Song duration*. The mean song duration was calculated from the total song duration of the 20 songs from each male. *Song output*. The total duration of singing pr. song was estimated by adding up the duration of each figure in a song and dividing this with the total duration of a song. *Minimum frequency*. The minimum frequency of the figure with the lowest frequency in a song was used as an estimation of the total minimum frequency of a complete song. *Maximum frequency*. The maximum frequency of a complete song. *Qualitative classification*. The defined figures were used to illustrate the degree of variation between the songs of one male. This is shown in Appendices 10-17.

#### 2.5. Statistics

The four parameters, song duration, song output, minimum and maximum frequency, were tested for homogeneity of variance by a  $F_{max}$ -test (see Table 1). If sample sizes are within a ratio of 4 to 1 and if  $F_{max} \le 10$ , then the data is said to show homogeneity of variance (TABACHNICK & FIDELL, 1996). In our data the sample size ratio is 1:1 for the six males in all four parameters. Normality was checked for each parameter and it was

found that, only song output was normally distributed. Two of the parameters could be transformed to approach normality and thus, we chose the square root transformation of song duration and the natural logarithm transformation of the minimum frequency parameter. The data, which followed the assumptions concerning normality, homogeneity of variance and independence, were used in a parametric one-way analysis of variance test (ANOVA). If there was a significant difference between the males, a Multiple Range Test (Fisher's LSD) was used to determine, which males were significantly different from each other.

The maximum frequency parameter could not be transformed to fulfil the assumption of normality and therefore was tested using a non-parametric Kruskal-Wallis test. This test does not allow conclusions to be drawn concerning, which males are different from each other, except those with the highest and lowest ranks (FOWLER et al., 1990). Therefore, we also used a multiple comparison test, the Kruskal-Wallis non-parametric one-way ANOVA, to test the pair wise differences (COLQUHOUN, 1971).

We also tested for differences in song duration between days, for males 5 and 7. This data was not normally distributed and could not be transformed to approach normality. Therefore the non-parametric Mann-Whitney U-test was used to compare the mean song duration between days.

The computer program, Statgraphics Plus 4.0, was used to calculate the parametric and non-parametric tests apart from the Kruskal-Wallis non-parametric one-way ANOVA.

# **3. RESULTS**

## **3.1. Identification of males**

Since, males 1, 2 and 3 were recorded simultaneously, there was a risk of confusing the songs of these three males. We have used two controls to assure, that this did not happen. First of all, when males 1 and 3 were singing alternately, it was observed that they kept singing the same individual characteristic song and did not begin to match each other exactly. Furthermore males 5, 7 and 8, which were recorded separately, repeated the same individual characteristic for the individual, were omitted from the analyses and new songs were used.

Since all the males were found to be very individual characteristic in their song, it was possible to see, that males 5 and 5a actually were the same male. This was also the case for males 7 and 9. Furthermore, the males were observed at the same locations, respectively. To make an objective choice between males 5/5a and 7/9, it was decided to use the songs from the day, when the male was first recorded, in the analysis. This means that there were only six different males for the analysis.

## 3.2. Qualitative classification

The figures were given consecutive numbers, beginning with male 1 (see Figure 2 and Appendices 3-9). Figures were given the same number even though they might show some variation in appearance and frequency. Appendices 10-17 show the order of all figures from the 20 songs of each male. These appendices might illustrate how stereotypic the songs are. A black box drawn around the recurring figures in all the songs shows what we call the "motif" of the song. This motif can be found in 95% of the songs i.e. in 19 out of 20 songs. The size of the box indicates the degree of stereotypy of the songs. The box of male 7 contains twelve figures (see Appendix 15), indicating high stereotypy, whereas the box of male 2 contains only four figures (see Appendix 11) indicating low stereotypy. The variation in the songs consists of either adding particular figures before or after this recurring motif, but primarily figures are added in the last part of the song. Between the 20 songs of each male, there are many similarities regarding the use of figure types, the recurring motifs and the sequences of the figures following this motif (see Appendices 10-17). We therefore conclude that all the males only have one song type each. Each male uses different motif sizes and

figures. Nevertheless, males 1, 3, 5 and 7 more or less all share the sequence of figures: "1, 2, 3, 1". Furthermore, all males share figure no. 3.

	Song duration (s)	Song output (s)	Minimum frequency (kHz)	Maximum frequency (kHz)
F <sub>max</sub> -test	9.66*	2.91*	8.53*	19.88

**Table 1.** Calculated  $F_{max}$ -values for the four parameters. The values marked with \* in the table show homogeneity of variance. The  $F_{max}$ -test is done on the square root transformation of song duration and on the natural logarithm transformation of minimum frequency.

# **3.3. Song duration**

It was found that there was an overall significant difference between the males (ANOVA:  $F_{5,114}=20.65$ , P<0.0001). Table 2 shows which males in pairs were found to be significantly different in The Multiple Range Test. The test showed that the mean song duration of males 1, 2, 3 and 5 was significantly different from the mean song duration of males 7 and 8. Furthermore, males 7 and 8 were also significantly different from each other. Figure 3 shows mean values of song duration for the six males. Furthermore, the standard error bars show the variation within each individual and they also indicate, which males are significantly different from each other. The standard error bars show the same results as The Multiple Range Test, except that males 1 and 3 seem different although they are not different statistically.

As we realised, that males 5 and 5a, as well as males 7 and 9, were the same individuals, it was possible to examine the difference in mean song duration between days. This is depicted in Figure 4a and 4b, for male 5 and male 7, respectively. We found that there was no significant difference between the days for male 5 (Mann-Whitney U-test: U=140, N<sub>1</sub>=N<sub>2</sub>=20, P=0.10) but there was a significant difference between the days for male 7 (Mann-Whitney U-test: U=40, N<sub>1</sub>=N<sub>2</sub>=20, P=0.00002). In Figure 4a and 4b, it seems that the mean song duration is longer on the first day of recording, than on the second day of recording for both males. The standard error bars indicate that there is a difference between days for both males.

# 3.4. Song output

Another measure of song duration is song output. It was found that there was a significant difference in mean song output between the males (ANOVA:  $F_{5,114}=25.77$ , P<0,0001). The Multiple Range Test showed that there was a statistically significant difference in song output between all males, except between males 1 and 3 and also males 5 and 7 (see Table 2). Figure 5 shows the mean song output for each male and the standard error bars show the same results as the Multiple Range Test.

# 3.5. Minimum frequency

It was found that there was a statistically significant difference in the mean minimum frequency between the males (ANOVA:  $F_{5,114}$ =10.89, P<0.0001). The Multiple Range Test showed that male 1 was significantly different from males 2, 3 and 5. Furthermore males 2, 3 and 5 were significantly different from male 7 and males 3, 5 and 7 were significantly

different from male 8 (see Table 2). Figure 6 shows the mean minimum frequencies for the six males and the standard error bars indicate the same results as The Multiple Range Test.

# **3.6. Maximum frequency**

It was found that there was a significant difference in the median maximum frequency between the males (Kruskal-Wallis: K=36.60, P<0.0001). Furthermore, the Kruskal-Wallis multiple comparison test showed that males 1, 2 and 5 were significantly different from male 7 and male 5 was also significantly different from males 3 and 8. Figure 7 shows a box-whiskers plot of the maximum frequencies from each male. The top and the bottom "whisker" show the largest and the smallest value (the extreme values), respectively. The upper- and the bottom line of the box represent the 75% and the 25% quartiles, respectively. The line in the middle of the box shows the median value. It seems that only data from males 1, 2 and 7 are relatively normally distributed, which supports our conclusion about the lack of normality. Males 5 and 8 seem to have extreme values that differ greatly from the other males and this contributes to the lack of normality of the entire data (see Figure 7). If the boxes do not overlap, it is likely that the males differ significantly different from males 2, 3 and 5. Furthermore, male 5 seems significantly different from males 2 and 3 as well as male 2 seems different from male 3.

