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Distribution, Reproduction, and Transport of Zooplankton in the Western Arctic

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DISTRIBUTION, REPRODUCTION, AND TRANSPORT OF ZOOPLANKTON IN THE WESTERN ARCTIC

By

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This dissertation focuses on the distribution, reproduction, and transport of zooplankton in the Chukchi and Beaufort seas and adjacent Canada Basin. Specifically, it analyzes 1) the species-specific distribution of copepod nauplii as it is forced by the surface layer mesoscale circulation and physical properties, 2) the reproduction of the dominant copepod *Calanus glacialis* in the western Arctic, and 3) the effects of eddy transport on the zooplankton community in the Chukchi and Beaufort seas and Canada Basin. To achieve this I adapted a molecular identification method to work with small crustaceans. The method was successfully used to generate a sequence database from adult copepods of species present in the region. Differences in the D1/D2 domains of the large subunit ribosomal were sufficient for the identification of all species present in the western Arctic with the exception of two sibling *Calanus* copepods which were discriminated using the mitochondrial cytochrome oxidase I gene. The application of this semi-robotic protocol to selected surface samples collected from the USCGC *Healy* in summer 2004 revealed that four copepod species dominated the naupliar community: *Oithona similis*, *C. glacialis*, *Pseudocalanus minutus*, and *P. mimus*. Each species had different abundance and distribution patterns related to their specific life cycles and environmental affinities. The molecular identification method was then applied to study the egg production rates of *C. glacialis* and differentiate it from *C. marshallae*. This work

validated reported spatial and seasonal variations in egg production rates in *C. glacialis* and showed that an increase in primary production in summer 2004 compared to summer 2002 did not result in an increase in secondary production of this copepod. The last component of this study is the result of a unique sampling design to study shelf-basin interactions. The results provided evidence of on-shelf transport of basin organisms by a wind-induced upwelling event and of eddy-mediated advection of zooplankton from the surrounding shelves into the Arctic basin. Overall, this study integrated new molecular tools and unusual sampling opportunities to advance our understanding of the role of zooplankton in this Arctic ecosystem.

Les questions les plus intéressantes restent des questions. Elles enveloppent un mystère.

A chaque réponse, on doit joindre un «peut-être».

Il n'y a que les questions sans intérêt qui ont une réponse définitive.

Éric-Emmanuel Schmitt

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CHAPTER 1: AN INTRODUCTION TO ZOOPLANKTON IN THE WESTERN ARCTIC

BACKGROUND

Some of the greatest advances in understanding the structure and functioning of natural systems occur when unprecedented opportunities or newly developed methods unlock scientific doors (Hansell and Carlson, 2001). Unparalleled circumstances offer unique opportunities to answer important questions. New developments that overcome older methodological constraints provide the tools to advance our knowledge. The present study results from the convergence of unusual field conditions during the Western Arctic Shelf-Basin Interactions (SBI) Project and a molecular identification technique previously reported to work on billfish larvae (Richardson et al., 2006) and adapted for the identification of small crustaceans at Rosenstiel School of Marine and Atmospheric Science (RSMAS). The results significantly advance our understanding of the distribution, reproduction, and transport of zooplankton, particularly copepods, in the western Arctic Ocean.

The SBI program was designed to investigate the impact of global change on the physical, biological and geochemical processes occurring over the shelf, slope and basin regions in the western Arctic Ocean (Grebmeier, 2003). This multidisciplinary project included field seasons in 2002, 2003, and 2004. In 2002, the Arctic sea ice extent reached its lowest level recorded since satellite tracking began 1978 (Serreze et al., 2003) and hence the summer 2002 field season was completed under record ice-free conditions. In the past, a low ice year was generally followed by a rebound to near-normal conditions;

however, 2002 was followed by two more low-ice years, both of which almost matched the 2002 record (Meier et al., 2005). These unprecedented summer low-ice conditions in 2002, 2003 and 2004 were coupled with weak wintertime recoveries and rapid ice melt in spring. Furthermore, in 2004 an increased inflow of warm Alaskan Coastal Water raised surface temperatures in the Beaufort Sea area ~ 5ºC higher than in previous years (Codispoti et al., submitted). As a result, sea ice cover continued shrinking and a new minimum in ice cover was observed in 2005 (Serreze et al., 2007). Our results are fortuitously embedded in the Arctic-wide trend of reduction in ice coverage and provide a unique opportunity to study ecosystem responses to climate warming (IPCC, 2007).

The sensitivity of the western Arctic to climate change has important consequences for human society. Reduced ice cover adversely impacts higher trophic-level animals, particularly marine mammals, and the native communities that depend on them. Physical forcing can also alter food webs through bottom up effects such as changes in species composition. One of the major goals of the SBI program was to investigate the biogeochemical modifications of North Pacific and Arctic waters over the Chukchi and Beaufort shelf and slope areas, with an emphasis on the role of key organisms in the ecosystem (Grebmeier and Harvey, 2005). This work focuses on the zooplankton community in the ecosystem of the western Arctic, which is the principal link between primary producers and higher trophic levels. The zooplankton community on the Chukchi and Beaufort shelves reflects the mixture of water masses of Pacific and Arctic origin. Small-sized copepods numerically dominate the zooplanktonic community of these continental shelves. Dominance by these small copepod species subsequently leads to proliferation of benthic organisms because most of the primary production sinks

to the bottom. The advection of large-bodied copepods from the adjacent basins onto the shelves has complex effects on the fate of primary production depending on the zooplankton structure (Wassman, 1998; Pastemak et al., 2002). Large copepods ingest the slowly sinking phytoplankton cells and release faster sinking fecal pellets (Wassman et al., 1999). In contrast, strong grazing by mesozooplankton can retain a large part of the production in the surface layer and enhance the pelagic ecosystem (Wassman, 1998). Given the significant role of copepods in regulating the fate of shelf-derived primary production, alterations in zooplankton abundance and composition can potentially drive large ecosystem changes.

One of the prerequisites to investigating zooplankton populations is the correct classification of species. A persistent problem when investigating copepod communities, especially immature stages, is that accurate identification is not always possible (Hill et al., 2001; Lindeque et al., 2004). Each life history stage of a copepod species has a different morphological form, but species descriptions are based on the characteristics of the adult stages. Also morphologically similar species often co-exist. In the western Arctic alone, four *Pseudocalanus* species and two sibling *Calanus* species co-occur. *Pseudocalanus* have unusually weak interspecific divergence in morphological features (Frost, 1989) and the two *Calanus* species found in the region cannot easily be distinguished visually (Frost, 1974). This identification problem falls beyond the capabilities of traditional non-molecular observation and thus for this research I used molecular markers to overcome this constraint.

Over the past five years, I became familiar with the molecular tools used in marine ecology (reviews by Burton, 1996; Avise, 2004) and the various molecular

methodologies available to classify marine organisms. Through collaborative efforts at RSMAS, I explored how different technological developments such as hybridization assays (i.e. Kiesling, 2004) and species-specific polymerase chain reactions (i.e. Bucklin et al., 1999) could be adapted to identify to the species level any developmental stage of the copepods present in the western Arctic. In the end, our choice of methodology was based on the expected efficiency of the approach to our specific problem. Using a semi-robotic protocol to extract and sequence DNA, I are able to 1) generate a sequence database from adult copepods of species present in the western Arctic, 2) assay relatively small quantities of DNA (~ 10 ng) from ethanol preserved samples, 3) enhance the speed at which copepod taxa can be identified, and 4) reduce labor and operational costs. This automation was complemented with a bioinformatics program developed to analyze the large sequence output (Richardson et al., 2006). The result is an easily interpreted table with suggested species identification and sequence information, which considerably reduces the training required.

TOPICS AND GOALS

The topic addressed in this study, the ecology and structure of zooplankton populations in the Chukchi and Beaufort seas and the Canada Basin, is a scientifically challenging one. The western Arctic is an extensive region with varied shelf systems (the broad Chukchi Shelf and the narrow Beaufort Shelf) and the interactions within its marine ecosystems are complex and in some aspects still poorly understood. To analyze patterns of distribution and abundance of zooplankton and transport processes requires working across disciplines and the integration of varied data sets. The challenge has been met in each of the chapters in an effort to elucidate the physical, chemical and biological

processes that affect zooplankton in the Chukchi and Beaufort seas and adjacent Canada Basin. Throughout the various chapters, the main objective is to provide new tools and information to understand, model, and predict the effects of climate variation on the marine ecology of this Arctic ecosystem.

This dissertation provides reviews of what is known about the numerically dominant copepods in the Chukchi and Beaufort seas. Chapter 2 discusses the literature on molecular approaches for species-level identification of organisms with few morphological distinguishable characteristics, with a focus on arctic copepods. Chapter 3 reviews the literature on the life histories of four copepod species (*O. similis*, *C. glacialis* *P. minutus* and *P. mimus*) in the Chukchi and Beaufort seas, while the next chapter studies what is known about the reproductive strategy and egg production rates of *C. glacialis*. The last chapter – chapter 5 – summarizes the literature on eddies in the western Arctic and on the current understanding of circulation pathways in the western Arctic.

Chapter 2 describes the molecular protocol developed to identify small crustaceans including a brief account of the composition of the copepod nauplii community found in four regions of the western Arctic. Much of the chapter is devoted to detailing the methodology of how DNA is extracted, amplified, and sequenced from hundreds of organisms at a time. As part of this chapter I also provide suggestions on the application of this novel technique to large ecological studies. In the next two components, this method is applied to identify copepod nauplii to the species level and to distinguish between the two *Calanus* species that co-occur in the study area: *Calanus glacialis* and *C. marshallae*.

The abundance and distribution patterns of copepod nauplii in the western Arctic during summer are the topic of chapter 3. Here I study the species-specific distribution of copepod nauplii in the Chukchi and Beaufort seas as it is forced by the mesoscale circulation and characteristics of the surface layer. To accomplish this I draw from three disciplines: molecular biology, physical oceanography, and ecology. Special attention is paid to four dominant copepod species, *O. similis*, *C. glacialis P. minutus* and *P. mimus*, because they were usually present in all surface samples in significant numbers and their juvenile and adult stages dominate the mesozooplankton biomass and abundance in the region (Ashjian et al., 2003; Lane et al., 2007). The results provide new information on the life cycle strategies of each species in the region and their individual roles in the shelf and basin ecosystems.

