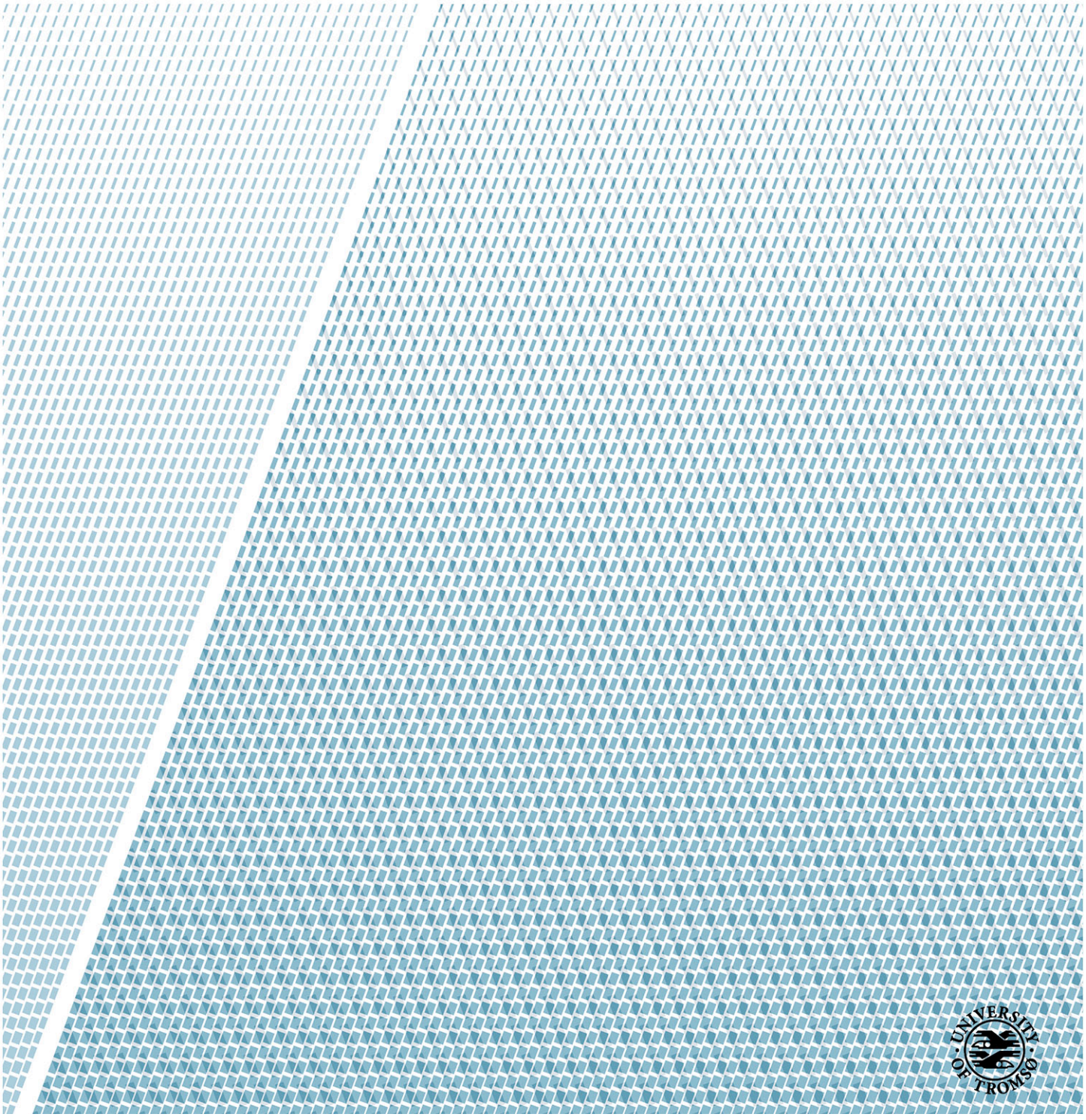
FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
DEPARTMENT OF ARCTIC AND MARINE BIOLOGY

Alternative prey choice in the pteropod *Clione limacina* (Gastropoda) studied by DNA-based methods

Ida Helene Funderud Kallevik

Master thesis in Biology
Field of study - Arctic Marine Ecology and Resource Biology
Bio-3950 (60 ECT)

November 2013



Alternative prey choice in the pteropod *Clione limacina* (Gastropoda) studied by DNA-based methods

Ida Helene Funderud Kallevik

Master`s Thesis in Biology

The Arctic University of Norway

Faculty of Biosciences and Economics

Department of Arctic and Marine Biology

and

The University Centre in Svalbard

Department of Arctic Biology

Supervisors:

Tove M. Gabrielsen Tove.Gabrielsen@unis.no

Jørgen Berge Jorgen.Berge@uit.no

Stig Falk-Petersen sfp@akvaplan.niva.no

Abstract

The gymnosome pteropod *Clione limacina* is regarded as a monophagous predator, feeding exclusively on the thecosome pteropod *Limacina helicina* in Arctic waters. Adult *L. helicina* is absent from the water column during late autumn, winter and early spring due to a short life span. Hence, *C. limacina* is adapted to survive periods of low food availability by long-term starvation. Although *L. helicina* is absent from the water, a number of other zooplankton species are present during this time. It may therefore seem surprising that *C. limacina* does not take advantage of these other food sources at times when their main prey is absent. DNA-based approaches have never been used to investigate *C. limacina* feeding habits previously. In this study, group-specific primers were used to analyse stomach content DNA in *C. limacina*. This is the first study to report that *C. limacina* feed on other types of prey than *L. helicina*. From the 138 *C. limacina* individuals investigated, 24 individuals had presence of amphipod DNA in their stomachs while three individuals were confirmed with traces of calanoid DNA. The traces of amphipod and calanoid DNA were positively identified by sequencing, suggesting that *C. limacina* is in fact a polyphagous predator. Predation on alternative prey may enable *C. limacina* to survive longer time periods of food scarcity. With *L. helicina* being susceptible to ocean acidification, utilising alternative prey may allow the continued existence of *C. limacina* if *L. helicina* populations decline.

Keywords: Pteropoda, *Limacia helicina*, monophagous, polyphagous , PCR, group-specific primers, DNA, Amphipoda, *Calanus* spp., *Parasagitta elegans*

Table of Content

1. Introduction

1.1 The Arctic marine ecosystem

Arctic marine ecosystems are influenced by high seasonal variability and fluctuations of physical and biological factors (Percy and Fife 1981; Cottier et al. 2005; Hop and Falk-Petersen 2006; Leu et al. 2011). Primary and secondary production are affected by seasonal changes in environmental factors, such as temperature, light conditions, ice cover, ocean currents and nutrient availability (Søreide et al. 2008; Søreide et al. 2010; Weydmann and Søreide 2013). Primary production can occur in high and rapid pulses over relatively short time periods, depending on the environmental conditions (Leu et al. 2011). As a response to the high fluctuations in food availability, zooplankton species have therefore developed life strategies and biochemical responses to cope with these changes (Percy and Fife 1981; Clarke 1983; Varpe et al. 2009). The long and dark polar night is often regarded as a period during which food availability is highly limited (Berge et al. 2012). The plankton community in Arctic waters is dominated by copepods in both abundance and biomass (Walkusz et al. 2003; Daase and Eiane 2007; Blachowiak-Samolyk et al. 2008; Falk-Petersen et al. 2009; Walkusz et al. 2009; Weydmann and Søreide 2013). However, other groups of organisms occur regularly in Svalbard waters, although with varying abundance: ctenophores, krill, pelagic amphipods, and pteropods (Søreide et al. 2003; Walkusz et al. 2003; Hop and Falk-Petersen 2006; Walkusz et al. 2009; Kwasniewski 2012; Weydmann and Søreide 2013). The abundance of Arctic pteropods, such as the sea angel *Clione limacina* and the sea butterfly *Limacina helicina* is closely associated with the variations in the abundance of their main food (Lalli and Gilmer 1989; Gilmer and Harbison 1991). This is particularly the case for the short-lived *L. helicina*, which goes through a complete life cycle during one single year (Gannefors et al. 2005). *Clione limacina* is regarded as entirely dependent on the availability of *L. helicina*(Lalli 1970; Conover and Lalli 1974; Hermans and Satterlie 1992; Böer et al. 2005). Further investigations to the interactions between *C. limacina* and other zooplankton species have not been attempted previously, or to the extent of the present study.

The pteropod *Clione limacina* (Phipps 1774) is one of 18 species in the family Clionidae (class Gastropoda, order Gymnosomata). It is the only species in this family occurring in the Arctic, and is the most abundant gymnosome in temperate waters (Morton 1958; Mileikovsky 1970; Suzuki et al. 2001; Böer et al. 2005). Several species in the order Gymnosomata are monophagous, feeding exclusively on thecosomes (Lalli and Gilmer 1989; Böer et al. 2005).

One example of a species generally being considered monophagous is the Arctic *C. limacina* (Lalli 1970; Conover and Lalli 1972; Lalli and Gilmer 1989; Gannefors et al. 2005; Böer et al. 2005), feeding exclusively on the thecosome *Limacina helicina* in the Arctic and on *Limacina retroversa* in temperate waters. As several types of Arctic zooplankton accumulate and biosynthesise lipids, serving as energy storage in periods of low food availability, *C. limacina* has developed similar strategies (Lee 1974; Lee 1975; Clarke 1983; Falk-Petersen et al. 1987; Larson and Harbison 1989; Kattner et al. 1990; Kattner et al. 1998; Scott et al. 1999; Falk-Petersen et al. 2000; Hagen and Auel 2001; Böer et al. 2005; Böer et al. 2006a; Böer et al. 2006b; Falk-Petersen et al. 2009). The lipid density found in *C. limacina* may make them an ideal energy source for other predators (Lebour 1931). In the Arctic, large amounts of *C. limacina* have been found in baleen whales, planktivorous fish and seabirds but data on predation on *C. limacina* is scarce (Lebour 1931; Lalli 1970). Triacylgycerols (TAG) and 1-*O*-alkyldiacylglycerol ethers (DAGE) are the major lipid classes in *C. limacina* (Falk-Petersen et al. 2001; Böer et al. 2006b). TAG is considered to be important for growth and development, while DAGE is suggested to be the main energy store for periods of food scarcity. Böer and colleagues (2006a,b) revealed that the overall size of the animals decrease during periods of starvation. During these starvation experiments, lipids in the digestive gland and the number of lipid droplets in the animals diminished, and muscle tissue eventually started to degenerate. Investigation of lipid content and dry mass revealed that catabolism of lipids was highest in the beginning of the starvation period, while in later stages of starvation using proteins becomes more prominent. This results in the lipid content remaining constant, while overall body size shrinks during the period of starvation. Reproductive organs degenerated during starvation, suggesting that *C. limacina* can use organs as energy storage for survival (Böer et al. 2006b). *Clione limacina* has a high assimilation rate, assimilating 90% of the carbon and close to 100% of the nitrogen from ingested prey (Conover and Lalli 1974). It has been proposed that assimilation efficiency is an advantageous adaptation of monophagy. Compared to generalist feeders, digestion and assimilation in specialist predators can be developed to maximise energy yield from the particular prey. Thus, Conover and Lalli (1974) stated that the assimilation efficacy resulting from monophagy would lead to increased ecological efficiency. The high assimilation rate, re-synthesis, storage and utilisation of TAG and DAGE lipids are important adaptations that enable *C. limacina* to accumulate the energy required for periods with low food availability (Conover and Lalli 1972; Conover and Lalli 1974; Lee 1974; Böer et al. 2005; Böer et al. 2006a). With the ability to delay protein catabolism which results in body shrinkage, and the capability to utilise

organs as an energy storage enables *C. limacina* to survive exceptionally long periods of starvation.

1.2 Breeding, development and feeding in *Clione limacina*

It has been suggested that *C. limacina* has at least a 2-year life-cycle in Svalbard waters (Böer et al. 2005). *Clione limacina* reaches a size of 70-85 mm, matures at a size of 30 mm and produce 0.12 mm eggs (Lebour 1931; Lalli and Gilmer 1989; Böer et al. 2005). Spawning of *C. limacina* is tightly correlated with the sea water temperature during the local spring and summer period and the peak abundance of phytoplankton (Mileikovsky 1970; Weydmann and Søreide 2013). *Clione limacina* was described as a protandric hermaphrodite, based on the observation of sperm maturing prior to egg development by Morton (1958). However, Boas (1886, as cited by Lalli 1970) described the copulation with both individuals connecting their penis to the vaginal part of the partner simultaneously. The synchronised copulation was observed in several paired individuals by Lalli and Gilmer (1989), suggesting simultaneous hermaphrodism existing in the species. They also observed how the accessory copulatory organ left a small scar on the body surface of the partner. Larger individuals were found to have up to four such scars, indicating that mating occurs multiple times during *C. limacinas* life span. Due to deformation during preservation, these scars cannot be investigated further on preserved individuals. Most of the developmental stages of *C. limacina* are thought to be dependent on the availability of *L. helicina* in polar waters (Conover and Lalli 1974; Lalli and Gilmer 1989; Böer et al. 2005). The earliest veliger larvae and the polytrochous larvae feed on phytoplankton, and it is not before the later larval stages that *C. limacina* start feeding on *L. helicina* (Conover and Lalli 1974; Lalli and Gilmer 1989; Böer et al. 2005). The predator-prey relationship is highly dependent on size. As *C. limacina* and *L. helicina* have parallel development following the polytrochous-larva stage, larval *C. limacina* continue to feed on prey of their own size (Conover and Lalli 1974). Larger *C. limacina* do not prey upon small *L. helicina,* larvae or veligers. In contrast, young *C. limacina* feed on the largest possible prey they are able to handle. When in contact with their prey, adult *C. limacina* extend six buccal cones, which attach to the shell of *L. helicina* (Figure 1) (Lalli 1970; Conover and Lalli 1972; Arshavsky and Deliagina 1989; Hermans and Satterlie 1992). The buccal cones carry sensory cells and small glands, which are assumed to secrete an adhesive mucus that ensures the grip on *L. helicina* (Pelseneer 1885; Morton 1958; Lalli 1970; Hermans and Satterlie 1992). The buccal cones twist the shell until the opening is aligned with the mouth opening of *C. limacina.* Chitinous hook sacs are protruded to grasp the *L. helicina* from within its shell

(Figure 1). *Limacina helicina* is pulled out of its shell and swallowed whole (Lalli 1970). According to Lalli (1970) *C. limacina* does not feed on dead *L. helicina*. Adult *C. limacina*, when given a selection of prey sizes, selected the largest prey to consume first, a behaviour that maximises energy yield from prey (Lalli and Gilmer 1989).