Comparison	Song duration (s)	Song output (s)	Minimum	Maximum
of male no.			frequency (kHz)	frequency (kHz)
1 and 2		*	*	
1 and 3			*	
1 and 5		*	*	
1 and 7	*	*		* *
1 and 8	*	*		
2 and 3		*		
2 and 5		*		
2 and 7	*	*	*	* *
2 and 8	*	*		
3 and 5		*		*
3 and 7	*	*	*	
3 and 8	*	*	*	
5 and 7	*		*	* *
5 and 8	*	*	*	* *
7 and 8	*	*	*	

**Table 2.** Pair wise comparisons from the Fisher's LSD Multiple Range Test on Song duration, Song output and Minimum frequency. Results from the multiple comparison test, the Kruskal-Wallis non-parametric one-way ANOVA on the Maximum frequency.\* indicates that the level of significance is 95%. \*\* indicates that the level of significance is 99%.



**Figure 1.** A spectrogram of a recorded snow bunting song, here exemplified by male 8, song no. 17. The box shows a figure type, where the two separate parts of the figure always appear together. Note: The scale is smaller compared to the original spectrograms.



**Figure 2.** A spectrogram, showing a consecutive numbering of figures in song no. 12 of male 1. Note: The scale is smaller compared to the original spectrograms.

## 4. DISCUSSION 4.1. Qualitative classification

A study by ESPMARK (1995) on Svalbard demonstrated that the songs of individual males were highly stereotypic, particularly regarding size of figure repertoire and of song type repertoire. This concurs with our observations on Disko Island. It can be concluded, that each male has only one song type, which in part is defined by the individual specific recurring motif, but also by the figures following this motif. This shows the variation between the males. Nevertheless, the songs also show some similarities between the males regarding figure types and their sequences, for example figure no. 1, 2, and 3, which are shared by males 1, 3, 5 and 7. This indicates that there could be a microgeographical variation, because these males are situated relatively close to each other. Furthermore male 8, which is farthest away only shares figure no. 3 with the other males. Having one song type does not necessitate that every song is identical. The intra-individual variation consists of leaving out or adding figures to the songs (see Appendices 10-17). However, this variation does not seem to be particularly large compared to the degree of stereotypy.

The above results are all dependent on how strict the definitions of figures, motifs and song types are. ESPMARK (1981) argues, that the figure types used in his study of the redwing were not unequivocal, but could vary in frequency and duration. This generalisation can be necessary, he argues, since the same figure type can vary in appearance on the spectrograms, due to variation in the volume of the song. A different definition of the figures may change the degree of inter-individual variation and to some extent also the intra-individual variation. If our definitions had been more detailed, regarding, for example, variation in pitch, the degree of inter- and intra-individual variation could have been even larger. Nevertheless, our general classification of figure types showed a significant difference between some males in all four parameters, which may indicate that a more detailed definition is not necessary for

song-based discrimination between individuals. This distinct visual difference between the males may also be apparent in the four parameters measured.

# 4.2. Song duration

To show the existence of microgeographical variation, we would expect that for instance male 8 would be different from the remainder of the males, because this male's territory is about 1.7 km away from the territories of the five other males. The Multiple Range Test showed a significant difference between male 8 and the other males, indicating that microgeographical variation might exist in this population. Males 1, 2 and 3 have adjoining territories within about 50 meters and therefore, we would expect only a small variation among them, if microgeographical variation can be seen in song duration. This is supported by the Multiple Range Test, where it was found that there was not a significant difference between these three males. Males 5 and 7 have territories that are situated relatively close to males 1, 2 and 3, hence we did not expect any of these males to be significantly different from each other. Despite this fact, male 7 was statistically significant different from the other males. Therefore, we cannot state that this parameter shows microgeographical variation. If the males are not recorded on the same day, then the comparison between the males may be complicated by variation in song duration between days. This variation in mean song duration between days was found in male 7 (see Figure 4b). However, we did not find any significant difference between the days for male 5, even though it seems as if there is a difference when looking at the standard error bars on Figure 4a. The lack of normality of the data can influence the reliability of the standard error bars and thus show a difference, that the statistical test does not support. Hence, the comparisons between males 1, 2 and 3 may be more reliable because they were recorded on the same day. This variation between days may be due to differences in motivation caused by weather conditions for example temperature differences (TINBERGEN, 1939), presence of other birds, the stage of the breeding cycle, etc. However, it may also be caused by physiological, genetic or morphological variation.

To avoid recording the same male several times, it is ideal to record one visible male at a time and stop recording, if the male moves out of sight (MUNDIGER, 1975). However, this method must be balanced by the need to collect sufficient recordings for later analysis (e.g. a minimum of 10 songs (ESPMARK, 1995)). Due to the period of the breeding season, only some males were singing, while we made the recordings, thus it was not possible to record as many males as we would have preferred. Furthermore, we chose to record any male even if we had been in the same area previously on a different day, to ensure that we would have a fair amount of data to analyse.

# 4.3. Song output

Song output gives a measure of the amount of actual singing pr. song but does not necessarily correlate with the song duration. This means that a song with a long duration does not have to contain many figures, but can contain long pauses between the figures. Thus, we compared the variation in song output between individuals and then determined, whether it showed similar levels of variation to that for song duration. We found considerable variation in song output between individuals. However, this variation was distributed differently to that of song duration, following our contention that the two measures were not correlated (see Table 2). For example, the mean song duration of male 7 is significantly longer and the song contains more figures than that of male 5 (see Figure 3 and Appendices 13 and 15), although they do not differ in song output. This may indicate that the songs of males 5 and 7 potentially contain the same information. On the contrary, males 1 and 2 do not show a significant difference in song duration but they differ in song output, i.e. male 1 has a greater song output than male 2



Figure 3. Mean song duration for males 1, 2, 3, 5, 7 and 8. Error bars indicate S.E. The figure is based on non-transformed data

(see Figure 5). This could indicate that the song of male 1 possibly contains more information than the song of male 2.

There is an agreement of the statistical outcome between song output and song duration in 9 out of 15 cases. Therefore, a correlation analysis could illustrate, whether the two parameters are dependent on each other or not. It could also have been interesting to correlate song duration and song output with the behavioural observations, in order to examine the function of the song. POESEL et al. (2001) suggest, that song output in the blue tit (*Parus caeruleus*) provides information about male quality, as males with a higher song output were paired to females that started laying earlier and laid larger clutches. It could be interesting to examine, if this is also the case for the snow bunting, among other things because it has been found that the song length (i.e. song duration) was positively correlated with number of fledglings for the snow bunting (HOFSTAD et al., 2002).

# 4.4. Minimum and maximum frequencies

The minimum and maximum frequencies do not show any signs of microgeographical variation because it seems arbitrary, which individual males are different from each other. Furthermore, the parameters do not indicate a great extent of inter-individual variation. The maximum frequency shows even less significant differences than the minimum frequency. The small inter-individual variation between the males may be caused by a physical constraint that limits the possible frequency range, within which the snow bunting, as a species, can sing. The box-whiskers plot of the maximum frequencies shows, that there are some extreme values in this data. To avoid the skewness of the data, we could have used the mean maximum frequency calculated from all the figures in a song. This would act to eliminate some of the extreme values. Differences in minimum frequency were not necessarily accompanied by differences in maximum frequency (see Table 2). Therefore, we can infer that both parameters ought to be analysed, if the inter-individual differences are to be illustrated. Nevertheless, it should be noted that the differences in the results could be due to



Figure 4a. Mean song duration for male 5 on two different days. Error bars indicate S.E. The figure is based on non-transformed data.



Figure 4b. Mean song duration for male 7 on two different days. Error bars indicate S.E. The figure is based on non-transformed data.

the use of different statistical tests. If the data had been normally distributed, then the Multiple Range Test could have shown more significant differences between the males. The minimum and maximum frequencies may not be correctly measured in the Avisoft program, since the values are dependent on a threshold, which has been set by us. Furthermore, there was some background noise, which had not been filtered out and this distorted the measurements. Especially the total minimum frequency (measured at a Hold time of 0.35 sec) was often estimated too low. We therefore tried to eliminate this measurement error by choosing the lowest and the highest frequency occurring in the figures of a song (measured at a Hold time of 0.022 sec). On the other hand, this method might obscure the objectivity of


Figure 5. Mean song output for males 1, 2, 3, 5, 7 and 8. Error bars indicate S.E.



