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Bio-3950 Master thesis in Biology
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Front page photo by Maria T. Antonsen

Photo of female *Microsetella norvegica*

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Tromsø, November 2014

Maria Terese Antonsen

Summary

Annual population dynamics and vertical distribution of the small (< 0.6 mm) hapacticoid copepod, *Microsetella norvegica*, was investigated through monthly sampling at station Svartnes in Balsfjord, Northern Norway from May 2013 to June 2014. *M. norvegica* is a pelagic particle feeder, and distributed from temperate waters to sub-arctic fjords, but frequently underestimated because of its small size. The species is therefore often overlooked and its biology poorly understood. In order to sample all stages of *M. norvegica*, from nauplii to adult appropriately, we used both a WP-2 net with 90 µm mesh size (175-50 and 50-0 m), and a 20 L Go-Flo water bottle (5, 20, and 50 m depth). Nauplii and copepodite stages from CI to adult were identified to determine total abundance, population structure, vertical and seasonal distribution. There were great differences in abundances and stage distribution dependent on sampling method. The Go-Flo bottle sampled all stages, from nauplii to adult stages, while the WP-2 net collected mostly adult stages. The discrepancy in sampling efficiency between the two gears is also clearly reflected when comparing the abundances. In June 2014 the total maximum abundance of *M. norvegica*, when integrating Go-Flo from 50-0 m, was 7.8×10^6 ind. m^{-2}. When sampling with WP-2 we found a total maximum abundance of 1.2×10^6 ind. m^{-2}. Minimum abundances of *M. norvegica* were found in January 2014. Females carrying egg-sac were observed in April to June and in August. Females carrying egg-sac peaked in June, with a total abundance of 754 270 ind. m^{-2}, when integrating Go-Flo from 0 to 50 m. Also, total abundance of females and egg-sacs in the upper 50 m was used to calculate the egg-sac:female ratio, where we found the highest ratio at 1.6 in May. Nauplii and small copepodite stages peaked in the upper 50 m in spring and summer, suggesting that their main reproductive period takes place in May and June. The older copepodite stages from CIV to adults dominated in winter from October to March. To investigate the body condition of females during winter, carbon content of *M. norvegica* was measured, and was found to have a strong seasonality. The lowest carbon content, when normalized to length, was found in January, and was highest in May. *M. norvegica* was highly abundant year-round in Balsfjord, but the sampling design is crucial for more reliable determination of their true abundance and population dynamics. Improved quality of abundance estimates may be a first step towards improving our knowledge about the biology and ecological role of this tiny but potentially important copepod.

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Introduction

Copepods belong to the successful sub-group Crustaceans that comprise more than 10 000 species, occupying all types of habitat from marine to some few, but more limnic (Huys & Boxshall 1991). In general, the free-living form of copepods is one of the reasons why they have such a high success, considering their abundance and diversity (Huys & Boxshall 1991). Overall, pelagic copepods have unique features that contribute to their numerical dominance. A torpedo shaped body and powerful movement in the water column allows the pelagic copepods to easily detect and escape predators, and their efficiency of mate finding provide a high productive rate for each generation (Kiørboe 2011).

Zooplankton also represent an important link between lower and higher trophic levels in the food web (Hopkins et al. 1984). In Balsfjord the composition of zooplankton is well studied (Davis 1976, Tande 1982, Hopkins et al. 1984, Pasternak et al. 2000). These studies found that species such as the calanoid *Calanus finmarchicus* (Tande 1982) and *Metridia longa* (Grønvik & Hopkins 1984), and the cyclopoid *Oithona similis* (Pasternak et al. 2000) are the most abundant. Their investigations showed that the population dynamics of these species varied through a year. Reproduction started in April, March or May, depending on the copepods reproductive strategy (Tande 1982, Hopkins et al. 1984). Early copepodite stages were present during summer, while older copepodite stages dominated the population during winter (Tande 1982. Hopkins et al. 1989). Species such as *C. finmarchicus* descends and hibernate through winter, while *M. longa* remained active in the water column (Tande 1982, Hopkins et al. 1984). Studies by Madsen et al. (2008) and Svensen et al. (2011) have shown that small copepod species could be important in the upper water strata, when *Calanus* spp. descends. However, the role of small copepods is not well understood.

Sampling of zooplankton, using recommended nets of 200 µm mesh size, has led to a severe under-estimation of small copepods, ranging from 200 µm to 800 µm in body length (UNESCO 1968, Riccardi 2010). The small copepod *Microsetella norvegica* (Boeck 1865) belong to the sub-group Harpacticoida (Huys et al. 1996), which is mainly bottom living, but *M. norvegica* is one of the few pelagic living species (Huys & Boxshall 1991). *M. norvegica* escape the net because of their small size (< 1 mm), and because of their elongated body (Pasternak et al. 2000).

Davis (1976) reported *M. norvegica* for the first time in Balsfjord, and concluded that *M. norvegica* was the most numerous copepod during winter. Unfortunately, Davis (1976) only presented a qualitative evaluation of species numbers. However, recent studies from the Inland Sea of Japan and from Godthåbsfjord in Greenland found *M. norvegica* to be highly abundant and dominating during late summer (Uye et al. 2002, Arendt et al. 2012, Koski et al. 2014). During winter, *M. norvegica* stayed active at deeper depths, and they ascended closer to the surface during spring to feed and reproduce (Uye et al. 2002, Koski et al. 2014). Compared to large calanoid copepods, there are few studies conducted with a focus on *M. norvegica*. Therefore the knowledge about their ecology and biology is limited. Further knowledge on activity and development of *M. norvegica* during different season in a sub-arctic fjord is also limited. As the species is highly abundant in the few other investigated systems it is most likely an important species, also in the Balsfjord zooplankton community.

Aim of study

The overall aim of this study was to increase the knowledge of the small and under estimated hapacticoid *M. norvegica*. The present study aims to sample and identify stages of *M. norvegica*, to increase the knowledge on this species population dynamics and abundance. To do this, two methods suitable for study of smaller zooplankton were combined. The seasonal population dynamics, as seen from abundance and stage distribution was investigated through a whole year, and the carbon content of the small harpacticoid copepod *M. norvegica* was investigated from October to March.

Biology and ecology of *Microsetella norvegica*

Systematics and distribution

Microsetella norvegica (Boeck 1865) belong to the order Harpacticoida, comprising more than 3000 species (Huys & Boxshall 1991). Harpacticoida is one of ten orders, belonging to the sub-class Copepoda (Huys et al. 1996). Other important groups include Calanoida and Cyclopoida. Marine harpacticoida are primarily bottom-living, but *M. norvegica* is one of the few pelagic species (Huys & Boxshall 1991). The genus Microsetella consists of two species, *M. norvegica* and *M. rosea*, where *M. norvegica* is the only one distributed in northern waters (Davis 1976, Boxshall 1979). *M. norvegica* has several synonyms, *Setella norvegica* (Boeck 1864), *M. atlantica* (Brady & Robertson 1873), *Ectinosoma atlanticum* (Brady & Robertso 1873), and *M. brevifida* (Giesbrecht 1891), although *M. norvegica* is the most used in literature today (Swadling 2013).

The two *Microsetella* species have similar features, but a few characteristics separate them. *M. norvegica* is smaller, ranging from 0.35 – 0.6 mm as adult, while *M. rosea* can become up to 0.8 mm. *M. rosea* has a caudal rami setae twice as long as its body length, where *M. norvegica* has a setae which is slightly shorter than its body lenght, but the setae may be broken due to handling. *M. norvegica* is dark and "chimney red", while *M. rosea* is pink (Swadling 2013). Common to the *Microsetella* spp. is their small size, their elongated body, and internal oil droplets that slows their rate of sinking (Huys & Boxshall 1991).

Distribution and abundance of *M. norvegica* has been investigated for several areas (Hirakawa 1974, Davis 1976, Uye et al. 2002, Arendt et al. 2012). The species have a world-wide distribution in marine and brackish water, and is known to be numerical in coastal waters, ranging from temperate to sub-arctic waters (Uye et al. 2002, Swadling 2013). Studies on *M. norvegica* were often carried out in fjords or coastal areas (Davis 1976, Arendt et al. 2012, Koski et al. 2014) where the species is highly abundant, but further studies is needed to improve our knowledge about their distribution and potentially important role in the ecosystem (Uye et al. 2002).

Life-cycle, seasonal distribution and feeding strategy

There is limited knowledge on the biology of *M. norvegica*, but a few previous studies provide some information about their life cycle, feeding strategy, abundance through seasons, and reproduction strategies (Hirakawa 1974, Huys & Boxshall 1991, Uye et al. 2002, Arendt et al. 2012, Koski et al. 2014).

The life cycle of *M. norvegica* has been studied in a few regions only. In Balsfjord, Northern Norway, their reproduction typically starts late in March to April, and they ascend closer to the surface (Davis 1976). Females are sac spawners, and carry their eggs until hatching in May and June, but Koski et al. (2014) suggest that they may detach the egg-sac before they hatched. Hirakawa (1974) described the stages of *M. norvegica*, as the species has 6 stages of nauplii and 6 stages as copepodites (hereafter referred to as CI to CV). The last copepodite stage is the adult stage (CVI), as male or female (Hirakawa 1974). Davis (1976) observed that *M. norvegica* reproduced a second time in late August and early September. A study from the Inland Sea of Japan (16.8 to 27.4 °C) report that *M. norvegica* evolved from nauplii to adult stage, from May to September (Uye et al. 2002). In fjords in the area of Tromsø, Northern Norway, *M. norvegica* was observed to survive and stay active during winter as stage CV and CIV, but mostly as adult (Davis 1976). During winter from October to February, the species is located in deeper water where the stay active until spring-bloom (Koski M. unpubl. in Koski et al. 2014). Based on current knowledge, it is not certain if *M. norvegica* is iteroparous or semelparous.

Because of their omnivorous feeding strategy, *M. norvegica* is frequently found close to the surface during spring bloom and summer, when they feed on motile and sinking particles (Uye et al. 2002, Koski & Kiørboe 2005). Nauplii are observed to be very active during feeding, by moving their appendages, while copepodites and adult stages are less active (Uye et al. 2002). Considering the high growth rate and omnivorous feeding of *M. norvegica*, they contribute considerably to the secondary production throughout the year (Uye et al. 2002, Arendt et al. 2012). The small copepod is predated by larger zooplankton, fish larvae, and fish (Arendt et al. 2012). Their mortality rate through the year has rarely been investigated, but is estimated from Greenland by Koski et al. (2014) who estimated rates for females to be low.

Arendt et al. (2012) investigated seasonal distribution of *M. norvegica* in a sub-arctic fjord in Greenland. *M. norvegica* was observed to dominate the mesozooplankton, representing up to 80 % of the total biomass from July to September (Arendt et al. 2012). The high average abundance (maximum: 408 125 ± 161 387 nauplii m^3 and 91 995 ± 6 864 copepods m^3) in this study indicate that the small copepods are very numerous (Arendt et al. 2012). The authors consider *M. norvegica* as one of the most important zooplankton species after *Calanus* spp. (Arendt et al. 2012), and similar quantity of *M. norvegica* has been observed in Japan (Hirakawa 1974).