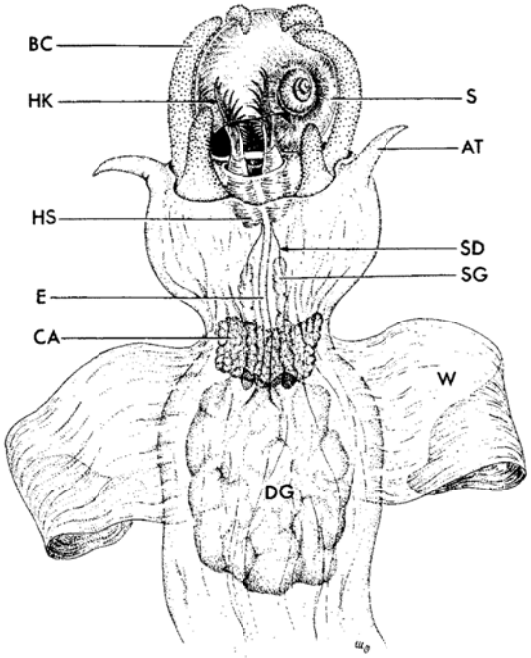


Figure 1: *Clione limacina* feeding on *Limacina helicina*.
Notes: AT: anterior tentacle, BC: buccal cone, CA: copulatory apparatus, DG: digestive gland, E: oesophagus, HK: hook sac, S: shell of prey, SD: salivary duct, SG: salivary gland, W: wing. Source of figure: Lalli (1970).

Norekian (1995) investigated neurone activity of *C. limacina* in response to *L. helicina* presence. The neurone activity in the buccal apparatus was high when *L. helicina* was in direct contact with the mouth opening of *C. limacina*. Swimming movements of *L. helicina* did not trigger neuron activity in the buccal apparatus, while the addition of homogenised *L. helicina* in the water did. This suggests that chemical responses are the main activation of buccal cone activity (Norekian 1995), also supported by the neural investigations performed by Arshavsky and Deliagina (1989). Neural responses triggered by other types of zooplankton were not investigated. *Clione limacina* is not cannibalistic, as the addition of small, swimming *C. limacina* resulted in avoidance behaviour in the swimming-pattern of observed individuals (Zakharov and Ierusalimsky 1992; Norekian 1995). This was also indicated in starvation experiments where *C. limacina* did not become cannibalistic even when starving individuals were grouped together (Böer et al. 2006a; Böer et al. 2006b).

1.3 Traditional methods of investigating feeding ecology

Traditionally, investigating gut contents by dissection and visual analysis have been the main means of investigating prey-predator relationships of different species (Karlson and Båmstedt 1994; Pompanon et al. 2012). This is a challenging method regarding soft digested matter, but ingested solid parts have been used to identify prey. Solid parts such as mandibles, telsons and other solid appendages can be identified to species or genus level, depending on available literature on mandible and telson structures (Karlson and Båmstedt 1994). Several calanoid copepod species and even one *Metridia longa*, have been identified in some amphipods, by using mandibles found in the digestive tracts of the amphipods (Dalpadado et al. 2008; Kraft et al. 2012). By such methods, Gilmer and Harbison (1991) could identify copepod thoraces and nauplii in *L. helicina* guts, and tintinnids, diatoms and dinoflagellates in the fecal pellets. Such studies have not revealed solid parts in *C. limacina* (Boas 1886, as cited by Lalli 1970). Only one study investigating *C. limacina* stomach content has been performed, and the soft, dark digested material was assumed to originate from *L. helicina* (Boas 1886, as cited by Lalli 1970). Other methods of investigating feeding ecology and trophic relationships include using fatty acid composition and stable isotope ratios (Falk-Petersen et al. 1987; Falk-Petersen et al. 2001; Scott et al. 2001; Falk-Petersen et al. 2002; Tamelander et al. 2006; Søreide et al. 2006; Layman et al. 2007; Nilsen et al. 2008; Post 2013). Fatty acids and stable isotopes are assimilated over long periods of time; hence an overall picture of trophic relations and trophic levels can be obtained. However, the fatty acids analysed in *C. limacina* do not correlate with the lipids found in *L. helicina* (Kattner et al. 1998; Hagen and Auel 2001; Böer et al. 2005). *Clione limacina* is re-synthesising lipids *de novo*, producing 17:1(n-18), 15:0, 16:1(n-7) and 18:1(n-7) fatty acids which are different lipid-compositions than those found in *L. helicina* (Kattner et al. 1998; Hagen and Auel 2001; Gannefors et al. 2005).

1.4 Molecular tools for studying feeding ecology

Molecular genetic analyses to investigate prey DNA in gut content or faeces of animals, is a growing field in ecological research (Jarman et al. 2002; Passmore et al. 2006; Jarman et al. 2006; King and Read 2008). The polymerase chain reaction (PCR) can be used to detect even the small fragments of prey DNA in predator organisms (Symondson 2002; Nejstgaard and Frischer 2003; Vestheim et al. 2005; Vestheim and Jarman 2008; Töbe et al. 2010). Such DNA-based approaches may be particularly relevant to investigate the feeding ecology of zooplankton, as visual inspection of zooplankton guts is challenging in small organisms and young stages. Different molecular techniques have so far been used to investigate prey items in e.g. copepods and krill (Jarman et al. 2002; Nejstgaard and Frischer 2003; Vestheim et al. 2005; Töbe et al. 2010; Cleary et al. 2012; Vestheim et al. 2013). DNA-based methods can also enhance feeding ecology data by identifying the soft, digested material found in zooplankton (Jarman et al. 2002; Dunshea 2009; Töbe et al. 2010; Pompanon et al. 2012). Different techniques are available to investigate presence of prey-DNA in predators (Passmore et al. 2006; Vestheim and Jarman 2008; King and Read 2008; Meekan et al. 2009; Pompanon et al. 2012). One method is using group-specific primers developed to detect and amplify DNA from certain groups of prey organisms. This method results in presence-absence data for the respective groups. The method of group-specific analyses was applied because it is an easy method to investigate presence of prey-DNA due to available primers. Another possibility is using general primers to amplify all prey in predator guts or faeces. In most cases this technique requires removal of predator-DNA, as general primers will amplify the predator-DNA as well. Alternatively, the amplification of predator DNA can be supressed by using blocking primers enabling a higher yield of prey-DNA (Vestheim and Jarman 2008). Independent of the technique selected, Next-Generation Sequencing (NGS) of predator and prey PCR amplicons gives a high yield of any DNA-sequences present in the sample, allowing the detection and possible identification of a broad range of prey-DNA in the sample (Luo et al. 2012; Pompanon et al. 2012; Bik et al. 2012). This method can give a relative abundance of the different DNA-sequences in a sample, thus enabling comparison of preferred prey and less consumed prey. The NGS-technique can be used directly on stomach content DNA, with or without the use of blocking-primers. This method was applied to compare the results of the group-specific primers and the NGS-results, and to assess the efficiency of NGS-methods without the use of blocking-primers.

1.5 Aim of study

Clione limacina is according to literature considered to be a monophagous species, feeding exclusively on *L. helicina.* Such a strategy is inconsistent with the current understanding of Arctic marine zooplankton, where no other examples of monophagous species can be found. As *L. helicina* appears to be more or less absent from the water column in late autumn, winter and early spring, *C. limacina* is faced with long periods of forced starvation. Although *C. limacina* can survive long periods of food scarcity, other zooplankton species are present in the water when the abundance of *L. helicina* is low. Hence, alternative prey items could potentially be consumed in periods of either low availability of *L. helicina* or in seasons with high density of other zooplankton species.

Based on this, I propose to test the hypothesis of monophagy in *Clione limacina:*

H_0: *Clione limacina* feed exclusively on *Limacina helicina* in Arctic waters

H_1: *Clione limacina* can consume other species of zooplankton

The main prediction from H_1 is that traces of prey items other than *L. helicina* will be detected in the guts of *C. limacina*. The main method of investigating alternative prey items in *C. limacina* was performed using molecular genetic tools. Group-specific primers targeting potential prey species were used to amplify DNA extracted from *C. limacina* stomachs. The group-specific primers gave qualitative data by indicating presence or absence of target organism DNA. In addition, a few samples were sent for NGS-analyses without using blocking-primers, to compare the results of the two molecular methods. Neither of these techniques has been used to investigate prey-consumption in *C. limacina* previously. A feeding experiment was attempted to investigate alternative prey consumption by direct observations.

2. Materials and Methods

2.1 Sample collection

Individuals of *C. limacina* were collected from Adventfjorden, Billefjorden, Hinlopen Strait, Kongsfjorden, Olga Basin, Rijpfjorden, Smeerenburgfjorden and at the polar ice edge forming North-East of Spitsbergen in 2012 (Figure 2). The samples were collected based on cruises of opportunity during University Centre in Svalbard (UNIS) courses or UNIS field campaigns using R/V Helmer Hanssen, R/V Lance, R/V Viking Explorer, and K/V Svalbard. Samples were collected throughout the year from October 2010 until September 2012 (Table 1).

Figure 2: Map of Svalbard covering the sample locations.
Notes: Map source Norwegian Polar Institute (http://svalbardkartet.npolar.no/Viewer.html?Viewer=Svalbardkartet), modified for this study.

Table 1: Overview of the 138 individuals of *C. limacina* that were analysed in this study.

Sample location	Latitude	Longitude	Season	Sampling date	Population ID	Equipment	No. Individuals
Adventfjorden	7815.713	01531.239	summer	15.06.12	AS	MIK-net	15
Adventfjorden	7816.34	01532.72	winter	14.12.11	AV	MIK-net	11
Billefjorden	7839.648	01642.605	autumn	07.10.10	BF	MIK-net	2
Billefjorden	7839.799	01641.283	autumn	28.08.12	BFH	WP2	1
Billefjorden	7839.799	01641.283	winter	13.12.11	BFV	WP3	1
Hinlopen Strait	7937.442	01852.780	autumn	02.10.10	H	MIK-net & WP3	12
Ice Edge	8029.534	01742.259	spring	25.04.12	IES	MIK-net	18
Kongsfjorden	7859.982	01141.863	autumn	01.10.10	KF	MIK-net & WP3	12
Olga Basin	7802.837	02644.912	autumn	28.08.11	OB	MIK-net	15
Rijpfjorden	8017.063	02219.156	autumn	11.09.12	RIH	MIK-net	16
Rijpfjorden	8017.451	02217.768	summer	16.07.12	RIS	Tucker-trawl	17
Rijpfjorden	8019.107	02214.478	winter	13.01.12	RIV	MIK-net	12
Smeerenburgfjorden	7940.872	01106.639	autumn	18.09.11	SB	MIK-net	6

2.2 Zooplankton net sampling

A MIK net (Havforskningsinstituttet, Norway) was mainly used to collect *C. limacina*. The MIK net has an opening of 3.15 m^2 with a mesh size of 1000 µm, and was trawled at 20-15 m depth at 1.5-2 knots for 15 minutes. During the sampling cruises, *C. limacina* were occasionally caught in a Tucker Trawl (Open Sea Instrumentation Inc., Canada), WP3 net (KC Denmark A/S, Denmark) or WP2 net (KC Denmark A/S, Denmark) and the specimens were retained for this study. The Tucker Trawl has an opening of 2.25 m^2 and a mesh size of 2000 µm. The Tucker Trawl was towed with a speed of 2 knots for 10 minutes. The WP3 net had a mesh size of 1000 µm and 1 m^2 opening. The WP2 net had a 0.25 m^2 opening with a 200 µm mesh size. Vertical net hauls were taken at depths of 180-150, 150-100, 100-50 and 50-0 m. Individuals of *C. limacina* for genetic analysis were washed in 70 % ethanol, and stored in 80 % ethanol (Rektifisert alkohol, Kemetyl Norway). The ethanol was changed after 2-3 days, and then changed again after 5-7 days before storing in room temperature until dissection and further analyses occurred.

2.3 Dissections and DNA extractions

The lab bench was cleaned with 96-100% ethanol before dissections started, and each sterile petridish was placed upon new sheets of aluminium foil. Samples for DNA extraction were individually dissected in sterile petridishes with a sterilised surgical blade (Swann-Morton®, England) and sterilised forceps. The scalpel was sterilised between the incisions of the outer epithelium and stomach wall lining. Visceral mass was carefully removed and stored at room temperature in 1.5 mL Eppendorf tubes (Eppendorf AG, Germany) in 80 % ethanol until DNA-extraction. Samples were kept in a fridge while rehydrated overnight prior to DNA-extraction, ethanol was removed with a 5 mL Pasteur pipette and Milli-Q® water (Millipore, Germany) was added. As the gut content of *C. limacina* was used for DNA-extraction, visual analysis was not performed prior to extraction to eliminate contamination sources. The colour and structure of the visceral mass did not allow distinction of full or empty stomachs.

