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Population structure, parasitism and prey preference in *Sclerocrangon boreas* and *S. ferox*, Svalbard

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Lise Kristin Bjørdalsbakke

Abstract

The aim of this study was to enhance the knowledge on ecological and life-history parameters of *Sclerocrangon boreas* and *Sclerocrangon ferox* in Svalbard waters, including sex ratio, population structure, parasitism and prey preference.

Little research has been done on *S. ferox* before. It has been subject to unprecedented research in the present study, considering size distribution, prey preference and DNA barcoding. The barcoding result gave a genetic distance of 13.7 % between *S. boreas* and *S. ferox*.

This study shows a clear difference in size distribution between males and females. Females are considerably larger than the males. This is an indication of the two species being protandric hermaphrodite. These findings support earlier research, showing the same pattern, suggesting *S. boreas* being a protandric hermaphrodite. It has not been found studies looking on *S. ferox* considering this, but the present study shows also this species follow this pattern and are most likely protandric hermaphrodite.

The largest groups of prey found in the stomach content were Crustacea, Echinoidea and Ophiuroidea. Individual specimens had mostly one type of prey in their stomach, but several types of prey were identified for both species. These findings suggest that *S. boreas* and *S. ferox* are specialists as individual specimens and generalists as species.

The genetic results indicate that the specimens of *S. boreas* from Smeerenburg are distinguished from Grønfjorden and Rijpfjorden, and there were also variation within the specimens sampled in Smeerenburg. However, the results do not give a basis to conclude whether the populations have different genetic signatures between the fjords where *S. boreas* is found. It can be concluded that DNA barcoding and the CO1 gene do not together give ample information of genetic differences within populations, but it will still be able to give a genetic signal on differences between populations.

Parasites were found on both *S. boreas* and on *S. ferox*. Genetic analyses, using CO1 and 18S, of the parasites conclude that they are cocoons from a piscicolid (fish) leech. The identity of this species has not been found.

1. Introduction

1.1 Oceanography

The northern and eastern parts of Svalbard are influenced by water masses originating from the Arctic Ocean. Rijpfjorden is situated on Nordaustlandet, on Svalbard, facing north and mainly influenced by arctic water masses, which are colder and less saline than Atlantic water. The East Spitsbergen current transport water masses south along the eastern coast (Ingvaldsen and Loeng 2009).

The western coast of Spitsbergen is strongly influenced by the west Spitsbergen current, an extension of the Norwegian Atlantic current, carrying warm and saline Atlantic water northwards (Ingvaldsen and Loeng 2009), finally entering the Eurasian Arctic Ocean (Aagaard et al. 1985). The west facing fjords on Spitsbergen exhibits large variations in topography and warm water influx, from the open and deep Kongsfjorden and Isfjorden, to the enclosed sill fjord Billefjorden producing "local Arctic water" (Gulliksen et al. 2009).

Hinlopen is a wide strait separating Spitsbergen and Nordaustlandet. Water masses from the West Spitsbergen current enters from the north and arctic water masses enter from the south (Berge, J. pers. com). This makes Hinlopen a transition zone for the water masses. There is, however, few oceanographic data from Hinlopen, but CTD snapshots of the oceanographic conditions provide some indications of a spatial and temporal variable environment. For sampling stations and map, see section 2.2.

1.2 Biology of *Sclerocrangon boreas* and *S. ferox*

Sclerocrangon boreas (Phipps, 1776) (Figure 1 and 2A) belongs to the family *Crangonidae* (Sainte-Marie et al. 2006). It is distributed in cold waters and is reported from the littoral zone down to 450 meters depth and is found on different substrates from sand to barren ground (Birkely and Gulliksen 2003a). It is found in coastal waters from the county of Nord-Trøndelag northward to Svalbard (Christiansen 1972). This shrimp is common in the fjords along the west coast of Spitsbergen. It is most abundant in temperatures up to 4˚C, on sand and gravel and prefers salinities above 33 PSU (practical salinity unit) (Birkely and Gulliksen 2003a).

Sclerocrangon boreas is a K-strategist, characterized by having few eggs, taking care of its offspring, having high survival rate and reaching maturity relatively late (Gosling and Sutherland, 2000). The females carry relatively small clutches of large eggs in spring/summer. These are held beneath the abdomen for about 9-12 months before they hatch in April to July (Lacoursière-Roussel and Sainte-Marie 2009). The eggs do not have a planktonic phase, but the juveniles develop through two larval stages holding on to their mother's pleopods (Figure

1) before they are released (Lacoursière-Roussel and Sainte-Marie 2009). The females grow faster and live longer than the males (Birkely and Gulliksen 2003a). Males can reach an age up to 4 years with a cephalothorax length (CL, Figure 1) at 17 mm, while the females can reach an age up to 6 years with a CL at 29 mm (Sainte-Marie et al. 2006). The sexes of *S. boreas* are distinguished according to the presence or absence of an appendix masculina on the second pleopod (Birkely and Gulliksen 2003b) (Figure 2C and 2D). There are, however, studies that suggest that *S. boreas* is a protandric hermaphrodite, i.e. that the shrimp matures as a male early in its life and then later develops into a female. This suggestion is based on the greater size of females relative to males and a perceived sex ratio bias in favor of males at small body sizes and in favor of females at the population level (Lacoursière-Roussel and Sainte-Marie 2009).

Due to its large size and palatability *S. boreas* has attracted interest from the fishing industry, but due to scattered distribution together with low catch rate and apparent scarcity of large shrimps, profitable fishing is unlikely (Sainte-Marie et al. 2006).

The diet of *S .boreas* consists mainly of polychaetes, gammaridean amphipods, mollusks, ophiuroids and to lesser degree hydroids (Sainte-Marie et al. 2006). It is a common prey to cod, sculpins, beluga whales and seals (Sainte-Marie et al. 2006). There is one other species of *Sclerocrangon* found in Svalbard waters, *Sclerocrangon ferox* (G. O. Sars, 1877) (Figure 2B), but the ecology of this species is not well known. Weslawski (1987) compared the two species with respect to distribution of water masses, where *S. boreas* is a species being an indicator of Atlantic water while *S. ferox* is an indicator of cold Arctic water.

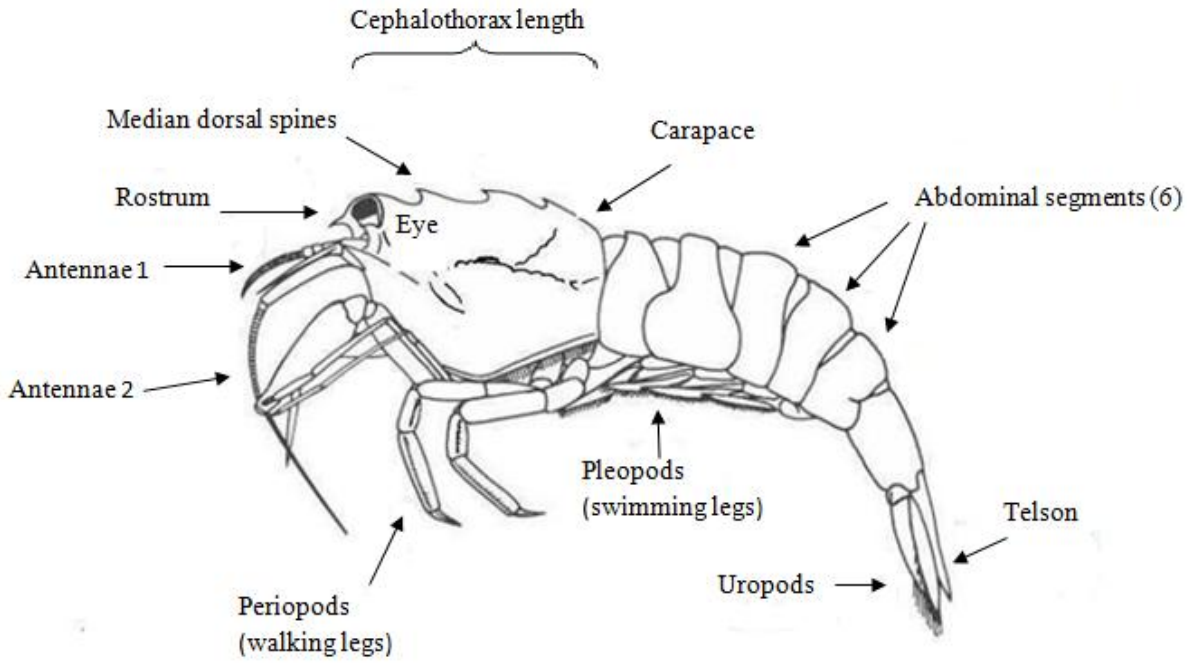

Figure 1: Schematic drawing of *Sclerocrangon boreas* (modified from Christiansen 1972).