A chapter on egg production rates of the copepod *Calanus glacialis* in the Chukchi and Beaufort seas – chapter 4 – uses molecular markers to validate previous studies in the region (Plourde et al., 2005) that were compromised by the difficulty of differentiating between the Arctic calanoid *C. glacialis* and the Pacific calanoid *C. marshallae*. Chapter 4 deals both with the hydrographic conditions of the region and the biological adaptations of the species, with a focus on the effects of the physical environment on reproducing *C. glacialis* females.

The last research chapter integrates physical and chemical properties with zooplankton distributions that were obtained during a survey of a cold core eddy in September 2004 in the Chukchi Sea. In chapter 5, I demonstrate that this feature originated at the shelf-break showing that biophysical properties in its core are similar to biophysical properties of the shelf-break region at the estimated time of formation. The

goal is to investigate the role of eddies in the distribution and transport of zooplankton from the Chukchi Shelf into the Canada Basin. Formation and subsequent migration of eddies have implications with respect to two regional issues: the transport of some zooplankton species from the periphery to the basin interior and the effects of this transport on local food webs.

A summary of the findings presented closes this dissertation. I integrate our results over the Chukchi and Beaufort shelves and adjacent slopes to facilitate extrapolation of the western Arctic to a Pan-Arctic perspective. To conclude, I discuss future research that needs to be done in the Arctic Ocean.

CHAPTER 2: A MOLECULAR METHOD TO RAPIDLY IDENTIFY SMALL CRUSTACEANS

INTRODUCTION

Correct species identification is a requisite to study marine communities and ecosystems. However, classification of individuals is not always possible because the early life stages of many species are morphologically indistinguishable and closely related species overlap in their distributions. This limitation is especially acute for certain taxonomic groups and within certain regions. For copepods, which pass through six naupliar and five copepodid stages before molting into adults, the earliest identifiable developmental stage is generally the first copepodid stage. As a consequence, most ecological studies of copepods do not consider the naupliar stages (Björnberg, 1984) or classify them into broad taxonomic groups (Kosobokova and Hirche, 2000). Other studies assume that the species composition is homogeneous across the development stages (Heath et al., 2000) ignoring that different environmental pressures act on each developmental stage of a species from egg to adult.

Over the last decade, research efforts have focused on molecular approaches for species-level identification of organisms with few morphologically distinguishable characteristics and/or developmental variability (Webb et al., 2006). Work with copepods includes allozyme variation, restriction fragment length polymorphism (RFLP), species-specific polymerase chain reactions (PCR), hybridization assays, and sequencing of mitochondrial and nuclear genes (Kann and Wishner, 1996; Bucklin et al., 1999; Lindeque et al., 1999; Kiesling et al., 2002; Goetze, 2005). Some of these identification methods were successfully applied to investigate copepod communities (e.g.,

Lindeque et al., 2006), while others have never been incorporated into large ecological studies. This is probably due to limitations of some technical component of each approach. RFLP and species-specific PCR are only appropriate for studies of a small number of species; hybridization probes have high development costs and can only identify targeted organisms; and DNA sequencing has historically been far too labor-intensive and expensive for most population level studies involving large sample sizes (Parker et al., 1998). An ideal method should use DNA markers that reliably reveal adequate genetic variation for a particular question, work with a minimum amount of effort and expense, and are applicable for the identification of a wide diversity of species.

Sequencing techniques in combination with PCR provide a method of collecting precise data for nucleotide sequences, and can be very powerful when used to analyze various regions of the mitochondrial and nuclear genomes (Parker et al., 1998). Sequencing efforts with copepods have focused on the mitochondrial cytochrome oxidase subunit I gene (mtCOI) and the large ribosomal (28S rDNA) nuclear gene. The slowly evolving 28S rDNA gene has been effectively used to investigate phylogenetic relationships among copepod orders (Braga et al., 1999; Kiesling, 2004; Tjensvoll et al., submitted). Lower taxonomic relationships among copepod species have been studied with the faster evolving mtCOI gene (Rocha-Olivares et al., 2001; Bucklin et al., 2003; Goetze, 2003). Other genes have also been successfully used to study molecular evolution in copepods (Willet and Burton, 2004; Machida et al., 2006). The conclusion from these studies is that different biological questions (e.g., population studies, species identification, phylogenies and evolution) can be addressed by sequencing adequate

genes for each question. However, in the past sequencing has been limited by cost and time requirements.

Our work with the ecology of copepods was an incentive to refine DNA extraction methods, integrate molecular techniques with the advent of automated equipment, and facilitate sequence analyses using bioinformatics software. The result is a rapid and simple molecular protocol to identify copepod species that is suitable for large scale population studies and can be adapted to address a variety of ecological and phylogenetic questions. The sequence-based identification method works consistently with small copepods (small amount of target DNA), and because the extraction, purification and sequencing steps have been automated, over 700 individuals can be analyzed per week.

In this study, I demonstrate the effectiveness of this molecular technique by extracting DNA from different developmental stages of the calanoid copepod *Calanus glacialis*. *Calanus glacialis* is one of the most abundant large-bodied copepods in the Arctic Ocean, and it often dominates the zooplankton community in terms of biomass (Kosobokova et al., 1997; Kosobokova, 1999; Auel and Hagen, 2002). I then show the applicability of this genetic technique for identifying copepod nauplii collected from different regions of the Chukchi and Beaufort seas and adjacent Canada Basin. Nauplii often dominate zooplankton assemblages in numbers, are a useful indicator of regional copepod reproduction, and no prior data exist on their species composition in the region.

MATERIALS AND METHODS

Field work

Sample collection

To collect surface plankton samples, I used a continuous-flow seawater system installed aboard the USCGC *Healy*. The intake of the system was located from the sea surface to 3 m depending on the speed of the ship and the sea conditions. The intake was clear polyvinyl hose weighted with a stainless steel bolt and deployed through the tube in the aft end of the ship designed for launching expendable bathythermograph sensors. The water inflow was then directed into a vortex debubbler (Ocean Instrument Laboratory). From the debubbler the water with bubbles flowed into a sink in the laboratory while a constant bubble-free seawater flow continued through a thermosalinograph (Seabird) and then into a SCUFA fluorometer. In the sink the water with bubbles coming from the debubbler and the outflow of seawater from the SCUFA fluorometer were used together to measure the flow rate of the continuous-flow system. After the flow rate had been measured, a 1-liter beaker with large drain holes covered with 35 μm mesh netting was placed under the outflow for a specified time period (generally 10 min) to collect the samples of zooplankton. The samples were rinsed with 95% ethyl alcohol to remove the seawater, then transferred into 22 ml vials for storage, and finally preserved in 95% ethyl alcohol for quantitative and molecular analysis. After two years of preservation, the samples were enumerated microscopically and the ethanol was changed before molecular analysis.

Laboratory analysis

Sample enumeration

Surface zooplankton samples were analyzed in the laboratory with a Leica MS5 microscope. The use of 35 μm mesh sieves permits quantitative estimates of copepod nauplii and early copepodids of most copepod species. Taxa enumerated for each sample included copepod categories such as copepod nauplii, calanoid copepodids, *Oithona similis* adults, *O. similis* copepodids, *Pseudocalanus* spp. adults and copepodids stages 4-5, *Acartia longiremis* adults and copepodids stages 4-5, and other zooplanktonic groups like appendicularians, barnacle nauplii, bivalve larvae, echinoderm larvae and eggs. Other taxa observed infrequently were also identified and their abundance noted. Each sample was counted in its totality, and the number of individuals counted was divided by the volume of water filtered to estimate abundance (No. m^{-3}).

Molecular analysis

I first established sequences for the dominant copepods inhabiting the Chukchi and Beaufort seas and the Canada Basin (Table 2.1) since no sequences of the D1/D2 domains of the large subunit ribosomal DNA (28S rDNA) or the mitochondrial cytochrome oxidase I (mtCOI) genes have been reported for most of them. The species were identified by a taxonomic expert. For each species a single consensus sequence was generated from four or more individuals to investigate any intra-specific variability.

The DNA extraction, isolation, amplification and sequencing reactions were performed in 96-well plates. During the extraction and isolation reactions, two wells were used: one as a positive control and the other as a negative control. The positive control well contained a previously identified adult copepod and the negative control well

contained only reagents without any specimen. An additional two wells were used as positive and negative controls in the amplification reaction (PCR). Instead of the isolated DNA the positive PCR control well contained DNA from an ammonium acetate extraction of a previously identified adult copepod. The negative PCR control well contained only PCR reagents without any DNA. The controls served as secondary identifiers of the orientation and identification of the plates for sequencing.

DNA extraction and isolation

To clean the copepods of contaminants, each individual was rinsed three times in sterilized nano-filtered water and transferred individually into wells filled with 60µl of 1X TE (pH 7.4) on a 96-well plate. After adding an extraction solution to each well, the plate, with 94 samples and the controls, was incubated at 57^{o}C and shaken at 1400 rpm on a Thermomixer (Eppendorf, Hamburg, Germany) for 3 hours or more. The extraction solution (modified from the proteinase K method in Kiesling 2004) consisted of 10 µl of cholic acid (sodium salt; 30% solution in water), 10 µl of buffer (1.6M Tris HCl, 0.2M EDTA, both at pH 8.0), 19 µl of proteinase K (20 mg/ml), and 1 µl of 0.1% sodium dodecyl sulfate (SDS). This incubation step ruptured the cell membranes and digested the proteins in the organism. To prevent cross-contamination during incubation, the plates were sealed with domed plastic tops (Bio-Rad, Hercules, CA, USA).

After the extraction, the DNA was separated from the digested membranes and proteins using magnetic beads that differentially bind to the DNA. This isolation protocol adapted from the Genfind kit (Agencourt, Beverly, MA, USA) to work with crustaceans and was automated on the Evolution P^3 liquid-handling robot (Perkin Elmer, Boston, MA, USA). The plate with the extraction products (DNA and digested proteins) was

centrifuged for 1 min at 800 rpm, the plastic tops were removed and the plate was transferred to the automated system. In the automated system, 50 µl of magnetic beads were added to each well, samples were thoroughly mixed, and then incubated without magnets for 5 minutes followed up with magnets for 5 minutes. After discarding the supernatant, 100 µl of protein wash was added, samples were mixed, and another incubation without (5 min) and with (5 min) a magnet was conducted. A second protein wash was carried out, and the samples were mixed and incubated as before. At this point, most proteins had been rinsed away and the DNA remained in the sample bound to the magnetic beads. To ensure that genomic DNA of high quality and purity was recovered, four washes with 100 µl of 70 % ethanol were carried out with the plate on the magnet. The resultant DNA-bead complex was air-dried for 15 minutes. The beads were resuspended in 30 µL of 0.5mM Tris pH 7.5. The eluted DNA was transferred to a final 96-well plate and the samples were stored at –20^{o}C until use.