DNA was extracted with the DNeasy® Blood & Tissue Kit (Qiagen, Germany) following the manufacturers protocol with an alteration of the last step in the protocol where eluation was repeated twice. For small *C. limacina* individuals (<2.5 cm), the whole visceral mass was used for extraction. For larger individuals (> 2.5 cm), up to 25 mg of stomach content was used for extraction. After removing the MilliQ water, 180 µL Buffer ATL and 20 µL proteinase K (Qiagen, Germany) were added in the 1.5 mL Eppendorf tube. The Eppendorf tubes were vortexed (VWR®, Germany) and placed in a heating block (Stuart®, England) at 56ºC for three hours. Samples were vortexed every 20 minutes during lysis time. At the end of lysis time, samples were vortexed for 15 seconds before adding 200 µL Buffer AL and 200 µL ethanol (96-100% Absolute alcohol, Kemetyl Norway). Samples were vortexed before the mixture, including precipitates, were pipetted into the DNeasy Mini spin columns with 2.0 mL collection tubes. The tubes were centrifuged (Eppendorf AG, Germany) at 8000 rpm for one minute, according to the protocol. The flow-through and collection tube were discarded, and the DNeasy Mini spin columns were placed in a new 2.0 mL collection tube. Then 500 µL Buffer AW1 was added, and centrifuged at 8000 rpm for one minute. The flow-through was discarded, and the collection tubes were re-used in the next step. Then 500 µL Buffer AW2 was added, and centrifuged at 14 000 rpm for three minutes. The flow-through and collection tube were discarded. Centrifugation was repeated if the DNeasy Mini spin column membrane had not dried properly. The DNeasy Mini spin column was placed in a 1.5 mL Safe-Lock Eppendorf (Eppendorf AG, Germany), and 100 µL Buffer AE was added and the mixture was incubated at room temperature for one minute. The tubes were centrifuged at

8000 rpm for one minute to elute the DNA. The 100 mL flow-through was pipetted onto the DNeasy Mini spin column for a second elution, to increase the DNA yield. The tubes were centrifuged at 8000 rpm for one minute.

Twenty µL of the DNA samples were pipetted into a new 1.5 mL Safe-Lock Eppendorf (Eppendorf AG, Germany) tube and 180 µL Milli-Q® water were added, to make a 1:10 diluted DNA working stock. The original and the working DNA stock were stored at -20°C. In total, DNA was extracted from 138 *C. limacina* individuals (Table 1). The number of *C. limacina* samples from each location varied from 1 to 18 individuals (Table 1). Each individual DNA sample was tested with the group-specific primers (Table 2).

Table 2: Group-specific primers used for prey detection in *C. limacina*.

Target group/organism	Primer name	Annealing temp. (°C)	Expected amplicon size (bp)	Gene	Primer sequence 5`-3`	Reference	Cross-amplification tested	Cross-amplification not tested
Amphipoda	AmphNSSf1	54	204-375	18S	CTGCGGTTAAAAGGCTCGTAGTTGAA	Jarman *et al* 2006	X	
	AmphNSSr1	54	204-375	18S	ACTGCTTTRAGCACTCTGATTTAC	Jarman *et al* 2006		
Calanus spp.	16SAR	54	~350	16S	CGCCTGTTTAACAAAAAC	Lindeque *et al* 2006		X
	16SB2R	54	~350	16S	ATTCAACATCGAGGTCACAAAC	Lindeque *et al* 2006		
Echinodermata	EchinNSSf1	54	157-163	18S	GCGTGCTTTTATTAGGA	Jarman *et al* 2006	X	
	EchinNSSr1	54	157-163	18S	CGACCATGRTARGCGCATAACG	Jarman *et al* 2006		
Euphausiacea	kLSUE9f	64	260-270	28S	TCTCAGCGCTGGCAAGGTGTCA	Jarman *et al* 2002	X	
	kLSUE9r	64	260-270	28S	CTCGGGGACGTTTTATCCGGGACGAG	Jarman *et al* 2002		
Pisces	FishF2	54	631	COI	TCGACTAATCATAAAGATATCGCGAC	Ivanova *et al* 2007		X
	FishR2	54	631	COI	CACTTCAGGGTGACCGAAGAATCAGAA	Ivanova *et al* 2007		
Parasagitta spp.	SagF	49	551	COII	GGAGCATCTCCTTTAATAGAACA	Peijnenburg 2004		X
	SagC2	49	551	COII	CCACAAATTTCTGAACATTGACCA	Peijnenburg 2004		

Notes: Annealing temperatures were optimised during this study, and may vary from source literature. The temperature listed was used during this analysis. Cross-amplification entails testing the potential for non-specific amplification of DNA-templates originating from other organisms than the target group of a particular primer.

2.4 Polymerase Chain Reaction

Polymerase Chain Reaction (Mullis et al. 1986) was carried out on an Eppendorf Mastercycler Ep Gradient S PCR cycler (Eppendorf AG, Germany). The PCR samples had a total volume of 25 µL, containing 2.5 µL 1x DreamTaq Buffer including 2 mM $MgCl_2$ (Fermentas, Germany), 2.5 µL dNTP mix with 0.2 mM of each dNTP (Fermentas, Germany), 0.25 µL of 10 mM for each primer, 0.20 µL DreamTaq DNA polymerase (Fermentas, Germany) and 2 µL template DNA (10x dilution). The following PCR program was used; initial denaturation at 94ºC for two minutes, 35 cycles of 94ºC denaturation for 30 seconds, 49-64ºC annealing for 30 seconds and 72ºC elongation for one minute with a final extension

step of 72ºC for five minutes before cooling down to 10ºC. The annealing temperature was optimised for each of the specific primers used in the analysis (Table 2). Primers were initially tested with the original annealing temperature from the source paper, with target DNA as template (Table 3). When a positive PCR-product occurred with use of the original annealing temperature, a higher temperature was also tested to eliminate the potential of unspecific amplification. If the primers did not produce a PCR-product at the original annealing temperature, the temperature was lowered until a positive product occurred (Table 2). Primers were also tested on pure *C. limacina* DNA prior to prey analyses, to investigate possible unspecific products. Although no by-products occurred while using group-specific primers on pure *C. limacina* DNA, by-products did occur with some primers during testing of stomach content DNA. The primers for amplifying *Calanus* spp., Echinodermata and *Parasagitta* spp. resulted in unspecific products giving miltiple bands in some of the PCR runs. The strength of the target-gene varied in each sample when using the *Calanus* spp. primer, although the target gene was in general a stronger product than the by-products. By-products occurred most often with the *Calanus* spp. and *Parasagitta* spp. primer, and to a lesser extent with weaker by-products for the Echinodermata primer. The Echinodermata by-products were much more prevalent following the second PCR prior to sequencing. The selected primers included both primers of distinct taxonomic groups designed for prey analyses (i.e. Amphipoda, Echinodermata and Euphausiacea) as well as general primers designed to amplify certain groups (i.e. *Calanus* spp., Pisces, *Parasagitta* spp.) (Jarman et al. 2002; Peijnenburg et al. 2004; Lindeque 2005; Jarman et al. 2006; Ivanova and Zemlak 2007). With respect to the zooplankton caught with *C. limacina* (Table A13, Appendix), these primers are of particular interest to use in the present study. The DNA from target organisms was used as positive PCR-controls for the different primers (Table 3). The primer pairs of the general primers had not been tested for cross amplification of related species. The PCR products were displayed on a 1 % agarose gel run on 90 V, 115 W for 1 hour. The agarose gel was stained with GelRed™ Nucleic Acid Stain (Biotium Inc, USA). Low Range DNA Ladder (Fermentas, Germany) and GeneRuler DNA Ladder (Fermentas, Germany) were used on the gel to assess the size of products.

Table 3: DNA from species used as positive controls for the group-specific primers.

Target group/organism	Primer name	Positive DNA control
Amphipoda	AmphNSSf1 AmphNSSr1	*Gammarus wilkitzkii* & *Themisto abyssorum*
Calanus spp.	16SAR 16SB2R	*Calanus finmarchicus*
Echinodermata	EchinNSSf1 EchinNSSr1	*Strongylocentrotus* sp.
Euphausiacea	kLSUE9f kLSUE9r	*Thysanoessa inermis*
Pisces	FishF2 FishR2	*Gadus morhua* & *Boreogadus saida*
Parasagitta spp.	SagF SagC2	*Parasagitta elegans*

2.5 Sanger sequencing

Representatives of all potential prey organisms identified in the stomachs of *C. limacina* using group-specific primers were attempted to be confirmed using Sanger sequencing. The positive PCR products were cleaned, re-amplified in a second PCR cycle with the same primers, and cleaned again before sequencing. The following PCR program was used for the second amplification; initial denaturation at 94ºC for two minutes, 25 cycles of 94ºC denaturation for 30 seconds, 49-64ºC annealing for 30 seconds, 72ºC elongation for one minute with a final extension step of 72ºC for five minutes before cooling down to 10ºC. The E.Z.N.A® Cycle-Pure Kit (Q-spin column) (Omega Bio-Tek, USA) was used to clean the PCR products according to the manufacturers recommendations. The samples were prepared according to the protocol of the ABI platform at the Department of Biosciences (University of Oslo,Norway); 3µL template, 1 µL 1 µM primer, and 6 µL MilliQ water. The primers used were the same primers as for the initial PCR (Table 2). The ABI platform used ABI 3730 DNA Analyser (Applied Biosystems, USA), with BigDye®Terminator v3.1 Cycle Sequencing Kit (Applies Biosystems, USA) and POP-7 polymer (Applied Biosystems, USA). From the BigDye®-kit, the pGEM Control DNA template and M13(-21) primer was used as a control.

The sequences were analysed using Sequencher® 5.1 (Gene Code Cooperation, USA). The resulting sequences were compared to the National Centre for Biotechnology Information (NCBI, USA) database (http://www.ncbi.nlm.nih.gov/). The Basic Local Alignment Search

Tool (BLAST®,USA) was used to compare the resulting sequences with the nucleotide databases (Altschul et al. 1990).

2.6 Quality control

A blank control was part of all DNA extractions and PCR tests using MilliQ-water as a substitute for tissue or DNA template, respectively. The DNA extraction control was also tested in a standard PCR reaction with the universal primers 28SF and 28SR, to investigate contamination during DNA extraction. In three separate DNA-extractions contaminations occurred and the extraction control exposed presence of DNA during PCR with universal primers 28SF (5`-GTGTAACAACTCACCTGCCG-) and 28SR (5`-GCTACTACCACCAAGATCTG-) (Vestheim and Jarman 2008). The extraction controls were then tested with all the group-specific primers and the Gastropod primer GastNLSf1 (5`-GCGGYAACGCAAACGAAGT-) and GastNLSr1 (5`-CGAAAWTMACACCGTCTCCG-) developed by Jarman et al. (2006). The group-specific primers gave no results, except for the Gastropod primer. Presuming a contamination of predator-DNA, analysis by group-specific primers continued under the assumption that with no apparent prey DNA being detected, prey-contamination was negligible.

2.7 Next-Generation Sequencing

To investigate the presence of other types of prey, including the main prey *L. helicina*, the stomachs of 10 individual *C. limacina* were analysed using Illumina MiSeq technology. The V9 region of the SSU rDNA was amplified using universal primers without the addition of a blocking primer (Stoeck et al. 2010). The amplicons were prepared for Ilumina sequencing using an internally developed library preparation (Nadeau et al. in prep) and sequenced on a MiSeq with V2 paired-end 150 bp reagents (Source Bioscience, Nottingham, UK). Sequences were de-multiplexed by sample using the basic toolbox on Galaxy (https://usegalaxy.org). Individual samples were then quality filtered and chimera-checked using *mothur* v.1.32.1 (Schloss et al. 2009). The sequences containing indefinite bases (N´s), with length <80 bp or >250 bp, or with an average quality score <35.8 were removed from the dataset. The remaining sequences in each sample were then subjected to *de novo* chimera checking using the uchime algorithm (Edgar et al. 2011), with an abundance skew threshold of 2.0 and a minimum of 1.0% divergence between recognised parental sequences. Sequences from all

samples were combined and clustered at a 98% identity level using the uclust algorithm as implemented in Qiime v.1.5.0 (Caporaso et al. 2010). Those operational taxonomic units (OTUs) represented by only a single sequence across the entire dataset were discarded as presumed sequencing errors. The most abundant sequence variant in each cluster was designated the representative sequence for each OTU and then taxonomy was assigned based on the top hit of BLAST searches against a custom database consisting of the SILVA (http://www.arb-silva.de) database v. 111 (Quast et al. 2013) combined with reference sequences from a variety of marine organisms present in Svalbard waters.

2.8 Feeding experiment

A small feeding experiment was attempted at the sampling cruise during September 2011. Single *C. limacina* (> 2.5 cm) were put in 5 L buckets with seawater, with one type of potential prey present. As a feeding behaviour control some buckets contained *L. helicina* as the available prey. In the experiment the chaetognath *Parasagitta elegans* and the copepod *Calanus glacialis* were used as potential prey. These species were chosen due to their high abundance at the location where the *C. limacina* were caught. The experiment was run in a cooling room at 4°C for 24 hours. Behaviour of *C. limacina* was recorded every 3-4 hours.

3. Results

3.1 Polymerase Chain Reaction analyses using group-specific primers

The PCR amplification using group-specific primers to identify prey in *C. limacina* stomachs identified at least some PCR products of the expected size of target DNA from all tested prey groups (Table 4). Positive PCR-products (Table 4) from stomach content DNA could be compared to the PCR-control with target animal DNA-template to assess the similarity and band length of the products (Figures 3-4).

Table 4: The number of *C. limacina* individuals where group-specific primers amplified a PCR product of expected size of target DNA (Detected PCR product), and the number of *C. limacina* individuals where no amplification occurred (No detected PCR product).