Figure 6. Mean minimum frequency for males 1, 2, 3, 5, 7 and 8. Error bars indicate S.E. The figure is based on non-transformed data.

these two parameters. Another method of measuring frequency could be to use 25%, 50% and 75% quartile parameters, which show the distribution of energy across the frequency spectrum (AVISOFT). These parameters are not dependent on the selected threshold and may therefore be more sufficient in showing the distribution of frequencies in a song.

The snow buntings have been mapped in previous studies from the Arctic Station, for instance in Blæsedalen (FINKE & BRANDT, 1999). By combining the registered locations with recordings of male songs, it would be possible to obtain a more exact estimate of the location of the territories and the size of the population around the Arctic Station, than by traditional mapping methods. As we have found, it is relatively easy to recognise individual males by their song. Furthermore, only 10 to 20 songs are necessary to identify a male because of the large degree of stereotypy in the songs. These two mapping methods, in combination, can be optimally used for conservation purposes. It would be optimal to record the snow buntings in their peak song period, but since the males do not cease singing after mating, it is still possible to record songs until the middle of July, when the Arctic Field Course takes place. Since a limited number of males were recorded, it is difficult to make general conclusions about a possible microgeographical variation around the Arctic Station. HOFSTAD et al. (2002) also conclude, that a short study period and a relatively small sample size may make it less likely to discover small, but significant biological differences.



Figure 7. A box-whiskers plot, showing the maximum frequencies for males 1, 2, 3, 5, 7 and 8. The top and the bottom "whisker" show the largest and the smallest value (the extreme values), respectively. The upper- and the bottom line of the box represent the 75% and the 25% quartiles, respectively. The line in the middle of the box shows the median value.

#### **5. CONCLUSION**

As we expected, the snow buntings had only one song type each and a very stereotypic song. The intra-individual variation consisted of adding figures or leaving out expected figures. This variation was considered minor, compared to the high degree of stereotypy, shown through the fixed motif of the song.

Following previous studies, we would expect to find a great inter-individual variation. Both the qualitative classification and the four quantitative parameters showed a distinct interindividual variation between the six males. We found that song output was the parameter,

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which was best in showing this variation. On the contrary, the maximum frequency was the least optimal parameter in showing inter-individual variation.

None of the four quantitative parameters could support an occurrence of microgeographical variation. Nevertheless, the type and sequence of figures, which were shared by males 1, 3, 5 and 7, may indicate a microgeographical variation.

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Date, 2002	Snow	L	Description of locations	GPS position	UTM
	bunting	oc			position
	male	at			-
		io			
		n			
9 <sup>th</sup> of July		1	A rocky plateau by the flagpole behind Arctic Station. Here the three snow bunting males were	N 69° 15,228 min.	22 400506
10 <sup>th</sup> of July			observed within a rather small area. The nest belonging to male 3 was identified.	W 53° 31,045 min.	W 7684710
9 <sup>th</sup> of July	1	1a	See location 1. A few meters from location 1.	N 69° 15,232 min.	22 400497
	1.2.3			W 53° 32,062 min	W 7684721
9 <sup>th</sup> of July,	, , -	1	A plateau below location 1 and 1a. From this location male 2 was observed.	N 69° 15,212 min.	22 400496
10 <sup>th</sup> of July		b		W 53° 31,049 min.	W 7684682
11 <sup>th</sup> of July	Fledglings	2	Near a water pipeline. Several fledglings and adults were recorded at random without observing	N 69° 15,402 min.	22 399844
,	adults	*	behaviour. The birds recorded were at a rocky area close to the cliff.	W 53° 32,064 min.	W 7685076
11 <sup>th</sup> of July	4	3	North-Northwest of location 2. Male 4 was observed from the foot of a cliff side.	N 69° 15,563 min.	22 399800
		-		W 53° 32,149 min.	W 7685383
11 <sup>th</sup> of July	4	3a	Twelve meters above location 3. It was presumably the same male as recorded at location 3.	N 69° 15,581 min.	22 399780
			Because we were not able to recognise any feather markings, we can only presume it is the same male, since it was seen in the same area as the one observed from location 3.	W 53° 32,189 min.	W 7685396
11 <sup>th</sup> of July	5*	4	At the top of the cliff, above locations 3 and 3a. From here male no. 5* was recorded, but the male was out of sight the entire recording period.	Not registered	Not registered
12 <sup>th</sup> of July	5 5a	5	Behind the house of the scientific leader. A small, flat area bordered by a low cliff side, where	N 69° 15,203 min.	22 400387
15 <sup>th</sup> of July	0, 0 <b>u</b>		male 5 was observed.	W 53° 31,208 min.	W 7684666
12 <sup>th</sup> of July	6.7	6	West of the "Stone yard" North of Arctic Station. Males 6 and 7 were recorded and observed on	N 69° 15,293 min.	22 400351
,	.,		the cliff side.	W 53° 31,287 min.	W 7684833
13 <sup>th</sup> of July	8	7	At the waste disposal site south of Qeqertarsuaq town. A rocky area surrounding a wet grass area.	N 69° 14,370 min.	22 399536
15 of tary	Ũ	ĺ	The two locations are situated within about 10 meters. Males 8 and 8a were observed on a rocky plateau.	W 53° 32,420 min.	W 7683148
16 <sup>th</sup> of July	8a	7a	See location 7.	N 69° 14,388 min.	22 399540
				W 53° 32,415 min.	W 7683184
16 <sup>th</sup> of July		8	East of the "Stone yard". Male 9 was observed and recorded both sitting and flying in the area.	N 69° 15,293 min.	22 400425
	9			W 53° 31,175 min.	W 7684826
16 <sup>th</sup> of July	9	8a	See location 8. The two locations are situated within a few meters.	N 69° 15,296 min.	22 400434
				W 53° 31,154 min.	W 7684847

#### Locations and recorded males.

Note: \* means the male is not visible from the place of recording and observing. Location 3a, 7a and 8a indicate that the male in question is recorded close to location 3, 7 and 8. Males 5a and 8a indicate that we have recorded a male on a different day but at the same location as males 5 and 8. Since we were not able to identify these individuals by feather markings, we were not certain, if it was the same male we recorded on both dates. Locations 2-4. A part of Lyngmarken: An open area surrounded by cliffs.



Spectrograms showing numbers of figure types in songs of males 1 and 2.



Spectrograms showing numbers of figure types in songs of male 3.



Spectrograms showing numbers of figure types in songs of male 5.



## Appendix 6

Spectrograms showing numbers of figure types in songs of male 5a.



Spectrograms showing numbers of figure types in songs of male 5a and 7



## Appendix 8

Spectrograms showing numbers of figure types in songs of male 8



Spectrograms showing numbers of figure types in songs of male 9

Male 1	Location	1 T	he 9th	of July	2002					
Song no.	Figure no									
3	1	2	3	1	4	5	6	7		
5	1	2	3	1	4	5	6	7		
6	1	2	3	1	4	5	6	7		
7	1	2	3	1	4	5	6	7	2	
8	1	2	3	1	4	5	6	7		
9	1	2	3	1	4	5	6	7	2	
10	1	2	3	1	4	5	6	7	2	
11	1	2	3	1	4	5	6	7	2	3
12	1	2	3	1	4	5	6	7	2	
13	1	2	3	1	4	5	6	7	2	
14	1	2	3	1	4	5	6	7	2	
15	1	2	3	1	4	5	6	7		
16	1	2	3	1	4	5	6	7		
17	1	2	3	1	4	5	6	7		
18	1	2	3	1	4	5	6	7		
19	1	2	3	1	4	5	6	7		
20	1	2	3	1	4	5				
23	1	2	3	1	4	5	6	7		
24	1	2	3	1	4	5	6	7		
25	1	2	3	1	4	5	6	7		