Polymerase chain reaction (PCR)

Sequences of the D1/D2 domains of the large subunit ribosomal DNA (28S rDNA) were determined for individual adults from PCR products amplified with primers F63 (5'- GCA TAT CAA TAA GCG GAG GAA AAG -3') and R635 (5'- GGT CCG TGT TTC AAG ACG G -3') from Secore (1996). Polymerase chain reactions had a final volume of 50 µL and contained 10 µL of the DNA template, 2 mM dNTP, 40 pmoles of each primer, 4µL of Taq, and a reaction buffer (final concentrations: 50 mM tris HCl pH 9.2, 16mM (NH4) 2SO4, 2.25 mM MgCl2, 2% DMSO, 0.1% Tween 20) (Paschallet al.2004). Reaction conditions were 30 s hot start at 80°C followed by 40 cycles of 15 s at 94°C, 1 min at 55°C and 1 min at 72°C. A final extension for 8 min at 72°C was carried

out after the PCR cycles. A negative (no template) and a positive control (validated standard) were included on every plate.

In an effort to distinguish between the morphologically similar species *Calanus glacialis* and *C. marshallae* additional samples were collected in areas where the populations of these species do not overlap, and mtCOI sequences were determined for these species. I used recently designed primers 44F (5' - CAG GGC TGA GTA TGA TTA TTC - 3') and 575R (5' - GTC AGA GAA GGT CGT ATT TAG -3'). PCR reactions were prepared as described for 28S rDNA with annealing temperatures at 50°C for 1 min.

Before sequencing, PCR products of all samples were separated on 1% agarose gels and were visualized under blue light after staining with GelStar (Cambrex, USA). The lengths of PCR products were compared to the positive PCR control length.

Cloning and sequencing

For adults used to establish consensus sequences, the pGEM-T Vector System (Promega, WI, USA) was used to clone the PCR products. Ligation reactions were carried out on purified PCR products as described by the manufacturer. PCR products were purified using the AmPure magnetic bead purification kit (Agencourt, Beverly, MA, USA). Following cleanup, PCR products were sequenced using dye terminator cycle sequencing protocols. Sequencing reactions consisted of 3.2 pmoles of BigDye Terminator (Applied Biosystems, Foster City, CA, USA), 1.9 µl of 5x buffer, and 1 µl of template in a final volume of 10 µl. After a sequencing thermocycling protocol of 40 cycles of 15 sec at 94°C, 20 sec at 50°C and 4 min at 60°C, sequences were purified on the Perkin Elmer Evolution P3 liquid-handling robot using the standard CleanSeq

protocol (Agencourt, Beverly, MA, USA). Finally, samples were run on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). For adults used to establish consensus sequences, PCR products were sequenced on both strands using primers F63 and R635 to sequence the 28S rDNA and primers 44F and 575R for mtCOI gene. To identify copepod nauplii, sequencing was unidirectional using the forward primer F63.

Consensus sequences

I determined the sequence of the D1/D2 domains of 28S rDNA for 22 copepod species observed in the western Arctic (Table 2.1). For each species, the consensus sequence was determined using Seqman (DNAstar, Madison, WI, USA) software and the consensus sequence was used for species identification. Four or more individuals per species were analyzed to quantify intraspecific variability for each species. MegAlign (DNAstar, Madison, WI, USA) was used to align all consensus sequences and calculate genetic divergence among copepod species (Table 2.2).

Naupliar identification

After establishing the consensus sequence for the most abundant copepod species in the study area, the identification of nauplii was carried out without the need to sequence both strands. For naupliar identification, I sequenced only a single strand of the PCR products with primer F63, and used a species identifier script designed to compare unidentified sequences to consensus (voucher) sequences. The script, developed by Richardson et al. (2006), uses MATLAB (Mathworks, Natick, MA, USA) and the bioinformatics toolbox MBEToolbox (Cai et al., 2005). Details of the program can be found elsewhere (Richardson et al. 2006). The script utilizes the Phred (Ewing and

Green, 1998) score to identify high quality sequences and the degree of similarity between the forward sequence of the specimen and each voucher sequence relevant to the study site. The Phred score provides quality score for each base-call (Ewing and Green, 1998). A base-call with a quality value of 20 has a 1.0 % probability of being incorrect; one with a quality value of 40 has a 0.01 % probability of being incorrect. The species identifier script compares each unidentified sequence with all voucher sequences and determines the number of nucleotide differences between them. It then generates three quality value groups (< 20, 20-40, and > 40) and determines the number of different nucleotides and the total number of nucleotides within each group for the comparison between the unidentified sequence and each voucher sequence.

The criteria for species identification are: 1) only bases with Phred scores of 20 or higher are included in the analysis, 2) 300 or more nucleotides are required for positive identification, 3) the unidentified and voucher sequence are less than 5% different, and 4) 80% or more informative sites must match the identified species for every possible comparison with another species (Richardson et al., 2006). For congeneric species with low sequence divergence (<5%) the bioinformatics script generates the most likely identification of the sample. The final output of the program is a text file with the results of two comparisons. The first compares the entire sample sequence against each consensus sequence (1st half of table 3). The results (# wrong/total) are broken by Phred scores. The second comparison uses only the informative nucleotides that separate pairs of voucher sequences (2nd half of table 3). The first number is the percentage of informative nucleotides in the sample sequence matching the species in the row heading *vs*. the species in the column heading. The second number in parentheses is the total

number of informative nucleotides that separate the two species. An example of this identification output is shown in table 2.3.

RESULTS

Sequence variation

I sequenced the D1/D2 domains of the large subunit ribosomal DNA for 22 copepod species observed in the western Arctic (Table 2.1). Differences in the 28S rDNA gene were sufficient for the identification of all species present in the western Arctic with the exception of the sibling species *C. glacialis* and *C. marshallae* (Table 2.2). These morphologically similar species were discriminated using mtCOI. The 28S rDNA sequence of *C. glacialis*/*C. marshallae* was remarkably divergent from the congeneric species *C. hyperboreus* (1.7%). In comparison, differences in the *Neocalanus* genera ranged from 0.6 – 1.0 % (Table 2.2). The number of diagnostics characters available to separate congeneric species was generally low but sufficient to identify each species (except for *C. glacialis* and *C. marshallae*).

The intraspecific variability for each species was quantified. Differences between individuals of the same species were low (<0.5%) for most species, in particular for species with long generation cycles such as *C. hyperboreus* (<0.3%), *N. cristatus* (<0.1%), *N. flemingeri* (<0.1%) and *N. plumchrus* (<0.1%). Two species with short generation cycles, *A. longiremis* and *O. similis*, had higher intraspecific variability than the other species (0.7% and 0.6% respectively). The relatively elevated intraspecific variability of these two species did not present a problem for species identification because the sequences of *A. longiremis* and of *O. similis* were very different from the

sequences of other species (Table 2.2). For all species identified using the 28S rDNA gene, intraspecific variability was lower than interspecies variability.

DNA yield

In order to quantify the yield of genomic DNA extracted, different developmental stages of the calanoid copepod *C. glacialis* were selected and the amount of DNA obtained from each individual was measured (Figure 2.1). Variance in prosome length was consistently less than variance in the amount of DNA extracted for each developmental stage. Total DNA yield ranged from 60 ng for nauplii to 2982 ng for adult individuals.

Identification success rates

To demonstrate the effectiveness of our molecular technique, over 1800 nauplii were analyzed, with more than 1550 successfully sequenced and identified. The success rate of extraction, amplification, and subsequent identification was 85%. The nauplii came from 20 stations, each collected at a different location. For each station, 92 nauplii were subjected to sequence identification using 96-well plates with positive and negative extraction controls, and positive and negative PCR controls. The plate success rate varied from 67% to 96%. Variability in success rate was not attributed to the smaller sizes of the specimens being identified but could be linked to storage conditions.

Naupliar communities

In order to study variations over large scales, I classified the locations where surface samples were collected into four regions based on water column depth: the inner shelf (<50 m), the outer shelf (50-200 m), the slope (200-2000 m), and the basin (>2000

m). The inner shelf region incorporated samples collected near the Alaska coast. The outer shelf region included four locations that formed a line running parallel to the 50m isobath of the Chukchi shelf and two locations at the shelf edge. Samples collected at the slope composed the slope region, and samples collected over the Canada Basin proper fell into the basin region. This approach ignores any variability not due to water column depth, such as the within-region variability of the outer shelf region due to the elevated primary production rates in Barrow Canyon (Codispoti et al., submitted) and the temporal difference between samples collected early in the cruise and those collected about a month later towards the end of the cruise. This approach however permits a broad analysis of the shelf to basin gradients of copepod reproduction and naupliar community composition going from the Chukchi and Beaufort seas to the Canada Basin.

The naupliar composition of the different regions observed in the Chukchi and Beaufort seas and adjacent Canada Basin was resolved with the molecular methodology (Figure 2.2). The samples identified to date indicate that some species which occurred as adults were not observed as nauplii in the region during our sampling in summer. This is probably due to a combination of factors including low abundances, temporally different reproductive cycles, and deeper vertical distribution of early developmental stages. The molecular identification of nauplii showed a low incidence of *C. glacialis* in the inner and outer shelf regions. *Calanus glacialis* nauplii were mostly found in shelfbreak/slope and basin regions, especially in the basin where 31% of the nauplii were identified as *C. glacialis*. This pattern is different from the distribution of *Oithona similis* nauplii. *Oithona similis* nauplii were consistently present in all regions and dominated the naupliar community of the inner shelf, outer shelf and slope regions. Occurrence of

Pseudocalanus species indicates that the naupliar distribution of congeneric species is dependent on the water column depth. A significant percentage (>15%) of the basin naupliar community were identified as *P. minutus* while only a small percentage (~4%) were classified as *P. mimus* and *Pseudocalanus* spp. In contrast, the relative importance of the *Pseudocalanus* species in the outer shelf and slope regions was similar.