	Amphipoda	*Calanus* spp.	Echinodermata	Euphausiacea	Pisces	*Parasagitta* spp.
Detected PCR product	24	30	29	4	3	22
No detected PCR product	114	88	109	114	115	116

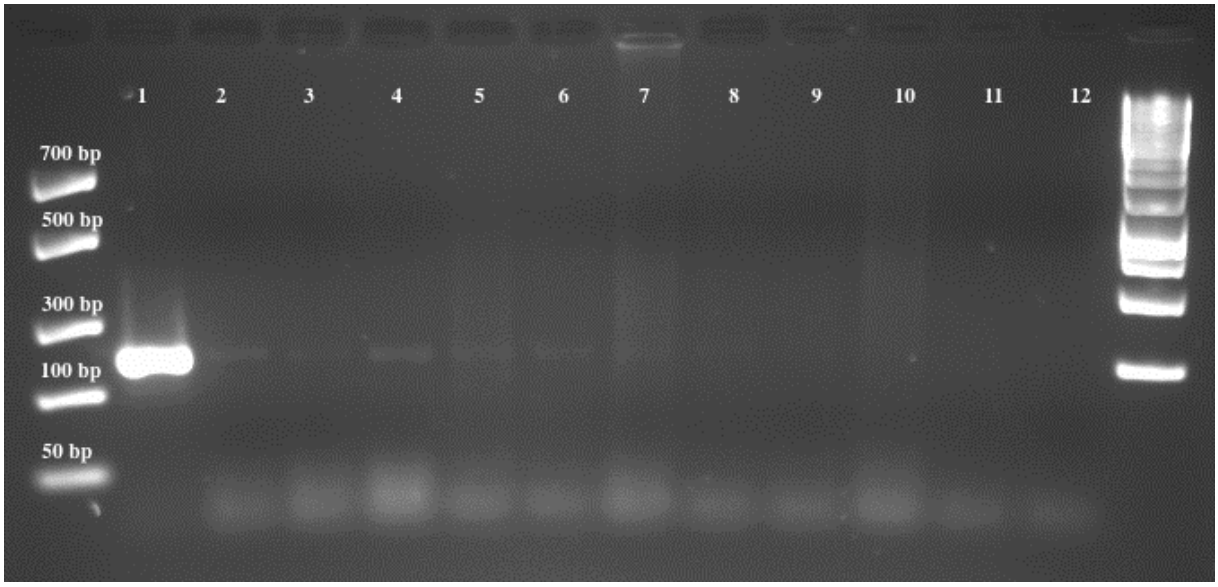


Figure 3: Picture of agarose gel electrophoresis used to separate PCR-products amplified from *C. limacina* stomach DNA using the amphipod-specific primers.
Notes: First and last lanes represent Low Range DNA Ladder and GeneRuler DNA Ladder respectively, and the labelled lanes contain samples. Lane 1; *Gammarus wilkitzkii* DNA. Lane 2-12: stomach DNA from *C. limacina* individuals OB1S, OB2S, OB4S, OB7S, OB8S, OB9S*, OB10S*, OB11S, OB12S, OB13S and OB14S respectively. * = PCR-products sequenced to check the identity of the DNA band (Table 5).

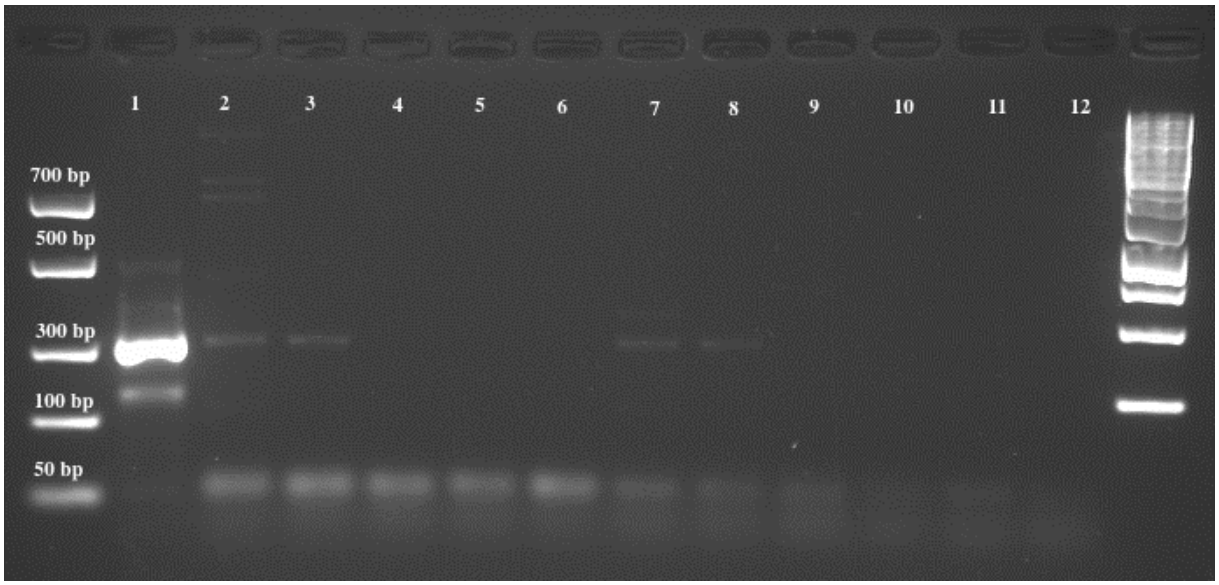


Figure 4: Picture of agarose gel electrophoresis used to separate PCR-products amplified from *C. limacina* stomach DNA using the *Calanus* spp. primers.
Notes: First and last lanes represent Low Range DNA Ladder and GeneRuler DNA Ladder respectively, and the labelled lanes contain samples. Lane 1; *Calanus finmarchicus* DNA. Lane 2-12; stomach DNA from *C. limacina* individuals AV1S, AV2S*, AV3S, AV4S, AV5S, AV6S*, AV7S*, AV8S, AV9S, AV10S and AV11S respectively. * = PCR-products sequenced to check the identity of the DNA band (Table 5).

3.2 Sanger sequencing and BLAST hits

It was not always possible to confirm the presence of the detected prey organisms in *C. limacina* stomachs by Sanger sequencing due to weak PCR products and/or multiple bands (Table 5). In some cases new PCR runs, using the PCR products of the first run as a template, produced enough PCR product to allow sequencing (Figure 5-6). A total of 50 PCR-products were sequenced aiming to confirm the presence of prey organisms, but only 13 of these received sequences were of high enough quality to be identified as target DNA (Table 5). From the amphipod PCR-products, 10 of the 11 samples could be identified as amphipod DNA. For the *Calanus* spp. PCR-products, however, only 3 of the 13 *Calanus* spp. PCR products could be identified as *Calanus glacialis*. The remaining sequences were not of a good enough quality to assign to taxa.

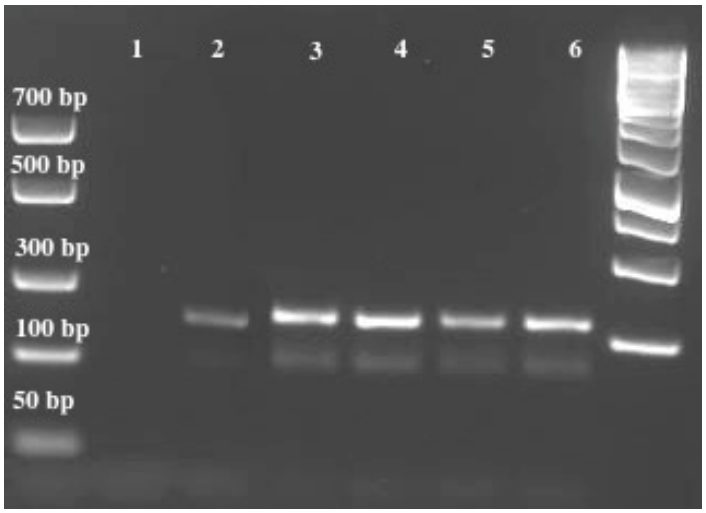


Figure 5: Picture of agarose gel electrophoresis used to separate positive PCR-products amplified from *C. limacina* stomach DNA using the amphipod-specific primers after a second PCR-run prior to sequencing.

Notes: First and last lanes represent Low Range DNA Ladder and GeneRuler DNA Ladder respectively, and the labelled lanes contain samples. Lane 1; PCR-control. Lane 2-6; stomach DNA from *C. limacina* individuals AS4S, RIS12S, AS1S, AS2S and AS3S respectively.

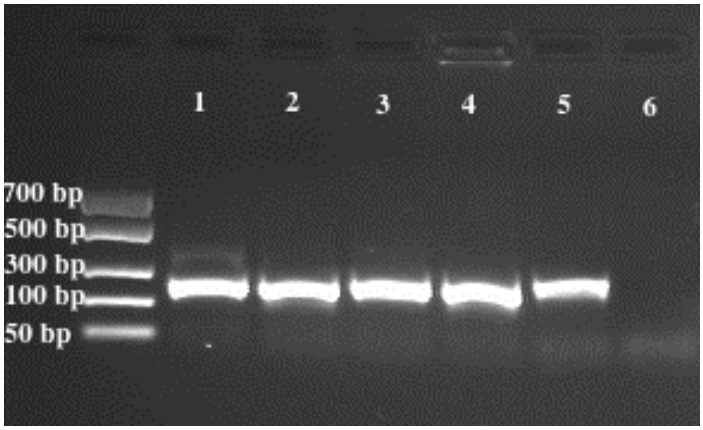


Figure 6: Picture of agarose gel electrophoresis used to separate positive PCR-products amplified from *C. limacina* stomach DNA using the amphipod-specific primers after a second PCR-run prior to sequencing.

Notes: First lane represents Low Range DNA Ladder, and the labelled lanes contain samples. Lane 1-5; stomach DNA from *C. limacina* individuals SB1S, SB3S, SB5S, OB9S, and OB10S respectively. Lane 6; PCR-control.

Table 5: PCR product, sequenced results and BLAST-hits related to individual *C. limacina* stomach content DNA.

Adventfjorden summer individuals	Positive pcr product	Pcr product comment	Readable sequence	Sequence-length	Blast hit	Query cover	E value	% similarity	Comment
AS1S	Amphipoda	Strong PCR product	X	232 bp	*Gammarus wilkitzkii*	100 %	2E-115	99 %	
	Calanus spp.	Strong PCR product,weak multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Echinodermata	No PCR product*	-	-	NA	NA	NA	NA	No usable sequence obtained
AS2S	Amphipoda	Strong PCR product	X	234 bp	*Gammarus wilkitzkii*	98 %	6E-116	100 %	
	Parasagitta spp.	Strong PCR product,multiple bands	(X)	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
AS3S	Amphipoda	Strong PCR product	X	244 bp	*Gammarus wilkitzkii*	98 %	1E-117	99 %	
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
AS4S	Amphipoda	Strong PCR product	X	235 bp	*Gammarus wilkitzkii*	98 %	1E-112	99 %	
AS5S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Echinodermata	Weak PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
Adventfjorden winter individuals									
AV1S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
AV2S	*Calanus* spp.	Strong PCR product,multiple bands	X	377 bp	*Calanus glacialis*	100 %	1E-161	99 %	
AV6S	*Calanus* spp.	Strong PCR product,multiple bands	X	366 bp	*Calanus glacialis*	99 %	2E-173	98 %	
AV7S	*Calanus* spp.	Strong PCR product,multiple bands	X	375 bp	*Calanus glacialis*	100 %	4E-162	99 %	
Hinlopen Strait autumn individuals									
H5S	Euphausiacea	Weak PCR product,weak multiple bands	(X)	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
H12S	Pisces	Strong PCR product	-	-	No hit	NA	NA	NA	
Ice Edge springbloom individuals									
IES14S	*Parasagitta* spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
IES15S	*Calanus* spp.		-	-	NA	NA	NA	NA	No usable sequence obtained
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
IES16S	*Parasagitta* spp.	Strong PCR product,weak multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
IES17S	*Parasagitta* spp.	Strong PCR product	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
Kongsfjorden autumn individuals									
KF7S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No usable sequence obtained
	Echinodermata	Strong PCR product	-	-	NA	NA	NA	NA	No usable sequence obtained
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	
KF8S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Echinodermata	Strong PCR product,2 strong bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
Olga Basin autumn individuals									
OB2S	Euphausiacea	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
OB4S	Euphausiacea	Weak PCR product,weak multiple bands	(X)	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
OB9S	Amphipoda	Strong PCR product	X	271 bp	*Gammarus wilkitzkii*	99 %	5E-133	99 %	
	Echinodermata	Strong PCR product	-	-	NA	NA	NA	NA	No sequence-data recieved
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
OB10S	Amphipoda	Strong PCR product	X	271 bp	*Gammarus wilkitzkii*	99 %	5E-133	99 %	
	Calanus spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Pisces	Strong PCR product	-	-	NA	NA	NA	NA	No usable sequence obtained
OB11S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No usable sequence obtained
	Echinodermata	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No usable sequence obtained
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No usable sequence obtained
Rijpfjorden autumn individuals									
RIH4S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No sequence-data recieved
	Echinodermata	Weak PCR product,weak multiple bands	-	-	NA	NA	NA	NA	No sequence-data recieved
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No sequence-data recieved
RIH7S	*Calanus* spp.	Strong PCR product,multiple bands	-	-	NA	NA	NA	NA	No sequence-data recieved
	Echinodermata	Weak PCR product	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
	Parasagitta spp.	Strong PCR product,multiple bands	-	-	No hit	NA	NA	NA	No usable sequence obtained from reverse-primer
RIH12S	Pisces	Strong PCR product	-	-	NA	NA	NA	NA	No usable sequence obtained
Rijfjorden summer individuals									
RIS4S	Amphipoda	No PCR product*	-	-	NA	NA	NA	NA	No sequence-data recieved
RIS12S	Amphipoda	Strong PCR product	X	245 bp	*Gammarus wilkitzkii*	97 %	3E-116	99 %	
Smeerenburgfjorden autumn individuals									
SB1S	Amphipoda	Strong PCR product	X	211 bp	*Gammarus wilkitzkii*	100 %	1E-102	100 %	
SB3S	Amphipoda	Strong PCR product	X	271 bp	*Gammarus wilkitzkii*	99 %	5E-133	99 %	
SB5S	Amphipoda	Strong PCR product	X	270 bp	*Gammarus wilkitzkii*	99 %	2E-132	99 %	

Notes: * = No PCR-product appeared in the 2nd PCR prior to sequencing. X = a sequence of good quality. (X) = a partial sequence.