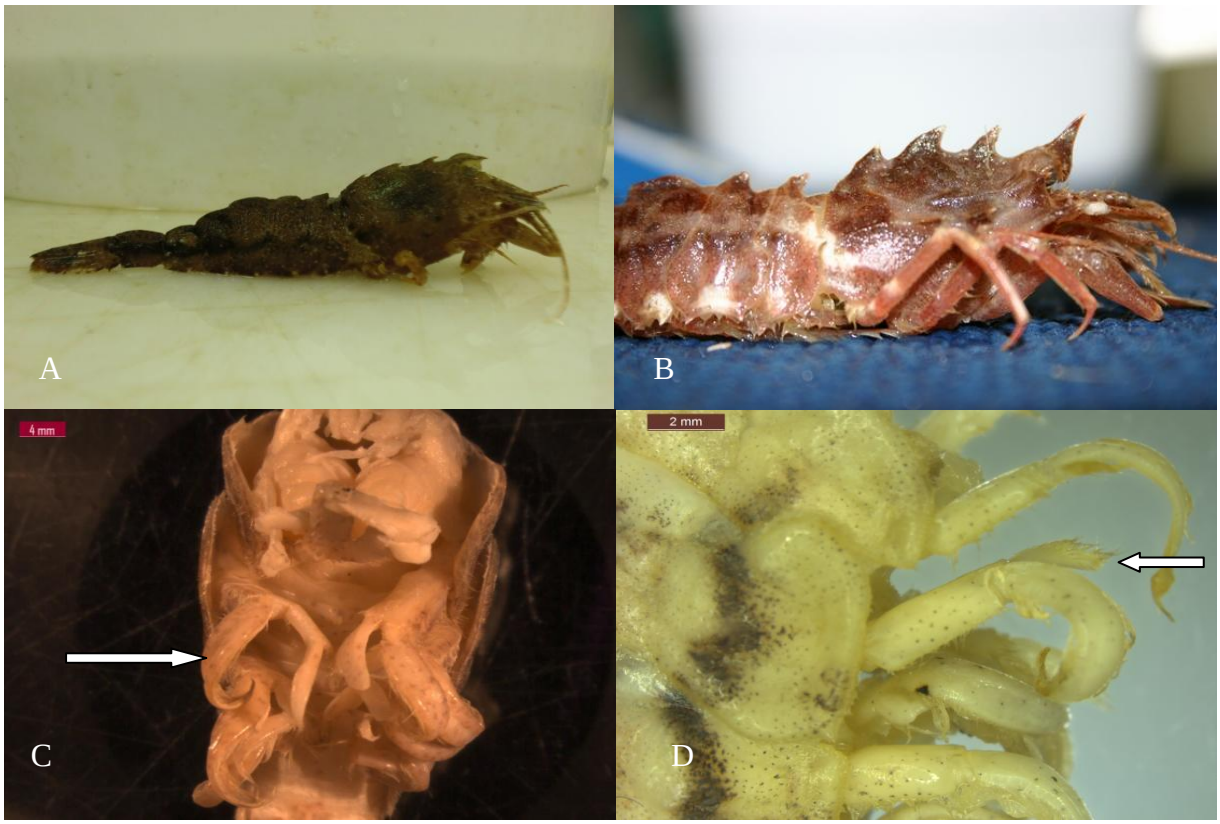

Figure 2A-D: A, *Sclerocrangon boreas*, B, *Sclerocrangon ferox*, separated from each other by the median dorsal spines (Figure 1). C, shows a female of *S. boreas*, while D shows a male, with an appendix masculina on the second pleopod (Birkely and Gulliksen 2003b). Arrows indicate second pleopod for both the female and the male. C, showing that the limbs on the second pleopod on the female are equal in size, D, shows the male where one of the limbs are much smaller, it is an appendix masculine. Photo 2A, 2C & 2D: Lise Kristin Bjørdalsbakke; 2B (www # 1).

1.3 Parasitism

An ecological interaction between different organisms which are in direct contact with each other is often referred to as symbiosis. Symbiotic relationships are divided into mutualism, commensalism or parasitism. In a mutualistic relationship, both organisms benefits from the cooperation, while in a commensalistic relationship only one of the organisms benefits from the cooperation, while the other organism is unaffected. Parasitism defines a situation where one organism benefits at the expense of the other. The parasite takes energy from and hurts its host in the process. Parasites that live inside its host are called endoparasites, while parasites that live on the outside of its host are called ectoparasites (Campbell and Reece 2005).

A familiar group of ectoparasites are leeches (Hirudinea), and about one-fifth of all leech species live in salt or brackish water (Sawyer 1986b). Leeches find a host and lay their eggs on the host, in form of cocoons. These cocoons look very much like the host species eggs,

only smaller. Chemically, the cocoon is comprised primarily of keratin-like protein and some species deposit these hard-stalked cocoons directly onto the exoskeleton of their hosts (Sawyer 1986a). Varying between leech species is that some stay with the same host their whole life, while others leave their host when they have eaten and reattach on a different host when hungry (Sawyer 1986b).

Crustaceans are often used as hosts for parasites. Cocoons of the piscicolid (fish) leech, *Notostomum cyclostoma*, have been found on different crab species in the deep fjords in the Portland Inlet system in northern British Columbia, Canada (Sloan et al. 1984). Piscicolid leeches are also found on blue crabs, *Callinectes* spp., in Brazil (Zara et al. 2009). The leeches deposit 14-18 cocoons, each containing only one egg, primarily attached to the first and second pairs of periopods (Figure 1) (Sawyer 1986b). Cocoons were in this study found on the pleopods (Figure 1) of both *S. boreas* and *S. ferox*, but to my knowledge there is no literature on what kind of species these cocoons belong to.

1.4 DNA Barcoding

Cloning in cells is the best method for preparing large quantities of a particular gene or DNA sequences. When the source of DNA is rare or impure the polymerase chain reaction (PCR) can be used, Figure 3. This technique will quickly amplify any specific target segment within one or many DNA molecules. PCR will in a couple of hours make billions of copies of a target segment of DNA (Campbell and Reece 2005).

The animal kingdom with its millions of species and their life-stage transformations gives a challenging target for taxonomy. A DNA-based identification system, founded on the mitochondrial gene, cytochrome *c* oxidase subunit 1 (CO1), have proven to aid the resolution of this diversity (Hebert et al. 2003a), and serve as the core of a global bioidentification system for animals (Hebert et al. 2003b). This is DNA barcoding, the way Hebert et al. (2003a & b) describes it. The universal DNA primers for PCR amplification of a 710 base pairs (bp) fragment of the mitochondrial CO1 gene were early found to work in 11 invertebrate phyla; Echinodermata, Mollusca, Annelida, Pogonophora, Arthropoda, Nemertinea, Echiura, Sipuncula, Platyhelminthes, Tardigrada and Coelenterata (Folmer et al. 1994).

A challenge for the CO1 gene was to separate closely allied species. An example is species of lepidoterans which tested the ability of CO1's diversity to resolve species boundaries. Hebert et al. (2003a) established that diversity in the amino acid sequences coded by the 5' section of CO1 was sufficient to reliably place species into higher taxonomic categories. They also found that diversity in nucleotide sequences in the same gene region regularly permitted the discrimination of closely related species. In total, sequence divergences were examined in more than 13000 congeneric pairs. Results from this supported that species-level diagnoses can routinely be obtained through CO1 analysis (Hebert et al. 2003a). DNA barcode data is

being assembled in the Barcode of Life System (BOLD). This is a large, rapidly growing, and searchable repository of CO1 DNA barcode sequences (Holmes et al. 2009).

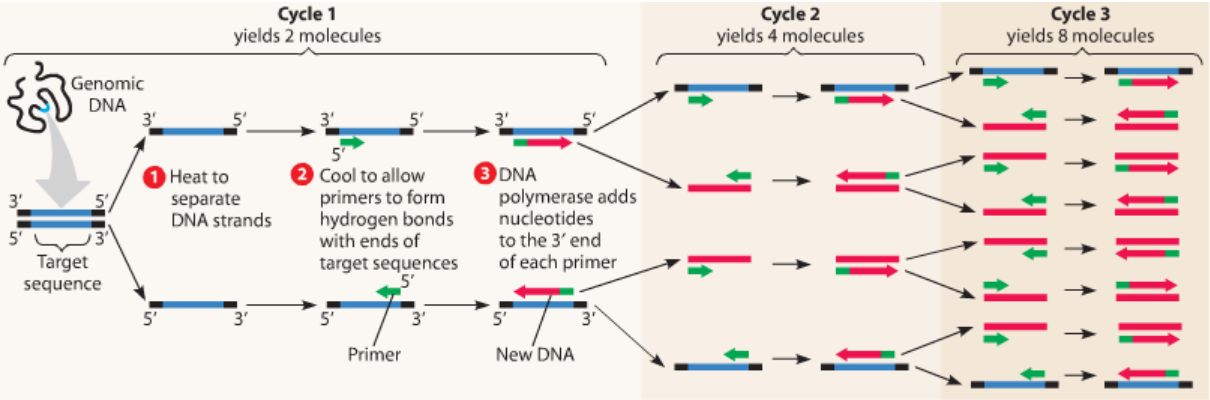

Figure 3: The polymerase chain reaction (from Campbell et al. 2009).

Figure 3 shows the principle of how the PCR works with the different steps in the cycles with denaturation, the DNA broken down to two single strands, annealing, the primers will align and bind to the segments, and extension, the building of DNA (Campbell et al. 2009). After the PCR is preformed it is common to run a gel electrophoresis. This is a method used to test the PCR result and is done by placing a small part, 4 µl, of the PCR product on a stained agarose gel and run the gel in a buffer under electrical current. The PCR products will have segments at different length. These segments will run at different speed through the gel and can be visualized in blue light. In this way we are able to see if the PCR reaction has been successful (Ekrem, et al. 2010).

Before the sequencing reaction can start the PCR products need to be purified. The reason for this is to remove all non-DNA additives and excess primers in the PCR cocktail, leaving only the amplified DNA segments of interest. The results from the sequencing are given in the form of chromatograms. Special software is needed to view the chromatograms and to make it possible to edit them. The preferred result is a clean signal with separate curves for each nucleotide. If there is a lot of noise in the signal the PCR have most likely amplified more than the segment of interest and the editing will be both difficult and time consuming (Ekrem, et al. 2010).

A common way to present results from sequencing is to construct trees. One method to construct trees is neighbor-joining (NJ). The NJ method is a method for re-construction of phenetic trees (phenograms) and computing the lengths of the branches of this tree (Saitou and Nei 1987). The method is widely used and preferred over other methods in large phylogenies because of its computational speed and the high accuracy in phylogenetic

inference as revealed in computer simulation studies (Kumar and Gadagkar 2000). The principle of the NJ method is to find pairs of operational taxonomic units which will minimize the total branch length (Saitou and Nei 1987).

1.5 18S rDNA

The nuclear 18S gene is approximately 1800 bp long (Dinapoli and Klussmann-Kolb 2010) and can be used in order to resolve phylogenetic relationships. It has been used within a range of organism groups as a genetic marker for a molecular systematic approach among higher ranks in relationships between groups (e. g. Vonnemann et al. 2005; Wollscheid and Wägele 1999).

1.6 Aim of the thesis

The aim of this study is to enhance the knowledge on ecological and life-history parameters of *Sclerocrangon boreas* and *Sclerocrangon ferox* in Svalbard waters, including sex ratio, population structure, parasitism and prey preference.

Specifically, the following hypotheses were tested:

- H1: *Sclerocrangon* spp. is a protandric hermaphrodite.
  Prediction: There is no overlap in size between males and females.
- H2: As species *Sclerocrangon* spp. is a food generalist, but individuals can be food specialists.
  Prediction: The stomach contents in individuals consist mainly of one type of prey.
- H3: *Sclerocrangon* spp. has no pelagic larval stage; the exchange between populations in different fjords is therefore low.
  Prediction: There is no isolated population in Isfjorden, but there are population signatures between fjords where we find *S. boreas*.