The ecological information gathered in this study could not have been obtained with the use of traditional microscopic identification techniques. The nauplii of the *Calanus* and *Pseudocalanus* are practically identical, and those of *Oithona* may be distinguished by the double-jointed endopod of the mandibule (Björnberg, 1984). In contrast to morphological classification methods, the molecular method reliably and rapidly identified large numbers of copepod nauplii to the species level. The technique was successfully used to determine which copepod species were present as nauplii in the western Arctic.

DISCUSSION

The accurate identification of individuals is the cornerstone of any ecological study, yet for zooplankton communities this fundamental step is not always possible. The inability to identify early life stages of planktonic organisms restricts the ecological questions that can be addressed, and limits our understanding of population structure and dynamics. To address this problem, I integrated molecular techniques with automated equipment and bioinformatics software. In this paper I described these adaptations and demonstrated their utility for high-throughput species identification. Although the technique described was developed for copepods, it can be used with other small crustaceans and it has already been applied to identify fish larvae (Richardson et al.,

2006). This technique could become a critical tool for rapid and accurate species identification across a wide range of taxa.

Advantages of this technique

The primary advantages of this technique are 1) minimal labor requirements, 2) high sample throughput, 3) high rate of success, and 4) simple species identification.

A minimum amount of labor is required because most laboratory steps were automated. Manual labor is required only to place specimens in individual wells and to make three mixes (the extraction, amplification and sequencing master mixes). The most labor intensive portion of the technique is the cleaning and transfer of single specimens into individual wells on the 96-well plate. Excluding the placement of specimens in the plates, less than 3 hours of manual labor are required to obtain identification and statistics for a 96-well plate over the course of two working days (<15 hours of unattended components).

The automation of this technique reduces the potential for human-related errors (e.g., pipetting mistakes, misplacement of samples, contamination). At the same time, it allows multiple plates to be run simultaneously with relatively minor increases in the labor and time requirements. The liquid-handling robot can accommodate up to 8 plates at a time for a total of 736 specimens (and 32 controls). The number of plates that can be identified is limited by the placement of individuals in the plates. In practice however, the technique is both time and labor efficient allowing for a high sample throughput of >700 individual identifications per week.

In addition to increased efficiency, the identification success rates from this technique are high. The 85% average success rate of the technique is higher than using

standard protocols (as low as 50%, Street and Montagna, 1996; Street et al., 1998), but lower than modifications to commercially available products (95%, Schizas et al., 1997). Comparable work with copepod nauplii in the Florida Bay (Kiesling, 2004) showed similar average success rates (87%) and variability. The success rate of our technique ranged from 67 to 96%. The primary source of this variation may be attributed to storage conditions. At locations where large amounts of phytoplankton cells were collected, the size of the container and the amount of ethanol used may not have been sufficient to properly preserve the organisms in the sample. Other studies have also observed that when ethanol does not penetrate the entire sample because of excess material, the DNA can be too damaged to allow successful amplification (Lindeque et al., 2006). At basin locations, where few phytoplankton cells were collected, success rates were consistently high (> 90%).

The final analysis of the sequence data is facilitated by the use of a MATLAB script developed by Richardson et al. (2006) and adapted to work with the consensus sequences of the dominant copepod species of the study area. This bioinformatics approach is effective for species identification and has three major advantages over other identification methods. First, no preprocessing of sequence data is required. Unlike other techniques that use tree-based methods to classify specimens (Huys et al., 2006), our identification script uses a character-based method. In other techniques, a tree is constructed using a distance-based neighbor joining method. The phylogenetic tree constructed is characterized by clusters of closely related individuals, and each cluster is considered a separate species (Dasmahapatra and Mallet, 2006). The results can be ambiguous if few individuals per species are included (underestimation of intraspecific

variability) or if sister taxa are not included (overestimation of interspecific variability). In contrast, our script relies on multiple characters to distinguish each species, and provides an easily interpreted output. The second advantage of the identification script is that it incorporates Phred scores and uses unidirectional sequencing. Unidirectional sequencing is both more time and cost effective than bidirectional sequencing, but less accurate. The use of Phred scores (base quality values) and strict identification criteria reduce the possibility of misidentifications. Third, the Matlab script is readily adaptable to a wide range of species identification. In this paper I demonstrated how this script, originally developed for the identification of fish larvae, worked for the identification of copepod species. The only step required was the generation of a molecular database of the copepod species present in the study area.

Application into large ecological studies

The development of this method was focused on enhancing our understanding of marine ecosystems through genetic analysis. This method is more expensive than traditional approaches but its application is justified when other types of data (e.g., microscopy) yield equivocal results. This molecular technique can recognize sibling species, explore diversity at the species level, and identify larval stages of marine crustaceans and fish. Our long term goal is to gain a better understanding of how dynamic larval processes (recruitment, survivorship, community structure) control populations of marine organisms, particularly crustaceans.

Marine crustaceans pass through several planktonic larval phases during their early life history. The success of the planktonic larval phase determines the dynamics and structure of adult populations and communities. In parasitic and sessile crustaceans,

adults live attached to their hosts and the substrate respectively and cannot move independently; hence the planktonic larval phase represents the only opportunity to colonize new habitats or hosts (Heuch and Karlsen, 1997; Thiyagarajan et al., 2006). For motile crustaceans including euphausiids, lobsters, crabs and shrimps variability in recruitment of early stages (nauplii and zoea) can affect economically important stocks. Early life stages of crustaceans are also of particular trophodynamic importance. For example, the naupliar life stages of copepods are the primary food resource of many larval fish (Dagg et al., 1984; Peterson and Ausubel, 1984; Hillgruber et al., 1995). Despite the economical and ecological roles of crustacean larvae, few studies include larval components. This is probably due to the limitations of morphological identification methods for larvae (Thatje et al., 2005; Goffredi et al., 2006) and the problematic isolation and amplification of DNA from small crustaceans (Street and Montagna, 1996). Our work demonstrated that the technique developed has the potential to resolve this issue when coupled with suitable genetic databases.

A first step towards molecular species identification is the generation of genetic databases, however very few genes have been characterized for marine crustaceans (Crawford, 1995; Bucklin et al., 2001). The issue was addressed here in the sequencing of the D1D2 region of the 28S rDNA from 22 copepod species in the western Arctic, and of the mtCOI gene of two morphologically similar but genetically distinct species. At present, there are several initiatives whose goal is to create nucleotide sequence databases for marine organisms, including crustaceans, as a tool for phylogenetic analysis and species identification (e.g., Barcode of Life, Census of Marine Life, ZooGene). The generation of large sequence databases and the application of rapid sequencing methods

will greatly expand the questions that can be addressed in large ecological studies. Likewise, these databases will make molecular identifications more readily available and facilitate biodiversity assessments (Smith et al., 2005).

CONCLUSIONS

In conclusion, the sequence-based method for the identification of small crustaceans is reliable, rapid, and relatively simple. It has the additional benefit that most steps are automated and manual labor is significantly reduced. As new DNA sequences become available the technique could potentially become widely applied to studies of taxonomic groups whose larval identification using microscopy is a time consuming, labor-intensive and inexact process.

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Table 2.1 Copepod species, collection region, collector, and GenBank accession number.

Species	Collection region	Collector	GenBank accession number
Acartia longiremis	Chukchi Sea	This study	EF460766
Chiridiella abyssalis	Canada Basin	This study	EF460767
Calanus glacialis	St. Lawrence River	S. Plourde	EF460768
Calanus hyperboreus	Canada Basin	This study	EF460769
Calanus marshallae	North Pacific	W. Peterson	EF460770
Halectinosoma finmarchicum	Chukchi Sea	This study	EF460771
Lubbokia glacialis	Canada Basin	This study	EF460772
Metridia longa	Canada Basin	This study	EF460773
Metridia pacifica	Chukchi Sea	This study	EF460774
Microcalanus pygmaeus	Chukchi Sea	This study	EF460775
Neocalanus cristatus	Chukchi Sea	This study	EF460776
Neocalanus flemingeri	Bering Sea	J. Napp	EF460777
Neocalanus plumchrus	Prince William Sound	T. Kline	EF460778
Oithona similis	Chukchi Sea	This study	EF460779
Oncaea borealis	Chukchi Sea	This study	EF460780
Oncaea media	Chukchi Sea	This study	EF460781
Paraeuchaeta glacialis	Canada Basin	This study	EF460782
Pseudocalanus mimus	Chukchi Sea	This study	EF460783
Pseudocalanus minutus	Chukchi Sea	This study	EF460784
Scaphocalanus brevicornis	Canada Basin	This study	EF460785
Spinocalanus elongatus	Canada Basin	This study	EF460786
Spinocalanus horridus	Canada Basin	This study	EF460787