Order of figure types from the 20 songs of male 1

## Appendix 11

Male 2	Locatior	1 1,b	т	ne 9th o	of July	2002						
Song no.	Figure no	<b>D</b> .										
6		Г	8	9	3	10	11	12	13	10	8	9
7			8	9	3	10	11	12	13	10		
8			8	9	3	10	11	12	13	10		
9			8	9		10	11	12	13	10		
10			8	9	3	10	11	12	13	10		
11			8	9	3	10	11	12	13			
12			8	9	3	10	11	12				
13			8	9	3	10						
14	8	9	8	9	3	10	11	12				
15	14	14	8	9	3	10	11	12	13	10	8	9
16	14	14	8	9	3	10	11	12	13	10	8	9
17			8	9	3	10	11	12	13	10		
18			8	9	3	10	11	12	13	10	8	
20			8	9	3	10	11	12	13	10	8	
21			8	9	3	10	11	8	9	3		
22			8	9	3	10	11	12	13	10		
23			8	9	3	10	11	12	13	10		
24			8	9	3	10	11	12	8	9	3	
25			8	9	3	10	11	12	13	10	8	9
26			8	9	3	10	11	12	13	10	8	9

Male 3	Location 1		The 9th o	f July	2002						
Song no.	Figure no.										
11	15	1	2	3	1	15	16	17	3	18	19
12	15	1	2	3	1	15	16	17			
13	15	1	2	3	1	15	16	17	3		
14	15	1	2	3	1	15	16	17	3		
15	15	1	2	3	1	15	16	17	3		
16	15	1	2	3	1	15	16	17	3		
17	15	1	2	3	1	15	16	17	3		
18	15	1	2	3	1	15	16	17			
19	15	1	2	3	1						
21	15	1	2	3	1	15	16	17	3		
22	15	1	2	3	1	15	16	17	3	18	19
23	15	1	2	3	1	15	16				
24	15	1	2	3	1	15	16				
25	15	1	2	3	1	15	16				
26	15	1	2	3	1	15	16				
27	15	1	2	3	1	15	16	17			
29	15	1	2	3	3	1					
30	15	1	2	3	3	1					
31	15	1	2	3	1	15	16	17	3	1	
34	15	1	2	3	1	15	16	17	3		

Order of figure types from the 20 songs of male 3

## Appendix 13

Male 5	Locati	on 5	The 12tl	h of July	2002								
Song no.	Figure	no.											
1		20	20	3	21	22	2						
3		20	20	3	21	22	2	3	1	20			
4		20	20	3	21	22	2	3	1	4	20		
5		20	20	3	21	22	2	3	1				
6		20	20	3	21	2							
7		20	20	3	21	22	2	3	1	2	3	1	
8		20	20	3	21	22	2	3	1	4	20	1	
9		20	20	3	21								
10		20	20	3	21	22	2	3	1				
11		20	20	3	21	22	2	3	1				
12		20	20	3	21	22	2	3	1	4			
13		20	20	3	21	22	2	3	1	4	20	1	
14		20	20	3	21	20	1	2					
15		20	20	3	21	22	2	3	18	19			
16		20	20	3	21	22	2	3	1	2	3	1	38
17	14	20	20	3	21	20	10	11	12	4			
18		20	20	3	21	1	2	3	1	20	10	11	12
19		20	20	3	21	22	2	3	1	4	20	1	2
20		20	20	3	21	22	2	3					
21		20	20	3	21	20	10	11	12	4			

Male 5a	Location	15 T	he 15th	n of Jul	y 2002				
Song no.	Figure no	<b>D</b> .							
1	20	20	3	21	22	2	3		
2	20	20	3	21	22	2	3	1	
3	20	20	3	21	22	2	20	1	2
4	20	20	3	21	22	2	3	1	
5	20	20	3	21	22	2	20	10	
6	20	20	3	21	22	2	3	1	4
7	20	20	3	21	22	2	3	1	
8	20	20	3	21	22	2	3	1	
11	20	20	3	21	22	2	3	1	
12	20	20	3	21	22	2	1		
13	20	20	3	21	22	2			
14	20	20	3	21	22	2	3	1	
15	20	20	3	21	22	2	20		
16	20	20	3	21	22	2	3	1	
17	20	20	3	21	22	2	20	1	
18	20	20	3	21	22	2	20		
19	20	20	3	21	1	2			
20	20	20	3	21	22	2	20	1	
21	20	20	3	21	22	2			
22	20	20	3	21	22	2	3	1	20

Order of figure types from the 20 songs of male 5a

## Appendix 15

Male 7	Locatio	n 6	The 12t	h of Jul	y 2002																
Song no.	Figure n	0.																			
1			20	23	24	25	3	1	26	27	17	28	29	30	24	20	23	24			
2	23	24	20	23	24	25	3	1	26	27	17	28	29	30	24						
3			20	23	24	25	3	1	26	27	17	28	29	30	24						
4			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3				
5			20	23	24	25	3	1	26	27	17	28	29	30	24	20	23	24			
6			20	23	24	25	3	1	26	27	17	28	29	30	24	25					
7				23	24	25	3	1	26	27	17	28	29	30	24	25	3	1	20	23	24
8			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3	1			
9			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3				
10			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3	1			
11			20	23	24	25	3	1	26	27	17	28	29	30	24	20	23	24			
12			20	23	24	25	3	1	26	27	17	28	29	30	24	25					
13			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3	1			
14			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3	1			
15			20	23	24	25	3	1	26	27	17	28	29	30	24	25					
16			20	23	24	25	3	1	26	27	17	28	29	30	24	25	3	1			
17	23	24	20	23	24	25	3	1	26	27	17	28	29	30	24	25					
18			20	23	24	25	3	1	26	27	17	28	29	30	24	25					
19				23	24	25	3	1	26	27	17	28	29	30	24	25	3	1	20	23	24
20			20	23	24	25	3	1	26	27	17	28	29	30	24	25					

Male 8	Location	n 7 1	The 13	h of Jul	y 2002																
Song no.	Figure n	0.																			
1			31	32	3	33	34	35	36	37	32	3	33	34	35	36	37	32	3	33	34
2			31	32	3	33	34	35	36	37	32	3	33	34							
3			31	32	3	33	34	35	36	37	32	3	33	34	35	36					
4			31	32	3	33	34	35	36	37	32	3	33	34	35						
5			31	32	3	33	34	35	36	37	32	3	33	34	35	36					
6	31	32	31	32	3	33	34	35	36	37	32	3	33	34	35	36					
7			31	32	3	33	34	35	36	37	32	3	33	34	35	36					
8				32	3	33	34	35	36	37	32	3	33	34	35	36					
9			31	32	3	33	34	35	36	37	32	3	33	34	14						
10			31	32	3	33	34	35	36	37	32	3	33	34	35	36					
11			31	32	3	33	34	35	36	37	32	3	33	34	35						
12			31	32	3	33	34	35	36	37	32	3	33	34	35	36	14	6	38		
13			31	32	3	33	34	35	36	37	32	3	33	34	35	36	14	6	38		
14		~ .	31	32	3	33	34	35	36	37											
15		31	39	32	3	33	34	35	36	37	32	3	33	34							
16			31	32	3	33	34	35	36	37	32	3	33	34							
17			31	32	3	33	34	35	36	37	32	3	33								
18			31	32	3	33	34	35	36	37	32	3	33	34	14	6					
19			31	32	3	33	34	35	36	37	32	3	33	34	35	36					
21			31	32	3	33	34	35	36	37	32	3	- 33	34	35	36					

Order of figure types from the 20 songs of male 8

## Appendix 17

Male 9	Location	8 Т	he 16th	of Ju	ly 2002																
Song no.	Figure no																				
1		20	23	24	25	3	1	26	27	17											
2					25	3	1	26	27	17	28	29	30	24	25	3	1	20	23	24	25
3		20	23	24	25	3	1	26	27	17	28	29									
4		20	23	24	25	3	1	26	27	17											
5	25	20	23	24	25	3	1														
6		20	23	24	25	3	1	26	27	17											
7		20	23	24	25	3	1	26	27	17											
8		20	23	24	25	3	1	26	27	17	28	29	23	24	20	23	24				
9		20	23	24	25	3	1	26	27	17											
10		20	23	24	25	3	1	26	27	17											
11		20	23	24	25	3	1	26	27	17	28	29									
12		20	23	24	25	3	1	26	27	17											
13		20	23	24	25	3	1	26	27	17											
14		20	23	24	25	3	1	26	27	17											
15			23	24	25	3	1	26	27	17											
16		20	23	24	25	3	1	26	27	17											
17		20	23	24	25	3	1	26	27	17											
18		20	23	24	25	3	1	26	27	17	28	29									
19		20	23	24	25	3	1	26	27	17	20	23	24	20							
20		20	23	24	25	3	1	26	27	17											

## **Diary of the Arctic Biology Field Course 2002**

Morten SMITH & Steen Wilhelm KNUDSEN

Department of Phycology, Botanical Institute, University of Copenhagen Department of Invertebrate Zoology, Zoological Museum of Copenhagen, Copenhagen, Denmark

#### Wednesday July 3<sup>rd</sup>

Expectations where high when the group of 12 students and 4 supervisors met in Kastrup. We met early in order to avoid any problems with check-in of the group and the two heavy microscopes. No problems occurred and we took off from Copenhagen at 1030.