2. Materials and methods

2.1 Sampling sites

The majority of the sampling was carried out from the 15th to the 28th of September 2011 on Svalbard during a cruise with the Research vessel R/V "Helmer Hanssen". The expedition was part of the course AB-321 "Marin Benthic fauna of Svalbard" held at the University Centre in Svalbard, UNIS. The rest of the samples were sampled in Grønfjorden in September 2010 and in Billefjorden in late August 2011.

The different locations for sampling are given in Figure 4. The main areas were fjords on the west coast, including Isfjorden, Grønfjorden, and Smeerenburg. In Isfjorden the sampling took place south of Sagaskjæret at the mouth of the fjord. In addition some sampling was done both in Hinlopen and in the northern part of Nordaustlandet, in Rijpfjorden. Methods used for sampling in these areas are given in Appendix 1.

2.2 Material

The total number of *Sclerocrangon boreas* specimens sampled on the cruise in 2011 was 150. Of these, 114 were from Grønfjorden, 3 from Smeerenburg, 5 from Rijpfjorden, 24 from Rossøya and 4 from Isfjorden (Figure 4). A total of 22 specimens of *S. boreas* were infected with parasites. The total number of *Sclerocrangon ferox* specimens was 76, all of which were sampled in Hinlopen, of which and 11 had parasites (Table 1). The parasites are most frequently found on the pleopods (Figure 12A) and are relatively large, up to 1 mm, and conspicuous and hence easy to see even without a stereomicroscope (Figure 12A-C).

All sampled specimens of both species were dissected and the stomach content analyzed onboard. The specimens for the genetic analysis were collected in Grønfjorden on Svalbard the autumn 2010 and some during the cruise in September 2011. From the 2011 cruise, specimens of *S. boreas* were collected from Smeerenburg and from Rijpfjorden, three from each location, whereas two specimens of *S. ferox* from Hinlopen were analyzed. These were preserved in ethanol and brought to NTNU Trondheim and analyzed at the Museum of Natural History and Archaeology.

The specimens of *S. boreas* collected in late September 2010 were from the same locations, depths and collected with the same gear (triangular dredge) in Grønfjorden as on the AB-321 cruise where the rest of the material were sampled (Appendix 1). A total of 126 specimens were collected in 2010.

There had also been sampled 17 specimens of *S. boreas* in Billefjordenin late august 2011 (Figure 4). They were kept in an aquarium at UNIS. They were only measured for length and weight and their sex were determined.

Figure 4: Map of Svalbard with the different locations for sampling marked (modified from www # 2).

2.3 Sampling

Most of the specimens were sampled using a triangular dredge and a bottom trawl.

Triangular dredge

Most of the samples were collected with a triangular dredge, 1x1x1 m, with a mesh size of 40 mm. Bottom time during towing was 5 minutes at 2-3 knots comprising a distance of approximately 200 m. The dredge was emptied on a sorting table on deck and the content was sorted.

Bottom trawl

The bottom trawl used was a Campelen1800. At the inner part of the trawl the mesh size was 22 mm, and increasing from 42, 60 to 80 mm at the opening. The trawl was towed with a speed of 3 knots for about 15 minutes, comprising a distance of approximately 500 m.

Diving

Additional specimens were collected by scuba divers. The divers were collecting for other projects, and were hence not actively looking for *S. boreas* and *S. ferox.* Specimens were collected by hand and kept in collection nets during the dive.

Processing of samples

After sampling, the obtained *S. boreas* and *S. ferox* specimens were measured for length and weight, sexed and visually inspected for parasites. The length was measured from the rostrum to the end of the telson (Figure 1). The sex was determined by the presence or absence of an appendix masculina on the second pleopod (Figure 2D) (Birkely and Gulliksen 2003b). The parasites are most frequently found on the pleopods (Figure 1), and looks like small eggs (Figure 12A-C). Measurements were taken with a caliper, with 1 millimeter precision. Each specimen was also weighed with a Mearl M2000 series scale. The scale used had an accuracy of ± 2 g.

The stomachs were extracted from the specimens as quickly as possible and put in labeled vials with 80 % ethanol, to stop the enzymatic processes from degrading the stomach content. The stomachs were then opened and the contents identified using a stereomicroscope. Prey organisms were identified to the lowest possible taxonomic level.

Data analysis

Microsoft Office Excel 2007 was used to make graphic figures of the sampled specimens, showing distribution in sex and size. Exponential trend curves were put on and R^2 values were measured. This is the coefficient of determination, the most common measure of how well a regression model describes the data. Values near 1 indicate that the equation is a good description of the relation between the independent and dependent variables. The value equals 0 when the values of the independent variable does not allow any prediction of the dependent variables, and equals 1 when you can perfectly predict the dependent variables from the independent variables (Rawlings 1988).

$$R^2 = \frac{SS(Regr)}{SS(Total)}$$

SS = sum of squares

Regr = total variation – not explained variation

Total = total variation

2.4 Genetic analysis

Tissue samples were collected from 11 specimens of *S. boreas*, of which five specimens sampled in 2010 and six in 2011 from different locations, and two specimens of *S. ferox*, sampled in 2011. One parasite was collected from each *S. boreas* holding parasites that was collected in 2010. All samples were stored in 96 % ethanol at -20°C before and after handling.

Cleaning

To avoid contamination from other organisms, such as bacteria on the surface of the parasite cocoons, the parasites were sterilized with a 10% solution (v/v) of commercial bleach (10% $NaOCl_3$) for 3 min and washed three times with de-ionized water.

Extraction

A small piece of muscle tissue from the shrimp specimens were extracted with MicroElute Genomic DNA kit. The same kit were used on the parasites, the whole parasite were used for the extraction. The extraction was done following the protocol from the manufacturer.

Polymerase chain reaction (PCR)

The standard DNA barcoding 710 basepairs (bp) fragment of the mitochondrial CO1 gene was used, the primers LCO1490, forward, and HCO2198, reverse, (Appendix 2) (Folmer et al. 1994). The following reagents were used for the PCR: 2.0 µl DNA template, 2.5 µl 10x buffer, 2.0 µl dNTP, 2.0 µl $MgCl_2$, 1.0 µl LCO1490, 1.0 µl HCO2198, 0.2 µl Hot Star Taq and 14.3 µl H_2O (total volume of 25.0 µl). This was done in room temperature, only the Hot Star Taq had to be on ice until it was used. The samples were then put on ice until they were placed in the PCR machine.

PCR cycle started with 95˚C for 15 minutes, for activation of Hot Star Taq, and 94˚C for 60 seconds. Five cycles of 94˚C for 30 seconds, 45˚C for 30 seconds and 72˚C for 60 seconds were followed by 35 cycles of 94˚C for 30 seconds, 51˚C for 30 seconds and 72˚C for 60 seconds, with a final extension of 72˚C for 5 minutes.

For the 18S program the following reagents were used for the PCR: 2.0 µl DNA template, 2.5 µl 10x buffer, 2.0 µl dNTP, 1.0 µl $MgCl_2$, 0.2 µl Hot Star Taq and 15.3 µl H_2O (total volume of 23.0 µl).

The primers used were 18A1, 1800, 1155R and 700F (Appendix 2) (Wollscheid and Wägele 1999; Vonnemann et al. 2005). This gave a PCR-product of 1800 bp, primers being divided up in three groups. 1.0 µl 18A1 and 1.0 µl 1800 were joined together to give the entire 18S sequence. 1.0 µl 18A1 and 1.0 µl 1155R were joined to give the first 1155 bp, and 1.0 µl 700F and 1.0 µl 1800 were joined to give the bp from 700 to the end of the fragment. This would give the entire sequence in pieces, which would overlap between 700-1155.

These three groups of primers were then added to the rest of the reagents, giving us three groups of reagents each consisting of a total volume of 25.0 µl. This was done in room temperature, except the Hot Star Taq which was stored on ice until it was used. The samples were then put on ice until placed in PCR machine.

PCR cycle started with 95˚C for 15 minutes, followed by 35 cycles of 94˚C for 30 seconds, 52.5˚C for 30 seconds and 72˚C for 1 minute and 30 seconds, with a final extension of 72˚C for 10minutes.

All samples, from both CO1 and 18S, were run on a C1000^{TM} Thermal Cycler (BIO-RAD). A negative control, a sample with H_2O not DNA template, was included in each round of PCR to check if the reagents were contaminated.

PCR product visualization

All samples were run on an agarose gel comprising 400.0 ml 1% TBE buffer, a solution of tris-borate-EDTA, and 4.0 g agarose. To make one gel 80 ml of this mix and 3.0 µl SYBR®Safe DNA gel stain, Invitrogen, were used. 4.0 µl PCR – product were mixed with 1.0 µl ReddyRun Gel Loading Buffer (10X Thermo Scientific) and applied to the gel. Also 4.0 µl Ladder, a calibration tool, was added to the gel for quantification of sequence length. The gels were run at 100mV or 120mV, depending on if one or two gels were run at the time, for 40 minutes. The bands were visualized by standard exposure to blue light and digitalized in the software GeneSnap 6.08 (Synoptics Ltd).

Purification of the PCR products

On PCR-products from both CO1 and 18S, diluted ExoSAP-IT was added together with water. The products were then placed in the PCR machine for 15 minutes at 37˚C followed by 15 minutes at 80˚C. The PCR products were then packed and sent together with diluted primers to Eurofins MWG Operon in Germany for sequencing.

Editing

The sequences were edited in Sequencher 4.8 and aligned by using ClustalW, implemented in MEGA5 (Tamura et al. 2011). MEGA5 was also used to perform a neighbor-joining (NJ) analysis. The Kimura-two-parameter (K2P) substitution model, being standard in DNA barcoding (Hebert et al. 2003b), was used to make the NJ analysis. Sequences were adjusted by eye in Sequencher after alignment. The sequence of one specimen of *S. boreas* from The Gulf of St Lawrence, Canada (Radulovici et al. 2009) was downloaded from GenBank (www # 3). This specimen was mainly included as a control and also as a comparison to the sampled *S. boreas* specimens.

The primer sites were cut away on both ends giving a fragment with a length of 658 bp for the shrimps and 652 bp for the parasites using CO1. For the parasites, using 18S, the sequence were 1717 bp after the primer sites were cut away.

Edited sequences were checked for similar sequences using Basic Local Alignment Search Tool (BLAST). This tool was used as a control to see which species the sequences tested were from, so both *S. boreas* and *S. ferox* was tested. Also the parasite sequences were tested similarly to test for a match. BLAST is provided from The National Center for Biotechnology Information (NCBI) and finds regions of local similarity between sequences. The search tool compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to find similar sequences as well as help identify members of gene families (www # 4).