Anatomy

The body of *M. norvegica* is divided into a prosome and an urosome (Figure 1). These body parts, however, are not easily distinguishable, because, typically for many Harpacticoida, the body of this species is fusiform and torpedo-shaped, tapering at each end (Figure 1) (Huys & Boxshall 1991). The prosome is divided into cephalothorax, bearing first pair of swimming legs, and three free prosomites. The urosome consists of five visible somites in females and six in males (Figure 2). *M. norvegica* develop one appendage of somite and/ or setae for each successive moult stage (Huys et al. 1996). Their rostrum is short and bent ventrally (Huys & Boxshall 1991).

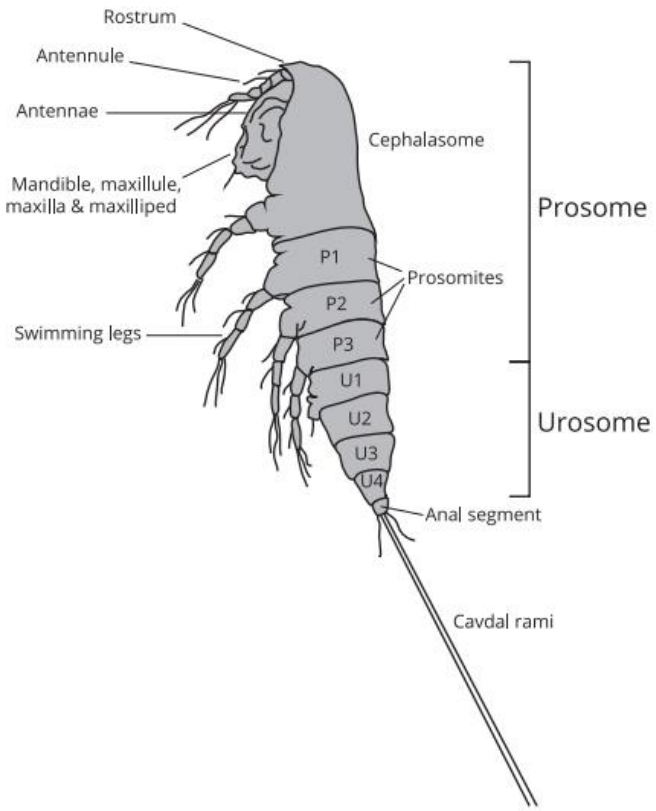


Figure 1: Illustration of *Microsetella norvegica* with taxonomical description of the body parts, modified from Hirakawa (1974).

The antennules (first antennae) in *M. norvegica* females are 6 segmented, and 14 segmented for males. The antennules in males are modified (geniculated) and are used for grasping females (Huys & Boxshall 1991). Antennae (second antennae) have a 3-segmented exopod (Huys & Boxshall 1991). The next, paired appendages in the head region include: the mandible, maxillule (first maxilla), maxilla (second maxilla) and maxilliped. These appendages have complex structures, with many limbs and spines, and are adapted for catching food (Huys & Boxshall 1991).

On the end of the last somite (last urosomal somite, anal somite) there are two pairs of caudal setae. The inner (longer) is approximately as long as the body. The outer setae are approximately 1/3 of the body. The two caudal setae are typically put close together, looking like one caudal setae. The caudal setae can also be broken, though it is not important for the identification.

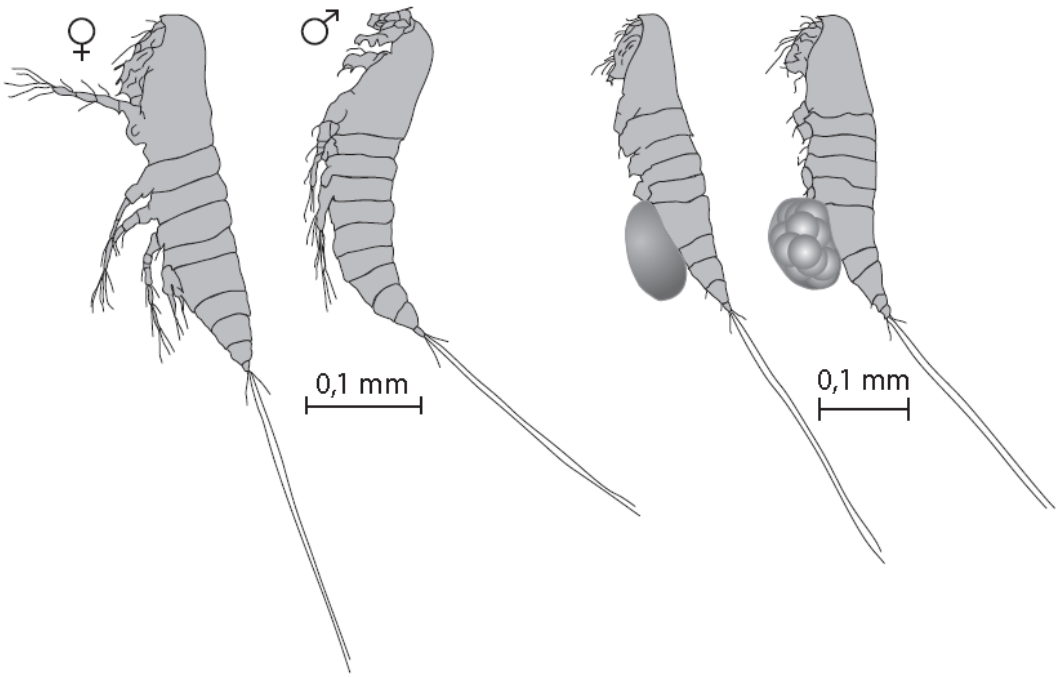


Figure 2: Adult *Microsetella norvegica*. Male and female to the left, and females bearing egg-sac to the right. Egg-sacs were observed to develop a bulky shape within a few days before hatching, modified from Hirakawa (1974).

M. norvegica has five pairs of swimming legs, and are developed to move the animal in the water column (Huys & Boxshall 1991). The first pair of swimming legs on the somite is associated with the cephalothorax (Figure 3). The three other swimming legs are placed on three free prosomites (Huys & Boxshall 1991).

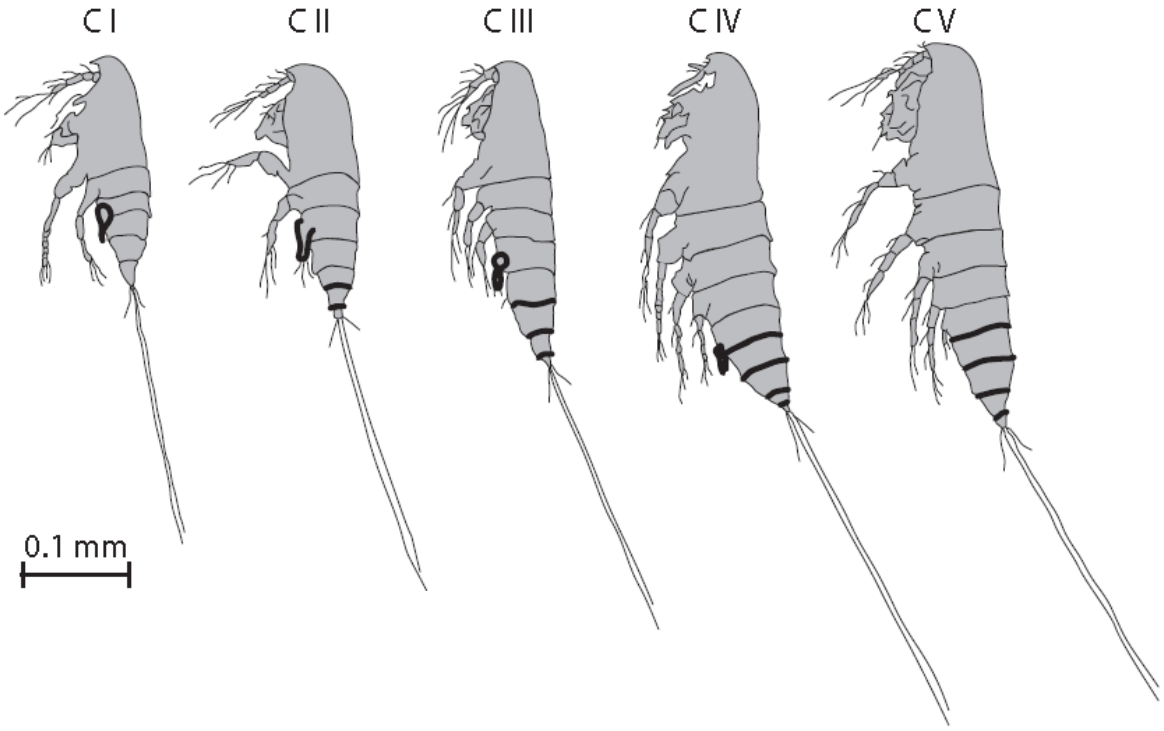


Figure 3: Illustration of copepodite stages of *Microsetella norvegica*, from stage CI to stage CIV. Somites and other features are highlighted, illustrating developmental differences between stages, modified from Hirakawa (1974).

Stages of *M. norvegica* nauplii range from 0.084 mm as NI to 0.187 mm as stage NVI (Figure 4). Nauplii stage NI have a red eye on the antennules and develop caudal setae, but no somites or swimming legs are developed (Hirakawa 1974). To identify the different stages of nauplii a microscope is required, and was considered to work demanding for the present investigation. Therefore, all stages of *M. norvegica* nauplii were counted as one group.

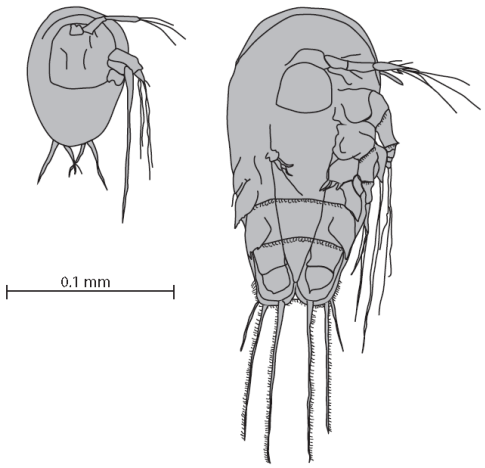


Figure 4: Illustration of *Microsetella norvegica* nauplii stages, as first nauplii stage (NI) and the sixth nauplii stage (NVI). Modified from Hirakawa (1974).