Our flight took us over Norway, North of the Faroe Islands and eventually North of Iceland. Since our party was seated on the port side of the plane we could see the North coast of Iceland in the distance -magnificent. As we came closer to Greenland the "great ice" became more and more dense and keeping in mind that we were cruising in about 10 km above sea level these icebergs must have been immense. The nunataks of Greenland's east coast also astonished us with their black rock in contrast to glacier ice, especially since the sun was shining from a clear sky. The huge inland glacier was not quite as big a sight to see -total white-out. We also got a look at the fjords of Western Greenland from the plane and here it is worth mentioning the emerald green melt water puddles on the glaciers. They were truly beautiful.

We landed safely in Kangerlussuaq at 1100 local time. With 2 hrs to kill before our DASH-7 connection to Ilulissat took off, we all went sightseeing. We toured the town and its immediate surroundings in small groups. After that, we met in the airport cafeteria for lunch and getting to know each other better.

Another safe landing in Ilulissat completed the air travel for the day. In Ilulissat we were first and foremost greeted by clouds of mosquitoes who also found Greenland a great place to spend the summer. Most of us saw no point in avoiding them, since we figured that we had better get used to them. So the mosquito nets were brought forward by those fortunate enough to have put them in their hand luggage. Nevertheless practically all of us got bitten -if there is a hole they'll find it! We checked in at the youth hostel for the night before leaving for Disko the next day. Most of the group had dinner at a restaurant before hiking to the Ilulissat Isbræ. The combination of perfect weather and a stunning view resulted in a hike which will never fade from our minds -it was unbelievably beautiful! As our hunger for adventure momentarily had become satisfied, most of us were beginning to feel more than a bit tired as it had been a long and eventful day.

#### Thursday July 4<sup>th</sup>

Breakfast at the youth hostel at 0800 and then a 7 km ride by taxi to the airport. There was no need to hurry, since the weather did not allow helicopters to fly. So we were delayed almost 3hrs. This was a reminder of how much the weather is in control up here for those of us who had forgotten. Then suddenly the sky cleared up a bit and a helicopter took off with the first group. We arrived at Qeqertarsuaq half an hour later after having a great ride and a great view of Disko from the helicopter. Frantz (the technical leader) was at the heliport to pick us up, and to drive our gear to Arctic Station. Here Barat, our kifaq (cooking and cleaning master) for the duration of our stay greeted us. The helicopter flights were still unstable and the rest of the group came

piece-meal for the rest of the afternoon, while the rest of us moved into our rooms. Dinner that night was quite an experience - whale steaks. Most of us had not tasted it before since it is virtually impossible to get anywhere else but in Greenland. After dinner we unpacked our scientific gear and got settled into the different laboratories. After that, most of us went to bed.

### Friday July 5<sup>th</sup>

P-day. First trip with the research vessel of Arctic Station: M/S Porsild, but the weather did not look too friendly. The sky was overcast and the visibility was bad. Fortunately, this did not last throughout the day. Porsild was scheduled to head out at 1230 because Reinhardt (the supervisor of the zoologists) was to meet with the mayor. This allowed the tantalocorids (Maja & Steen), the protists (Knud, Lone & Morten) and the tardigrades (Jesper & Agnete) incl. their supervisors (Reinhardt & Niels) and our guest Bjørn the geologist, to pack and prepare all morning. At 1300 we headed out onboard "Porsild" bound for Ippiq and at the same time the sun broke through the clouds and shined on us for the duration of the trip. The protists took a sample just off Qegertarsuag but they had some trouble with the CTD probe, which they thought they overcame. After sampling, we enjoyed the weather and the coastline until we came to the muddy seabed at Ippiq. Another sample was taken by the protists but this time the CTD probe was not cooperating at all! An alternate sampling method was used, and the sampling was completed. Then it was the zoologist's turn to sample and they hauled kilo after kilo of foul smelling mud aboard, and no problems were encountered, except maybe for the yawing created by the strong wind and waves. It was quite a bumpy ride home, but nobody got seasick. When we arrived at the harbour, Poul's seaweed group (Berit, Maiken & Anja and to some extent her son Alf) was ready to head out to collect samples.



A view of the coastline, on the way to Ippiq. Photo by M. Smith

While Porsild was out at sea, Anne Marie & Linda, working on their bird project, were trying to record the territorial singing of the Redpoll (*Carduelis flammea*) but they could not get a proper recording, and were therefore pretty frustrated. However, they decided to give it a few days more. We got home just in time for dinner -nice timing. Afterwards there was a lot of laboratory work with the samples. Maja & Steen were busy with their stereomicroscopes and the protists were supposed to centrifuge their samples. However, the centrifuge had a will of its own. It kept shattering the tubes so Knud hauled it into the workshop and gave the lid a good going over with a grinder. And then they were back in business. For their part it was a long night centrifuging and doing microscopy. The seaweed people also spent the night to sort out their material. We could not deny ourselves the joy of seeing the warm reddish rays of the midnight sun colouring the icebergs in the bay.

### Saturday July 6<sup>th</sup>

This day started like Friday but sadly there was no sunny weather in store for us. A shame the weather was not any better because Reinhardt was planning on giving us a guided tour of the town. He spent all morning telling us about the history, demography and culture. He also showed us the sights to see. As you can imagine it was very interesting but it just cannot be described properly here -watching is believing! We went back to the station for lunch and after that: Work. After dinner Bjørn gave a talk about Disko Island's geological history and birth. This was also a comprehensive presentation of the rocks one could find on the island. Later that night Reinhardt was serving chocolate horns and there was much rejoicing. But roses have thorns: One of them was without chocolate and the lucky owner was to be next in line to serve afternoon/night goodies. Poul was the winner! On this night we also got acquainted with the beer on Greenland at 15 DKK a bottle it was expensive but nevertheless a necessity.

## Sunday July 7<sup>th</sup>

This morning we were in for a cultural experience because summer is the time for first communion in Greenland. So some of us walked to the church hoping to see the Inuits in their traditional garments. We were not disappointed. Some of the women wore their colourful outfits and the men in their white anoraks - quite a spectacular sight. Most of us had a lot of work to do before going to Mellemfjord on Monday so we returned to the Station. Only Bjørn, Reinhardt and Majken attended the actual ceremony.

A walk to Kuanit was scheduled after lunch. Kuanit means "place of the kvan" (*Angelica archangelica*). During the walk Bjørn and Reinhardt stopped a lot and explained the geological and biological curiosities we encountered. On the way there we met Marianne Philip, a botanist from the University of Copenhagen who told us about her pollination examinations of moss campion (*Silene acaulis*). So what was thought to be a relatively short walk ended up taking more than 3 hrs. For the duration of the hike we were surrounded by thick fog, which made the landscape appear as were we walking in a Tolkien novel. After dinner Sunday night we worked a little and then we got together in the living room on the station for a beer.

### Monday July 8<sup>th</sup>

Clear blue sky and  $10^{\circ}$  C - perfect weather for the botanists' two-day trip to Mellemfjord. The weather only increased their excitement and they were pretty silly to be around.

The bird girls had now completely abandoned the Redpolls (*Carduelis flammea*) since they could not get a decent recording. Fortunately the Snow buntings (*Plectrophenax nivalis*) were still

singing and they were quite abundant around the station. So they were selected as a new subject for their project.

Since no work was scheduled on the way to Mellemfjord we just enjoyed the sun and scenery. Some were relaxing on the deck, especially Lone who were sleepy from her seasickness medication. Others were intensively looking for the seabirds (Knud & Morten). This resulted in the preliminary score of:

Mammals: None, sadly!