Table 2.2 Pairwise percentage differences in 28S rDNA for 22 copepod species present in the western Arctic.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*A. longiremis*																					
2	*C. glacialis*	24.4																				
3	*C. hyperboreus*	24.0	1.7																			
4	*C. marshallae*	24.4	0.0	1.7																		
5	*C. abyssalis*	24.3	8.7	9.8	8.7																	
6	*H. finmarchicum*	37.5	28.4	29.2	28.6	28.5																
7	*L. glacialis*	40.2	29.2	29.8	29.4	27.9	21.9															
8	*M. longa*	25.9	14.6	15.3	14.6	16.2	28.3	29.3														
9	*M. pacifica*	27.9	15.1	15.8	15.1	18.0	28.8	28.8	2.4													
10	*M. pygmaeus*	27.2	11.0	11.3	11.1	7.1	30.0	30.6	17.0	19.2												
11	*N. cristatus*	24.2	2.7	1.6	2.7	10.0	29.2	30.2	15.8	16.4	11.1											
12	*N. flemingeri*	24.0	2.6	1.4	2.6	10.2	29.4	30.0	15.1	15.7	11.3	0.7										
13	*N. plumchrus*	23.8	2.6	2.0	2.6	9.9	29.0	30.0	15.1	16.0	10.9	1.0	0.6									
14	*O. similis*	39.8	32.8	33.3	32.8	31.1	22.8	25.3	32.7	33.5	34.7	32.7	33.2	33.0								
15	*O. borealis*	39.7	32.1	32.7	32.3	31.5	24.3	25.5	32.1	33.6	34.1	32.5	32.5	32.0	27.1							
16	*O. media*	38.3	31.1	31.7	31.3	31.4	24.3	26.6	32.0	33.2	34.1	31.2	31.2	31.0	25.7	6.2						
17	*P. glacialis*	24.2	8.6	9.4	8.6	2.4	28.0	28.9	16.1	17.9	6.9	9.5	9.7	9.4	31.7	31.1	31.5					
18	*P. mimus*	24.1	8.7	9.5	8.7	5.4	27.9	29.3	15.8	17.5	6.6	9.7	9.8	9.5	31.4	32.4	32.2	5.3				
19	*P. minutus*	24.3	8.1	9.5	8.1	5.1	27.5	28.3	15.6	17.3	6.2	9.7	9.8	9.5	30.5	31.5	31.7	5.4	0.8			
20	*S. brevicornis*	22.9	9.5	10.2	9.5	5.9	29.2	30.7	15.7	16.8	9.4	10.3	10.5	10.2	33.3	34.5	33.5	6.7	7.0	6.8		
21	*S. elongatus*	26.0	12.3	13.1	12.5	9.4	28.2	29.2	18.5	20.2	11.0	13.0	13.2	12.8	34.2	32.6	32.5	9.3	10.6	9.7	11.1	
22	*S. horridus*	25.3	12.4	13.3	12.6	9.9	28.2	28.7	18.7	20.5	11.3	13.1	13.3	12.9	33.2	33.0	32.7	9.9	11.2	10.4	11.2	1.4

Table 2.3 Output of the bioinformatics program showing two sequence comparisons for species identification. The first half of the table shows the comparisons of the entire sample sequence against each consensus sequence. The results (# wrong/total) are broken by Phred scores. The second comparison uses only the informative nucleotides that separate pairs of voucher sequences (2nd half of table 3). The first number is the percentage of informative nucleotides in the sample sequence matching the species in the row heading *vs*. the species in the column heading. The second number in parentheses is the total number of informative nucleotides that separate the two species (table not show in its entire length).

Sample: EB1_E11 Most Likely ID: ***O. similis***

		Phred score											
	Species	0-20	21-40	41+	Total (>20)	Species	1	2	…	14	…	21	22
1	*A. longiremis*	74/149	105/271	61/238	166/509	*A. longiremis*		41%(85)	…	**0%**(169)	…	41%(86)	38%(86)
2	*C. glacialis*	68/148	98/273	55/240	153/513	*C. glacialis*	59%(85)		…	**0%**(156)	…	50%(44)	43%(46)
3	*C. hyperboreus*	67/149	97/273	57/240	154/513	*C. hyperboreus*	58%(89)	43%(7)	…	**0%**(157)	…	50%(50)	44%(52)
4	*C. marshallae*	68/149	98/273	55/240	153/513	*C. marshallae*	59%(86)	NaN%(0)	…	**0%**(156)	…	51%(45)	45%(47)
5	*C. abyssalis*	68/150	96/272	46/240	142/512	*C. abyssalis*	65%(86)	65%(34)	…	**0%**(145)	…	63%(32)	57%(35)
6	*H. finmarchicum*	55/149	79/272	35/239	114/511	*H. finmarchicum*	73%(115)	71%(92)	…	**0%**(117)	…	73%(80)	69%(84)
7	*L. glacialis*	57/147	77/274	37/239	114/513	*L. glacialis*	71%(129)	68%(110)	…	**0%**(116)	…	67%(107)	66%(108)
8	*M. longa*	64/150	96/272	55/240	151/512	*M. longa*	59%(92)	50%(48)	…	**0%**(155)	…	50%(64)	46%(67)
9	*M. pacifica*	64/150	98/271	54/240	152/511	*M. pacifica*	57%(98)	48%(50)	…	**0%**(156)	…	49%(72)	45%(75)
10	*M. pygmaeus*	67/150	98/272	52/240	150/512	*M. pygmaeus*	60%(88)	52%(42)	…	**0%**(154)	…	51%(39)	45%(42)
11	*N. cristatus*	66/145	95/273	56/240	151/513	*N. cristatus*	60%(88)	60%(10)	…	**0%**(154)	…	53%(47)	47%(49)
12	*N. flemingeri*	67/150	98/273	56/240	154/513	*N. flemingeri*	59%(87)	45%(11)	…	**0%**(157)	…	50%(50)	44%(52)
13	*N. plumchrus*	67/150	99/273	56/240	155/513	*N. plumchrus*	58%(84)	42%(12)	…	**0%**(158)	…	49%(47)	43%(49)
14	*O. similis*	34/151	0/280	0/240	**0/520**	*O. similis*	**100%**(169)	**100%**(156)	…		…	**100%**(154)	**100%**(151)
15	*O. borealis*	63/149	86/272	38/240	124/512	*O. borealis*	66%(131)	62%(117)	…	**0%**(127)	…	62%(108)	59%(111)
16	*O. media*	63/149	75/275	45/240	120/515	*O. media*	70%(125)	65%(114)	…	**0%**(123)	…	65%(111)	63%(112)
17	*P. glacialis*	68/149	98/271	46/240	144/511	*P. glacialis*	63%(89)	58%(36)	…	**0%**(148)	…	59%(34)	51%(37)
18	*P. mimus*	68/149	96/274	47/240	143/514	*P. mimus*	67%(84)	68%(31)	…	**0%**(146)	…	66%(35)	58%(38)
19	*P. minutus*	68/149	95/274	47/240	142/514	*P. minutus*	67%(85)	70%(30)	…	**0%**(145)	…	68%(34)	59%(37)
20	*S. brevicornis*	69/151	100/272	52/240	152/512	*S. brevicornis*	60%(86)	50%(34)	…	**0%**(155)	…	50%(36)	44%(41)
21	*S. elongatus*	66/148	103/272	47/237	150/509	*S. elongatus*	59%(86)	50%(44)	…	**0%**(154)	…		14%(7)
22	*S. horridus*	67/150	98/273	49/240	147/513	*S. horridus*	62%(86)	57%(46)	…	**0%**(151)	…	86%(7)	

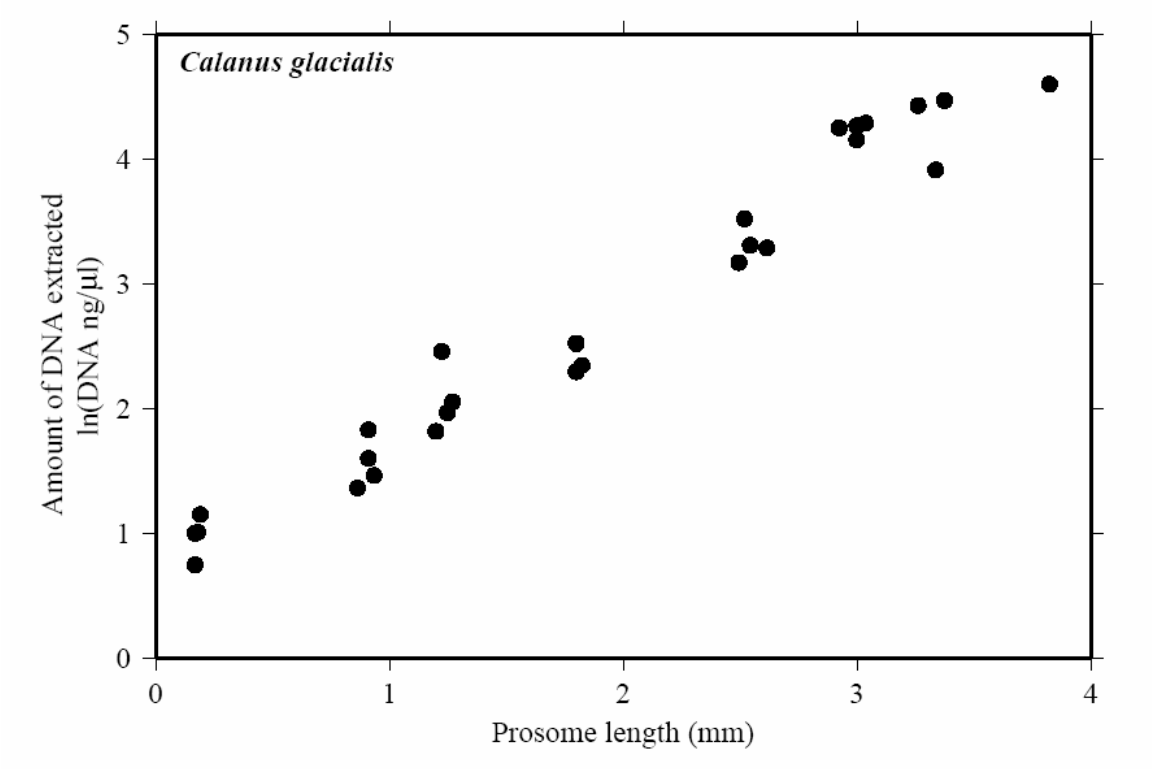


Figure 2.1 Amount of DNA obtained *versus* prosome length for different developmental stages of the calanoid copepod *Calanus glacialis*.