Table 6: Overview of the number of *C. limacina* individuals where prey DNA was detected, and the number of samples sent to and confirmed by sequencing in relation to the sampling location.

	Target group:	Amphipoda			*Calanus* spp.		
Area/season	**Total number of *C. limacina* individuals**	PCR-product	Sent to sequencing	Confirmed by sequencing	PCR-product	Sent to sequencing	Confirmed by sequencing
Adventfjorden/summer	15	11	4	4	3	2	0
Adventfjorden/winter	11	0	-	-	4	4	3
Billefjorden/autumn	2	0	-	-	0	-	-
Billefjorden/autumn	1	0	-	-	0	-	-
Billefjorden/winter	1	0	-	-	0	-	-
Hinlopen Strait/autumn	12	0	-	-	0	-	-
Ice Edge springbloom	18	0	-	-	1	1	0
Konsfjorden/autumn	12	0	-	-	8	2	0
Olga Basin/autumn	15	7	2	2	6	2	0
Rijpfjorden/autumn	17	0	-	-	7	2	0
Rijpfjorden/summer	16	2	2	1	1	-	-
Rijpfjorden/winter	12	1	-	-	0	-	-
Smeerenburgfjorden/autumn	6	3	3	3	0	-	-
Total:	138	24	11	10	30	13	3

Notes: – = no PCR-products were sent for sequencing, 0 = no detection from the PCR and the group-specific primers.

In total, amphipod DNA was confirmed to be present in 17 % of the total number of *C. limacina* stomachs analysed (24 *C. limacina* stomachs; Table 6). A potential occurrence of *Calanus* spp. DNA was identified in 21 % of the *C. limacina* stomachs analysed (30 *C. limacina* stomachs), although only three of these were confirmed by sequencing due to weak PCR products or multiple bands. A few individuals of *C. limacina* were found to have preyed upon more than one food item (Table 7). One individual of *C. limacina* from Rijpfjorden, three from Adventfjorden and five individuals from Olga Basin had fed both on amphipods and *Calanus* spp. (Table 7).

Table 7: *Clione limacina* individuals with DNA traces of multiple prey species, represented with the individual *C. limacina* ID according to sampling area and season.

Area/season	Amphipoda	*Calanus* spp.
Adventfjorden/summer	AS1S	AS1S
	AS5S	AS5S
	AS9S	AS9S
Olga Basin/autumn	OB4S	OB4S
	OB7S	OB7S
	OB8S	OB8S
	OB9S	OB9S
	OB10S	OB10S
Rijpfjorden/summer	RIS4S	RIS4S

3.3 Next-Generation Sequencing

A total of 10 individuals of *C. limacina* were analysed using Illumina MiSeq sequencing. From the 1 797 526 reads generated across the 10 samples, a total of 1 711 771 reads remained after filtering, chimera checking, clustering, and removal of singleton and unidentifiable OTUs. An average of 171 177 sequences and 21 OTUs were recovered per sample, where the majority (99.9983% or 1 711 740 reads) could be recognised as Gastropoda and presumably represent *C. limacina.* The OTUs were compared to a custom database including the SILVA reference sequences and those sequences of *C. limacina* and *L. helicina*-generated during this study. One *C. limacina* stomach showed a distinct prey signal from *L. helicina* with 5 525 reads, while 6 other individuals showed traces of *L. helicina* as a small number of reads (Table 8). The remaining 31 reads detected were assigned to protists belonging to Chloroplastida, Alveolata, Rhizaria, and Stramenopiles. Two *C. limacina* individuals had each one read of the chaetognath *Parasagitta elegans* (Table 8).

Table 8: Overview of the number of reads obtained from the NGS-results for the 10 *C. limacina* individuals analysed.

C. limacina individual:	H6S	H7S	H8S	H10S	H12S	IES15S	IES16S	IES17S	IES18S	KF8S
C. limacina	132 571	190 978	205 862	197 695	131 641	159 663	220 056	174 695	126 901	165 859
Limacina helicina	0	95	127	54	0	5	10	0	5 525	1
Parasagitta elegans	1	0	0	0	1	0	0	0	0	0
Protist	0	13	2	0	0	0	2	0	11	0

Notes: The *L. helicina* reads were a match to the reference sequence generated in this study, while the *Parasagitta elegans* and the protists reads represent matches to accessions in the SILVA database. As a full length sequence for *C. limacina* has not successfully been obtained, the best match in the SILVA database was the gastropod *Aiteng ater*. However, this match was presumed to represent *C. limacina* sequences, as the highest abundance of gastropod sequences was expected to be *C. limacina.*

3.4 Feeding experiment

Clione limacina buccal cones were observed extended with all 3 types of prey present, however, at the end of the experiment no prey had been ingested. The experiment was repeated with the same results. No further feeding-experiments were attempted on board the ship, in case engine vibrations, noise or other factors on board were stressing the animals as not even the *L. helicina* were predated. In the aquarium containing the all of the live *C. limacina*, one individual was observed while attempting to ingest a dead *Parasagitta elegans*, which accidentally occurred in the aquarium after changing seawater. The *C. limacina* individual attempted to feed on the *P. elegans* for more than 20 minutes. Conover and Lalli (1972) observed how feeding-time ranged from 2 minutes up to 45 minutes. It was not possible to confirm success or failure of this attempt at the time. Due to the challenges of keeping the *C. limacina* alive in the aquarium, no further feeding experiments were attempted in the lab. According to Böer et al. (2006b), live *C. limacina* were treated with an aquarium antibacterial treatment (Sera Baktopur direct, Germany) to decrease the possibility of the animals getting bacterial infections. Sera Baktopur was ordered for this study; however, it did not arrive in time to treat the live *C. limacina* caught in autumn 2011. The *C. limacina* suffered from bacterial infections and did not survive. No further feeding experiments were attempted.

3.5 Zooplankton identified from the sampling stations

A number of other zooplankton species, i.e. copepods, krill, chaetognaths, ctenophores, amphipods and young stages of fishes were among the organisms that were caught in the same sampling locations as *C. limacina* (Table A13, Appendix). In addition to these potential prey species for *C. limacina*, larvae and unidentified eggs from several taxa occurred at a few stations.

4. Discussion

4.1 Rejection of the H_0

During this study amphipods, *Calanus* spp. and possibly *Parasagitta elegans* were identified as part of the *C. limacina* diet. Whereas the NGS analysis suggests that *L. helicina* is the dominant prey in at least a few of the analysed stomachs of *C. limacina,* the detection of in particular amphipods and *Calanus* spp. DNA suggests that *C. limacina* is not a monophagous predator. Therefore, the results from this study enable the H_0 hypothesis to be rejected: *Clione limacina* is not feeding exclusively on *Limacina helicina*. The DNA-based methods revealed traces of non-*L. helicina* prey DNA throughout the year, undeniably disputing the traditional view of *C. limacina* being strictly monophagous.

4.2 Alternative prey consumption linked to season or dependent on *Limacina helicina* presence?

There was no obvious difference in the prey detected in the stomachs of *C. limacina* collected in different seasons (Table 6), suggesting that *C. limacina* feeds on alternative prey throughout the year. Although the number of individuals sampled varies across the seasons, from 18 individuals caught in the spring to a total of 53 individuals caught in the autumn, traces of non-*L. helicina* DNA were detected in all seasons. Interestingly, most of the *C. limacina* (11 of 15 individuals) caught in Adventfjorden during summer had ingested amphipods. Such a high frequency may be unexpected in a season when *L. helicina* should occur with a high abundance. However, this is a season with high density of zooplankton. If *C. limacina* actively hunt for food in this season, and feed on other types of prey, it is not surprising to find alternative prey in their stomachs. Juvenile amphipods were found in Adventfjorden during summer (Table A13, Appendix), and as DNA does not distinguish between juveniles and adults, *C. limacina* may have fed on the present juveniles. The Rijpfjorden summer population had a lower frequency of amphipod-traces with only 2 positive PCR-products in 16 samples, nevertheless supporting that alternative prey consumption occurs in *C. limacina*. The consumption of *Calanus* spp. by *C. limacina* was confirmed in Adventfjorden winter samples (Tables 5-6), where calanoid copepods may serve as an alternative and more reliable food source in periods when *L. helicina* is absent. Calanoid copepods overwinter in deeper water layers (Conover 1988; Søreide et al. 2003; Falk-Petersen

et al. 2008), the depth depending on species and area. The copepods occur in relatively high densities at overwintering depths, and feeding on calanoid copepods would be rather easy if *C. limacina* is migrating to these depths. A strong Dial Vertical Migration (DVM) or Seasonal Vertical Migration (SVM) pattern has not been investigated for *C. limacina*, although it is able to migrate up and down the water column (Mileikovsky 1970). *Clione limacina* actively maintains its position in the water column, and observation of ceased swimming followed by sinking to a lower depth has been recorded (Satterlie et al. 1985; Lalli and Gilmer 1989; Norekian and Satterlie 1996). There are indications that the main prey of *C. limacina*, namely *L. helicina*, performs DVM, although this has not been properly investigated (Falk-Petersen et al. 2008). At several sampling locations where traces of *Calanus* spp. DNA were detected, copepod nauplii were present in the water (Table A13, Appendix). The buccal cones could potentially catch nauplii or small organisms when *C. limacina* is hunting or successfully catching *L. helicina*, hence copepods and small zooplankton may be ingested sporadically in contrast to deliberately. The glands surfacing the buccal cones can secrete an adhesive mucus, and accidental ingestion has appeared to happen when specimens are being preserved (Lalli 1970; Hermans and Satterlie 1992). Lalli (1970) described how during the preservation procedure, buccal cones were protruded due to stress and any small object nearby could be attached and appear to be an ingestion-attempt. Accidental ingestion cannot be excluded in this study.

The NGS-results revealed consumption of *L. helicina* in 7 of the 10 *C. limacina* individuals analysed, although the number of reads varied greatly. For instance, for two individuals both caught at the ice edge during the spring bloom, the number of reads varied from 5 to 5525. This implies that consumption of *L. helicina* has started during the spring bloom. Three individuals caught in the Hinlopen Strait contained 54 and up to 127 reads of *L. helicina* sequences, while only one read for *L. helicina* was detected in the individual from Kongsfjorden. Thus, not surprisingly, feeding on *L. helicina* still happen in the autumn. However, alternative prey consumption occurred both during the presence and absence of *L. helicina* (Tables A1-A11, Appendix). In the individuals of *C. limacina* caught in Kongsfjorden during autumn, when *L. helicina* was absent, traces of *Calanus* spp. DNA were detected (Table 6; Table A6, Appendix). Thus, it appears that with the absence of *L. helicina*, feeding on copepods occurs instead. Four sampling stations are missing data on presence or absence of *L. helicina*, which makes it difficult to properly assess if alternative predation occur more often when *L. helicina* is absent or present. With indications of alternative prey

being consumed by *C. limacina*, further studies to assess if this occurs dependent or independent of *L. helicina* presence would be of scientific interest.

4.3 Arctic zooplankton and potential prey

Perennial zooplankters occur in smaller numbers during the Arctic winter, although copepods, chaetognaths, ctenophores, krill and amphipods can be found in the water masses co-occurring with overwintering *C. limacina* (Conover 1988; Weslawski et al. 1991; Søreide et al. 2003; Falk-Petersen et al. 2009). Therefore, although adult *L. helicina* is absent, potential food items are available during winter. The zooplankton species in the Arctic are adapted to survive highly fluctuating food availability (Hagen and Auel 2001; Ji et al. 2012). Overwintering strategies with lipid storage and hibernation are well known for Calanoid copepods (Conover 1988; Hagen and Auel 2001; Clark et al. 2012). However, several species are active hunters or opportunistic feeders even in times of low food abundance, such as *Metridia longa*, *Mertensia ovum*, *Themisto libellula* and *Parasagitta elegans* (Falk-Petersen et al. 2002; Hop and Falk-Petersen 2006; Kraft et al. 2012; Vestheim et al. 2013). Although these species are omnivorous, they feed on several different species and stages of copepods. In general, monophagy is rare in plankton-species. With such fluctuating abundance of *L. helicina* it is remarkable that *C. limacina* feed exclusively on one specific member of the zooplankton community, which previous studies have suggested (Lalli 1970; Conover and Lalli 1972; Hermans and Satterlie 1992).

4.4 The role of *Clione limacina* in the Arctic marine ecosystem

The evidence suggesting that *C. limacina* is polyphagous challenges the traditional view that *C. limacina* feeds exclusively on *L. helicina*. Although *C. limacina* is a highly specialised predator adapted to feed on *L. helicina* (Lalli 1970; Conover and Lalli 1972; Conover and Lalli 1974; Hermans and Satterlie 1992; Norekian 1995; Norekian and Satterlie 1996; Böer et al. 2005), it appears to be able to feed on other organisms as well. This result suggests that *C. limacina* is not explicitly dependent on the availability of *L. helicina* as its only food source. The ecological implications of a polyphagous *C. limacina* should be studied in more detail; nevertheless this implies an Arctic zooplankton food web with previously unknown predator-prey links. Another important ecological implication of a polyphagous *C. limacina* is the

possibility for this species to survive in a possible future Arctic ocean with lowered abundance of *L. helicina*. Due to its thin aragonite shell, *L. helicina* has been hypothesised to be susceptible to ocean acidification, particularly the overwintering larval stages (McNeil and Matear 2008; Lischka and Riebesell 2012). Levels of $CaCO_3$ are naturally low in wintertime, and are assumed to decrease further by climate change (Orr et al. 2005; McNeil and Matear 2008). As larval stages of *L. helicina* are most concentrated during winter, and with the shell degradation caused by $CaCO_3$ undersaturation, an effect in the population structure of *L. helicina* may be expected possibly leading to a decline of *L. helicina* in Arctic waters (Lischka and Riebesell 2012). In such a scenario, *C. limacina* probably stand a better chance of survival as a polyphagous rather than a monophagous predator.