Birds:

Eider (Somateria mollissima), King eider (Somenteria spectabilis), Fulmar (Fulmarus glacialis), Iceland gull (Larus glaucoides), Glaucous gull (Larus hyperboreus), Cormorant (Phalacrocorax carbo), Arctic skua (Stercorarius paraciticus) & Black guillemot (Cepphys grylle)

Savouring the impressions of the day we came to Mellemfjord where both group took samples. The protists were sampling from Porsild and the seaweed girls from the dinghy. As there where only 4 bunks onboard Porsild we had to camp on a plain just beyond the beach. However, before camping, we enjoyed the dinner that the girls had made - well reheated. After dinner we were shipped to the beach with everything needed for an overnight stay. The courteous mosquitoes made it painfully clear to us that they were thrilled to have us visiting. A camp was established and the evening coffee was made. With these duties aside we enjoyed our coffee, biscuits and chocolate in the polar summer night.

## Tuesday July 9<sup>th</sup>

In the morning Knud & Morten bitterly regretted the fact that they had chosen to sleep like real men (I.e. with no inner tent). This, of course, was of much amusement to the rest of the group. We were picked up by Lars at 0800 and brought back to the ship where we had breakfast. Then the course was set for Arctic Station and on the way home the protists had to take a lot of samples. This was, however, made difficult by the fact that the CTD-probe finally chose to stop working at all. Much to our dismay, we found that this was due to a faulty cable. This was indeed more problematic than the initial notion of worn out batteries. So now the plankton net was used as a Secchi disc. This concluded our lesson nr. 1 in arctic science: Adapt, improvise and overcome! Between the sampling stations we could also add a couple of birds to yesterdays record: Puffin (*Fratercula arctica*) and Brünnich's guillemot (*Uria lomvia*).

At the station it was business as usual; work in front of the microscopes. Maja and Steen had difficulties extracting their crustaceans so Reinhardt brought them some tadpole-shrimps (*Lepidurus arcticus*) and fairy shrimps (*Branchinecta paludosa*) for them to draw. The botanists arrived back at the station just in time for dinner and then afterwards they went to the laboratory building for another late night.

## Wednesday July 10<sup>th</sup>

We got up to a wet and cold morning (4°C). The botanists had a lot of work to do on their collected samples. The protists in particular had a long day ahead of them with 6x2 samples to centrifuge equalling 13 hrs. Steen and Maja had been through their first samples and were looking forward to their trip to Mudderbugten at Thursday.



Inuits in their traditional garments. Photo by M. Smith



Hikers resting in the mystery fog of Kuanit. Photo by M. Smith

### Thursday July 11<sup>th</sup> and Friday July 12<sup>th</sup>

With a 2-day trip to Mudderbugten expectations were running run high. The tardigrade group (Jesper and Agnete), the tantalocorids (Steen and Maja), Bjørn, Reinhardt and the lonely botanist Morten were on the passenger list. Originally all of the protists should have accompanied him but since they were not done with the centrifuging straws had to be drawn. We sailed out in cold weather but reasonably good visibility. The travel time was a good 8 hrs and some of us took advantage of this to prepare themselves for the coming night's hike to the Lymnaea Lake by sleeping. The objective of this trip was to collect tardigrades at Isunguaq, collect the snail (*Lymnaea vahlii*) in the lake and on the way home to collect water samples for the protists as well as some mud for Maja and Steen hopefully containing their tantalocorids.

We arrived at Mudderbugten at around 1800 and sailed ashore in the Zodiac. Our dinner was prepared near an old hunter's hut but soon this became our hut since the mosquitoes were more than plentiful. The downside to this was that the hut, together with the trash outside, smelled of rotten fish and the mosquitoes appeared to be as disgusted with the smell as we were and stayed outside. We did not! We actually preferred this sickening dump to the mosquitoes -there were that many. We started out at 2000 with a 30+ kilometres to hike before we were to be picked up at 1100 the next morning. The idea of walking in the night was to avoid the mosquitoes, since the relative coldness of the night would make them less active. This does not imply that there were no mosquitoes at all, but Reinhardt postulated that it would be impossible to walk at day. We were tempted to agree with him. We all happy and merry strode along and we all enjoy the different landscapes. We crossed two rivers, which were hardly rivers more like a stream because we could cross it in wellies without getting too wet. About half way to the lake we saw and heard a blue fox (Alopex lagopus) in the distance. After approx. 4 hrs (i.e. around midnight) of walking we were supposed to be close to the lake and we started looking for it. But if Reinhardt had not remembered that the lake received water from 3 warm springs we would have walked right by it. After localizing the lake we set up a mini camp to make some well deserved coffee meanwhile Reinhardt was running berserk at the lake collecting the snails and shouting about the pintails (Anas acuta) and the Red-necked phalaropes (Phalaropus lobatus) and their chickens. After bird watching we enjoyed the best half a cup of coffee of our lives. After the rest we returned to our tent camp by a 150 years old reindeer track (the last native reindeer was shot 150 years ago), which actually was quite distinct. The track was an improvement over the cross-country hike so our speed soared considerably and we almost ran home to the camp. On our way we saw a number of Ptarmigans (Lagopus mutus) and a dead Gyr falcon (Falco rusticulus). The running came to a stop when we were to cross the two rivers again. After the crossing we were all pretty tired but rushed forward by the thought of the we've-done-very-well-whiskey Reinhardt had promised upon return. We returned to the camp at 0600 and Steen was man of the day when he brought a small iceberg from the beach. We enjoyed the whiskey and spent a cosy hour before going to bed at around 0700.

As we were to be picked up at 1200 we rose at 1100 and started packing the camp. The weather had deteriorated while we slept and the wind had picked up speed. When we got to the beach Lars was already on his way in the Zodiac to pick us up and after several round trips and in spite of the rough weather we all were brought safely aboard. Everybody except Reinhardt and Morten slept the whole way home but Agnete had her finest hour when she reheated Maja's meat sauce for lunch. On the way home Morten and Bjørn collected water samples and Maja and Steen

collected their mud samples at Ippiq. We returned to the station at approx 2030 wanting only: food, shower and sleep -and lots of the latter.

### Saturday July 13<sup>th</sup>

The expedition crew were allowed to sleep in but were most unpleasantly awoken by Knud, Lone & Anja banging pots and pans. To their defence they had prepared a nice brunch for us -lovely. The rest of the day was spent on the collected material.

### Sunday July 14<sup>th</sup>

The day started with an early breakfast because of the planned trip hike to Engelskmandens Havn. The weather was not as good as it could have been for a hike: light rain and 4°C. Everybody including Marianne's botanists was going but a few whose bodies were still marked after the Lymnaea trip stayed home. It was a marvellous trip and Marianne showcased the Greenland flora including a couple of orchids. Engelskmandens Havn is an area heavily influenced by the 5 homothermal springs running there. The springs are the reason why the flora and fauna here are much more "southern". Upon return work on the projects continued. After dinner Bjørn gave a talk on his work with stable isotopes of oxygen and the origin of water in the springs and sea of Greenland. After the talk the seaweed group started to prepare for their trip tomorrow and so did the protists. The packing was interrupted when Agnete spotted the water blast from an exhaling whale. It is probably not worth mentioning that everybody was on the porch in an instant with binoculars trying to spot it too. Most of us did see it and the experts deemed it a finback (*Balaenoptera physalis*) and this is done on the shape of the water blast and the small triangular fin.

### Monday July 15<sup>th</sup>

Fine weather and light winds perfect setting for the botanists trip to Kitsigsut (Kronprinsens Eilande), a group of islands just south of Qeqertarsuaq: Along with them went two of French/Canadian cormorant (*Phalacrocorax carbo*) research team who had finally returned from Qeqertaq. We expected to see a lot of seabirds since these islands are a refuge for these. Our expectations were fulfilled and on the way to the islands we saw:

Birds: Eider (*Somateria mollissima*), Fulmar (*Fulmarus glacialis*), & Black guillemot (*Cepphys grylle*), Little auk (*Alle alle*), Puffin (*Fratercula arctica*), Brünnich's guillemot (*Uria lomvia*), Razor billed auk (*Alca torda*) & Great black-backed gull (*Larus maritimus*)

Mammals: Seals (species not identifiable) and Porpoise (Phocoena communis)

Poul's and the seaweed girls went out in the dinghy to collect their algae with a triangular scraper. They went in close under land to shallow water. The weather and their success made it lovely experience. The ever bird interested Knud followed them in the dinghy and he made an amphibious landing to take a closer look at the nesting seabirds (not knowing that landing on these islands is illegal). The protists got their samples too and Frederik the skipper turned the boat around to head for home. A shame because these islands were really a treat but acceptable because the weather had deteriorated. They got home just in time for dinner and the rest of the evening was spent on the collected samples.