Material and methods

Study site

Balsfjord is characterized as a cold-water fjord with temperature ranging from 1-7° throughout the year (Eilertsen et al. 1981). Strong estuarine circulation in the fjord is driven by seasonal stratification and wind (Wassmann et al. 2000). A shallow sill of 35 m at the mouth of the fjord, limits water inflow into Balsfjord (Eilertsen et al. 1981). However, because of the tide there is an advection of species from the outer coastal water (Wassmann et al. 2000). The fjord is also influenced by a yearly inflow of warmer and salter Atlantic water, starting in the spring. This leads to a significant water exchange into the deep-water basin (Eilertsen et al. 1981).

Balsfjord is a suitable sampling site for several reasons. Balsfjord is a well-studied fjord, and background information is available on ecosystem processes and throughout the year (Eilertsen et al. 1981, Tande 1982, Grønvik & Hopkins 1984, Hopkins et al. 1984, 1989, Bax & Eliassen 1990, Pasternak et al. 2000). The proximity to UiT allows one-day cruises. Balsfjord is located in Northern Norway, ca. 10 km south of Tromsø (Figure 5). The fjord is 5 km on its widest, and stretches 46 km between higher mountain that ranges up to 1500 m

(Forwick & Vorren 1998). Balsfjord is made up by two basins, where the outermost basin is 130 m deep, and the innermost basin is 190 m deep (Forwick & Vorren 1998).

The material was collected at station Svartnes (69°22'N, 19°06'E) with RV *Hyas*, approximately once per month. Station Svartnes is located in the innermost basin (Figure 5), which is the deepest part of the fjord.

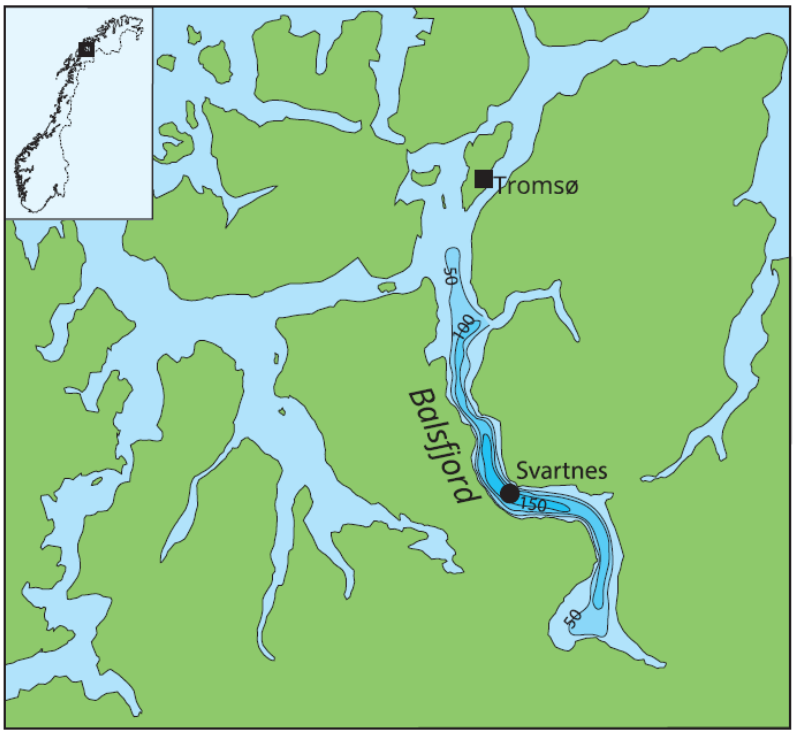


Figure 5: Map of Balsfjord, Northern Norway and station Svartnes, where *Microsetella norvegica* was sampled monthly from May 2013 to June 2014.

Sampling of *Microsetella norvegica*

Seasonal population dynamics of *Microsetella norvegica* in Balsfjord was investigated by monthly sampling using RV *Hyas* at station Svartnes. A total number of 13 sampling events were spread throughout the year, from May 2013 to June 2014 (Table 1).

Tabel 1: One-day cruises were carried out from May 2013 to June 2014. Sea temperature (°C) is presented as a mean from 0 to 50 m. Fluorescence was measured from 0 to 175 m.

Cruise	Date	Temperature °C	Fluorescence max
1	27.05.2013	NO CTD	NO CTD
2	28.06.2013	5.6	7.6
3	23.08.2013	8	1.9
4	19.09.2013	8.6	1.4
5	15.10.2013	8.4	3.4
6	19.11.2013	6.4	0.2
7	08.01.2014	4.5	0.2
8	30.01.2014	3.2	0.2
9	03.03.2014	2.6	0.2
10	25.03.2014	2.4	0.8
11	29.04.2014	3.2	12
12	06.05.2014	3.4	15
13	16.06.2014	6.7	NO DATA

I tested two methods to sample *M. norvegica* appropriately: a WP-2 net (175-50 m, 50-0 m) with 90 µm mesh size and a 20 or 30 liter Go-Flo bottle (5, 20, 50 m) (Figure 6). The WP-2 net was 57 cm in diameter and had attached a filtering cod-end. Before the net was taken onboard it was rinsed with seawater from a hose, to include all specimens that were attached on the net. Before transferring the sample to a 10 L plastic bucket, the cod end was rinsed carefully to avoid damage on the specimens. The samples were concentrated using a 90 µm

sieve, and transferred to a 250 ml PVC plastic bottle. The WP-2 net was equipped with a closing mechanism to allow discrete sampling from 175 to 50 m. A weight was dropped when the net was at 54 m to ensure closing at 50 m. Clogging during sampling was somewhat problematic during spring bloom both years. The filtration volume was estimated from WP-2 diameter and the total sampling depth.

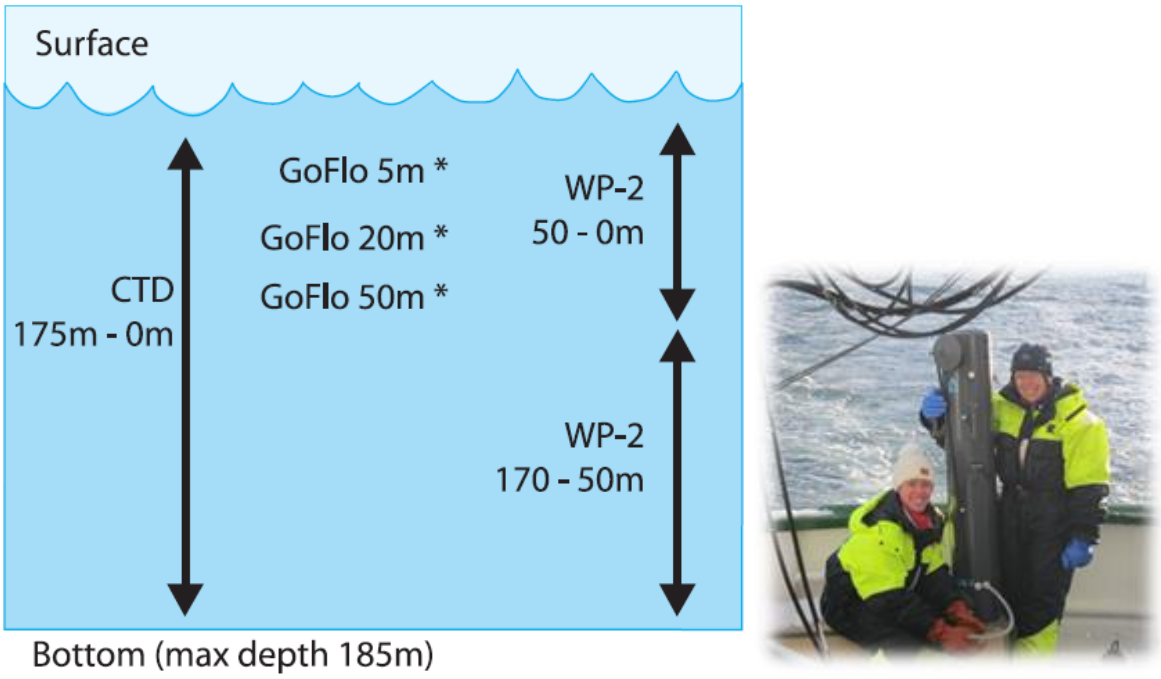


Figure 6: Illustration of sampling strategy at station Svartnes (left). The CTD recorded temperature (°C), fluorescence, density, and salinity from 0 to 175 m. To collect *Microsetella norvegica* we used a Go-Flo bottle (5, 20, 50m), and a WP-2 net (0.3 m/s) at two depth intervals (175-50 m, 50-0 m). The photo to the right illustrates sampling when using a Go-Flo.

The Go-Flo collected eggs and copepodite stages of *M. norvegica*, and was to obtain quantitative data of all stages. A 20 L Go-Flo was normally used to ease handling, except from one cruise when we used a 30 L Go-Flo (15.10.2013). The content of the Go-Flo bottle was carefully concentrated over a 20 µm sieve lowered in seawater, using a silicone tube. The concentrated sample was transferred to a 100 ml PVC plastic bottle. In the laboratory, approximately 3 hours after sampling, all samples were fixed with Zoofix (buffered formaldehyde, hexamethylenetetramine and propandiol) at 4 % final concentration. All handling of the samples was performed in a ventilated hood using gloves and lab coat.

Enumeration and species identification

Before enumeration and identification, the samples were transferred from Zoofix by sieving the sample through a 20 µm sieve. The sample was transferred to filtered sea water before microscopical analysis. The plastic bottle that contained the sample was carefully rinsed with filtered seawater to collect all content. All samples were aerated approximately 24 hours to reduce fumes from the buffered formaldehyde, making it less hazardous to work with. Waste water from the samples was collected in a bottle and transferred to a suitable container. All treatment of the samples was conducted in a ventilation hood, using gloves and lab coat.

Dilution of samples with filtered seawater was often necessary with WP-2 samples due to high densities, but not with the Go-Flo samples. Before taking out subsamples from a WP-2 sample, it was important to homogenize the whole content. When homogenizing a sample it was stirred in a figure eight, using a stirring rod. Subsamples were collected by using a pipette, set to 3 ml. Each subsample was transferred to a counting chamber that could contain four subsamples. Identification and enumeration continued until at least 300 individuals were counted, for all stages combined. The same procedure was used for the Go-Flo samples. Though, the Go-Flo samples seldom contained more than 300 individuals, and the whole sample was counted. Occasionally, a few samples contained more than 300 individuals, and the Go-Flo sample was diluted, using the same procedure as for the WP-2 samples.

Different stages of *M. norvegica* were identified using a stereo microscope (Leica MZ 16 at 40 – 100 x magnification). Stages was enumerated and identified as nauplii, stage CI and up to CV, and for adult stages of female or male copepods (Hirakawa 1974, Boxshall 1979). Stage CIV and CV was counted as one group because these two stages require dissecting of their first leg pair and analysis of these in the microscope (Table 2). The procedure was too demanding for the present investigation.

Table 2: Table of *Microsetella norvegica* leg development in different copepodite stages (Hirakawa 1974).