4.5 Evaluation of the molecular methods used

The molecular approaches used to investigate *C. limacina* feeding ecology represent new and promising tools to investigate predator-prey relationships. The use of group-specific primers is a cheap and rather easy method to investigate feeding ecology. However, the efficiency of this approach depends on the availability and specificity of relevant primers. Designing group-specific primers, testing their accuracy and excluding potential cross-amplification is time-consuming (King and Read 2008), although when these primers have been developed they can be used in a wide range of similar studies. The data obtained from this method is qualitative; only presence and absence data can be acquired. Primers cannot distinguish DNA originating from eggs, juveniles or adults. However, in cases where no solid traces of adults have been found during visual gut content investigation, or predation on adults is less likely, DNA-traces from certain groups of organisms can originate from eggs or juveniles (Meekan et al. 2009). Therefore, DNA-based approaches could be a method to reveal predation on eggs or larvae by *C. limacina* if this indeed occurs. Juveniles, larvae and eggs from target organisms were present at some sampling-stations (Table A13, Appendix).

In this study, available group-specific primers developed for similar studies were used (Jarman et al. 2006). However, the specificity of these primers varied; while the amphipod primers produced strong PCR products resulting in good quality sequences that were identified as amphipod DNA, the echinoderm primers produced weaker products that could not be confirmed by sequencing. Group-specific primers developed for this type of investigations are still scarce, hence general primers designed to amplify certain groups were

also tested in this study (Jarman et al. 2002; Peijnenburg et al. 2004; Lindeque 2005; Ivanova and Zemlak 2007). Except for the Euphausiacea-primer developed by Jarman et al. (2002), these primers have not been tested for cross-amplification previously. The occurrence of by-products may explain the problems encountered when sequencing these products; the by-products might interfere with the sequencing process, resulting in low-quality sequences. In the samples that tested positive for presence of *Calanus* spp. DNA, only three were confirmed by sequencing (Table 5). The *Parasagitta* spp. primers appeared to amplify bacterial DNA, and hence the positive products from this primer were disregarded (Table A1-A11, Appendix for all results). Although the general primers for Euphausiacea and Pisces gave strong PCR-products, the sequences could not be identified as target DNA. The Pisces primer also appeared to produce bacterial products and sequences. The specificity of these primers does not appear to be high enough to only amplify target DNA when it occurs in small amounts, without unspecific amplification of host-DNA. Therefore, these primers cannot be used in a mixture of DNA, which is inevitable when extracting stomach content. A factor contributing to failed primer specificity can be the annealing temperature. A relatively low annealing temperature can compromise the specificity of both the group-specific primers and the general primers, leading to nested PCR runs (Table 2). Although the annealing temperatures were optimised during the study, the relatively low temperatures could induce formation of PCR by-products, especially for the general primers.

One disadvantage of using group-specific primers is that the relative abundance of prey DNA cannot be assessed. Next-Generation Sequencing is a promising approach for assessing relative abundance of different sequences (Bik et al. 2012). In this study, the NGS-method revealed presence of *L. helicina* in the stomach content of *C. limacina*. Predictably, the NGS-results exposed a high content of DNA originating from *C. limacina*. The relative abundance of *L. helicina* sequences obtained enables a comparison of ingested material between the analysed individuals. The number of reads varied greatly in these individuals, from 5 to 5525 reads in two individuals both caught at the ice edge during the spring bloom. However, this being the first *C. limacina* stomach DNA analysed by NGS-methods, the results cannot be used to assess if individuals have fed on different numbers of *L. helicina*, as the *C. limacina* could have been at different stages of digestion. To investigate this, stomach content DNA from newly fed *C. limacina*, individuals fed several *L. helicina*, individuals at different digestion stages and starving individuals must be analysed with NGS-methods to relate the amount of *L. helicina* reads to the feeding or digestion stage of *C. limacina.* The NGS-results

detected little alternative prey, which is inconsistent with positive PCR-products from the group-specific primers for the same *C. limacina* individuals (Table 8; Table A12, Appendix). For instance, *Calanus* spp. and Echinodermata were detected in the *C. limacina* individuals IES15S and KF8S with group-specific primers (Table A5-A6, Appendix), but these prey items were not identified using Illumina sequencing. Nevertheless, *L. helicina* DNA was confirmed in these individuals by NGS-sequencing (Table 8), indicating that they are active feeders. Two *C. limacina* individuals caught in the Hinlopen Strait revealed traces of *P. elegans*, with one read each (Table 8). These traces from *P. elegans* suggest that chaetognaths could be a potential prey for *C. limacina*, but this requires further analyses. The protist reads from the NGS-results may be regarded as coincidental ingestion from surrounding water, as phytoplankton is presumably too small to be actively preyed on by a carnivore such as *C. limacina* (Lalli and Gilmer 1989). The accuracy of species-identification from sequences highly depends on the DNA-libraries, which influences both the sequenced results of group-specific primer products and the NGS-results (Bik et al. 2012). Therefore, it is important to improve and expand the DNA-libraries if DNA-based methods are to be a significant part of ecological studies.

The NGS analyses were run without blocking-primers or other suppression of the predator DNA, to assess the effectiveness of this approach on its own. Next-Generation Sequencing has the advantage of producing a large amount of sequences and a substantial amount of data in a very short time compared to group-specific primers (Luo et al. 2012; Pompanon et al. 2012). The inability to detect anything but the predator, and to some degree the most abundant prey, indicates that it is necessary to remove or suppress predator DNA prior to sequencing to be able to reliably detect prey DNA in *C. limacina* stomachs. Developing blocking primers to suppress the amplification of host-DNA may enhance the detection rate of prey-DNA (Vestheim and Jarman 2008). However, designing, testing and optimising blocking-primers is a very time-consuming process. As my results suggest that *C. limacina* is not as dependent on *L. helicina* as previously assumed (Lebour 1931; Morton 1958; Lalli 1970; Conover and Lalli 1972; Arshavsky and Deliagina 1989; Hermans and Satterlie 1992; Norekian 1995; Falk-Petersen et al. 2001; Böer et al. 2005), further investigations into the polyphagous nature of *C. limacina* would profit from the development of blocking primers against *C. limacina*.

4.6 Feeding experiment

The feeding experiments did not give any results; surprisingly the *L. helicina* were not ingested. Hermans and Satterlie (1992) stated that inducing a feeding response in *C. limacina* was challenging, as more than one day could pass without *C. limacina* reacting to the presence of *L. helicina*. If the *C. limacina* had been treated with Sera Baktopur immediately, it could have been possible to keep them alive for a longer time period. Then it could have been possible to perform a more elaborate feeding-experiment. Animals caught with *C. limacina* individuals could have been kept alive in separate tanks, and presented as potential prey. Krill, amphipods, ctenophores, and any kind of larvae could have been presented, as well as a new attempt with copepods and chaetognaths. It might be necessary to keep the *C. limacina* without food for a longer period of time, to ensure a higher probability of feeding-response. Keeping several *C. limacina* in the same container during feeding experiments could trigger faster feeding responses. Lalli (1970) observed that the feeding response from one individual seemed to trigger buccal cone actions in other *C. limacina* as well. There appear to be high intraspecific competition between *C. limacina* individuals. When one individual attempted to feed on a *L. helicina*, *C. limacina* in the near vicinity would either attack the feeding *C. limacina* or attempt to get the prey (Lalli 1970). Due to the challenges associated with keeping multiple *C. limacina* alive, a second approach could be used to investigate feeding responses. As in the neural investigations performed by Norekian (1995), the method of adding homogenised prey to the water and measuring neural activity could be an alternative. Preparation for this type of study would be rather simple, and the experiment could definitely be replicated.

4.7 Limitations and restrictions of the study

The methods used in this investigation cannot give a proper assessment of how frequently *C. limacina* is consuming prey. It was not possible to assess which individuals had fed on *L. helicina*, the amount of prey ingested or if stomachs were empty, by the morphology of the visceral mass during dissection. There was a relatively low sample number from each location, which contributes to the uncertainty of assessing consumption-frequency of alternative prey.

Preferably, a number of associated samples would have been collected at every sampling location. However, gathering enough *C. limacina* individuals was prioritised. Therefore, a proper collection of zooplankton community data comprised of plankton in different size ranges could not be gathered. To determine if the presence of prey DNA in *C. limacina* stomachs was due to ingestion of eggs or juveniles, it would be necessary to confirm the presence of these in the water column. WP11-net sampling would be necessary for sampling eggs and juveniles, as the WP3 and WP2 nets have too large mesh-size to catch eggs or juveniles. The WP3 and WP2 nets are not sufficient to confirm abundance of the larger organisms such as amphipods and krill as these organisms are able to escape these sample nets (Ohman 1988). During the *C. limacina* sampling by MIK-net trawling, organisms present were noted down while handling the sampled animals (Table A13, Appendix). There was no time for detailed screening of the samples. Any quantitative investigations could not be performed, as the MIK-net was trawled to catch *C. limacina*. Only qualitative data for present potential prey could be noted, with varying accuracy.

5. Conclusion

Ecological studies improve our understanding of ecosystems, enabling us to better predict effects of changes in the environment. Visual gut content analyses in combination with biochemical analyses are powerful tools to enhance the knowledge of marine ecosystems. By using DNA-based analyses of gut content from *C. limacina*, the present study challenges the traditional view of *C. limacina* being a monophagous feeder. Compared to previous knowledge of the narrow food selection of *C. limacina*, the present study has revealed new and essential food web linkages that will be of importance for future modelling efforts of the high Arctic food chains. The ecological significance of these results can be regarded with respect to climate change. As *L. helicina* is susceptible to ocean acidification, utilising alternative prey could allow the continued existence of *C. limacina* in case of declining *L. helicina* populations. With new knowledge adding to the complexity of Arctic marine food-web interactions, further studies would be of interest to properly investigate the role of *C. limacina* in the ecosystem. As the dependence of *C. limacina* on the presence of *L. helicina* is not as tightly linked as previously considered, the fate of *C. limacina* in a changing Arctic environment must be re-evaluated.

6. Acknowledgements

I wish to thank my supervisors, Associate professor Tove M. Gabrielsen, Professor Jørgen Berge and Dr. Scient Stig Falk-Petersen, for giving me the opportunity to carry out this idea. Thank you for all your guidance, support and patience. Thanks to Post Doc Marie Louise Davey and Lab Engineer Courtney Nadeau for helping me handling the Illumina-data. I am grateful to Dr. Scient Anna Vader, PhD-candidate Archana Meshram, Lilith Kuckero and Stuart Thomson for always having advice at hand. Special mention is given to librarian Berit Jakobsen for never-ending detective-work in the search for literature. To my friends and colleagues at UNIS. I appreciate the assistance from the helping hands and the crewmembers on the R/V Helmer Hanssen, R/V Lance, K/V Svalbard, and R/V Viking Explorer during sample collection. I would like to thank Henrik Bjørklund, MSc. Carl Ballantine and Stuart Thomson for help with MatLab and Photoshop.

I need to thank my parents for endless support, always believing in me and encouraging me to pursue my curiosity in natural sciences.

To MSc. Marie K. Føreid for keeping me alive.

I appreciate the help from PhD-candidate Pernilla Carlsson, PhD-candidate Charmain Hamilton, Aquatic Biologist Terina Hancock, MSc. Lena Geitung, PhD-candidate Eike Stübner, Mark Dornbach and Anne Paa to make me see when my own words blinded me.

I have to thank Asbjørn Solheim for the supply of excellent coffee to keep me going. The hiding places which Alexander Pilditch and PhD-candidate Pernilla Carlsson provided were invaluable. Thanks to my friends, for all support, for always picking up the phone and making me laugh when I needed it.

To Maximilian Hinkel, for making me smile again.

Finally, I acknowledge the generous financial support from The Arctic University of Norway (UiT), The University Centre in Svalbard (UNIS), the CirCA-project, the Arctic Field Grant, the Fram Centre Flagship “Effects of climate change on fjord and coastal ecosystems in the North” and the Jan Christensen-fund.

References

Altschul S, Gish W, Miller W (1990) Basic local alignment search tool. J Mol Biol 215:403–410.

Arshavsky Y, Deliagina T (1989) Control of feeding movements in the pteropod mollusc, Clione limacina. Exp Brain Reseach 78:387–397.

Berge J, Varpe Ø, Moline M (2012) Retention of ice-associated amphipods: possible consequences for an ice-free Arctic Ocean. Biol Lett. doi: 10.1098/rsbl.2012.0517

Bik HM, Porazinska DL, Creer S, et al. (2012) Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol Evol 27:233–43. doi: 10.1016/j.tree.2011.11.010

Blachowiak-Samolyk K, Søreide JE, Kwasniewski S, et al. (2008) Hydrodynamic control of mesozooplankton abundance and biomass in northern Svalbard waters (79–81°N). Deep Sea Res Part II Top Stud Oceanogr 55:2210–2224. doi: 10.1016/j.dsr2.2008.05.018

Boas JE V. (1886) Zur Systematik und Biologie der Pteropoden. Zool Jb 311–340.