The snail (Lymnaea vahlii). Drawing by S. W. Knudsen

## Tuesday July 16<sup>th</sup>

Everybody was working on the projects all day. After dinner the zoologists' trip to the Disko Fjord was planned. They spent the rest of the evening packing for the voyage in the most beautiful midnight sun so far.

### Wednesday July 17<sup>th</sup>

The weather was not perfect for a trip to Disko Fjord: 5°C and clouded but on the other hand it did not make it impossible and that is always good up here. "Porsild" headed out at 0900 and it was not until "Porsild" reached the Disko Fjord the sun broke through the clouds. After passing Qeqertaq Bjørn spotted a whale to the North. This time it was a humpbacked whale (*Megaptera boops*). Bjørn and Reinhardt had planned to collect some water samples from a radioactive spring at Eqallúngiut so they were put ashore. Reinhardt collected some moss samples hoping to find some tardigrades, as the tardigrades here are heavy influenced by the radioactive source (e.g. they do not develop legs). Maja and Steen collected mud samples at Kuanit and when done there the ship turned for home with an estimated time of arrival of 2400. In the meantime two scientists from Reinhardt's department at the Zoological Museum of Copenhagen had arrived; Martin who works with meiofauna and Katrine who works with polychaetes. So the ones not too tired shared a nightcap with the newly arrived.

### Thursday July 18<sup>th</sup>

The rain poured down already when got up and continued though the day. So there was not much else to do than to work on the projects. The protists got through their samples and committed sacrilege by claiming the sofas and the VCR for the remainder of the day. Not something easy excused in Greenland. They were somewhat excused by the foul weather, though. After dinner the seaweed girls are packing for their trip to Mudderbugten at Friday, and Reinhardt gave a lecture about warm springs.

## Friday July 19<sup>th</sup>

It was cold and foggy when Reinhardt, Niels, Martin, Katrine, Poul and the seaweed girls headed out the port bound for Mudderbugten. Today was Barat's 60<sup>th</sup> birthday and remaining residents on the Arctic Station was invited to a "kaffemik". Kaffemiks are held at any festive occasion and the word means coffee and cosiness so we gladly accepted the invitation. The actual party runs as follows: you come at around the time you are invited and you are treated with coffee, tea and an enormous selection of cakes. You stay and chat for around half an hour until the next group arrives and this goes on for the whole day. For Barat's kaffemik Steen had prepared a new etiquette for a bottle of wine with a drawing of a seal and a text: "Seal Wine 2002 mis en bouteille par Arctic Station" and Maja had given the bottle a seal skin scarf. Before we went to Barat's we visited the local "bazaar" in order bring home some souvenirs and some of us found just that. We went to Barat's house the kaffemik to follow the above lay out. We were all very grateful to be invited to participate in an old Greenland tradition. So thank you for that Barat! After around half an hour we said goodbye to Barat and headed home. On the way there we had to buy some dinner since Barat naturally had the day off.

The tourists from Mudderbugten returned at around midnight not much of an experience richer as the weather had remained poor throughout the day.

### Saturday July 20<sup>th</sup>

Still not too lucky about the weather we found that it rained already from the morning. Of course there was nothing to do about it so everybody just worked on the projects quietly accepting the fact. The kitchen team found themselves in another kaffemik as they returned with Barat from the supermarket. Luckily the rain eased off before lunch and Knud, Lone, Linda, Anne-Marie, Morten and two of Marianne's master students: Elisabeth and Gry was determined to seize the

top of Skarvefjeld, which is 861 metres above sea level. The weather improved as they went along on this marvellous hike. The view over Qeqertarsuaq was magnificent to the East and the valley of Røde Elv to the North. It must be said that it was not a picnic there was times when they were brought into the red area as the path steepened as we went along. But the summit was reached after  $3\frac{1}{2}$  hrs and they had a look on the cairn erected by Knud Rasmussen. He supposedly left a spent rifle cartridge in it and they looked for it but in vain. About 5 min. after the top was reached it started to snow and the visibility was reduced to about 5 metres. This, of course, did not add to the pleasure of picnicking there so they gulped down their lunch to get out of there. On the way back they saw a helicopter in the valley of Røde Elv from above! It was a team of geologists who had been working at a surging glacier in the Disko Fjord. The hikers returned to the station an hour faster than the out trip. Bente the scientific leader of Arctic Station gave a talk on her work with seed dispersal. After the days hardship most of us went early to bed trying to prepare ourselves for the hike to Lyngmarks Bræen (a glacier North of the town) Sunday.

### Sunday July 21<sup>st</sup>

It was perfect weather for the hikers with 10°C and the sun was shining bright. But with the sun comes the mosquitoes -so no roses without thorns. Everybody was going except Linda, Bjørn and Morten. Linda and Bjørn would rather hike to Røde Elv and Morten had promised to take some pictures of Linda and Anne-Marie's snow buntings (*Plectrophenax nivalis*) and the weather was just perfect for that. The hike to the top of the glacier was stunningly beautiful and it became even better when top was reached as they now could see the mountains surrounding the Disko Fjord. That could not see the water though. The hikers returned in small groups in the hour before dinner. After dinner a Belgian research team was giving a talk on testate amoebae as bio indicators. The Belgians were followed by the geologists who gave a talk on their work at the glacier.

### Monday July 22<sup>nd</sup>

We found it to be no coincidence that the sun shined from a blue sky right from the morning. It was no coincidence because July 22<sup>nd</sup> was the day that the Arctic Station was founded by Morten Porsild 96 years ago. So we had a birthday party on our hands. The station's birthday coincided with the Arctic Field Course going away party so this was a day we had looked forward to. But before we could commit ourselves to the party preparations we all had to pack our laboratory gear because it was to be sent to Denmark the following day. Some of the groups had a hard time making their gear fit in to the boxes it was brought in. The packing continued through the morning into the afternoon and eventually everybody finished. By then everybody was participating in the party preparations. The scientific leader of Arctic Station Bente and her family, Frantz and family as well as the crew of "Porsild" and their wives was invited to the party. The dinner itself was an extravaganza of Greenland food so for starters we had shrimps and Greenland crab (*Chinoecetes opilio*) and a main course of reindeer with greens and potatoes followed by a dessert of home made ice cream with a sauce of raspberries and blackberries. We all enjoyed the lovely food and the party continued through to the wee little hours.

### Tuesday 23<sup>rd</sup>

For obvious reasons we did not the earliest start of the course on this morning but through the morning more and more sleepy people turned out. Because we took care of the packing yesterday it was no problem for Reinhardt and Frantz to hand in the boxes at the port, which had to be done

before 1300. We also said goodbye to Anja, Alf and Bjørn who was flying to Ilulissat because there was not room for us all in the helicopters for Wednesday. So they were to wait for us in Ilulissat for the following day. We spent a cosy afternoon of light reading and socializing. This apathy was completely ruined by Knud who really wanted to see a gyr falcon and he organized an expedition consisting of Anne-Marie, Linda, Martin, Berit and Maiken. They returned at 0300 without seeing a single falcon. The rest of us spent reminding each other what a marvellous experience this course had been. On one side everybody was sad we had to leave in the morning but on the other some of us was beginning to miss girlfriends, boyfriends, friends and families.

### Wednesday July 24<sup>th</sup>

We woke up fair weather and it was just as well since we all had to leave Disko today by helicopter and the machines do not agree with bad weather. Since only 7 people could fly at one time (and we were not the only ones who left Disko on this day) several helicopter trips was necessary. That meant we came in little group at different times to Ilulissat. We were all checked in to the youth Hostel for the night. One group got the ride of their lives when Anne-Marie (who sat in the co-pilots seat) asked: "Don't you get bored with the autopilot once in a while?" To which the pilot replied: "hmm yearh but it is easy to turn it off" and so they got a low level ride over Ice Fjord and the Ilulissat glacier. We spent the rest of the day sightseeing in small groups. As we had to check by 0730 the following day most of us went to bed early.