3. Results

3.1 Population structure

During sampling we saw that the distribution of *S. boreas* was quite scattered. The location with most sampled specimens was in Grønfjorden with 114 specimens, relative to 4 specimens in Isfjorden (south of Sagaskjæret at the mouth of the fjord), 3 specimens in Smeerenburg, 5 from Rijpfjorden and 24 specimens at Rossøya. *Sclerocrangon ferox* were sampled in Hinlopen, 76 specimens. Table 1 shows the number of specimens from the different locations and also the distribution between the sexes and number of specimens infected with parasites.

Table 1: Total number of specimens sampled at the different locations, with information regarding sexes, and total number infected specimens with parasites.

Sclerocrangon boreas	**Total number**	**Males**	**Females**	**Infected males**	**Infected females**
Isfjorden	4	3	1	0	0
Billefjorden	17	11	6	0	1
Grønfjorden	114	99	15	11	7
Smeerenburg	3	3	0	1	0
Rijpfjorden	5	3	2	2	0
Rossøya	24	7	17	1	0
Total number	167	126	41	15	8

Sclerocrangon ferox					
Hinlopen	76	53	23	8	3

Figure 5 shows the distribution of females and males of *Sclerocrangon boreas* sampled from Grønfjorden in 2010. An exponential curve and the R^2 values are shown. The curve for the females has a R^2 value of 0.95 while the one for the males have a R^2 value of 0.85. For Figures 5-9 all the female R^2 values are marked with red and the R^2 values for males are marked with blue.

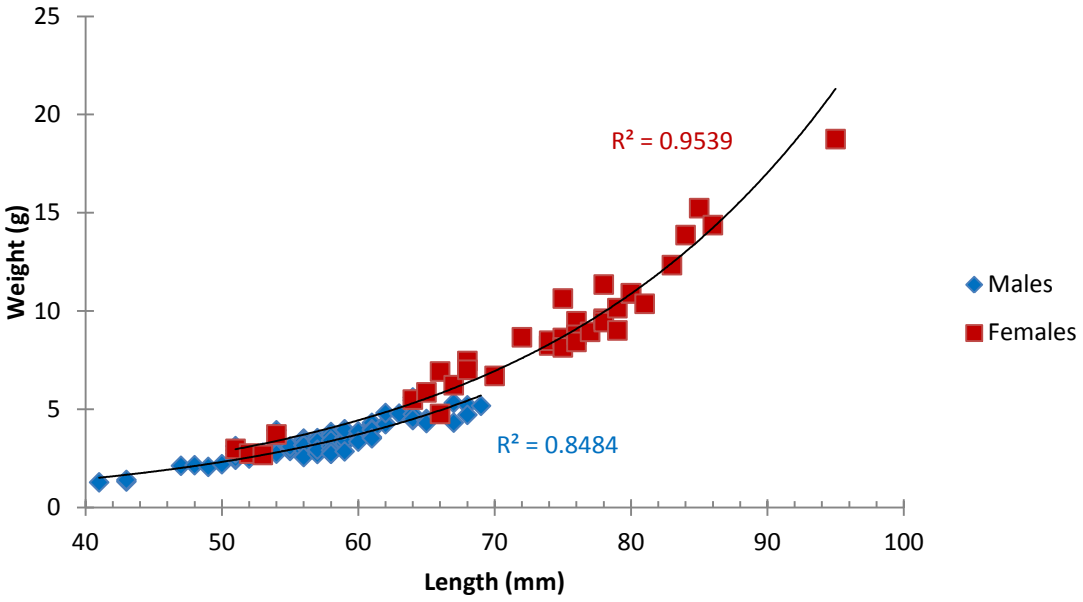

Figure 5: Distribution of males and females of *Sclerocrangon boreas* collected from Grønfjorden on a cruise in September 2010. Lines denote the exponential curve fit with R^2 values for males and females.

A combined overview of all the specimens sampled in Isfjorden, south of Sagaskjæret at the mouth of the fjord, in Grønfjorden and also the specimens sampled in Billefjorden is given in Figure 6. Exponential curves are in the figure, and the R^2 value for the males is 0.72 and for the females the value is 0.86.

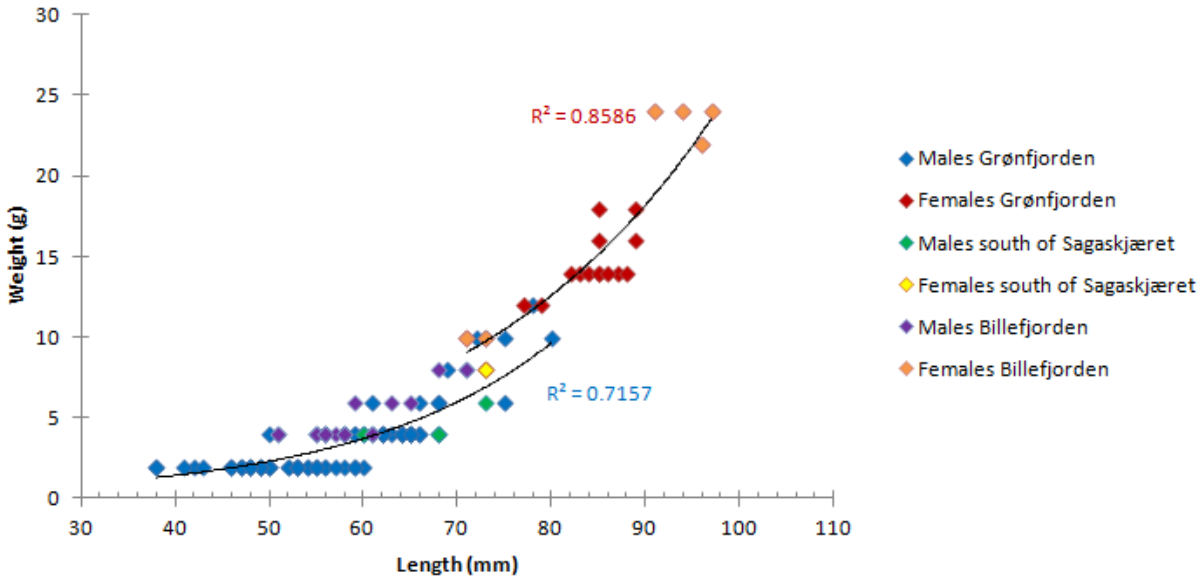

Figure 6: Distribution of males and females of *Sclerocrangon boreas* collected from entire Isfjorden in August and September 2011. Lines denote the exponential curve fit with R^2 values for males and females.

The specimens sampled in Grønfjorden are shown in Figure 7, they are also included in Figure 6, but are taken out in an own figure since a quite high number of specimens were sampled there. The R^2 value for the males is 0.73, while for the females it is 0.69.

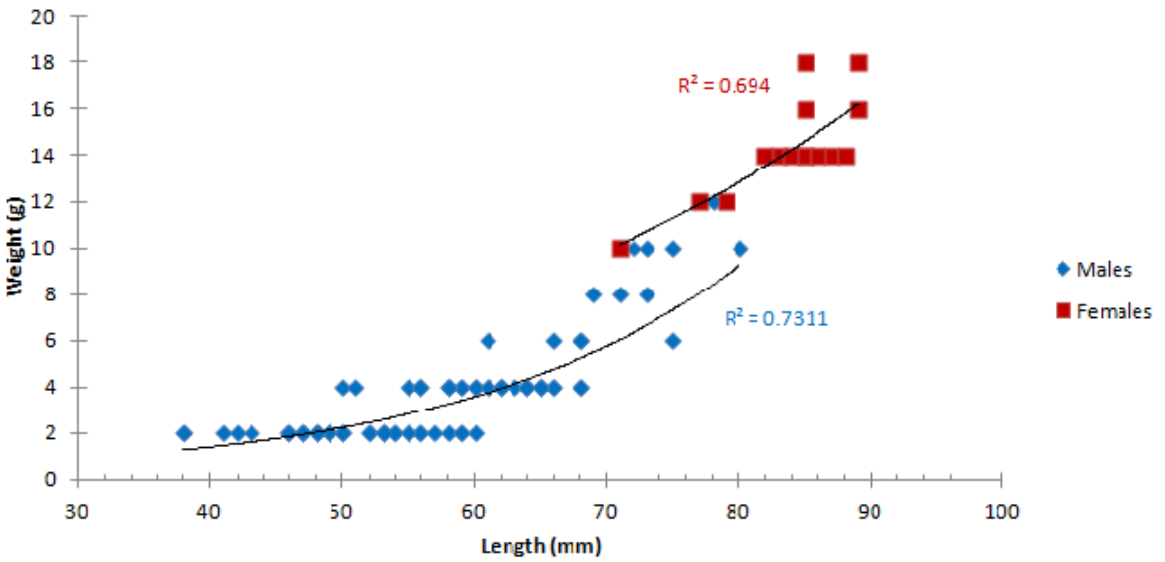

Figure 7: Distribution of males and females of *Sclerocrangon boreas* collected from Grønfjorden on the AB-321 cruise in September 2011. Lines denote the exponential curve fit with R^2 values for males and females.

Figure 8 shows the distribution of males and females of *Sclerocrangon boreas* sampled at Rossøya. The R^2 value is 0.83 for the females. For the males no value is calculated since four out of five specimens has the same weight.

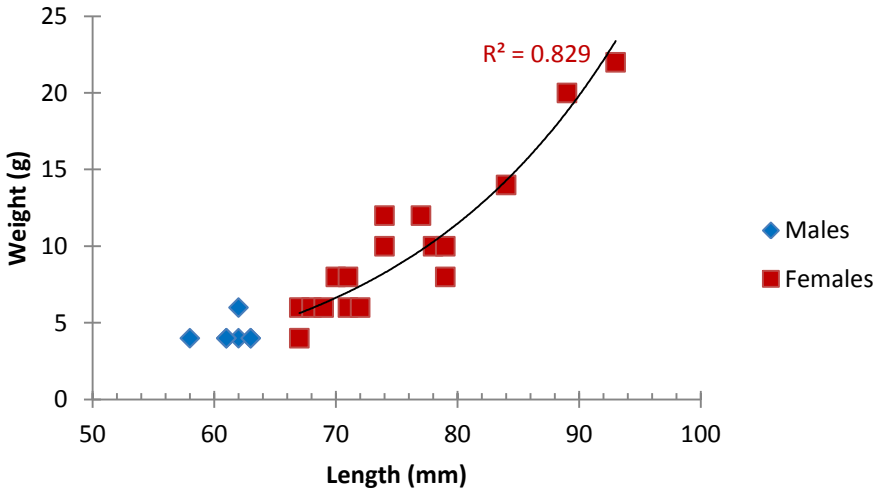

Figure 8: Distribution of males and females of *Sclerocrangon boreas* collected from Rossøya on the AB-321 cruise in September 2011. The line denotes the exponential curve fit with R^2 value for females.