Stage	Leg 1		Leg 2		Leg 3		Leg 4		Leg 5	
	Exopod	Endopod	Exopod	Endopod	Exopod	Endopod	Exopod	Endopod	Exopod	Endopod
CI	1	1	1	1	Rudimentary	Rudimentary	0	0	0	0
CII	2	2	2	2	1	1	Rudimentary	Rudimentary	0	0
CIII	2	2	2	2	2	2	1	1	Rudimentary	Rudimentary
CIV	2	2	2	2	2	2	2	2	Rudimentary	Rudimentary
CV	3	3	3	3	3	3	3	3	1	1
Female	3	3	3	3	3	3	3	3	1	1
Male	3	3	3	3	3	3	3	3	1	1

Nauplii were counted as one group, and not identified for their different stages, because of its small size (Arendt et al. 2012). Females with egg-sac and egg-sacs that were not attached to a female were counted in separated groups. To identify the different stages of *M. norvegica* the somites were counted, because they grow one somite from one stage to the next (Table 3), except from stage CIV to CV. The total body lenght of *M. norvegica* could also be used for stage identification.

Table 3: Somites on the prosome and urosome of *Microsetella norvegica* (Hirakawa 1974).

Stage	Prosome	Urosome	Total somites	No. caudal satea
CI	3	2	5	2
CII	4	2	6	2
CIII	5	2	7	4
CIV	5	3	8	6
CV	5	3	8	6
Female	5	4	9	6
Male	5	5	10	6

After identification and enumeration of *M. norvegica* stages in the sub-samples, total abundance of the samples was calculated to a concentration of m^{-3} and integrated to m^{-2}. In order to compare Go-Flo samples with the WP-2 sample taken at 50-0 m, the vertical distribution was integrated as m^{-2} to represent the vertical distribution. Integrating the Go-Flo

samples was done by multiplying the calculated m^{-3} as a trapezoid integration of each depth, assuming the sample depths represented the mid-point in each interval.

Determination in carbon content of females

In October 2013, early January, February, and May 2014, an additional WP-2 sample for live animals was taken from 50-0 m. Samples were transferred to a 10 liter plastic bucket, and the cod end was rinsed carefully, but never sieved or fixed. The bucket was covered with a black plastic bag, and carefully transported back to the laboratory. The bucket was kept in a refrigerator overnight, to ensure low temperature and no light, to imitate their natural environment.

Each time 600 female individuals (without egg-sac) were collected and divided into two group's á 300. By using suitable tools (Figure 7), females were scooped out and rinsed in a petri-dish containing filtered seawater, and repeated twice. The purpose is to remove particles and algae in the ambient water (Satapoomin 1999, Arendt et al. 2012).

To relate the carbon content to individual size of the females it was necessary to measure the total body length of 50 females, from each of the sampling dates. I used a stereo microscope (model Zeiss Discovery V20, Eyepiece 10x) to measure the females in µm. Further, the content of the petri dish, containing 300 females, were filtered onto a pre-combusted GF/F filter. When rinsing the petri dish, it was used a glass pipette and MilliQ water. MilliQ water was purified through UV radiation and the amount of total organic carbon was reduced, and the sample would not get contaminated. GF/F filters, containing 300 *M. norvegica* females each, was folded, packed in aluminums foil, and placed in separate zip lock bags. The samples were frozen at -20 °C until analysis.

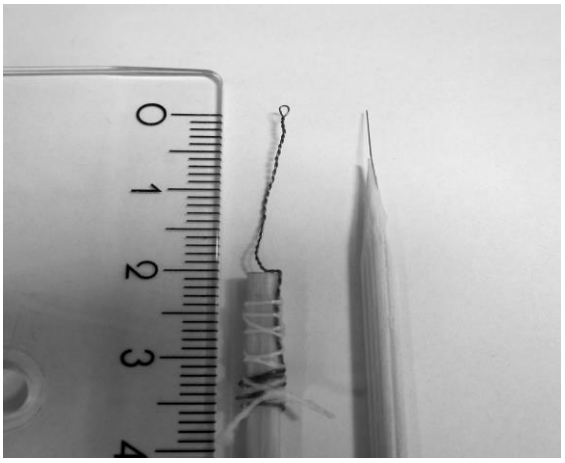


Figure 7: Handmade tools for handling of *Microsetella norvegica*. Tools were used to scoop out live specimens, and to transfer females from subsamples to petri dishes. The ruler illustrates the size of the tools.

A CHN analyzer (Lab Leeman Elemental Analyzer 440) was used to determine particulate organic carbon (C) and nitrogen (N) of the females. Filters were placed in test tubes in a dry heater (60 °C) for 24 hrs. to remove moist. To remove inorganic carbon the GF/F filters were fumed with concentrated HCl for 24 hrs. before 24 hrs. in a dry oven (60 °C). The sample was analyzed with the CHN analysor, and C and N content was calculated using acetanlilide as standard. The average carbon weight (C, µg) of female *M. norvegica* were plotted to provide a length and carbon relationship.

As a comparison to own measured carbon content of female body carbon weight (C, µg) for *M. norvegica*, I used an equation based on the relationship between their total body length (BL, µm) and carbon body content (Uye et al. 2002);

(1): $C\ (\mu g) = 2.65 \times 10^{-6} \times BL\ (\mu m)^{1.95}$

Statistics

Principal component analysis (PCA) was used to look into the patterns of stage distribution of *M. norvegica*. PCA is a type of a multivariate analysis. The aim for a PCA was to reduce variables, finding patterns in the data, and represent biological dissimilarity, by being less sensitive to outliers. When applying a PCA the data should be normally distributed. The data were not normally distributed, so the data were ranked. Ranking data resulted in equal differences from the raw-data matrix. Outliers and other information could have been lost, but the most significant patterns were revealed (Quinn & Keough 2002). SYSTAT 13 (Cranes Software International Ltd, Chicago, IL, USA) was used to apply PCA.

Software used

Calculations were done in Microsoft ® Excel 2010 ® for Windows (Microsoft Corp. Redmond. WA, USA). Graphs were made by using SYSTAT 13 (Cranes Software International Ltd, Chicago, IL, USA). CTD data was converted using SEATERM © (Sea-Bird Electronics, Inc. Washington, USA). SBE Data Processing-Win32 (Sea-Bird Electronics, Inc. Washington, USA) was used for treatment of data.

Results

Hydrography

Hydrographic profiles were obtained monthly from June 2013 to June 2014 from 0 to 175 m at Svartnes, Balsfjord. Julian day was calculated on the x-axis and present measurements of salinity, temperature (°C) and fluorescence (µg/L) from June 2013 to June 2014. At station Svartnes there was observed three periods with lower salinity, respectively in June 2013, January 2014 and June 2014. Between the three periods, there were periods with higher salinity. The salinity (0-50 m) ranged from a maximum of 33 in March 2014 to a minimum of 22 in February 2013. Late in January 2014 it was observed fresh water in the surface layer (Figure 8A).

During summer, from May to September, warmer surface temperatures were measured, reaching a maximum surface temperature at 12 °C in June 2013. During winter, from January to April 2014, the temperature was approximately 4 °C (Figure 8B).

Fluorescence showed two periods of increased fluorescence, with a strong bloom in May 2014. Data from June 2013 indicated remains of a bloom. A fluorescence maximum of 15 at 13 m was measured at Julian day 500 to 550, and is equivalent to May and June 2014 (Figure 8C).

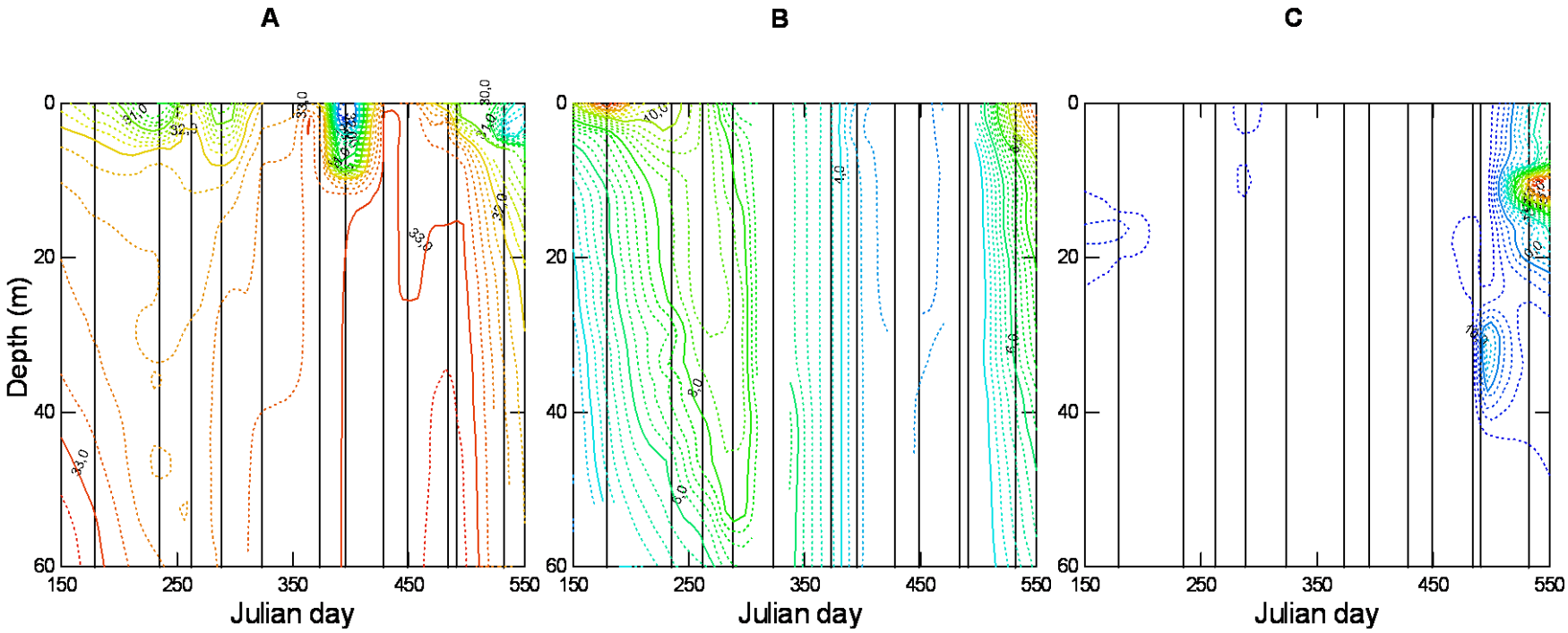


Figure 8: Salinity (A), temperature °C (B) and fluorescence (µg/L) (C) profile from station Svartnes, Balsfjord. Black lines present the monthly measurements, from June 2013 to June 2014 (no cruise in July 2013). Julian day is calculated from the first to the last day of measurement. Julian day 150 represents May 2013, Julian day 250 represent September 2013, Julian day 350 represent December 2013, Julian day 450 represent March 2014, and Julian day 550 represent June 2014. No data from available from July 201

Comparison of WP-2 net and Go-Flo for sampling *Microsetella norvegica*

Integrated Go-Flo (m^{-2}) and WP-2 net (m^{-2}) from 50 to 0 m was compared to investigate the sampling efficiency of both methods. Go-Flo samples resulted in general in higher abundances (m^{-2}) and sampled nauplii and all copepodite stages of *M. norvegica* (Figure 9). WP-2 net sampled poorly on nauplii and small copepodite stages, and the samples were dominated by adult stages.