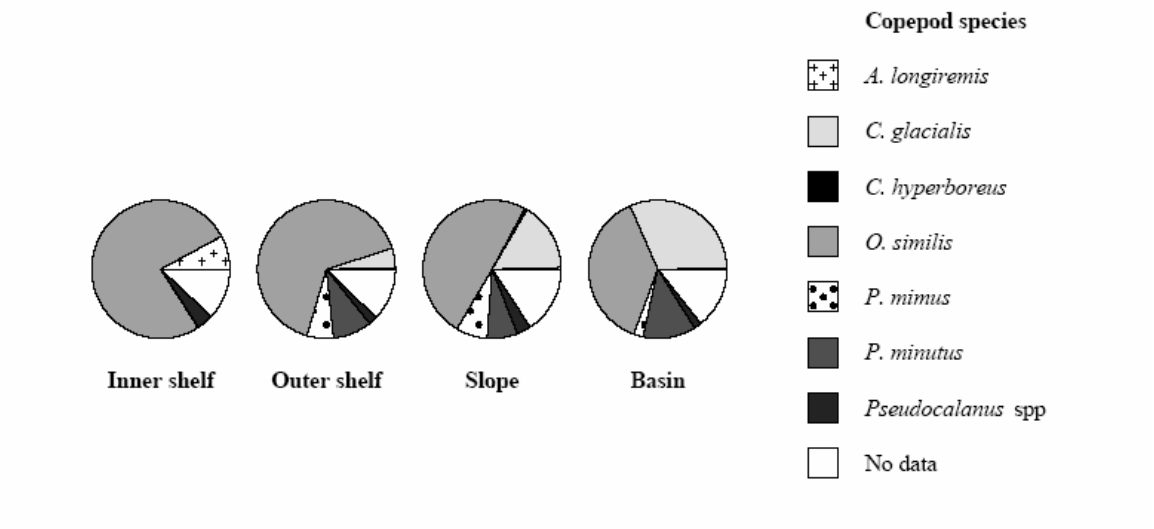


Figure 2.2 Naupliar composition of the different regions in the Chukchi and Beaufort seas and adjacent Canada Basin. "No data" is the portion of samples where the DNA extraction, amplification or sequencing was unsuccessful and the species could not be determined.

CHAPTER 3: ABUNDANCE AND DISTRIBUTION OF NAUPLII OF THE FOUR DOMINANT COPEPOD SPECIES IN THE WESTERN ARCTIC DURING SUMMER 2004

INTRODUCTION

The zooplankton of the western Arctic are composed by a mixture of species of different origin. *Oithona similis, Acartia longiremis* and *Pseudocalanus* spp. often dominate in the neritic (< 200 m) regions of the Chukchi and Beaufort seas (Lane et al., 2007), while *Calanus glacialis, C. hyperboreus, Microcalanus pygmaeus, Metridia longa* and *Oncaea* spp. of arctic waters dominate areas that are more oceanic (> 2000 m) (Ashjian et al., 2003; Lane et al., 2007), with some oceanic species also associated with slope (200 – 2000 m) regions (Plourde et al., 2005; Llinás et al., submitted a). At different times of the year, these species may co-exist in continental shelf waters and over the basin, although their origins and life histories differ. Each copepod species has a unique life cycle with adaptations tied to its reproduction, development and growth strategies (see reviews by Corkett and McLaren, 1978; Paffenhöffer, 1993; Mauchline, 1999). As a result of different life histories and abundances, the roles of these copepod species in the regional food webs are also different.

To determine the significance of these copepods in the ecosystem, it is necessary to understand their life history and identify all developmental stages (Siefert, 1998). It is also important to distinguish between different species because they have different distributions and environmental affinities, yet they co-occur and compete interspecifically. However, one of the persistent problems when investigating copepod communities, especially immature stages, is the correct identification of species (Bucklin et al., 1999; Lindeque et al., 2004). Each life history stage of a copepod species has a

different morphological form, but species descriptions are based on the characteristics of the adult stages. Diagnostic keys for the other five copepodid and six naupliar stages of copepod species are uncommon.

Due to their great variety and abundance, copepod nauplii undoubtedly play an important role in pelagic food chains (Hopcroft et al., 2005). For example, many fish taxa prey upon *Acartia* and *Pseudocalanus* nauplii (Dagg et al., 1984; Montelone and Peterson, 1986; Takatsu et al., 1995). In the Barents Sea, first feeding capelin larvae are generally thought to prey upon *Calanus* nauplii (Karamushko and Reshetnikov, 1994). Furthermore, copepod nauplii generally dominate by number the mesozooplankton community in arctic ecosystems and account for a small percentage of the mesozooplankton biomass. The contribution of each species varies. *Calanus* nauplii have higher carbon content than other copepod species nauplii such as *Acartia* because of their larger size (Berggreen et al., 1988; Hygum et al., 2000). Despite their numerical importance and key role as food for larval fish, investigation of stage specific phenomena and studies of naupliar biology are hampered by a lack of descriptions of diagnostic characters for nauplii.

Molecular techniques have the potential to provide definitive identification for copepods, thereby overcoming the taxonomic difficulties faced by morphometric classification methods (Lindeque et al., 1999; Lindeque et al., 2006). In the case of copepod nauplii and early copepodids, the use of molecular markers for identification purposes is needed given the limitation of conventional (microscopic) identification methods. Recent studies with copepods have demonstrated that the nuclear gene encoding the large subunit of ribosomal DNA (28S rDNA) shows considerable nucleotide

divergence among copepod species (Braga et al., 1999; Kiesling et al., 2002) and that to differentiate between closely related species, the mitochondrial cytochrome oxidase 1 gene (mtCO1) can be used (Bucklin et al., 2003; Goetze and Bradford-Grieve, 2005). Utilizing both genes, a molecular method has been adapted to provide definitive identification of individual copepods (Llinás et al., submitted b). Using this method it is possible to unambiguously identify large numbers of copepods to the species level at any developmental stage. Here, this semi-robotic protocol was employed to answer three important ecological questions: 1) what are the species-specific patterns of abundance and distribution of copepod nauplii during summer in the western Arctic, 2) are these patterns associated with the physical properties of the surface water where the nauplii develop, and 3) can this information elucidate the life cycle strategies of each species in the region and their individual roles in the shelf and basin ecosystems.

The first objective of this paper is to determine the patterns in abundance and distribution of nauplii of the four dominant copepod species in the Chukchi and Beaufort seas and Canada Basin during summer 2004. Our analysis focuses on distributions of nauplii of *O. similis*, *C. glacialis P. minutus* and *P. mimus* because their juvenile and adult stages dominate the mesozooplankton biomass and abundance in the region (Ashjian et al., 2003; Lane et al., 2007). I also chose to illustrate these species because given their different origins (*O. similis*, *P. minutus*, and *P. mimus* are shelf species and *C. glacialis* is an arctic basin species; Lane et al., 2007), I expected them to be strong indicators of distinct water sources and hydrographic processes. Other species commonly found in the region were not included in the study due to either their low standing stocks (e.g. *Acartia longiremis*; Lane et al., 2007) or because spawning occurs earlier (e.g.

Calanus hyperboreus has a life cycle in which spawning occurs in spring; Smith and Schnack-Schiel; 1990). Information on the species composition of the community of copepod nauplii is a prerequisite to study the role of individual copepod species in this arctic food web, the second objective of this work. I begin with a description of the general mesoscale circulation of the upper layer and the physical properties of the surface water where the nauplii were collected in the western Arctic during summer 2004. This is followed by a description of the distribution of copepod nauplii observed that summer. Finally, I use our results on the naupliar distribution and community structure to discuss the roles these copepod species can have in the shelf and basin food webs.

MATERIALS AND METHODS

Sample collection and microscopic enumeration

To collect surface plankton samples, I used a continuous-flow seawater system installed aboard the USCGC *Healy*. The intake of the system was located from the sea surface to 3 m depending on the speed of the ship and the sea conditions. The intake was clear polyvinyl hose weighted with a stainless steel bolt and deployed through the tube in the aft end of the ship designed for launching expendable bathythermograph sensors. The water inflow was then directed into a vortex debubbler (Ocean Instrument Laboratory). From the debubbler the water with bubbles flowed into a sink in the laboratory while a constant bubble-free seawater flow continued through a thermosalinograph (Seabird) and then into a SCUFA fluorometer. In the sink the water with bubbles coming from the debubbler and the outflow of seawater from the SCUFA fluorometer were used together to measure the flow rate of the continuous-flow system. After the flow rate had been measured, a 1-liter beaker with large drain holes covered with 35 μm mesh netting was

placed under the outflow for a recorded period of time (generally 10 min) to collect the samples of zooplankton. The samples were rinsed with 95% ethyl alcohol to remove the seawater, then transferred into 22 ml vials for storage, and finally preserved in 95% ethyl alcohol for quantitative and molecular analyses. After two years of preservation, the samples were enumerated microscopically and the ethanol was changed using a 35 μm mesh sieve before molecular analysis.

Surface zooplankton samples were analyzed in the laboratory with a Leica MS5 microscope. The use of 35 μm mesh sieves permits quantitative estimates of copepod nauplii and early copepodids of most copepod species. Each sample was counted in its totality to determine the number of copepod nauplii in each sample (Table 3.1). To estimate abundance (No. m^{-3}), the number of copepod nauplii counted was divided by the volume of water filtered. A total of 34 samples from a cruise during summer 2004 in the Chukchi and Beaufort seas were enumerated and the species composition was determined for half of the enumerated samples. The species composition was not determined for 17 samples located on the EB line because the samples were only used to investigate the effects of small-scale circulation processes on the abundance of copepod nauplii. Using a molecular identification method, the species composition of the copepod nauplii was determined for a subset of 17 samples: 4 samples on the EB line, 4 samples on the BC line, 4 samples on the WHS line, and 5 samples on the EHS line (Table 3.1). The subset of molecularly analyzed samples was selected to cover the shelf, slope and basin regions and to facilitate a broad analysis of the distribution of copepod nauplii (Figure 3.1).

Surface circulation and hydrographic characteristics

I used three different data sets to describe the mesoscale circulation and hydrographic properties of the surface water in the region. The surface layer circulation was provided by Drs. J. Clement and W. Maslowski using the Pan-Arctic ice-ocean model (http://www.oc.nps.navy.mil/NAME/name.html). The distributions of temperature and salinity at 2 m depth for the cruise during summer 2004 were plotted using data collected with the continuous-flow seawater system. Finally, ice cover was recorded by the bridge crew of the USCGC *Healy*. I used the meteorological logs of the ship to reconstruct the ice conditions encountered at each station.

Molecular identification

The DNA extraction, isolation, amplification and sequencing reactions were performed in 96-well plates, with four wells used as positive and negative controls and as secondary identifiers of the orientation and identification of the plates for sequencing. Details of the DNA extraction and isolation technique are in Llinás et al., submitted b. In brief, to clean the nauplii of contaminants, each individual was rinsed three times in sterilized nano-filtered water and transferred individually into wells filled with a extraction solution consisting of 60µl of 1X TE (pH 7.4), 10 µl of cholic acid (sodium salt; 30% solution in water), 10 µl of buffer (1.6M Tris HCl, 0.2M EDTA, both at pH 8.0), 19 µl of proteinase K (20 mg/ml), and 1 µl of 0.1% sodium dodecyl sulfate (SDS). After extraction, the DNA was isolated using a magnetic beads protocol adapted from the Genfind kit (Agencourt, Beverly, MA, USA).