The following figure (Figure 9) indicates the distribution of males and females of *Sclerocrangon ferox* sampled in Hinlopen. Also on these the exponential curve fit have been put on, the R^2 values are 0.85 for the females and 0.77 for the males.

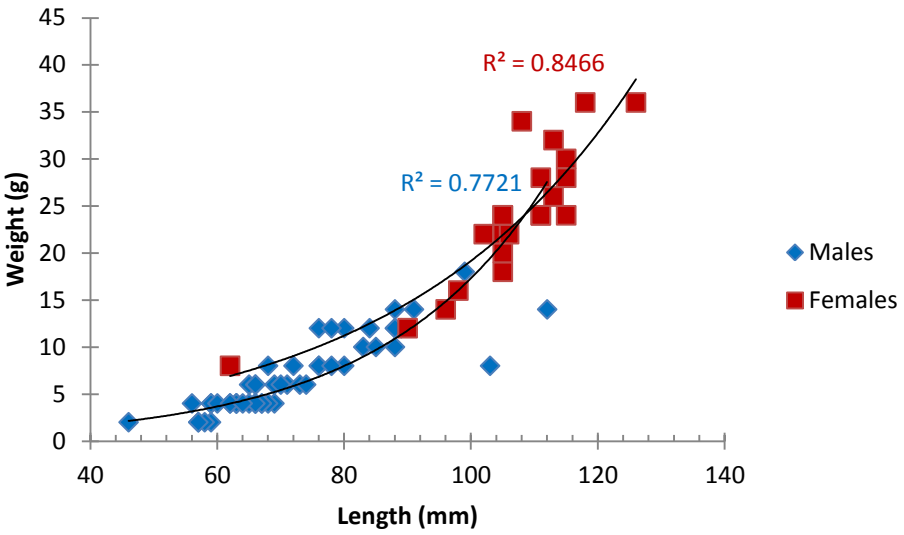

Figure 9: Distribution of males and females of *Sclerocrangon ferox* collected from Hinlopen on the AB-321 cruise in September 2011. Lines denote the exponential curve fit with R^2 values for males and females.

What all these figures (Figures 5-9) have in common is that they have the same trend. The trend is that the males are relative smaller than the females. The sampling from both 2010 and 2011 shows the same pattern, this support the first hypothesis, H1, confirming the species being protandric hermaphrodite.

The lowest R^2 value is 0.69 and the highest 0.95. Most values are between 0.72-0.85, which indicates that the equations are a good description of the relation between the independent and the dependent variables.

3.2 Gut content

For the analyses of the gut contents every specimen of both *S. boreas* and *S. ferox* from all locations were analyzed (Figure 10). Several specimens had empty stomachs or had content that was impossible to identify (Table 2). The results from the stomachs where the contents were possible to identify are given in Figure 10, the empty and unidentified are not included in the figure.

Table 2: An overview, showing the percentage of stomachs it was possible to identify the content from on each location.

	Total number	**Identified stomachs content**	**Empty and Unidentified stomachs**
Isfjorden	4	75 %	25 %
Grønfjorden	114	22.8 %	77.2 %
Smeerenburg	3	100 %	0 %
Rijpfjorden	5	40 %	60 %
Rossøya	24	62.5 %	37.5 %
Hinlopen	76	44.7 %	55.3 %

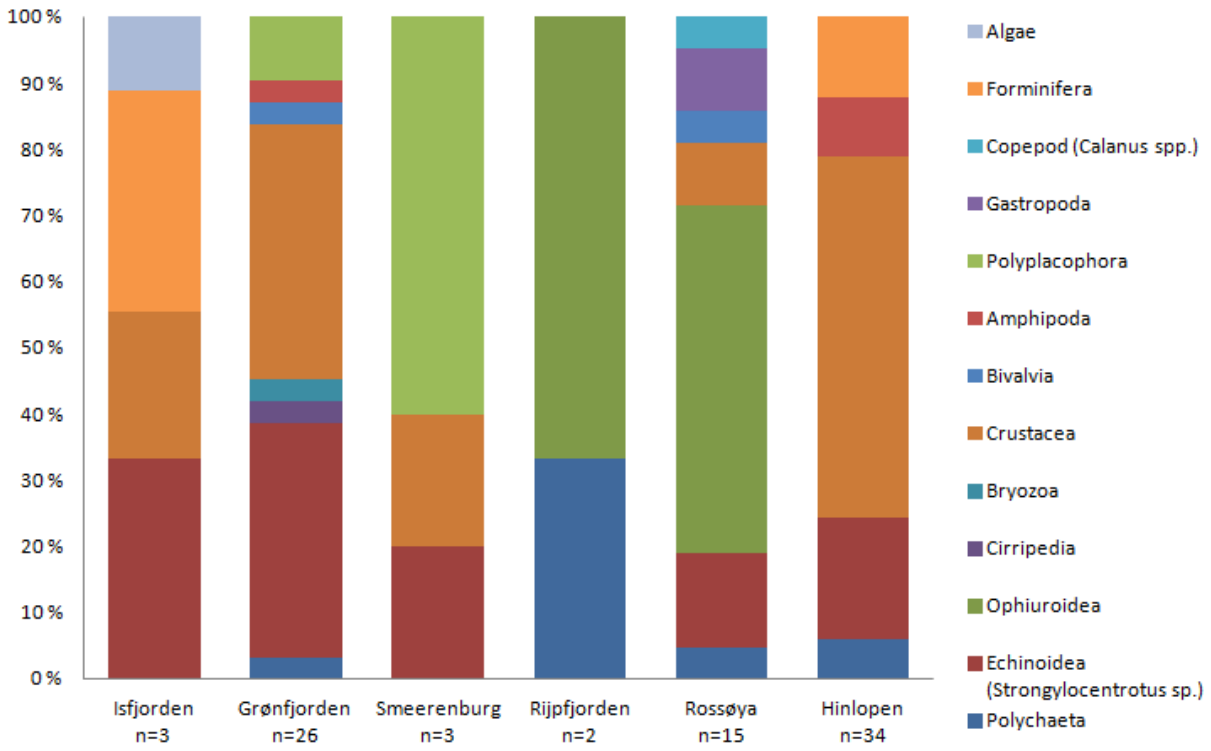

Figure 10: Gut content from *Sclerocrangon boreas* and *Sclerocrangon ferox*, all location sites are *Sclerocrangon boreas*, except Hinlopen which is *Sclerocrangon ferox* data. N are the number of specimens with positive identification of stomach content. All empty stomachs and stomachs where the prey was impossible to identify are not included.

The largest groups of prey, found in *S. boreas* and *S. ferox*, are Crustacea, Echinoidea and Ophiuroidea. At first also Foraminifera, Polyplacophora and Bivalvia appear substantial, but that is on the locations with only 2 and 3 specimens with identifiable stomach content. They rather they belong to the rarely occurring groups together with algae, copepods, gastropods, Amphipoda, Bryozoa, Cirripedia and Polychaeta (Figure 10).

In Isfjorden, south of Sagaskjæret at the mouth of the fjord, the specimens had a rather equal proportion of *Strongylocentrotus* sp., Foraminifera, crustaceans and algae in their stomachs. In Grønfjorden the dominating groups of prey were different crustaceans and *Strongylocentrotus* sp. In a lesser degree Polyplacophora, Cirripedia, Bryozoa, Bivalvia, Amphipoda and Polychaeta were a part of their content.

The stomach content was identified in all three specimens of *S. boreas* from Smeerenburg. These three had eaten most ophiuroids and a quite equal share of *Strongylocentrotus* sp. and crustaceans. The two specimens from Rijpfjorden had eaten ophiuroids and polychaetes. The dominating group of prey in the specimens from Rossøya was Ophiuroidea. Smaller groups that were found in even numbers are *Strongylocentrotus* sp., crustaceans, polychaetes, gastropods and bivalves also one *Calanus* spp. was identified. In Hinlopen the dominate group were crustaceans. Then *Strongylocentrotus* sp. and foraminifera followed while amphipoda and polychaetes were the least eaten prey.

What is not shown in Figure 10 is whether or not specimens had one or several types of prey in their gut. That information was given just as observations during the analyses. Some specimens of both species had several types of prey in their stomachs, but quite many of them had only one type of prey. This observation was done at all the sampling stations, not just at one, showing the same pattern at every location, supporting the second hypothesis, H2. Unfortunately there are no numbers to support this.

The specimens of *S. boreas* that were sampled in Billefjorden, were not dissected to look at the stomach content since they had been starved in the aquarium at UNIS.

3.3 Genetic analyses

Results from the DNA barcoding is presented as a neighbor-joining tree (Figure 11). The tree shows the five specimens of *S. boreas*, sampled in Grønfjorden 2010, denoted as Groenfjorden1-5 in the figure. The other six specimens of *S. boreas* are marked Smeerenburg1-3 and Rijpfjorden1-3, three specimens from each location sampled on the 2011 cruise in September. The last *S. boreas* specimen (FJ581901.1) from the Gulf of St Lawrence, Canada, was downloaded from GenBank. It is included for control and comparison to the other *S. boreas* specimens. Two specimens of *S. ferox* are marked as Hinlopen1-2 (Figure 11).