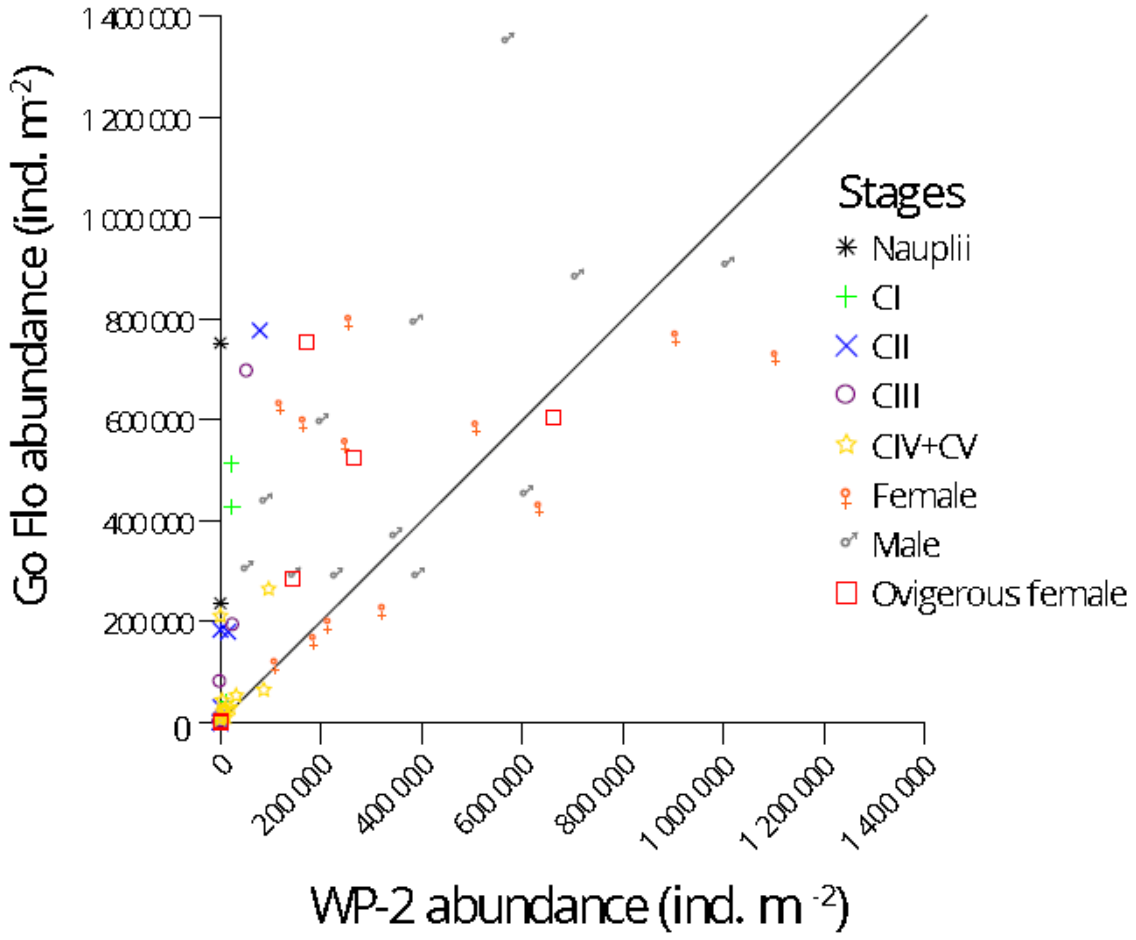


Figure 9: Comparison of WP-2 net and Go-Flo sampling from integrated Go-Flo (m^{-2}) and WP-2 net from 50 to 0 m. *Microsetella norvegica* was sampled from June 2013 to June 2014 at Svartnes, Balsfjord. Each symbol represents abundance and developmental stage.

Go-Flo sampled both nauplii and copepodite stage CI to CIII, while the WP-2 net sampled little or none of these stages (Table 4). Females, ovigerous females, males and CIV/CV were a few times sampled in higher abundance by using WP-2.

A ratio (G:W) of organisms collected with the Go-Flo to WP-2 was calculated, and the numbers illustrate the difference in sampling efficiency between the methods (Table 4). A ratio of 1 signifies that Go-Flo and WP-2 sampled *M. norvegica* equally. We found that Go-Flo sampled up to 8 times as much as the WP-2 for total abundances.

Go-Flo represents better abundances (m^{-2}) and developmental stages of *M. norvegica*. The WP-2 net from 0 to 50 m under-sampled and did not sample all developmental stages, and will therefore not be presented further. WP-2 from 50 to 175 m will however be presented because the result show the relative distribution of large stages below 50 m in the water column.

The integrated Go-Flo from 0 to 50 m provided a pattern for when the stages are present throughout one year (Table 4). Nauplii and ovigerous females were present from April to August. Stage CI was present from June to August, while CII and CIII were present from June to September. Adult stages and CIV/CV are presented throughout the year.

Table 4: Integrated (0-50 m) abundance (10^3 ind. m^{-2}) of *Microsetella norvegica* in Svartnes, Balsfjord, June 2013 to June 2014, sampled using a Go-Flo bottle (G) and WP-2 net, 90 µm, (W) from 50 to 0 m. A comparison of abundance obtained from the Go-Flo and WP-2 is given as G:W ratio, for all developmental stages, where the abundance of Go-Flo is divided on the abundance of the WP-2. Stages that were not present are given as 0.

Mth	**Tot m^{-2}**			**Nauplii**			**CI**			**CII**			**CIII**			**CIV, CV**			**F**			**M**			**F egg**		
	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W	G	W	G:W
Jun13	5223	629	8,3	1824	9	202	512	21	24	776	78	10	695	53	13	28,5	20	1,4	799	255	3,1	303	49	6,2	283	143	2
Aug	2156	1538	1,4	10	0	10	39	10	3,9	179	15	12	192	26	7,5	211	0	211	729	1102	0,7	792	385	2,1	2,7	0	2,7
Sept	1791	2002	0,9	0	0	-	2	0	2	30	0	30	19	8	2,5	64	86	0,7	268	904	0,8	907	1004	0,9	0	0	-
Oct	1933	826	2,3	0	0	-	0	0	-	0	0	-	0	0	-	27	10	2,7	555	248	2,2	1351	567	2,4	0	0	-
Nov	560	539	1	0	0	-	0	0	-	0	0	-	0	0	-	24,5	9	2,6	167	185	0,9	369	345	1,1	0	0	-
Dec	523	710	0,7	0	0	-	0	0	-	0	0	-	0	0	-	7	0	7	226	322	0,7	290	388	0,7	0	0	-
Jan14	423	254	1,7	0	0	-	0	0	-	0	0	-	0	0	-	12,7	2	6,5	119	108	1,1	291	143	2	0	0	-
Feb	498	452	1,1	0	0	-	0	0	-	0	0	-	0	0	-	9,5	11	0,9	199	214	0,9	289	227	1,3	0	0	-
Mar	1237	366	3,4	0	0	-	0	0	-	0	0	-	0	0	-	43,5	3	13	598	164	3,6	595	198	3	0	0	-
Apr	1695	1532	1,1	236	0	236	0	0	-	0	0	-	0	0	-	53	31	1,7	429	633	0,7	452	604	0,7	525	264	2
May	3093	1971	1,6	752	0	752	0	0	-	0	0	-	0	0	-	264	96	2,8	590	508	1,2	883	705	1,3	604	661	0,9
Jun	7840	1276	6,1	5306	870	6,1	427	24	18	183	0	183	80	0	80	21	5	4,1	631	118	5,4	438	86	5	754	173	4,4
Mean	2248	1008	2,5	677	73	9,2	82	5	16	97	7,8	12,4	82	7	12	64	23	2,7	484	397	1,2	580	392	2,2	181	103	1,7

Annual stage composition and abundance of *Microsetella norvegica*

Variation in annual stage composition and variation in depth distribution showed a clear pattern during a year (Figure 10). During summer, the abundance of *M. norvgica* increased substantially, with the highest abundances in June and August 2013, and with maximum abundance in June 2014 at 5 m depth (750 000 ind. m^{-3}, June 2013). From April to September the total average abundance using WP-2 (50-175 m) sampled 1440 ind. m^{-3}, while abundance from Go-Flo at 50 m sampled 20 000 ind. m^{-3}, 20 m sampled 52 300 ind. m^{-3}, and 5 m sampled 237 600 ind. m^{-3}. In winter, the total average abundance from October to March using a WP-2 (50-175 m) was 3150 ind. m^{-3}, while Go-Flo at 50 m sampled 20 900 ind. m^{-3}, 20 m sampled 21 160 ind. m^{-3}, and 5 m sampled 6500 ind. m^{-3}. The difference in abundance between the seasons indicates differences in depth distribution of *M. norvegica* from April to September and from October to March. The stage distribution of *M. norvegica* is also presented as percentage (Figure 10).

Nauplii and all copepodite stages of *M. norvegica* were present during summer, and the population of *M. norvegica* was found to have the highest abundance above 20 m. In June, both years, nauplii represented a large part of the total abundance of *M. norvegica*. Nauplii represented up to 46 % of the population in June 2013, and 70 % in June 2014. Early copepodite stages represented up to 6.5 % of the population in June 2013.

In winter, the population of *M. norvegica* decreased considerably. Adult stages from CIV/CV, females, and males were present during winter. The older stages were present in the whole water column, but with the highest abundance at 20 m and deeper. During winter the sex ratio of females and males was approximately 1:1. Males were the most abundant stage in October, presenting up to 70 % of the population, from 0 to 50 m. On average males represented up to 61 % of the population from October to Mach, from 0 to 50 m. Females represented 48 % of the population in March, from 0 to 50 m. The PCA is presented in appendix I because the results show the same trend of stage distribution as in Figure 10.