Böer M, Gannefors C, Kattner G, et al. (2005) The Arctic pteropod Clione limacina: seasonal lipid dynamics and life-strategy. Mar Biol 147:707–717. doi: 10.1007/s00227-005-1607-8

Böer M, Graeve M, Kattner G (2006a) Impact of feeding and starvation on the lipid metabolism of the Arctic pteropod Clione limacina. J Exp Mar Bio Ecol 328:98–112. doi: 10.1016/j.jembe.2005.07.001

Böer M, Graeve M, Kattner G (2006b) Exceptional long-term starvation ability and sites of lipid storage of the Arctic pteropod Clione limacina. Polar Biol 30:571–580. doi: 10.1007/s00300-006-0214-6

Caporaso JG, Kuczynski J, Stombaugh J, et al. (2010) QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. Nat Publ Gr 7:335–336. doi: 10.1038/nmeth0510-335

Clark K a. J, Brierley a. S, Pond DW (2012) Composition of wax esters is linked to diapause behavior of lanus finmarchicusn a sea loch environment. Limnol Oceanogr 57:65–75. doi: 10.4319/lo.2012.57.1.0065

Clarke A (1983) Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr Mar Biol 21:341–453.

Cleary A, Durbin E, Rynearson T (2012) Krill feeding on sediment in the Gulf of Maine (North Atlantic). Mar Ecol Prog Ser 455:157–172. doi: 10.3354/meps09632

Conover R (1988) Comparative life histories in the genera Calanus and Neocalanus in high latitudes of the northern hemisphere. Hydrobiologia 167:127–142.

Conover R, Lalli C (1972) Feeding and growth in Clione limacina(Phipps), a pteropod mollusc. J Exp Mar Bio Ecol 9:279–302.

Conover RJ, Lalli CM (1974) Feeding and growth in Clione limacina (Phipps), a pteropod mollusc. II. Assimiliation, metabolism, and growth efficiency. J Exp Mar Bio Ecol 16:131–154.

Cottier F, Tverberg V, Inall M, et al. (2005) Water mass modification in an Arctic fjord through cross-shelf exchange: The seasonal hydrography of Kongsfjorden, Svalbard. J Geophys Res 110:C12005. doi: 10.1029/2004JC002757

Daase M, Eiane K (2007) Mesozooplankton distribution in northern Svalbard waters in relation to hydrography. Polar Biol 30:969–981. doi: 10.1007/s00300-007-0255-5

Dalpadado P, Yamaguchi A, Ellertsen B, Johannessen S (2008) Trophic interactions of macro-zooplankton (krill and amphipods) in the Marginal Ice Zone of the Barents Sea. Deep Sea Res Part II Top Stud Oceanogr 55:2266–2274. doi: 10.1016/j.dsr2.2008.05.016

Dunshea G (2009) DNA-based diet analysis for any predator. PLoS One 4:e5252. doi: 10.1371/journal.pone.0005252

Edgar RC, Haas BJ, Clemente JC, et al. (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–200. doi: 10.1093/bioinformatics/btr381

Falk-Petersen S, Dahl T, Scott C (2002) Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. Mar Ecol Prog Ser 227:187–194. doi: 10.3354/meps227187

Falk-Petersen S, Hagen W, Kattner G, et al. (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178–191.

Falk-Petersen S, Leu E, Berge J (2008) Vertical migration in high Arctic waters during autumn 2004. Deep Sea Res II 55:2275–2284. doi: 10.1016/j.dsr2.2008.05.010

Falk-Petersen S, Mayzaud P, Kattner G, Sargent (2009) Lipids and life strategy of Arctic Calanus. Mar Biol Res 5:18–39. doi: 10.1080/17451000802512267

Falk-Petersen S, Sargent JR, Kwasniewski S, et al. (2001) Lipids and fatty acids in Clione limacina and Limacina helicina in Svalbard waters and the Arctic Ocean: trophic implications. Polar Biol 24:163–170. doi: 10.1007/s003000000190

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 8:115–120. doi: 10.1007/BF00297065

Gannefors C, Böer M, Kattner G, et al. (2005) The Arctic sea butterfly Limacina helicina: lipids and life strategy. Mar Biol 147:169–177. doi: 10.1007/s00227-004-1544-y

Gilmer R, Harbison G (1991) Diet of Limacina helicina(Gastropoda: Thecosomata) in Arctic waters in midsummer. Mar Ecol Prog Ser Oldend 77:125–134.

Hagen W, Auel H (2001) Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology 104:313–26. doi: 10.1078/0944-2006-00037

Hermans CO, Satterlie R a. (1992) Fast-Strike Feeding Behavior in a Pteropod Mollusk, Clione limacina Phipps. Biol Bull 182:1. doi: 10.2307/1542175

Hop H, Falk-Petersen S (2006) Physical and biological characteristics of the pelagic system across Fram Strait to Kongsfjorden. Prog Oceanogr 71:182–231. doi: 10.1016/j.pocean.2006.09.007

Ivanova N, Zemlak T (2007) Universal primer cocktails for fish DNA barcoding. Mol Ecol Notes 7:544–548. doi: 10.1111/j.1471-8286.2007.01748.x

Jarman S, Gales N, Tierney M (2002) A DNA-based method for identification of krill species and its application to analysing the diet of marine vertebrate predators. Mol Ecol 11:2679–90.

Jarman S, Redd K, Gales N (2006) Group-specific primers for amplifying DNA sequences that identify Amphipoda, Cephalopoda, Echinodermata, Gastropoda, Isopoda, Ostracoda and Thoracica. Mol Ecol Notes 6:268–271. doi: 10.1111/j.1471-8286.2005.01172.x

Ji R, Ashjian CJ, Campbell RG, et al. (2012) Life history and biogeography of Calanus copepods in the Arctic Ocean: An individual-based modeling study. Prog Oceanogr 96:40–56. doi: 10.1016/j.pocean.2011.10.001

Karlson K, Båmstedt U (1994) Planktivorous predation on copepods. Evaluation of mandible remains in predator guts as a quantitative estimate of predation. Mar Ecol Prog Ser 108:79–89.

Kattner G, Graeve M, Ernst W (1990) Gas-liquid chromatographic method for the determination of marine wax esters according to the degree of unsaturation. J Chromatogr A 513:0–5.

Kattner G, Hagen W, Graeve M, Albers C (1998) Exceptional lipids and fatty acids in the pteropod Clione limacina(Gastropoda) from both polar oceans. Mar Chem 61:219–228.

King R, Read D (2008) INVITED REVIEW: Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol Ecol 17:947–63. doi: 10.1111/j.1365-294X.2007.03613.x

Kraft A, Berge J, Varpe Ø, Falk-Petersen S (2012) Feeding in Arctic darkness: mid-winter diet of the pelagic amphipods Themisto abyssorum and T. libellula. Mar Biol 160:241–248. doi: 10.1007/s00227-012-2065-8

Kwasniewski S (2012) Interannual changes in zooplankton on the West Spitsbergen Shelf in relation to hydrography and their consequences for the diet of planktivorous seabirds. ICES J Mar Sci 69:890–901. doi: 10.1093/icesjms/fss076

Lalli CM (1970) Structure and function of the buccal apparatus of Clione limacina (Phipps) with a review of feedin in gymnosomatous pteropods. J Exp Mar Bio Ecol 4:101–118.

Lalli CM, Gilmer RW (1989) Pelagic Snails, The Biology of Holoplanktonic Gastropod Mollusks, 1st Edition. 58–213.

Larson R, Harbison G (1989) Source and fate of lipids in polar gelatinous zooplankton. Arctic 42:339–346.

Layman C, Arrington D, Montaña C, Post D (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48.

Lebour M V. (1931) Clione limacina in Plymouth Waters. J Mar Biol Assoc United Kingdom 17:785–795. doi: 10.1017/S0025315400051973

Lee R (1974) Lipids of Zooplankton from Bute Inlet, British Columbia. J Fish Board Canada 31:1577–1582.

Lee RF (1975) Lipids of Arctic zooplankton. Comp Biochem Physiol B 51:263–6.

Leu E, Søreide JE, Hessen DO, et al. (2011) Consequences of changing sea-ice cover for primary and secondary producers in the European Arctic shelf seas: Timing, quantity, and quality. Prog Oceanogr 90:18–32. doi: 10.1016/j.pocean.2011.02.004

Lindeque PK (2005) Integrating conventional microscopy and molecular analysis to analyse the abundance and distribution of four Calanus congeners in the North Atlantic. J Plankton Res 28:221–238. doi: 10.1093/plankt/fbi115

Lischka S, Riebesell U (2012) Synergistic effects of ocean acidification and warming on overwintering pteropods in the Arctic. Glob Chang Biol 18:3517–3528. doi: 10.1111/gcb.12020

Luo C, Tsementzi D, Kyrpides N, et al. (2012) Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample. PLoS One 7:e30087. doi: 10.1371/journal.pone.0030087

McNeil BI, Matear RJ (2008) Southern Ocean acidification: a tipping point at 450-ppm atmospheric CO2. Proc Natl Acad Sci U S A 105:18860–4. doi: 10.1073/pnas.0806318105

Meekan MG, Jarman SN, McLean C, Schultz MB (2009) DNA evidence of whale sharks (Rhincodon typus) feeding on red crab (Gecarcoidea natalis) larvae at Christmas Island, Australia. Mar Freshw Res 60:607. doi: 10.1071/MF08254

Mileikovsky S (1970) Breeding and larval distribution of the pteropod Clione limacina in the North Atlantic, Subarctic and North Pacific Oceans. Mar. Biol. 334:

Morton J (1958) Observations on the gymnosomatous pteropod Clione limacina (Phipps). J Mar Biol Ass UK 287–297.

Mullis K, Faloona F, Scharf S, et al. (1986) Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol 51:263–273.

Nejstgaard J, Frischer M (2003) Molecular detection of algal prey in copepod guts and fecal pellets. Limnol Oceanogr Methods 1:29–38.

Nilsen M, Pedersen T, Nilssen EM, Fredriksen S (2008) Trophic studies in a high-latitude fjord ecosystem — a comparison of stable isotope analyses (δ 13 C and δ 15 N) and trophic-level estimates from a mass-balance model. Can J Fish Aquat Sci 65:2791–2806. doi: 10.1139/F08-180

Norekian TP (1995) Prey capture phase of feeding behavior in the pteropod mollusc Clione limacina: neuronal mechanisms. J Comp Physiol A 177:41–53.

Norekian TP, Satterlie RA (1996) Whole Body Withdrawal Circuit and Its Involvement in the Behavioral Hierarchy of the Mollusk Clione limacina. J Neurophysiol 75:529–537.

Ohman M (1988) Behavioral responses of zooplankton to predation. Bull Mar Sci 43:530–550.

Orr JC, Fabry VJ, Aumont O, et al. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–6. doi: 10.1038/nature04095

Passmore AJ, Jarman SN, Swadling KM, et al. (2006) DNA as a dietary biomarker in Antarctic krill, Euphausia superba. Mar Biotechnol (NY) 8:686–96. doi: 10.1007/s10126-005-6088-8

Peijnenburg KTC a., Breeuwer J a. J, Pierrot-Bults AC, Menken SBJ (2004) Phylogeography of the Planktonic Chaetognath Sagitta Setosa Reveals Isolation in European Seas. Evolution (N Y) 58:1472–1487. doi: 10.1554/03-638

Pelseneer P (1885) Memoirs: The Cephalic Appendages of the Gymnosomatous Pteropoda, and especially of Clione. Q. J. Microsc. Sci.

Percy JA, Fife FJ (1981) The Biochemical and Energy Marine Content of Arctic Composition Macrozooplankton. Arctic 34:307–313.

Phipps CJ (1774) A Voyage Towards the North Pole. 275 pp.

Pompanon F, Deagle BE, Symondson WOC, et al. (2012) Who is eating what: diet assessment using next generation sequencing. Mol Ecol 21:1931–50. doi: 10.1111/j.1365-294X.2011.05403.x

Post DM (2013) Using Stable Isotopes to Estimate Trophic Position : Models , Methods , and Assumptions. Ecol Soc Am 83:703–718.

Quast C, Pruesse E, Yilmaz P, et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. doi: 10.1093/nar/gks1219

Satterlie RA, LaBarbera M, Spencer AN (1985) Swimming in the pteropod mollusc, Clione limacina. J Exp Biol 116:189–204.

Schloss PD, Westcott SL, Ryabin T, et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–41. doi: 10.1128/AEM.01541-09

Scott C, Falk-Petersen S, Gulliksen B, et al. (2001) Lipid indicators of the diet of the sympagic amphipod Gammarus wilkitzkii in the Marginal Ice Zone and in open waters of Svalbard (Arctic). Polar Biol 24:572–576. doi: 10.1007/s003000100252

Scott C, Falk-Petersen S, Sargent J (1999) Lipids and trophic interactions of ice fauna and pelagic zooplankton in the marginal ice zone of the Barents Sea. Polar Biol 65–70.

Stoeck T, Bass D, Nebel M, et al. (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 19 Suppl 1:21–31. doi: 10.1111/j.1365-294X.2009.04480.x

Suzuki A, Yuki M, Mawatari K (2001) Biological features of a pelagic snail, Clione limacina (Class Gastropoda). Mombetsu Okhotsk Sea Cold Ocean Res Assoc 16:163–173.

Symondson WOC (2002) Molecular identification of prey in predator diets. Mol Ecol 11:627–41.