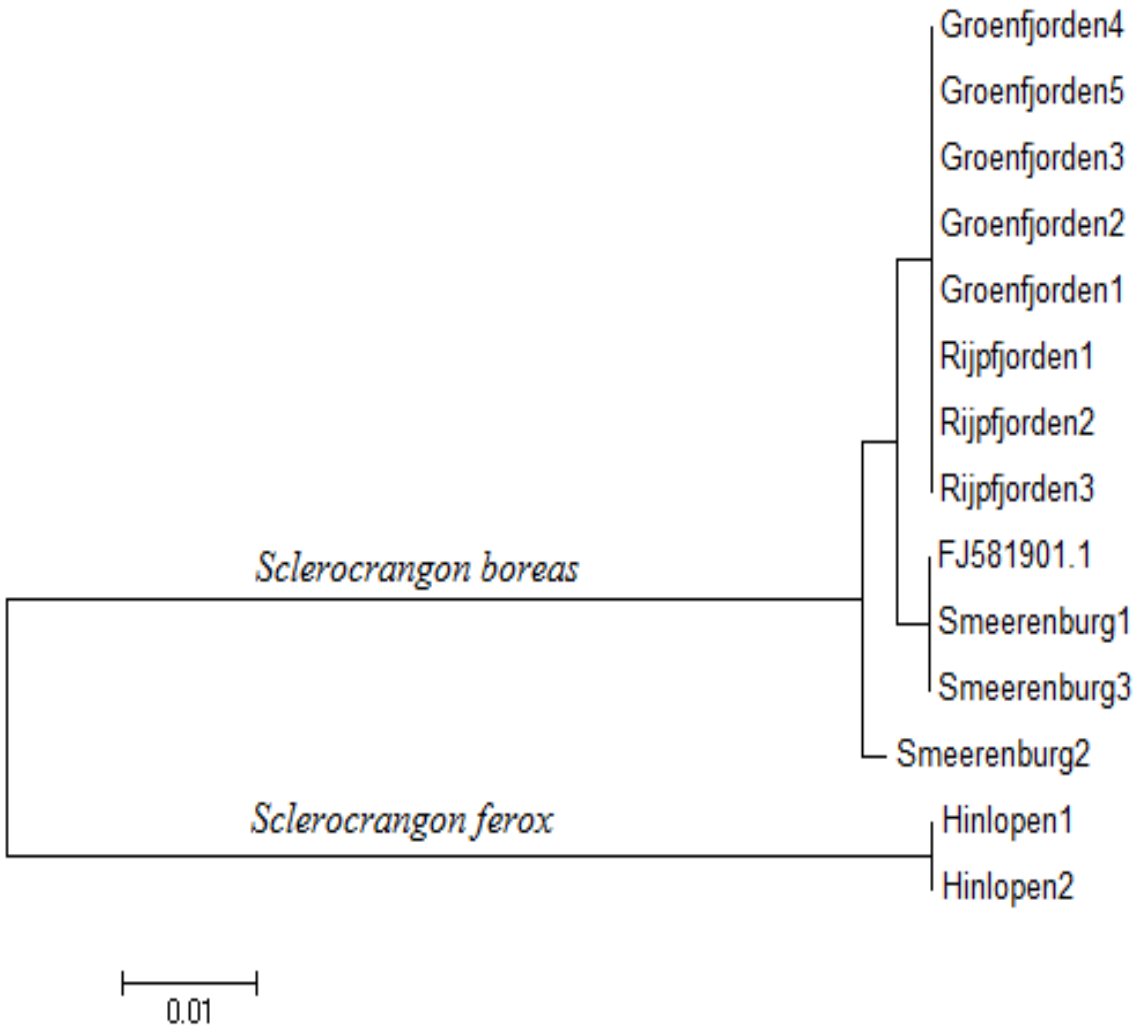

Figure 11: DNA barcoding results of *Sclerocrangon* spp. from Svalbard presented as a Neighbor-Joining tree. Scale bar 0.01, gives a genetic distance of 1 %. This gives an actual genetic distance between *S. boreas* and *S. ferox* on 13.7 %.

The sequences, used to make the tree (Figure 11) had a length of 658 basepairs after the primers were cut off. The scale bar of the figure is 0.01 which gives a genetic distance of 1 %. The results show that the genetic distance between different populations of *S. boreas*, give population signal in three groups, but they show no system between the fjords.

The specimen from the Gulf of St Lawrence, taken from GenBank, is identical to two of the *S. boreas* specimens sampled in Smeerenburg.

Between the two species, *S. boreas* and *S. ferox*, there is a genetic distance of 13.7 %. This value was calculated in MEGA5.

3.4 Parasites

The parasites were most frequently abundant on the pleopods, Figure 12A-C.

Figure 12A-C: A, *Sclerocrangon boreas* female with eggs and parasites on the pleopods marked with arrows, B, and C, shows pictures of the parasites on the pleopods, with a 1 mm scale on B, and a 500 µm scale on C. Photos, Lise Kristin Bjørdalsbakke.

After the genetic analysis was done, the parasite sequences gave one hit in BLAST. 18S were used as an attempt to find which species the parasite represented. BLAST gave the same results for both CO1 and 18S where CO1 gave the species while 18S gave the same family. This hit was *Crangonobdella spitzbergensis*, with a 99 % match for CO1 (Appendix 3) citing an unpublished article "A new arctic *Pisicolidae* from Svalbard waters" by Kolb, J. B. 18S gave a hit with 97 % match on the same family *Piscicolidae* (Appendix 4). Most likely the species name given as match in GenBank is a "nomen nudum", a species that is not formally described.

Figure 13 and 14 show all the specimens of *S. boreas* and *S. ferox* collected and how many of them that was infected with parasites. About 14 % of both species were infected (Figure 13 and 14).

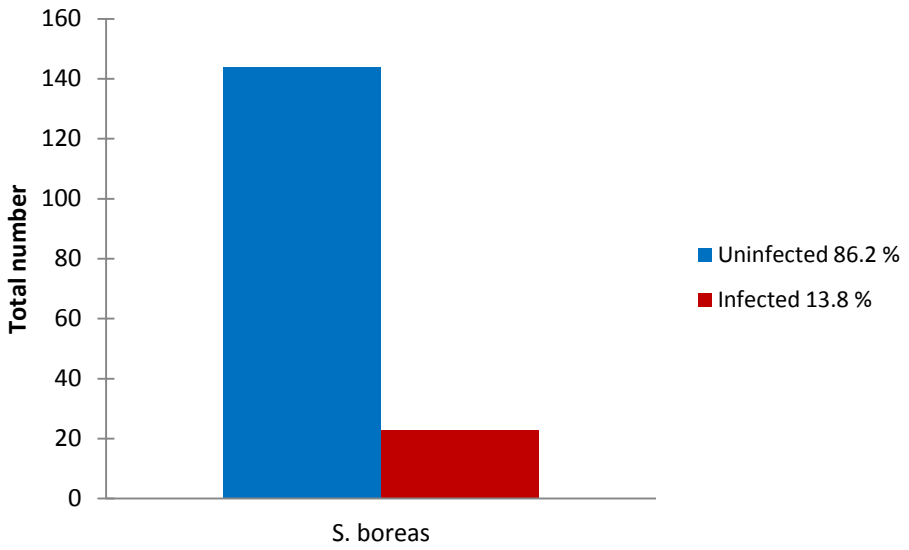

Figure 13: The percentage of infected specimens of *Sclerocrangon boreas*.

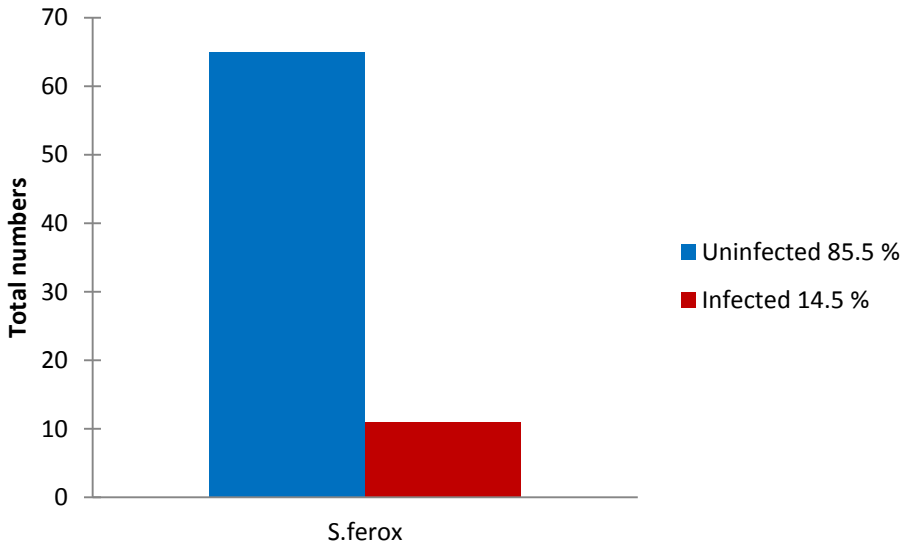

Figure 14: The percentage of infected specimens of *Sclerocrangon ferox*.

Figure 15 and 16 shows which specimens that are infected with parasites. It is the same distribution of the specimens as in the other figures, (Figure 5-9) the females being larger than males. Only difference is that all the *S. boreas* species are put together in the same graph in Figure 15. Figure 15 shows that for *S. boreas* the parasites are found in the entire size distribution, in both males and females, except in the smallest males and largest females. The prevalence of parasites is higher in males, 15 in total, compared to eight specimens of females (Table 1). Figure 16 shows that when it comes to *S. ferox* there are also most parasites on the males, eight in total (Table 1). Parasites are also found on the larger females, on three specimens (Table 1), but are most prevalent on the smaller males.

For both species parasites are most prevalent on males, 62.5 % for *S. boreas* and 72.7 % for *S. ferox*.

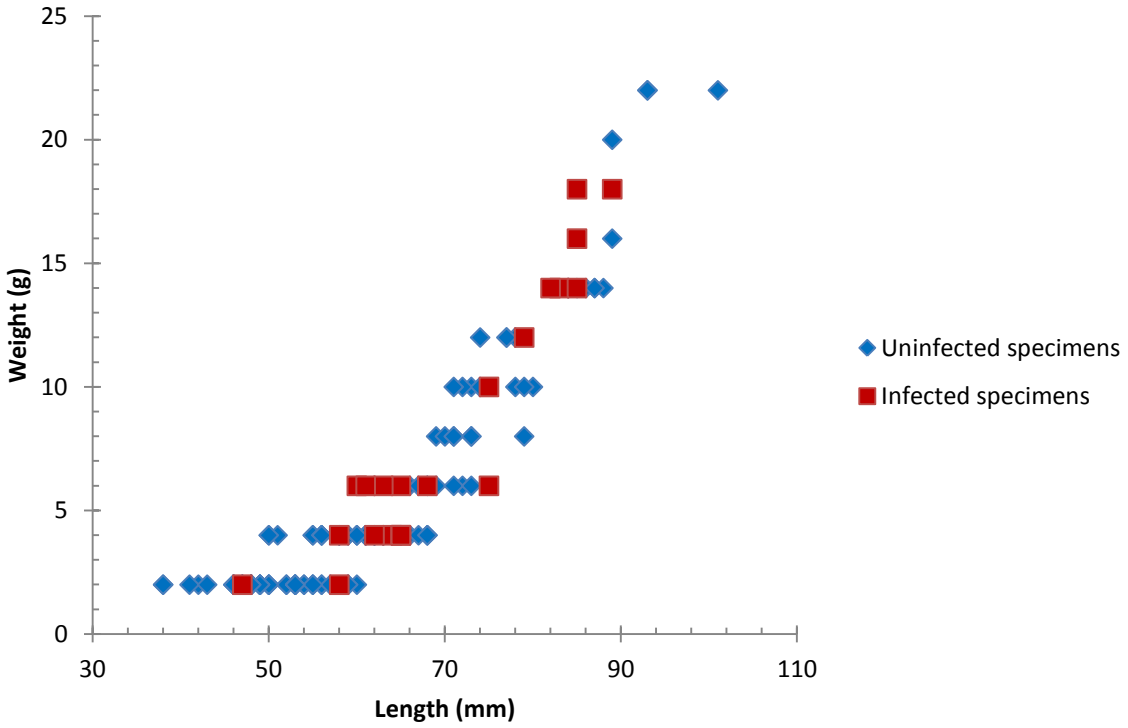

Figure 15: Uninfected and infected *Sclerocrangon boreas* as a function of length and weight.