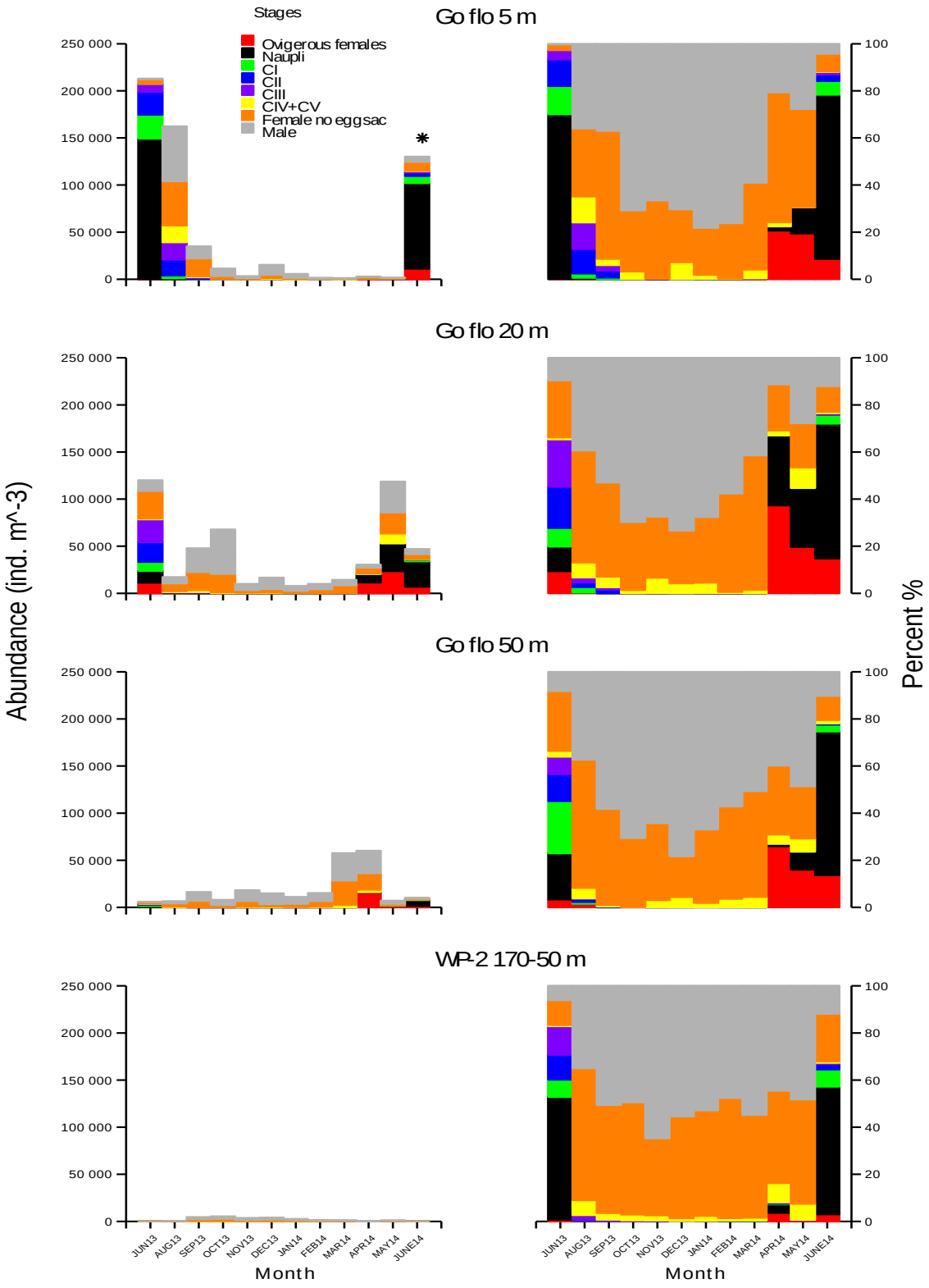


Figure 10: Stage composition and total abundance (m^{-3}), of copepodite stages and nauplii of *Microsetella norvegica*. Abundance is given on the left panel, while relative stage composition on the right panel. Because of high abundance in June 2014 at Go-Flo 5 m (*), the abundance of all stages at this date were divided by 5. No data from July 2013.

Female fecundity

Females of *M. norvegica* (n=30) was measured by their total body length (µm), and eggs per egg-sac were counted. Total body length of females ranged from 480 to 620 µm, and eggs per egg-sac ranged from 6 to 13 eggs. There was no correlation between female body length and the number of eggs per female (p = 0.9) (Figure 11).

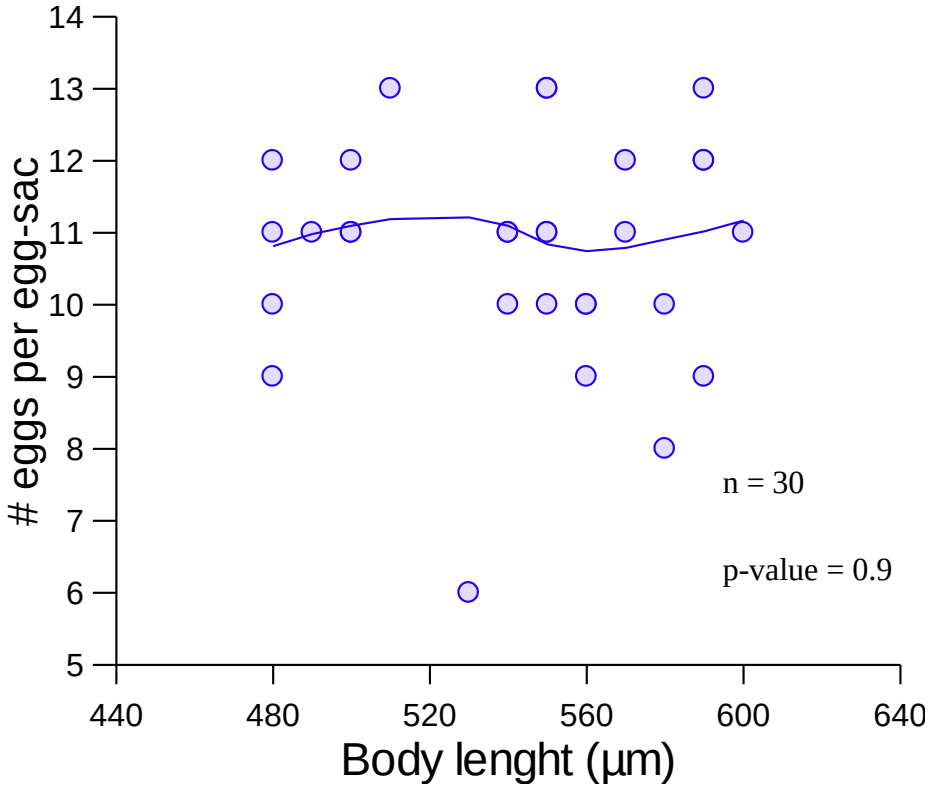


Figure 11: Body length (µm) and number of eggs per egg-sac for female *Microsetella norvegica*.

The total abundance of females and egg-sacs in the upper 50 m was used to calculate an egg-sac:female ratio (Table 5). A ratio above 1 indicates a higher number of egg-sacs than females in the water column. The number of egg-sacs was more than one and a half time higher than the number of females in May (1.62), and equal in April (1.05). Also, in June the number of egg-sacs was close to equal of the number of females (0.80).

Table 5: Abundance of integrated Go-Flo (10^3 ind. m^{-2}, 0-50 m) of females of *Microsetella norvegica*. Ratio indicates relationship between total egg-sacs and females.

Month	Female	Egg-sac	Ratio
January	119	0	0.00
February	199	0	0.00
March	598	1	0.00
April	954	1007	1.05
May	1193	1934	1.62
June	1385	1178	0.80
August	731	69	0.09
September	768	18	0.02
October	555	5	0.01
November	167	0	0.00
December	226	0	0.00

Carbon content of females

In October 2013, January, March and May 2014 female *M. norvegica* were sampled for analyses of organic carbon (C, µg ind.$^{-1}$) and nitrogen (N, µg ind.$^{-1}$). The individual carbon (C µg ind^{-1}) was highest in October (0.39 ± 0.01), and lowest in March (0.18 ± 0.04) (Table 6). The C:N ratio is given as atomic ratio, and ranged from 11.35 in October to 5.89 in May. To correct the individual carbon content for a variable in length, the carbon content was corrected for length by estimating a µg C/µg length (Table 6). The maximum and minimum carbon content was found in May (9.60 x 10^{-4}) and January (8.55 x10^{-4}), respectively.

Table 6: Carbon content (C, µg ind.$^{-1}$) and nitrogen content (N, µg ind.$^{-1}$) of *Microsetella norvegica*. All values are given as mean ± SD. C:N ratio is given as atomic ratio. The individual carbon content, corrected for length, is given as µg C/ µm lenght of *M. norvegica.*

Month	Carbon	Nitrogen	C:N	µgC/µm lenght
October	0.39 ± 0.01	0.04 ± 0.00	11.35 ± 0.04	9.15 x 10^{-4}
January	0.30 ± 0.04	0.04 ± 0.01	6.64 ± 1.53	8.55 x 10^{-4}
March	0.18 ± 0.04	0.03 ± 0.01	7.60 ± 0.95	9.00 x 10^{-4}
May	0.26 ± 0.01	0.05 ± 0.00	5.89 ± 0.46	9.60 x 10^{-4}

Carbon content (C, µg ind^{-1}) was measured directly for *M. norvegica* (Figure 12 A). As a comparison, carbon content was also estimated based on a length-carbon relationship (Uye et al. 2002) (Figure 12 A). The estimated carbon content was higher compared to the direct measurements, especially in March (0.41 µg C ind^{-1}) and in May (0.47 µg C ind^{-1}).

Total average body length of 100 *M. norvegica* females decreased from October to January, and increase in March and May (Figure 12B). Length of females of *M. norvegica* ranged from 340 µm to 590 µm. Average minimum length of 427 µm was observed in January, and average maximum length of 503 µm was observed in May.