## Thursday July 25<sup>th</sup>

After a little confusion about our tickets we were all checked in and had a nice flight to Kangerlussuaq. It was clouded and pretty cold when we arrived in Kangerlussuaq but in spite of this most of us wanted to see the Inland Ice. Agnete who had worked in Kangerlussuaq previously got hold of her old boss and he agreed to drive us the 25km in his passenger truck for 2500 DKK. The Inland Ice expedition went shopping for food before departure. On the way there we saw a young reindeer (*Rangifer tarandus*) quite close to the truck.

After an hour or so of driving we arrived at the campsite and the just across the river the Inland Ice rose over 20 metres -impressive. Only Anja, Alf, Reinhardt, Maja and Bjørn went back to Kangerlussuaq. The rest of us sat up a camp of 4 tents fairly quickly as we were all eager to explore the surroundings. We left the camp as one group all determined to find a place to climb the Inland Ice. Unfortunately we could not find a suitable place to cross the river so we want on in minor groups trying to savour the scenery. When we arrived we saw a small group of musk oxen including a couple calves. Some of us decided to get a closer look on these. On the way back Niels and Morten surprised a bull on the North side of the river (where the oxen were not supposed to be) and this resulted in some decent pictures and quite an experience. Pretty hungry after all the adventures of the day it was time to the barbeque fry up in only half decent weather. We sat very close to stay warm, as it was pretty cold. After our arctic barbeque we were visited by a young Arctic fox (*Aloxpex lagopus*) that appeared to be very curious or simply hungry. Probably the latter as it was very interested in our sausages and against all sensibility we threw it a couple. Finally it got too cold and we had a nightcap in our tents.

## Friday July 26<sup>th</sup>

The downside of being driven out here was that the only way to get back to the airport, and our plane home, was walking the 25 km there. So we tore down the camp and headed for the airport in quite good weather. We naturally divided in to several group: the almost running and the

lingering and everything in between. We all returned in good time for the check-in at around 1800. SAS brought us all safely back to Copenhagen to which we arrived early in the morning Saturday (0600 local time).

## List of Birds, Mammals and Fish seen on Arctic Field Course 2002

#### **BIRDS** (aves)

Arctic skua (Stercorarius paraciticus) DK: Alm. Kjove Black guillemot (Cepphys grylle) DK: Tejst Brünnich's guillemot (Uria lomvia) DK: Polarlomvie Cormorant (Phalacrocorax carbo) DK: Skarv Eider (Somateria mollissima) DK: Ederfugl Fulmar (Fulmarus glacialis) DK: Mallemuk Glaucous gull (Larus hyperboreus) DK: Gråmåge Great black-backed gull (Larus maritimus) DK: Svartbag Gyr falcon (Falco rusticulus); Dead. DK: Jagt falk Iceland gull (Larus glaucoides) DK: Hvidvinget måge King eider (Somenteria spectabilis) DK: Kongeedderfugl Lapland bunting (Calcarius lapponicus) DK: Laplandsværling Little auk (Alle alle) DK: Søkonge Pintail (Anas acuta) DK: Spidsand Ptarmigan (Lagopus mutus) DK: Fjeldrype Puffin (Fratercula arctica) DK: Lunde Purple sandpiper (Calidris maritima) DK: Sortgrå ryle Raven (Corvus corax) DK: Ravn Razorbilled auk (Alca torda) DK: Alk Red-necked phalarope (Phalaropus lobatus) DK: Odinshane Redpoll (Carduelis flammea) DK: Gråsisken Snow bunting (Plectrophenax nivalis) DK: Snespurv

#### MAMMALS (Mammalia)

Arctic fox (*Aloxpex lagopus*); var. white and blue, DK: Polar ræv Porpoise (*Phocoena communis*) DK: Marsvin Finback (*Balaenoptera physalis*) DK: Finhval Humpback (*Megaptera boops*) DK: Pukkel hval Various seals Reindeer (*Rangifer tarandus*) DK: rensdyr Musk ox (*Ovibos moschatus*) DK: Muskusokse

FISH (Osteichtyes) Uvak (*Gadus ogac*) DK: uvak Arctic char (*Salvelinus alpinus*) DK: Fjeldørred

# **Programme - Arctic Field Course 2002**

## 3. juli – 27. juli 2002

3. juli:	Afrejse fra København til Kangerlussuaq og direkte videre til Ilulissat. Overnatning
	på Ilulissat's Vandrehjem. Vandretur til Jakobshavns Isbræ.
4. juli:	Videre til Qeqertarsuaq. Indlogering på Arktisk Station. Dagen blev brugt til
	udpakning, klargørelse af laboratorier m.m. og rundvisning i Qeqertarsuaq, om
	aftenen blev der givet en kort introduktion til kursus.
5. juli:	Kort tur med "Porsild" til Iqpik og permanent CTD-station. Afprøvning af den nye
	trekantskraber om aftenen
6. juli:	Feltforelæsning om Disko's botanik. Tur til Kuanit. Aftenforelæsning om Diskos
	geologi.
7. juli (sønda	ag): Konfirmation i kirken. Tur til Østerlien og Røen sø. Pakning til sejlads.
89. juli:	Tur til Diskofjord og Mellemfjord (botanik, 9 pers). Gråsisken synger ikke.
10. juli:	Regnvejr med store dønninger hele dagen. Tre snespurve hanner synger.
11. juli:	Tur til Mudderbugten og Kvandalen. Gik ind til Lymnaea-søen (Sten, Maja, Jesper,
	Agnete, Reinhardt, Morten + Bjørn)
12. juli:	Hjem fra Mudderbugten. CTD-prøver ved Flakkerhuk, Skansen og Iqpik.
	Mudderprøver ved Iqpik.
13. juli:	Afhentning af geograf (Dave) ved galopperende gletscher og David Gremillet (plus
	3 pers) ved Qeqertaq.
14 juli (sønd	ag): Ingen Porsild. Tur til Røen sø. Lyngmarken og Engelskmandens havn.
	Aftenforedrag om isotoper i vand. Problemer med Porsild's ror.
15. juli:	Tur til Brændevinsskærene og Kronprinsens Ejlande (botanik).
16. juli	Ingen Porsild. Aften forelæsninger om skarv (Gremillet)
17. juli	Besøg ved de varme kilder, Tarajungitsoq og Anguujaartuutit i Disko Fjord.
	Mudderprøver ved Kuanit (Bjørn, Reinhardt, zoologer (6 pers.)).
18. juli:	Ingen Porsild. Hjemmedag og dårlig vejr. Aftenforelæsning om varme kilder.
19. juli:	Martin, Katrine, Reinhardt, Linda, Anne Marie + PMP'gruppe (3 pers) studenter til
	Mudderbugten Prøvetagning undervejs tilbage ved Flakkerhuk, Skansen og Iqpik.
	Retur til Arktisk Station. Barats fødselsdag.
20. juli:	Hjemmedag, udsortering af prøver. Foredrag om Bentes spiringsforsøg.
21. juli (sønd	lag): Ingen Porsild. Tur til Lyngmarksgletscheren. Strålende vejr.
	Aftenforelæsninger af Professor Louis Beyens (Antwerpen) om jordbundsfauna,
	samt af geografer om den galloperende gletcher.
22. juli	Tur til Fortune Bay med den nye speedbåd. Reje og krabbe-party.
23. juli:	Pakning og mundtligt fremlæggelse af projektarbejde. Anja, Alf og Bjørn rejser.
24. juli:	Afrejse fra Arktisk Station. Overnatning i Ilulissat i regnvejr
25. juli:	Videre til Kangerlussuaq. Ekskursion til arktiske, aride lokaliteter i Unimox. Niels
-	og studenterne overnattede inde ved indlandsisen, andre på campingplads.
26. juli:	Prøvetagning i Store Saltsø. Afrejse fra Kangerlussuaq om aftenen
27. juli	Hjemkomst til København, planmæssigt kl. 6:00.