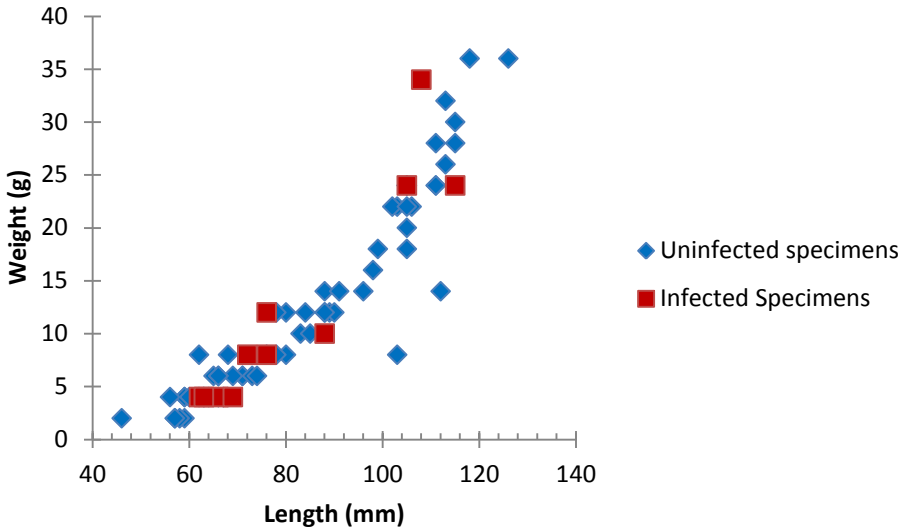

Figure 16: Uninfected and infected *Sclerocrangon ferox* as a function of length and weight.

4. Discussion

Weslawski (1987) described *S. boreas* as an indicator of Atlantic water masses and *S. ferox* as an indicator of Arctic water masses. This description implies where it is expected to find these two species in Svalbard waters. The west Spitsbergen current influence the entire west coast of Spitsbergen and all the way north and east Nordaustlandet, bringing warm Atlantic water masses to these locations (Figure 4). In Hinlopen there is a mix of warm Atlantic-, and cold Arctic water masses. Based on this it was expected to find specimens of *S. boreas* along the west coast of Spitsbergen and north to Nordaustlandet, following the west Spitsbergen current, and *S. ferox* in the colder areas in Hinlopen.

On the cruise in September 2011 these expectations proved to be correct when *S. boreas* were mostly found on the west coast of Spitsbergen and also at Rossøya and in Rijpfjorden further north, all of the locations following the West Spitsbergen current. *Sclerocrangon ferox* were only found in Hinlopen. The median dorsal spines (Figure 1 and 2) were used to distinguish between the two species. In accordant with Sainte-Marie et al. (2006) were the distribution of *S. boreas* quite scattered which also were registered during the sampling on the cruise in September 2011. The location where we obtained most specimens of *S. boreas* was in Grønfjorden, on a locality which have been sampled before, by Jørgen Berge in the autumn 2010, being a promising indicator that there are specimens of *S. boreas* in at least some amount.

Some of the females in the present material sampled at Svalbard in late September had eggs. The eggs were relatively large, between 2-3 mm. (Figure 12A-C), compared to the parasites which are between 0.5-1 mm. Lacoursière-Roussel and Sainte-Marie (2009) stated that the females carry relatively small clutches of large eggs in spring/summer, held beneath the abdomen, for about 9-12 months before they hatch in April to July. Their material was sampled in the Gulf of St. Lawrence, Canada.

4.1 Protandric hermaphrodite

All specimens sampled from each location gave one common result for both *S. boreas* and *S. ferox*. All of the figures (Figures 5-9) in the result part show the same pattern. All the largest specimens are females and the smaller ones are males. The sampling from both 2010 and 2011 showed the same pattern. Lacoursière-Roussel and Sainte-Marie (2009) suggested that *S. boreas* is a protandric hermaphrodite, based on the greater size of females relative to males and a perceived sex ratio bias in favor of males at small shrimp sizes and in favor of females at the population level. The result from the present study confirms that the males are relatively smaller than the females, supporting the findings done by Lacoursière-Roussel and Sainte-Marie (2009).

As shown in Figure 5 there is a clutch of four females together with smaller males. Also in Figure 9 one female is smaller than the other females. These specimens can be a source of error; they might be sexed wrong. Another explanation might be that these specimens have

had an early sex transitional phase. These specimens are the only findings challenging the suggestion that this species is a protandric hermaphrodite.

However, due to the clear pattern shown in the distribution of males and females it can be concluded that these species are protandric hermaphrodite as stated in the first hypothesis, H1.

In the present study the specimens were measured from the rostrum to the telson, while Sainte-Marie et al. (2006) measured the cephalothorax length (CL) (Figure 1). This makes comparing the measurements difficult. Sainte-Marie et al. (2006) suggested that males may be ≥ 4 years old with a CL at 17 mm, while the females may be ≥ 6 years old with a CL length at 29 mm. This also supports the suggestion that *S. boreas* is a protandric hermaphrodite, with the females both older and larger than the males.

Important information to bring to the fishing industry is that the result, from the cruises in 2010 and 2011, support the suggestion about the species being a protandric hermaphrodite. This means that the large profitable specimens are females, and in some cases with roe. Considering this is a species with few eggs, a K-strategist, and it takes 6-7 years for the specimens to grow into "catchable size" it is easy to destroy the population due to overfishing. This is important considering the survival of the population if the fishing industry again will show interest and try a profitable fishery on these species. These findings are supported by Sainte-Marie et al. (2006) who claims *S. boreas* has attracted interest from the fishing industry due to its large size and palatability, but due to its scattered distribution and low catch rate and apparent scarcity of large shrimps making profitable fishing unlikely.

4.2 Generalist vs. specialists

All specimens sampled of both *S. boreas* and *S. ferox* were dissected and the stomach content was analyzed. Figure 10 shows the results from the gut content analysis for both *S. boreas* and *S. ferox* at the different locations. It shows the frequency of occurrence of the different food items. For all locations the stomachs that were empty and the ones with prey that was unidentifiable are taken out of the figure (Figure 10). As Table 2 shows, a large percentage of the specimens had either empty stomach or unidentifiable stomach content.

Figure 10 show that the largest groups of prey are crustaceans, echinoids and ophiuroids in Grønfjorden, Rossøya and Hinlopen, locations where most of the specimens were sampled. On the other locations there were very few specimens, and some of the stomachs were empty. In Rijpfjorden only two specimens had stomach content possible to analyze. These two gives the result that ophiuroids and bivalves had been eaten and is not a very credible result for an entire population. The figure also shows that both species are eating several types of prey.

In Birkely and Gulliksen (2003a) *S. boreas* was found to be the most opportunistic species of the two species examined, when eight out of ten prey categories were recorded. The highest prey intake was polychaetes, and it was only in *S. boreas* infaunal mollusks appeared. They also found that females fed more on infaunal organisms, while males used epibenthic

organisms as a food source. In the 2011 sampling analyses on the stomach content were not preformed considering which sex the specimens were. Therefore a comparison with these results is not possible. Birkely and Gulliksen (2003a) also concluded that differences in the diet in *S. boreas* were primarily because of depth and secondarily by sex.

In the report written by the students during course AB-321 at UNIS in 2009 (Rabindranath et al. 2009), they obtained some of the same results as in this study. They sampled both *S. boreas* and *S. ferox* and respectively 38 and 33 stomachs were analyzed. The analyses showed that crustaceans dominated the diet of *S. boreas* while polychaetes dominated the diet of *S. ferox*. They concluded that *S. boreas* is a food generalist, while *S. ferox* is a food specialist.

In the present study it was observed during the analyses that individuals of both species mostly had one type of prey in their stomachs, as stated in the second hypothesis, H2. Hence to this observation it can be said that as species they are generalists, while as individual specimens they are specialists. Unfortunately there are no numbers to support this. This was only an observation made during the analyses. At the locations were most specimens were sampled, Grønfjorden, Rossøya and Hinlopen, several types of prey were identified. Showing that the species has quite a variable diet, but in all locations the pattern that the specimens in most cases only had one type of prey in their stomachs were observed.

Sources of error, potentially compromising the output of the results, are the following: They were done by six students during course AB-321, inducing variation, since some were precise while others were not in the identification, observations and cataloging of the specimens. There were also differences in taxonomic knowledge, giving variations in how well the identification of prey in the content was done. This is the reason there are no results showing both the degree of fullness in the stomachs, and if there were one or several types of prey in the stomachs.

4.3 Genetic analyses

The purpose of the genetic analyses was to obtain a genetic signal to see if there are different population signatures at the different locations.

The results from the genetic analyses are shown in Figure 11, and are quite surprising. The expectation was that it would be quite a difference, genetically, between the populations sampled at different locations. Due to these species don't have a pelagic larva stage, as suggested in the third hypothesis, H3.

That was not the result, instead they showed that the specimens sampled in Grønfjorden and Rijpfjorden, roughly 540 km following the west Spitsbergen current (Figure 4) (www # 2), were an exact match with identical sequences. In the specimens from Smeerenburg there were more differences. Two specimens, Smeerenburg1 and Smeerenburg3 (Figure 11), were identical and they were also similar to the specimen from The Gulf of St Lawrence, Canada (Radulovici et al. 2009). The fact that the specimen from Canada is an exact match to two

specimens from Smeerenburg supports the findings showing a lack of a clear population structure on Svalbard.