I sequenced the D1/D2 domains of the large subunit ribosomal DNA (28S rDNA) using primers F63 (5'- GCA TAT CAA TAA GCG GAG GAA AAG -3') and R635 (5'-

GGT CCG TGT TTC AAG ACG G -3') from Secore (1996). Polymerase chain reactions had a final volume of 50 µL and contained 10 µL of the DNA template, 2 mM dNTP, 40 pmoles of each primer, 4µL of Taq, and a reaction buffer (final concentrations: 50 mM tris HCl pH 9.2, 16mM (NH4) 2SO4, 2.25 mM MgCl2, 2% DMSO, 0.1% Tween 20) (Paschallet al.2004). Reaction conditions were 30 s hot start at 80°C followed by 40 cycles of 15 s at 94°C, 1 min at 55°C and 1 min at 72°C. A final extension for 8 min at 72°C was carried out after the PCR cycles. A negative (no template) and a positive control (validated standard) were included on every plate. PCR products were purified using the Ampure magnetic bead purification kit (Agencourt, Beverly, MA, USA), on the liquid-handling robot. Following cleanup, PCR products were sequenced using 3.2 pmoles of primer, 1µL of BigDye Terminator (Applied Biosystems), 1.9 µL of reaction buffer, and 1µL of template in a 10µL reaction. The sequencing thermocycling protocol consisted of 40 cycles at 94°C for 15 s, 50°C for 20 s and 60°C for 4 min.

For identification of the nauplii, PCR products were sequenced unidirectionally (primer F63) and a species identifier script was used to compare unidentified sequences to consensus (voucher) sequences. The script, developed by Richardson et al. (2006), uses MATLAB (Mathworks, Natick, MA, USA) and the bioinformatics toolbox MBEToolbox (Cai et al., 2005). The species identifier script compares each unidentified sequence with all voucher sequences and provides the most likely identification of the sample (see Richardson et al., 2006).

The 28S rDNA gene did not differentiate between *Calanus glacialis* and *C. marshallae*, whose nucleotide sequences were 100% identical. For individual nauplii initially identified as *Calanus glacialis*/*marshallae* using the 28S rDNA gene, a second

set of PCR reactions was carried out to sequence the mitochondrial cytochrome oxidase subunit I gene (mtCOI). I used recently designed primers 44F (5' - CAG GGC TGA GTA TGA TTA TTC - 3') and 575R (5' - GTC AGA GAA GGT CGT ATT TAG -3') (Llinás et al., submitted b). PCR reactions were prepared as described for 28S rDNA with annealing temperatures at 50°C for 1 min. For *Calanus glacialis*/*marshallae* nauplii identification, PCR products were sequenced unidirectionally (primer 44F) and the species identifier script was used to compare the unidentified sequences to mtCOI consensus sequences.

RESULTS

Surface circulation

A schematic of circulation in the upper 10 m during our sampling in the Chukchi and Beaufort seas is shown in Figure 3.2. Surface flow over the Chukchi Shelf was generally northward, with intensification occurring in both Herald and Barrow Canyons and in the Central Channel. Hence these surface shelf waters were dominated by Pacific water advected into the Chukchi Sea through Bering Strait. Upon reaching the shelfbreak the flow turned eastward. Along the Chukchi slope the flow was eastwards at ~0.10 m s^{-1}, while north of Barrow Canyon and on the Beaufort slope the flow was significantly stronger (~0.20 m s^{-1}). In addition, the modeled flow shows a convergence zone at the head of Barrow Canyon that corresponds to our sampling station BC1 (see Figure 3.1). This convergence zone was generated by the inflow of three distinct surface waters: 1) waters flowing northward in Barrow Canyon, 2) waters in the Central Channel deflected eastward before reaching the shelfbreak, and 3) slope surface waters that flow southward following the 50-m isobath. Lastly, upper layer flow was much less organized and

weaker over the basin compared to the shelf and slope regions. These surface waters offshore of the shelf originate to the north, as part of the Arctic Ocean.

Physical characteristics of surface water

The temperature and salinity characteristics of the surface waters in which the copepod nauplii were collected are shown in Figure 3.3. In the Chukchi and Beaufort seas during summer 2004, the 0 and 1°C isotherms were located over the basin. These "cold" isotherms ran parallel to the 3000 m isobath (Figure 3.3A). The 2°C isotherm was generally located over the slope region, but extended inshore between Hanna Shoal and Barrow Canyon, probably under the influence of the surface waters of the slope flowing south in that area. The warmer isotherms (3 and 4°C) ran parallel to the Alaska coastline (Figure 3.3A). In the Chukchi Sea, the isohalines also ran parallel to the isobaths, but east of Barrow Canyon they abruptly turned southward (Figure 3.3B). The result of this difference in orientations is that surface waters on the western side of the Chukchi Shelf had lower temperatures and higher salinities than surface waters on the eastern side of the Chukchi Shelf and the Beaufort Shelf, when considered at similar water column depths.

The surface water mass characteristics of the Chukchi/Beaufort shelf and slope are primarily influenced by the northward advection of Pacific Ocean waters through Bering Strait and polar mixed layer waters. The characteristics of these water masses vary seasonally due to the formation and ablation of sea ice. In summer, the surface waters of the northeastern Chukchi Shelf are relatively warm (>2 °C) with the warmest waters associated with the Alaskan Coastal Current. That current flows northeastward along the Alaskan coast and exits the shelf through Barrow Canyon. At the head of the canyon, the warm Alaskan Coastal waters converge with cooler waters flowing eastward from the

central Chukchi shelf and possibly with even colder surface waters derived from the shelfbreak or slope that flow southward along the western wall of Barrow Canyon (as suggested in Figure 3.2). Surface water salinities at this time are <28 and reflect dilution by melting ice and/or advection of runoff-influence waters within the Alaskan Coastal Current. Our sampling was carried out under unusual ice-free conditions (Figure 3.4). Heavy ice conditions (>5/10) were observed early in the cruise and only at the most offshore stations of our eastern section EB and BC. Ice maps of our study area show that just prior to our sampling, the EB section was ice covered, while during our sampling the region was practically ice-free. The ice maps also reveal that the sea ice retreat was not uniform due to the flow of warm Alaskan Coastal water through Barrow Canyon. The rapid retreat of the ice edge and the warm Alaskan Coastal water controlled the salinity and temperature of the surface water in our EB section, and likely influenced the abundance and composition of copepod nauplii as well.

Distribution of copepod nauplii

The abundance of copepod nauplii showed important spatial variability related to the temperature of the surface waters and circulation patterns. The highest abundances of nauplii were observed in the eastern side of the study area associated with the warm isotherms (Figures 3.5 and 3.3A). Stations with high nauplii abundances were also in regions of strong surface flow (Figure 3.2), making it difficult to discern the separate effects of temperature and convergence on the increased abundance of copepod nauplii in this area. However, the nearly four-fold increase in abundance of nauplii observed at BC1 compared with the other stations having warm surface water is best explained by the convergence of the three different surface water sources (Figure 3.2). This convergence

likely entrained both copepod adult females and nauplii. Outside the influence of the warm surface waters, abundances of nauplii were consistently lower, in particular at stations in the Canada Basin.

The EB line (Figure 3.1) was used to investigate the small-scale horizontal distribution of copepod nauplii. Sea surface temperature, salinity and fluorescence were examined relative to distance from the shelf to the basin (starting at EB1 and ending at EB8) to detect frontal systems overlaid by plots of abundance of nauplii. The role of circulation processes on the distribution and abundance of copepod nauplii was studied by defining fronts as abrupt changes in physical properties and relating variations in abundance to the presence/absence of such fronts. However, this approach provided little information on the degree to which the abundance of copepod nauplii is affected by fronts in the western Arctic. There was no concordance between fronts as determined by changes in temperature, salinity or fluorescence and changes in abundances of nauplii (data not shown), indicating that copepod nauplii do not have a common response to these environmental variables.

Distribution of copepod nauplii using molecular markers

Molecular analyses of the subset of samples selected showed that 42% of the nauplii found in the Chukchi and Beaufort seas and Canada Basin were *O. similis*, 26% were *C. glacialis*, 10% were *P. minutus*, and 4% were *P. mimus*. These four copepod species comprised 82% of the nauplii analyzed and dominated the copepod nauplii community during summer 2004. Other species such as *A. longiremis* and *Pseudocalanus* sp. were present but rare, accounting for 4% of the community of nauplii. The portion of

samples where the DNA extraction, amplification or sequencing was unsuccessful and the species could not be determined was 14%.

The surface distribution of copepod nauplii of the small cyclopoid copepod *O. similis* (Figure 3.6A) followed the same trends observed in the total abundance of nauplii. Highest abundances were observed at stations under the influence of warm surface waters and strong upper layer flows. In general, *O. similis* nauplii were more abundant over the shelf than over the slope and in the basin. There was a negative relationship between abundance of *O. similis* nauplii and water column depth, which suggests that the adults may have laid more eggs and were more abundant in shallower water. With the exception of stations influenced by convergence zones (BC1 and BC3), I observed a west-to-east increase in abundances of nauplii for this species.

Molecular identification showed a different distribution of nauplii for the Arctic calanoid copepod *C. glacialis* (Figure 3.6B). Ignoring the high abundances at convergence zones such as Barrow Canyon, *C. glacialis* nauplii were more abundant over the slope than the shelf and basin regions. Highest abundances were observed at the shelfbreak in Barrow Canyon and over the Chukchi slope. In the Chukchi Sea, the slope is a region of enhanced biological activity (primary and secondary production) where the mixture of nutrient-rich shelf water and deep basin waters contribute to the reproductive success of this Arctic copepod (Plourde et al., 2005). In contrast, *C. glacialis* nauplii were practically absent over the Beaufort shelf and slope regions, suggesting that egg production may have been hindered by environmental factors such as increased temperatures and low chlorophyll concentrations. In this survey, no *C. marshallae* nauplii were found.