Søreide J, Hop H, Falk-Petersen S, et al. (2003) Macrozooplankton communities and environmental variables in the Barents Sea marginal ice zone in late winter and spring. Mar Ecol Prog Ser 263:43–64. doi: 10.3354/meps263043

Søreide JE, Falk-Petersen S, Hegseth EN, et al. (2008) Seasonal feeding strategies of Calanus in the high-Arctic Svalbard region. Deep Sea Res Part II Top Stud Oceanogr 55:2225–2244. doi: 10.1016/j.dsr2.2008.05.024

Søreide JE, Hop H, Carroll ML, et al. (2006) Seasonal food web structures and sympagic–pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. Prog Oceanogr 71:59–87. doi: 10.1016/j.pocean.2006.06.001

Søreide JE, Leu E, Berge J, et al. (2010) Timing of blooms, algal food quality and Calanus glacialis reproduction and growth in a changing Arctic. Glob Chang Biol 1–10. doi: 10.1111/j.1365-2486.2010.02175.x

Tamelander T, Søreide JE, Hop H, Carroll ML (2006) Fractionation of stable isotopes in the Arctic marine copepod Calanus glacialis: Effects on the isotopic composition of marine particulate organic matter. J Exp Mar Bio Ecol 333:231–240. doi: 10.1016/j.jembe.2006.01.001

Töbe K, Meyer B, Fuentes V (2010) Detection of zooplankton items in the stomach and gut content of larval krill, Euphausia superba, using a molecular approach. Polar Biol 33:407–414. doi: 10.1007/s00300-009-0714-2

Varpe Ø, Jørgensen C, Tarling G, Fiksen Ø (2009) The adaptive value of energy storage and capital breeding in seasonal environments. Oikos 119:363–370. doi: 10.1111/j.1600-0706.2008.17036.x

Vestheim H, Brucet S, Kaartvedt S (2013) Vertical distribution, feeding and vulnerability to tactile predation in Metridia longa (Copepoda, Calanoida). Mar Biol Res 9:949–957. doi: 10.1080/17451000.2013.793806

Vestheim H, Edvardsen B, Kaartvedt S (2005) Assessing feeding of a carnivorous copepod using species-specific PCR. Mar Biol 147:381–385. doi: 10.1007/s00227-005-1590-0

Vestheim H, Jarman S (2008) Blocking primers to enhance PCR amplification of rare sequences in mixed samples–a case study on prey DNA in Antarctic krill stomachs. Front Zool 5:12. doi: 10.1186/1742-9994-5-12

Walkusz W, Kwasniewski S, Falk-Petersen S, et al. (2009) Seasonal and spatial changes in the zooplankton community of Kongsfjorden, Svalbard. Polar Res 28:254–281. doi: 10.1111/j.1751-8369.2009.00107.x

Walkusz W, Storemark K, Skau T (2003) Zooplankton community structure; a comparison of fjords, open water and ice stations in the Svalbard area. Polish Polar Res 24:149–165.

Weslawski J, Kwasniewski S, Wiktor J (1991) Winter in a Svalbard fiord ecosystem. Arctic 44:115–123.

Weydmann A, Søreide J (2013) Ice-related seasonality in zooplankton community composition in a high Arctic fjord. J Plankton Res 00:1–12. doi: 10.1093/plankt/fbt031

Zakharov IS, Ierusalimsky VN (1992) The neuroanatomical basis of feeding behavior in the pteropod mollusc, Clione limacina (Phipps). J Comp Physiol A 170:525–32.

Appendix

Table A1: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Adventfjorden during summer.

Adventfjorden summer individuals	AS1S	AS2S	AS3S	AS4S	AS5S	AS6S	AS7S	AS8S	AS9S	AS10S	AS11S	AS12S	AS13S	AS14S	AS15S
Sample depth (m)	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	15-0	15-0	15-0
Presence of *L. helicina* in water	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Amphipoda	1*	1*	1*	1*	1	1	1	1	1	0	1	1	0	0	0
***Calanus* spp.**	1*	0	0	0	1*	0	0	0	1	0	0	0	0	0	0
Echinodermata	1*	0	0	0	1*	1	1	0	0	0	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	1*	1*	1	1	0	0	0	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A2: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Adventfjorden during winter.

Adventfjorden winter individuals	AV1S	AV2S	AV3S	AV4S	AV5S	AV6S	AV7S	AV8S	AV9S	AV10S	AV11S
Sample depth (m)	45-0	45-0	45-0	45-0	45-0	45-0	45-0	45-0	45-0	45-0	45-0
Presence of *L. helicina* in water	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆
Amphipoda	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	1*	1*	0	0	0	1*	1*	0	0	0	0
Echinodermata	0	0	0	0	0	0	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = not detected. * = individuals sequenced to confirm identity of PCR-product. ◆ = presence of juvenile *L. Helicina*. Information on sequence-data is given in the results (Table 5).

Table A3: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Billefjorden during autumn and winter.

Billefjorden autumn/autumn/winter individuals	BF1S	BF4S	BFH1S	BFV1S
Sample depth (m)	50-0	50-0	50-0	180-100
Presence of *L. helicina* in water	1	1	NA	0
Amphipoda	0	0	0	0
***Calanus* spp.**	0	0	0	0
Echinodermata	0	0	0	0
Euphausiacea	0	0	0	0
Pisces	0	0	0	0
***Parasagitta* spp.**	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. Information on sequence-data is given in the results (Table 5).

Table A4: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Hinlopen Strait during autumn.

Hinlopen Strait autumn individuals	H1S	H2S	H3S	H4S	H5S	H6S	H7S	H8S	H9S	H10S	H11S	H12S
Sample depth (m)	150-0	150-0	150-0	150-0	150-0	180-0	50-0	50-0	50-0	100-0	100-0	150-0
Presence of *L. helicina* in water	1	1	1	1	1	1	1	1	1	1	1	1
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	0	0	0	0	0	0	0	0	0	0	0	0
Euphausiacea	0	0	0	0	1*	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	1*
***Parasagitta* spp.**	0	0	0	0	1	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A5: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught at the ice edge during the springbloom.

Ice Edge springbloom individuals	IES1S	IES2S	IES3S	IES4S	IES5S	IES6S	IES7S	IES8S	IES9S	IES10S	IES12S	IES13S	IES14S	IES15S	IES16S	IES17S	IES18S
Sample depth (m)	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0	50-0
Presence of *L. helicina* in water	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆	1◆
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0
Echinodermata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	0	0	0	0	0	0	0	1*	1*	1*	1*	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. ◆ = presence of juvenile *L. helicina*. Information on sequence-data is given in the results (Table 5).

Table A6: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Kongsfjorden during autumn.

Kongsfjorden autumn individuals	KF1S	KF2S	KF3S	KF4S	KF5S	KF6S	KF7S	KF8S	KF9S	KF10S	KF11S	KF12S
Sample depth (m)	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0
Presence of *L. helicina* in water	0	0	0	0	0	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	1	0	1	1	1	0	1*	1*	1	0	1	0
Echinodermata	1	1	1	1	1	0	1*	1*	1	1	1	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	0	1*	1*	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A7: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Olga Basin during autumn.

Olga Basin autumn indiviuals	OB1S	OB2S	OB4S	OB7S	OB8S	OB9S	OB10S	OB11S	OB12S	OB13S	OB14S	OB15S	OB16S	OB17S	OB18S
Sample depth (m)	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m	170-0m
Presence of *L. helicina*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Amphipoda	1	1	1	1	1	1*	1*	0	0	0	0	0	0	0	0
***Calanus* spp.**	0	0	1	1	1	1	1*	1*	0	0	0	0	0	0	0
Echinodermata	0	0	0	1	1	1*	0	1*	0	0	0	0	0	0	0
Euphausiacea	0	1*	1*	1	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	1*	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	1	1	1	0	1*	0	1*	0	0	0	0	1	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A8: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Rijpfjorden during summer.

Rijpfjorden summer individuals	RIS1S	RIS2S	RIS3S	RIS4S	RIS5S	RIS6S	RIS7S	RIS8S	RIS9S	RIS10S	RIS11S	RIS12S	RIS13S	RIS14S	RIS15S	RIS16S	RIS17S
Sample depth (m)	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	20-0	200-0	170-0	170-0	170-0	170-0
Presence of *L. helicina* in water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Amphipoda	0	0	0	1*	0	0	0	0	0	0	0	1*	0	0	0	0	0
***Calanus* spp.**	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A9: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Rijpfjorden during autumn.

Rijpfjorden atuumn individuals	RIH1S	RIH2S	RIH3S	RIH4S	RIH5S	RIH6S	RIH7S	RIH8S	RIH9S	RIH10S	RIH11S	RIH12S	RIH13S	RIH14S	RIH15S	RIH16S
Sample depth (m)	25-0	25-0	25-0	25-0	25-0	25-0	25-0	25-0	25-0	25-0	25-0	25-0	50-0	50-0	50-0	20-0
Presence of *L. helicina* in water	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	1	1	0	1*	1	1	1*	1	0	0	0	0	0	0	0	0
Echinodermata	1	0	1	1*	1	1	1*	1	0	0	1	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	0
***Parasagitta* spp.**	0	0	0	1*	1	0	1*	0	0	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A10: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Rijpfjorden during winter.

Rijfjorden winter individuals	RIV1S	RIV2S	RIV3S	RIV4S	RIV5S	RIV6S	RIV7S	RIV8S	RIV9S	RIV10S	RIV11S	RIV12S
Sample depth (m)	75-0	75-0	75-0	75-0	75-0	75-0	75-0	75-0	75-0	75-0	75-0	75-0
Presence of *L. helicina* in water	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Amphipoda	1	0	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	0	0	0	0	0	0	0	0	0	0	0	0
Echinodermata	0	0	0	0	0	0	0	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	0	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A11: Direct PCR-results according to the individual *C. limacina* stomach content DNA, from individuals caught in Smeerenburgfjorden during autumn.

Smeerenburgfjorden autumn individuals	SB1S	SB2S	SB3S	SB4S	SB5S	SB6S
Sample depth (m)	10-0	15-0	15-0	15-0	15-0	20-0
Presence of *L. helicina* in water	1	1	1	1	1	1
Amphipoda	1*	0	1*	0	1*	0
***Calanus* spp.**	0	0	0	0	0	0
Echinodermata	0	0	0	0	0	0
Euphausiacea	0	0	0	0	0	0
Pisces	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	0

Notes: 1 =Detected presence, 0 = Not detected. * = individuals sequenced to confirm identity of PCR-product. Information on sequence-data is given in the results (Table 5).

Table A12: Potential prey DNA detected based on group-specific priemrs (Direct PCR-results) and the Illumina run (NGS-results) for the 10 individuals investigated utilising NGS.

C. limacina individual	H6S	H7S	H8S	H10S	H12S	IES15S	IES16S	IES17S	IES18S	KF8S
Direct PCR-products:										
Amphipoda	0	0	0	0	0	0	0	0	0	0
***Calanus* spp.**	0	0	0	0	0	1	0	0	0	1
Echinodermata	0	0	0	0	0	0	0	0	0	1
Euphausiacea	0	0	0	0	0	0	0	0	0	0
***Parasagitta* spp.**	0	0	0	0	0	1	1	1	0	1
NGS-results:										
L. helicina	0	1	1	1	0	1	1	0	1	0
Parasagitta elegans	1	0	0	0	1	0	0	0	0	0
Protist	0	1	1	0	0	0	1	0	1	0

Notes: 1 =Detected presence, 0 = Not detected. NGS-results is converted to presence-absence data, the NGS-number of reads are given in the results (Table 8).

Table A13: Zooplankton identified at the *C. limacina* sampling locations. The zooplankton was collected as a part of UNIS student projects, and the species list were made available to this study.

Organisms	Adventfjorden summer	Adventfjorden winter	Billefjorden autumn	Billefjorden winter	Hinlopen strait autumn	Ice Edge springbloom	Kongsfjorden autumn	Olga Basin autumn	Rijpfjorden autumn	Smeerenburgfjorden autumn
Copepoda	*Calanus* sp. Other copepods	*Calanus* sp. Other copepods	*Calanus* sp. Other copepods	*Calanus* sp. Other copepods	*Calanus* sp. Copepod nauplii Other copepods	*Calanus* sp. Copepod nauplii	*Calanus* sp. Copepod nauplii Other copepods	*Calanus* sp. Copepod nauplii Other copepods	*Calanus* sp. Copepod nauplii Other copepods	*Calanus* sp. Other copepods
Amphipoda	Juvenile *Onisimus* sp. Juvenile *Themisto libellula*		*Gammarus wilkitzkii* *Themisto abyssorum* *Themisto libellula*	*Gammarus wilkitzkii*	*Apherusa glacialis* *Gammarus wilkitzkii* *Onisimus glacialis* *Onisimus nanseni* *Themisto abyssorum* *Themisto libellula*	*Apherusa glacialis* *Gammarus wilkitzkii* *Hyperia galba* Juvenile amphipods *Onisimus glacialis* *Onisimus nanseni* *Themisto libellula*	*Themisto abyssorum* *Themisto libellula*	*Hyperia galba* *Themisto abyssorum* *Themisto libellula*		
Euphasiacea	Juvenile *Thysanoessa* sp.	*Thysanoessa* sp.	*Thysanoessa inermis* *Thysanoessa longicaudata*		Euphasiid larvae *Meganyctiphanes norvegi* *Thysanoessa inermis* *Thysanoessa longicaudata*	*Meganyctiphanes norvegica* *Thysanoessa inermis* *Thysanoessa longicaudata*	*Meganyctiphanes norvegica* *Thysanoessa inermis* *Thysanoessa longicaudata*	*Thysanoessa* sp.	Euphasiid larvae *Thysanoessa inermis*	
Other organisms	Chaetognaths Crustacea larvae Fish larvae Hydrozoans *Limacina helicina*	Chaetognaths Hydrozoans Juvenile *Limacina helicina* *Limacina retroversa*	Chaetognaths Crustacea larvae Hydrozoans *Limacina helicina*		Chaetognaths Hydrozoans *Limacina helicina*	Chaetognaths *Crustacea larvae* Ctenophore-larvae Eggs indet. Fish eggs Hydrozoans Juvenile *Limacina helicina* Oikiopleura larvae Polychaete larvae	Chaetognaths Hydrozoans	Hydrozoans Chaeognaths Crustacea larvae Echinoderm larvae Gastropod veliger *Limacina helicina*	Gastropod larvae *Limacina helicina*	Chaetognaths Hydrozoans *Limacina helicina*