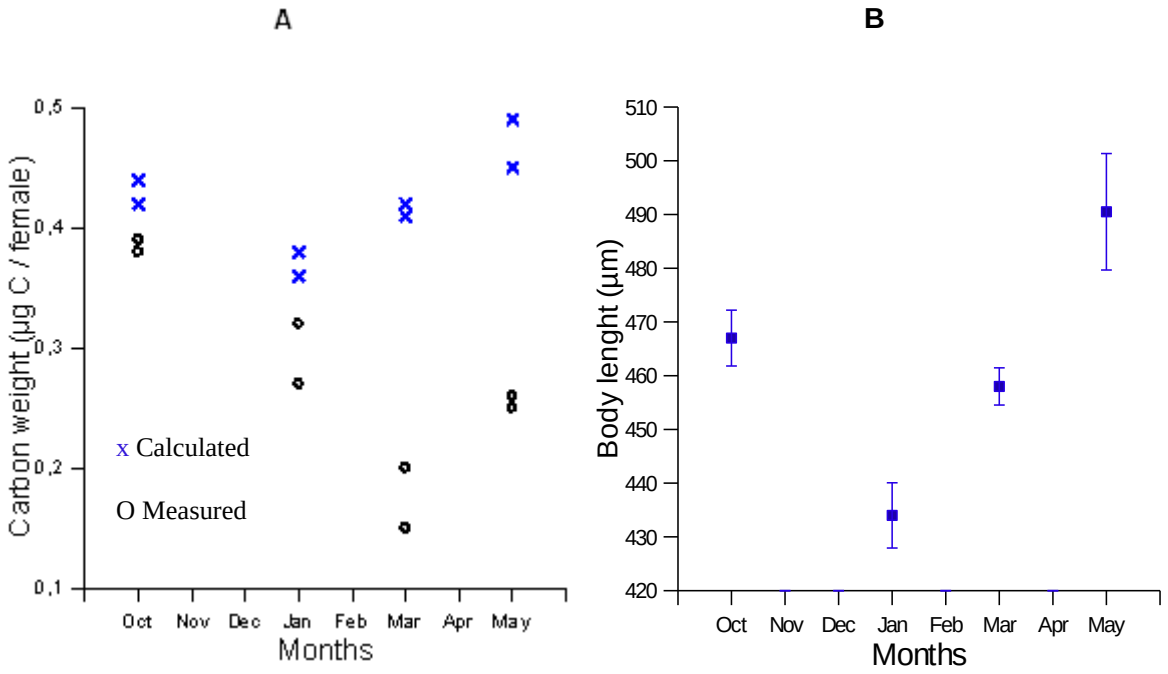


Figure 12 A: Relationship between mean carbon content (µg C) and mean total body length (µm) of females without egg-sacs of *Microsetella norvegica*. The blue crosses represent calculated carbon as a mean of 50 females by their total body length (Uye et al. 2002), and black circles represent the average measured carbon of 2* 300 females. B; A mean of total body length of 100 females are represented for each month.

Discussion

The study was conducted to gain knowledge on one of the less studied copepod species *Microsetella norvegica*. More specifically, the annual population dynamics of *M. norvegica* was investigated monthly from May 2013 to June 2014 in a sub-arctic fjord, Balsfjord.

Comparison of Go-Flo bottle and WP-2 net

For sampling mesozooplankton, the ICES zooplankton methodology manual (Harris et al. 2000) recommends a WP-2 with 200 µm mesh size in accordance with UNESCO (1968). Several studies, e.g. Gallienne & Robins (2001), found that small copepods, ranging from 200 µm to 800 µm in body length, are significantly under-estimated when sampling with 180-200 µm mesh size. As a result, ecosystem dynamics has been evaluated incorrect because of the

use of inappropriate methods (Gallienne & Robins 2001, Riccardi 2010). The sampling strategy should be designed in relation to the aim of the sampling, and with the organisms size in focus (Riccardi 2010). Small copepods, such as *M. norvegica* , < 1 mm, has consequently been underestimated when sampled with a WP-2 with 180-200 µm mesh size.

Recent studies suggest that 80 µm mesh size is suitable for sampling of small copepods in the marine environment (Gallienne & Robins 2001, Riccardi 2010). However, Koski et al. (2014) used 50 µm mesh size when sampling for small copepods, while Uye et al. (2002) sampled with 94 µm mesh size to study *M. norvegica*. Due to dense spring bloom in Balsfjord, I used a WP-2 with 90 µm mesh size to avoid clogging of the zooplankton net.

A large Go-Flo bottle is most often used to sample phytoplankton and mikrozooplankton. Nonetheless, in a study by Svensen et al. (2011), all stages of *Oithona similis* was sampled successfully by using a Go-Flo bottle. Due to previous reports of under sampling of small copepods, and to compare sampling efficiency, both Go-Flo (20 L) and a WP-2 (90 µm) were used to sample *M. norvegica* in Balsfjord in the present study.

The results showed that the WP-2 net under-sampled the total abundance of all stages of *M. norvegica*, and sampled poorly on both nauplii and early copepodite stages despite 90 µm mesh size (Table 4). The Go-Flo bottle sampled all stages of *M. norvegica*, with an average of 2.5 times higher, while the WP-2 net also under-sampled the adult stages. This was also found for small copepods in general by Svensen et al. (2011). For this reason, the results from the WP-2 net with 90 µm mesh size, do not represent seasonal abundance or stage distribution appropriately, and a large 20-30 L Go-Flo bottle is therefore recommended when sampling for small copepods such as *M. norvegica*.

Annual population dynamics and reproduction

Calculating the abundance and studying a species stage distribution is the first step towards gaining knowledge about a species potential importance, population structure, and life cycle. Estimating the species abundance is important, because we get an understanding of which species that are dominating in an ecosystem, also, the abundance could be used to estimate production in a system (Arendt et al. 2012). The species stage distribution reflects the

population structure and can describe species life cycle in relation to the environment and pelagic community. This information can later be used to improve our understanding of the food web in an ecosystem (Hopkins et al. 1989).

Davis (1976) did a study on overwintering copepods in Balsfjord. He used a WP-2 net with 64 µm mesh size, and samples were taken from the upper water column from September to March. He reported species such as *Calanus finmarchicus*, *Pseudocalanus minutus*, *Acartia longiremis*, *Oithona similis*, and *M. norvegica*, and concluded that *M. norvegica* was the most numerous copepod in Balsfjord. In terms of stages, there were mostly large copepodite stages, adult and ovigerous females presented in his study. Unfortunately, Davis (1976) only presented a qualitative evaluation of species numbers, and did not present the abundance. Also Pasternak et al. (2000) identified *M. norvegica*, but the abundance data was pooled with *Oithona* spp. The presented work was therefore motivated by the need for abundance data for this species in Balsfjord.

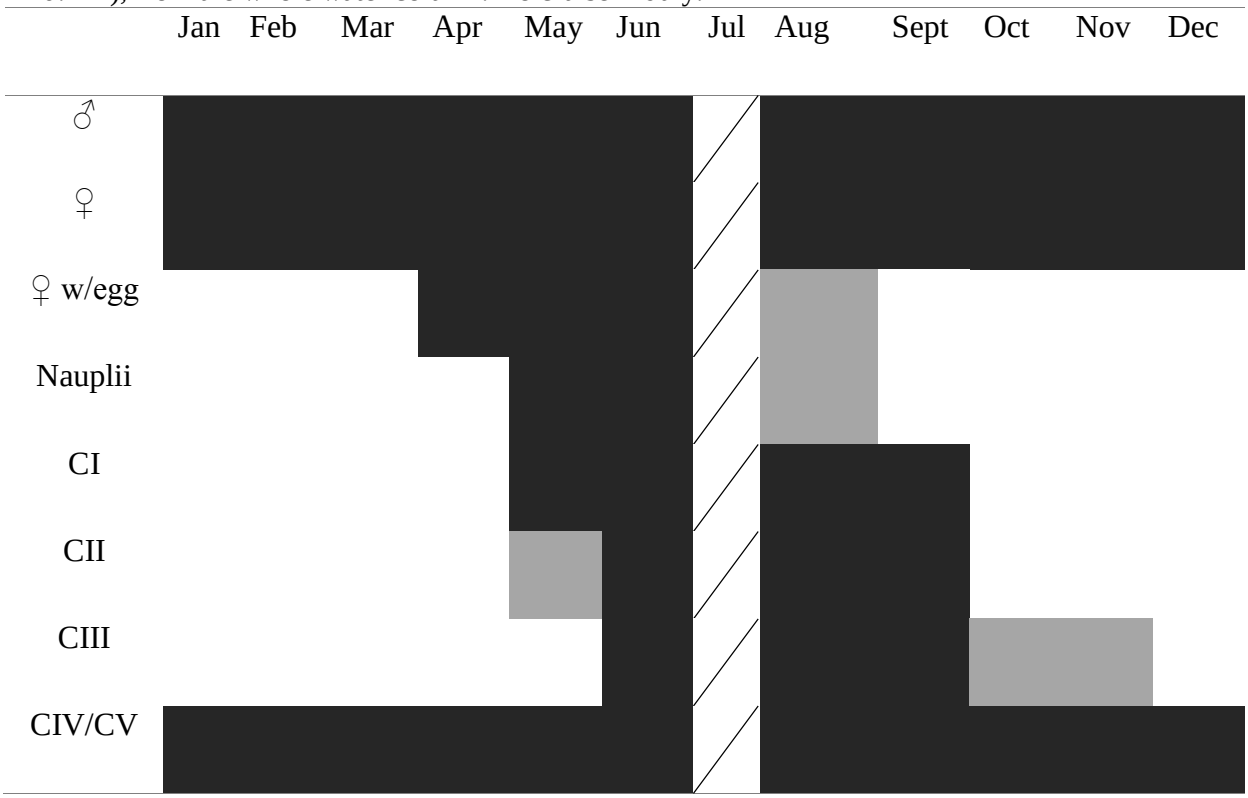
In the present study *M. norvegica* was found throughout the whole year in Balsfjord, and a considerable variation in seasonal population dynamic was found. The integrated (0 to 50 m) abundance of *M. norvegica* peaked in June 2014 at 5 m (253 x 10^4 copepodites m^{-2} and 530 x 10^4 nauplii m^{-2}) and the abundance remained high from May to September. During winter (October to April) the abundance of *M. norvegica* decreased, but was still high (Figure 10). A comparable study of copepodites of *Metridia longa*, found that the abundance from 0 to 175 m was lower in June (7200 ind. m^{-3}), and they peaked later in September (20 500 ind. m^{-3}) (Grønvik & Hopkins 1984). The present study supports the previous suggestions of *M. norvegica* being a highly abundant species. *M. norvegica* was highly abundant during the whole study period, and is most likely the most abundant species in Balsfjord. Abundance data on *C. finmarchicus* from Balsfjord was not available, as all published data present biomass found.

The abundance of copepods in Balsfjorden are high, and is compared with abundance of *M. norvegica* that was found in Godthåbsfjord in Greenland. Arendt et al. (2012) conducted a 5-year study on mesozooplankton in the sub-Arctic fjord in Godthåbsfjord on Greenland. The abundance of *M. norvegica* in Godthåbsfjord was high (~90 000 copepodites m^{-3} and ~400 000 nauplii m^{-3}) during the same period of sampling, but abundance of different stages

were not presented in the study by Arendt et al. (2012). *M. norvegica* dominated the copepod community 7 months of the year, while *Calanus* spp. dominated in May and June (Arendt et al. 2012). Species such as *O. similis* and *Pseudocalanus* spp. were also abundant (Arendt et al. 2012). The results from the study by Arendt et al. (2012) together with the present study support the suggestion of *M. norvegica* being a dominant species in coastal sub-arctic ecosystem.

To the best of my knowledge, the present study is the first to investigate annual stage distribution of *M. norvegica* in Balsfjord. Nauplii and early copepodites were most abundant in the upper 20 m depth during summer (> 80 % of total population). Adult stages were found at all sampled depths during the entire year (Table 7), but the majority of the population was distributed below 20 m during winter (> 60 % of total population).

Table 7: Presence of nauplii and copepodite stages of *Microsetella norvegica* during the investigated period. Black indicates high abundance (> 900 ind. m^{-3}), and grey indicates lower abundance (< 900 ind. m^{-3}), from the whole water column. No cruise in July.