The last specimen from Smeerenburg, Smeerenburg2 stood out in the NJ tree (Figure 11). It had four different bp from the other two from this location. These differences were manually double-checked.

The two *S. ferox* specimens sampled in Hinlopen, were an exact match, with identical sequences, and gave the same results when put into BLAST. Both came back with an 87 % match to the species *Sclerocrangon boreas*. After searching for *S. ferox* in BLAST, several times, the conclusion were that it is most likely not been done genetic analyses on this species. The NJ tree (Figure 11) shows the two specimens of *S. ferox* joined together as a distinct group away from the rest, with a genetic distance of 13.7 % to *S. boreas*.

For both *S. boreas* and *S. ferox*, the CO1 gene was used for the analyses. An explanation for these results is that DNA barcoding and the CO1 gene is good to separate between species, but not adequate to separate specimens within the same species (Hebert et al. 2003a). The results from Hebert et al. (2003a) showed that intraspecific divergences rarely were greater than 2 %, and in most cases less than 1 %. Since most nucleotide positions are constant in comparisons of closely related species a modest rate of 2 % of sequence change will cause 12 diagnostic nucleotide differences in a 600 bp comparison of species (Hebert et al. 2003b).

It can be concluded that DNA barcoding and the CO1 gene do not together give ample information of genetic differences within populations, but it will still be able to give a genetic signal on differences between populations. Another explanation for the results is that even though *S. boreas* has no pelagic larva stage, the specimens can follow the west Spitsbergen current along the west coast of Spitsbergen, all the way to Nordaustlandet, to Rijpfjorden and Rossøya (Figure 4) and therefore mix the populations.

The results indicate that the specimens of *S. boreas* from Smeerenburg are distinguished from Grønfjorden and Rijpfjorden, and there were also variation within the specimens sampled in Smeerenburg. The specimens from Grønfjorden and Rijpfjorden were an exact match with identical sequences, but due to DNA barcoding and the CO1 gene not being adequate, the results do not give a basis to conclude whether the populations have different genetic signatures, as suggested in H3.

4.4 Parasites

Genetic analysis for the parasites was only done on parasites taken from *S. boreas* specimens. Parasites were also observed on *S. ferox*, but none were analyzed.

The CO1 sequences from the parasites returned a match on a species from BLAST, most likely being a “nomen nudum”, an undescribed species. However it cannot be ruled out that it is a known species, were genetic analysis never has been done. The parasites were found on

both *S. boreas* and on *S. ferox*, on both males and females and on both large and small specimens. For both species they were most prevalent on the males, 62.5 % for *S. boreas* and 72.7 % for *S. ferox*. Considering the result from this study it is reasonable to conclude that this parasite is eggs from a piscicolid (fish) leech.

5. Conclusions and future aspects

5.1 Conclusions

- Due to the clear pattern shown in the distribution of males and females it can be concluded that these species are protandric hermaphrodite.
- In the present study it was observed during the analyses that individuals of both species mostly had one type of prey in their stomachs. Hence to this observation it can be said that as species they are generalists, while as individual specimens they are specialists. Unfortunately there are no numbers to support this.
- The results indicate that the specimens of *S. boreas* from Smeerenburg are distinguished from Grønfjorden and Rijpfjorden, and there were also variation within the specimens sampled in Smeerenburg. The specimens from Grønfjorden and Rijpfjorden were an exact match with identical sequences, but due to DNA barcoding and the CO1 gene not being adequate, the results do not give a basis to conclude whether the populations have different genetic signatures.
- Parasites were found on both *S. boreas* and on *S. ferox.* Genetic analyses, using CO1 and 18S, of the parasites conclude that they are cocoons from a piscicolid (fish) leech. The identity of this species has not been found.

5.2 Future aspects

The size distribution should be done measuring the cephalothorax length, to be able to compare results with other studies, both when it comes to protandric hermaphrodite and also looking at the age of the specimens.

To study the prey preference the analyses should be done more adequate methods, for both species, being more consequent in the performance and in the identification, observations and cataloging of the specimens.

New methods must be established which are more specific than DNA barcoding and the CO1 gene to be able to look more thoroughly at population difference within species.

The parasite encourages more research focusing on biology and long time effects on the host.

6. References

6.1 Literature

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3. GenBank (accessed the 22th of November 2011). http://www.ncbi.nlm.nih.gov/genbank/

4. BLAST® (accessed the 6th of November 2011). http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome

7. Appendix

7.1 Appendix I

Appendix I: Sampled locations and methods used in each of them.

Date	Location	Stn.	Log	Latitude	Longitude	Depth	Equipment
17.09.11	Grønfjorden	491	8345.539	7801.56374 N	01409.11498 E	62.52	Triangular dredge
17.09.11	Grønfjorden	492	8346.228	7802.09877 N	01407.48941 E	63.78	Triangular dredge
17.09.11	Grønfjorden	493	8346.701	7802.50135 N	01406.40468 E	39.19	Triangular dredge
17.09.11	Grønfjorden	494	8348.457	7801.52373 N	01408.99970 E	49.43	Triangular dredge
17.09.11	Grønfjorden	495	8348.996	7801.98827 N	01407.83323 E	58.62	Triangular dredge
17.09.11	Grønfjorden	496	8349.448	7802.34087 N	01406.61113 E	44.54	Triangular dredge
17.09.11	Grønfjorden	497	8351.194	7801.52615 N	01409.21100 E	62.44	Triangular dredge
17.09.11	Grønfjorden	498	8351.631	7801.89904 N	01408.16820 E	63.08	Triangular dredge
17.09.11	Grønfjorden	499	8352.043	7802.22730 N	01407.04393 E	59.15	Triangular dredge
18.09.11	Smeerenburg	506	8460.311	7941.34037 N	01105.72348 E	191.19	Diving
18.09.11	Smeerenburg	506	8461.914	7941.31677 N	01105.02736 E	145.22	Diving
20.09.11	Hinlopen	522	8667.706	7937.90082 N	01856.23096 E	323.33	Bottom trawl start
20.09.11	Hinlopen	522	8668.422	7938.32835 N	01853.05764 E	323.57	Bottom trawl stop
24.09.11	Rijpfjorden	563	9036.407	8011.57969 N	02211.91762 E	36.59	Triangular dredge
24.09.11	Rijpfjorden	564	9036.987	8011.22704 N	02212.13537 E	45.05	Triangular dredge
24.09.11	Rijpfjorden	565	9037.596	8011.60396 N	02211.65454 E	37.33	Triangular dredge
24.09.11	Rijpfjorden	568	9066.551	8022.66527 N	02203.41023 E	263.29	Bottom trawl start
24.09.11	Rijpfjorden	568	9066.927	8022.31738 N	02204.24163 E	260.28	Bottom trawl stop
25.09.11	Rossøya	573	9127,937	8040.83683 N	01940.80084 E	90,67	Bottom trawl start
25.09.11	Rossøya	573	9128,490	8040.54408 N	01943.61700 E	93,91	Bottom trawl stop
27.09.11	Isfjorden	588	9510,543	7834.91786 N	01628.59655 E	160,85	Bottom trawl start
27.09.11	Isfjorden	589	9511,221	7835.48767 N	01630.36805 E	162,22	Bottom trawl stop

7.2 Appendix II

Appendix II: Primers used, with name and length.

Primer	Fragment length	Sequence 5' → 3'	Direction	Reference
LCO1490	Ca 700 bp	GGT CAA CAA ATC ATA AAG ATA TTG G	Forward	Folmer et al. 1994
HCO2198		TAA ACT TCA GGG TGA CCA AAA AAT CA	Reverse	Folmer et al. 1994
18A1	Ca. 1800 bp	CCT ACT TCT GGT TGA TCC TGC CAG T	Forward	Wollscheid & Wägele 1999
1800		TAA TGA TCC TTC CGC AGG TT	Reverse	Wollscheid & Wägele 1999
700F		GTC TGG TGC CAG CCG CG	Forward	Vonnemann et al. 2005
1155R		CCG TCA ATT CCT TTA AGT TTC AG	Reverse	Wollscheid & Wägele 1999

7.3 Appendix III

Appendix III: The 99 % match given using CO1 on the parasite.

Crangonobdella spitzbergensis cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial

GenBank: DQ889681.1

FASTA Graphics

Go to:

```
LOCUS       DQ889681                 788 bp    DNA     linear   INV 22-
AUG-2006
DEFINITION  Crangonobdella spitzbergensis cytochrome c oxidase subunit I
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ACCESSION   DQ889681
VERSION     DQ889681.1  GI:112434103
KEYWORDS    .
SOURCE      mitochondrion Crangonobdella spitzbergensis
  ORGANISM  Crangonobdella spitzbergensis
            Eukaryota; Metazoa; Annelida; Clitellata; Hirudinida;
Hirudinea;
            Rhynchobdellida; Piscicolidae; Crangonobdella.
REFERENCE   1  (bases 1 to 788)
  AUTHORS   Kolb,J.B.
  TITLE     A new artic Piscicolidae from Svalbard waters
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 788)
  AUTHORS   Kolb,J.B.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-2006) Institute of Biology, University of
Kassel,
            Heinrich-Plett-Str. 40, Kassel 34132, Germany
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7.4 Appendix IV

Appendix IV: The 97 % match given using 18S on the parasite.

Malmiana buthi 18S small subunit ribosomal RNA gene, partial sequence

GenBank: DQ414276.1

FASTA Graphics Popset

Go to:

```
LOCUS       DQ414276                1762 bp    DNA     linear   INV 26-
OCT-2006
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            sequence.
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VERSION     DQ414276.1  GI:89520944
KEYWORDS    .
SOURCE      Malmiana buthi
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            Rhynchobdellida; Piscicolidae; Malmiana.
REFERENCE   1  (bases 1 to 1762)
  AUTHORS   Williams,J.I. and Burreson,E.M.
  TITLE     Phylogeny of the fish leeches (Oligochaeta: Hirudinida:
            Piscicolidae) based on nuclear and mitochondrial genes and
            morphology
  JOURNAL   Zool. Scr. 35 (6), 627-639 (2006)
REFERENCE   2  (bases 1 to 1762)
  AUTHORS   Williams,J.I. and Burreson,E.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-FEB-2006) Environmental and Aquatic Animal
Health,
            Virginia Institute of Marine Science, PO Box 1346,
Gloucester
            Point, VA 23062, USA
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                     /product="18S small subunit ribosomal RNA"
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