Pasternak et al. (2000) suggest that large copepods, such as *Calanus finmarchicus*, reproduce in April to June in Balsfjord. Small copepods, such as *Oithona similis*, probably reproduce

later, as their nauplii and small copepodite stages are found in May and late June. Copepod species such as the calanoid *C. finmarchicus* and *Metridia longa*, and the cyclopoid *O. similis* have a comparative pattern of stage distribution during summer (Tande 1982, Grønvik & Hopkins 1984, Pasternak et al. 2000). *C. finmarchicus* nauplii were present in late April and May in the surface, while early copepodite stages were present in May and early June (Tande 1982). This could indicate an earlier development of stages than of *M. norvegica* nauplii and early copepodite stages. Grønvik & Hopkins (1984) reported that copepodite stage CI of *M. longa* was present at the end of May and June, and in August the population was dominated by stage CV. This is comparable with stage distribution found in the present study. I found nauplii of *M. norvegica* present in May and June and with low abundance in August (Table 6). In Balsfjord copepodite stage CI was present from May to August, and stage CII and CIII from June to September. Compared to *C. finmarchicus* the developmental time of copepodite stage CI to CIII of *M. norvegica* was one month earlier for *C. finmarchicus.* The result suggest that the hibernating *C. finmarchicus*, reproduce earlier than the winter active *M. longa* and *M. norvegica*, indicating difference in strategies or need of energetic resources prior to reproduction.

Females and males of *M. norvegica* had an approximately equal abundance during the winter season. A sex ratio of 1:1 of males and females of *M. norvegica* are very different from the sex ratio of other copepods, but is not unheard (Tande 1982, Grønvik & Hopkins 1984, Koski et al. 2014). Many copepods, such as *C. finmarchicus*, hibernate as stage CIV or CV, and males were only present in late winter (Tande 1982). *C. finmarchicus* males appeared in lower abundance than the females during the reproductive period in March and April (Tande 1982). The copepod *M. longa* does not hibernate as adult (Grønvik & Hopkins 1984). The males of *M. longa* dominated in November, while females dominated in April when reproduction started (Grønvik & Hopkins 1984). *M. norvegica* had no such shifts in the proportions of females and males during winter months. The ecological advantages of having a sex ratio of 1:1, is that it opens up for a longer reproductive period. And this indicates that other conditions for reproductions were not sufficient. However, reproduction was not observed.

In Balsfjord there are two peaks in primary production, first in April and May and a second in August and September (Eilertsen et al.1981). Davis (1976) and Koski et al. (2014) found that *M. norvegica* generally reproduces in April and May in the surface, where they feed and grow

until late summer. It is common to observe that copepods ascends closer to the surface during early spring and summer, and it is probably because of feeding (Madsen et al. 2008). Grønvik & Hopkins (1984) reported that the winter active *M. longa* starts their reproduction in May in Balsfjord. Davis (1976) observed females of *M. norvegica* with egg-sac in March and April, and again in September and suggested that *M. norvegica* reproduced twice a year in Balsfjord.

In this study, *M. norvegica* females with egg-sac were first observed in April and were present until June, and again in August. Females with egg-sacs peaked in May, and were mainly present in the upper 50 m (Figure 10). In August the abundance of female *M. norvegica* with egg-sacs was very low (150 ind. m^{-3}), compared to May (38 000 ind. m^{-3}). However, no nauplii or early copepodite stages was observed in autumn. This may indicate failed reproduction in autumn in Balsfjord. Difference in time for observation of females with egg-sac in spring in Balsfjord may be due to different dates of sampling or an inter-annual difference in reproductive onset.

Koski et al. (2014) found that *M. norvegica* reproduced more than one egg-sac, based on the observation that they had a higher egg production rate than expected based on hatching time. Koski et al. (2014) suggested that *M. norvegica* releases their egg-sacs before they have hatched, and produce another egg-sac before the previous are hatched (Koski et al. 2014). A similar strategy is found within a calanoid copepod *Eurytemora affinis* (Koski et al. 2014). Also, in this study we found that egg-sacs of *M. norvegica* exceeded the number of females, with a ratio of 1.8 in May (Table 5). Apparently, females produced high number of egg-sacs rather than a large clutch size. Koski et al. (2014) reported that free egg-sac was found to have a higher mortality rate than females, but since the egg-sac had a ratio at 4, 5 higher than females they were still able to reach high abundances (Koski et al. 2014). The reproductive strategy of decrease in egg mortality relative to broadcast spawners, but increasing egg production compared to sac spawners may be the reason why *M. norvegica* reach high abundances (Koski et al. 2014).

Previous study have reported that the reproductive rate of *M. norvegica* was dependent on food availability and temperature (Uye et al. 2002). The suggestion originated form the belief that *M. norvegica* was a pure sac-spawner, and not releasing their egg-sacs as found in the study by Koski et al. (2014). Based on more recent studies it has been suggested that *M.*

norvegica have a behavioral adaption of reproduction, rather than physical adaption (Koski et al. 2014). The reproduction rate of *M. norvegica* is most likely dependent on food availability, and not temperature (Koski et al. 2014).

Large females were expected to produce more eggs than smaller females, because the larger females would potentially be in a better condition for reproducing. In our study female length varied from 480 to 610 µm in June, and eggs per egg-sac ranged from 6 to 13 (mean of 10.7 eggs per egg-sac) (Figure 11). This is almost identical to eggs per egg-sac found by Koski et al. (2014) from Greenland. Koski et al. (2014) reported that the clutch size of females ranged from 6 to 14 eggs per egg-sac. Compared to a study by Uye et al. (2002), his females were longer and varied between 600 to 700 µm, with a mean of 15.8 eggs per egg-sac. Despite longer females and more eggs in the study by Uye et al. (2002) no correlation between female length and number of eggs was found within the Balsfjord population. We therefore conclude that body length of *M. norvegica* is not directly related to reproductive success in *M. norvegica*.

Seasonal variation in body condition

Carbon content is interpreted as a measure of the condition of copepods, because it is related to the protein and lipid reserves (Conover & Corner 1968). To obtain information on the condition of the small sized copepod *M. norvegica*, the carbon was both measured and estimated by a length-carbon regression published by Uye et al. (2002). Davis (1976) observed *M. norvegica* in the upper water column during winter, indicating that *M. norvegica* was active during winter. Since food availability fluctuated with season, it was expected that the carbon content of *M. norvegica* would vary accordingly.

We found that the carbon content of female *M. norvegica* did show a seasonal variation. In October the average carbon content was 0, 38 µg C ind.$^{-1}$, to 0, 17 µg C ind.$^{-1}$ in March, and increased to 0, 25 µg C ind.$^{-1}$ in May (Table 5). Hence, the carbon content of *M. norvegica* was lower during winter and spring than in summer. By calculating the µg C/µm body length, the carbon content was corrected for the length of females. Length corrected carbon content of *M. norvegica* was then lowest in January and highest in May (Table 5). The results from this

study showed a decrease in carbon content, and this means that *M. norvegica* had limited access to food during winter.

Compared to previous studies of carbon content in other copepods such as *O. similis*, *M. longa*, *C. finmarchicus*, and *C. glacialis* (Tande 1982, Grønvik & Hopkins 1984, Kiørboe & Sabatini 1995), *M. norvegica* has a low carbon content. The large difference in carbon content is naturally a function of body size. It is however interesting to note that *C. finmarchicus* and *M. longa* showed a similar relative decrease in carbon content as *M. norvegica* from January to March in Balsfjord (Hopkins et al. 1984). Also C/N ratio decreased in a comparable manner (Table 6), indicating decrease in the lipid (C/N=100) to protein (C/N=3) composition of the copepods during the food limited winter, where also reproduction is prepared (Tande 1982).

During summer in the Inland Sea of Japan the individual female carbon content of *M. norvegica* was estimated to approximately 0.85 µg C ind.$^{-1}$ (Uye et al. 2002). Our measured females ranged from 0, 39 to 0, 18 µg C ind.$^{-1}$, through the autumn-winter-spring period (Table 6). In this study we also estimated the individual carbon content of *M. norvegica* in Balsfjord, using the length-carbon regression that was established by Uye et al. (2002). The estimated individual carbon content of females was higher compared to the measured carbon content, especially from January to March (Figure 12A).

The regression by Uye et al. (2002) was established under conditions with warm temperatures and during a period where the access to food was good. During winter in Balsfjord, the copepods were starving and used their energy reserves. The females of *M. norvegica* showed a decrease in individual carbon content, and also the average length of females decreased in the population (Figure 12A and B).

Decrease in body length of copepods have been seen previously, as within *C. finmarchicus* (Hopkins et al. 1984). However, *M. longa* has shown to have a constant body length through the year in Balsfjord (Grønvik & Hopkins 1984). Within the population of *M. norvegica* there was approximately a 60 % decrease in copepodite stage CIV/CV from December to January. The decrease may be due to moulting from stages CIV/CV to female or male, given that these females are shorter than older females. Decrease in the length of *C. finmarchicus* has been shown to be due to a "trade off", where energy is invested in gonadal growth instead of

somatic growth (Hopkins et al. 1984). Nevertheless, this strategy is not investigated for the population of *M. norvegica*. In addition, we also suggest that predation on the largest individuals during this period could be one explanation to why we observed a decrease in the mean body length of *M. norvegica*. It is speculated that the largest individuals of *M. norvegica* may have been grazed upon more substantially during winter months when other species are less abundant (Davis 1976, Arendt et al. 2012, Koski et al. 2014).

Conclusions

To sample small copepods, such as *M. norvegica*, the present study recommends using a Go-Flo bottle, because this method sampled nauplii and all copepodite stages, when the WP-2 under-sampled. This study showed that *M. norvegica* was present all year, and was also the most abundant species in Balsfjord. Females with egg-sac were present from April to August, and had a successful reproduction once a year. In addition, by calculating the ratio between number of females and number of egg-sacs, we found that there were almost one and a half times as many egg-sacs than females in May. Females of *M. norvegica* appeared to detach their egg-sac, and produce another egg-sac before the previous egg-sac had hatched. This findings suggests the previous findings that the reproductive strategy of *M. norvegica* is neither truly a sac- or broadcast-spawner. Nauplii and early copepodite stages were present from May to September. Older copepodite stages were present all year, but dominated through winter. Carbon content of *M. norvegica* decreased during winter, with the lowest carbon content in January. Also, the average total body length decreased through winter, with the shortest length in January.

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Appendix I

Figure A1: Principal component analysis plot (PCA) of *Microsetella norvegica* and their stage distribution from May 2013 to June 2014 in Balsfjord. Factor I and II relates to the methods used (Go Flo and WP-2 net) respectively, months, and species stages calculated to m^{-3}. Stage CIV/CV, males and females without egg-sac were placed closely, such as those are present in Balsfjord through some of the seasons. Nauplii, CI and females with egg-sac were placed closely.