In the case of the neritic *Pseudocalanus* species, *P. minutus* and *P. mimus*, their distributions showed some similarities. Highest abundances of nauplii were found at stations with temperatures above 3 - 4°C (Figures 3.6C and 3.6D). On the eastern sections (BC and EB) there was a general shelf-to-basin decrease in *Pseudocalanus* nauplii whereas in the western sections (WHS and EHS) no clear pattern emerged. Our analysis also revealed that the nauplii of these species differed in their geographic distribution in summer 2004. *Pseudocalanus mimus* nauplii were not found over the basin of the western Arctic and were less abundant than *P. minutus* nauplii in surface water over the slope. The abundance of *P. minutus* nauplii decreased with water column depth, but nauplii of the species were detected offshore in all samples over the Canada Basin.

A fifth species, *A. longiremis*, was abundant in some coastal samples but absent in most samples (data not shown).

DISCUSSION

During summer, the physical properties of surface waters in the western Arctic are determined by their source. Continental shelf waters are dominated by Pacific water transported into the region through Bering Strait. Deep basin waters offshore of the continental shelf represent water masses of the Arctic Ocean proper, although the upper halocline layer is composed of Pacific water. The summer hydrography of surface waters is also influenced by ice melt and wind-driven coastal upwelling. One purpose of this work was to determine the community of copepod nauplii in the Chukchi and Beaufort seas and Canada Basin. I found that abundances of nauplii were greater in shelf waters than over the basin. The main reason for this difference may be related to differences in primary production rates and chlorophyll concentration. Primary production decreased

significantly with increasing bottom depth in summer 2004 (Kirchman et al., submitted), and thus higher copepod production in shelf waters would be expected.

Not only were there spatial variations in abundances of nauplii, I also observed differences in the distribution of nauplii of various copepod species. I suggest that these differences in species composition derive from the different sources of the surface waters for the shelf, slope, and basin regions. For example, *P. mimus* nauplii were not found in the Canada Basin. The naupliar distribution demonstrated that compared to *P. minutus*, which is a more oceanic/basin species, *P. mimus* is a more neritic/shelf species (Morgan et al., 2003). This suggests that *P. mimus* plays a minor role in the Arctic Ocean proper. From our work it can also be concluded that copepod nauplii as a whole do not have a "mean" response to environmental variables. Each species has unique nauplii abundance and distribution patterns and more study of individual species variation is needed to understand and predict the ecosystem response to climate variability.

Morphological versus molecular identification

The microscopic identification of copepod nauplii can be done using complex identification keys based on body shape, position of specific appendages, and segment numbers of the appendages (i.e. Dahms, 1993; Siefert, 1998). This process is time consuming and often unreliable. For example, species of the genus *Pseudocalanus* have unusually weak interspecific divergence in morphological features (Frost, 1989). The majority of studies therefore classify copepod nauplii as a single group or broad taxonomic groups (i.e. Kosobokova and Hirche, 2000; Thor et al., 2005). Our study demonstrated the limitations of this approach. When I treated the copepod nauplii as a single group, I observed broad spatial variability related to hydrographic characteristics

of the surface waters, yet little information was obtained on the smaller scale horizontal distribution of copepod nauplii and the environmental factors influencing the composition of the community of nauplii.

Calanus glacialis

The free-spawning calanoid *C. glacialis* is a highly motile copepod that performs extensive ontogenetic (occurring through the sequential developmental stages of its life cycle) migrations (Smith and Schnack-Schiel, 1990). Its high fecundity and feeding rates (Plourde et al., 2005, Campbell et al., submitted) allow it to exploit the transient phytoplankton blooms that develop during spring in Arctic waters. These spring blooms, which result from stabilization of the water column by ice melt, exposure to increasing light seasonally, and nutrient-rich water held in the stabilized upper layer when the ice retreats, are the principal food source for the reproduction, growth, and accumulation of lipid reserves in this species (Sargent and Falk-Petersen, 1988). Hence, the behavior, reproduction and life cycle strategy of *C. glacialis* are clearly coupled to the spatio-temporal distribution patterns of primary production (Kosobokova and Hirche, 2001; Plourde et al., 2005), especially in regions with large fluctuations in ice cover such as the Chukchi and Beaufort Seas. This is supported by previous studies showing that *C. glacialis* is unable to persist under warmer conditions (Tande et al., 1985; Kosobokova, 1993; Niehoff and Hirche, 2005).

Our sampling in the western Arctic occurred during the post-bloom period (Codispoti et al., submitted) so the high concentrations of *C. glacialis* nauplii may represent the reproductive peak of the species in the region. Two findings support this contention. First, experiments with *C. glacialis* revealed that the highest egg production

rates recorded for the species occurred in spring prior to our observations in this study (Plourde et al., 2005, Llinás et al., in prep.). Second, development times from egg to copepodid stage 1 ranged from approximately 50 to 150 days over the range of temperatures expected in the region (-2.0 to 1.0 ºC) (Ashjian et al., 1992). Our observations may show the maximized reproductive output for this species in all regions sampled during summer. On a broad scale, I found uneven distribution patterns with high concentrations of *C. glacialis* nauplii over the Chukchi slope region and in Barrow Canyon and few *C. glacialis* nauplii over the Beaufort shelf and slope. The elevated abundances over the slope provide additional evidence of the ecological importance of the eastward flowing shelf-break jet (Pickart, 2004; Ashjian et al., 2005). The high abundances of *C. glacialis* nauplii and other copepod species in Barrow Canyon support the occurrence of a biological hot spot at the head of Barrow Canyon (Hill and Cota, 2005; Codispoti et al., submitted) where three different surface waters converge (waters flowing northward in Barrow Canyon, waters in the Central Channel deflected eastward before reaching the shelfbreak, and slope surface waters that flow southward following the 50-m isobath). The low abundance of *C. glacialis* nauplii over the Beaufort shelf is more difficult to explain. I believe it may related to the presence of warm surface layers in summer 2004 (Codispoti et al., submitted) and the effects of warming on the termination of *C. glacialis* reproduction (Kosobokova, 1999).

Oithona similis

Oithona similis is probably one of the most ubiquitous and abundant copepods in the Arctic Ocean and surrounding shelves. It is adapted to a wide range of habitats and can maintain populations under more disadvantageous conditions than more specialized

and larger calanoid copepod species (Paffenhöfer, 1993). In recent years a strong interest in this and other small copepod species has developed with the increased awareness of their high abundance and biomass (Gallienne and Robins, 2001; Turner, 2004; Hopcroft et al., 2005). In the western Arctic, *O. similis* numerically dominated the zooplankton community (Ashjian et al., 2003; Lane et al., 2007; Smith, unpublished data). It was also the most abundant copepod species in the community of nauplii, with nearly ten-fold more *O. similis* nauplii in some areas (e.g. head of Barrow Canyon) than other copepod nauplii. This has significant implications for estimates of zooplankton-mediated fluxes and for modeling of marine ecosystems (Gallienne and Robins, 2001). For example, in the Baltic Sea the seasonal variation in biomass of *Oithona* spp. is much less than the variations in the biomass of the co-occurring calanoid genera (Kiørbe and Nielsen, 1994). Low metabolic rates may account for the year-round persistence and high abundance of *O. similis* over calanoid copepods, particularly when food resources may be limiting for calanoid copepods (Castellini et al., 2005). Consequently, *O. similis* may contribute relatively more to zooplankton biomass in the Arctic during less productive seasons (Sabatini and Kiørbe, 1994), especially winter when most of the Arctic Ocean is ice covered.

Despite its important role in ecological processes, information on the biology of *O. similis* in the Arctic is still scarce. To our knowledge there are only a few studies that provide insight into this species. Work in the Svalbard Archipelago demonstrated that *O. similis* reproduced almost continuously throughout the year, but with two main reproductive periods in May/June and August/September (Lischka and Hagen, 2005). Our results confirm the existence of the summer reproductive period in the western

Arctic, but evidence from previous surveys is lacking to support a fall peak. During an eddy survey in fall 2004, Llinás et al (submitted a) reported high abundances of *O. similis* adult females and copepodids in surface waters over the slope region. Zooplankton abundance data obtained during the same cruise showed high abundances of this cyclopoid copepod in the Chukchi and Beaufort seas (Smith, unpublished data). While this information is limited, it suggests that *O. similis* may also have two principal reproductive peaks in the western Arctic. This biannual reproductive cycle and the low mortality rates observed after the early naupliar stages (Eiane and Ohman, 2004) may account for the numerical dominance of *O. similis* in the western Arctic region.

Pseudocalanus minutus

Pseudocalanus species co-occur extensively in the Arctic region (Corkett and McLaren, 1978; Frost, 1989). According to Frost (1989), four species have been reported in the Chukchi and Beaufort seas and adjacent Canada Basin: *P. acuspes, P. mimus, P. minutus*, and *P. newmani*. Two species, *P. acuspes* and *P. newmani*, accounted for less than 3% of the copepod nauplii in the subset of samples analyzed using molecular techniques and therefore have not been considered further. Adults of *P. minutus* were common in spring and summer (Smith, unpublished data) and nauplii of the species showed a wide geographical distribution and in many parts co-occurred with two or three other species of *Pseudocalanus*. In an effort to understand the life history of the species in the study area I used what is known about its adaptations to similar Arctic environments. In the Svalbard Archipelago, reproduction of *P. minutus* starts in May and lasts until June (Lischka and Hagen, 2005). In the northern Labrador Sea, females in the spring spawn two broods in succession and produce two generations per year (Carter,

1965). Given the elevated numbers of *P. minutus* nauplii in summer, I believe the reproduction of this species in the western Arctic is also timed to take advantage of the spring bloom. Hence, *P. minutus* nauplii are important components of the post-bloom community of copepod nauplii over the Chukchi and Beaufort shelves.

Although the timing of its reproduction may correspond to the increase in phytoplankton concentrations, I suspect that *P. minutus* is also able to exploit an alternate food source for growth and reproduction when open water chlorophyll *a* levels are low. Over deep water (>2000m), underice pelagic copepods may feed on primary production derived from the sea-ice. This hypothesis is supported by the presence of *P. minutus* nauplii in surface waters over the Canada Basin and by previous reports of *Pseudocalanus* species feeding under ice (Conover et al., 1986). Furthermore, Runge and Ingram (1991) observed that algae growing at the ice-water interface were an important source of nutrition for *P. minutus*. Like most members of the sub-ice community, *P. minutus* could be an important trophic link between sea-ice derived primary production and higher trophic levels in the water column (Grainger and Hsiao, 1990; Werner, 2006).

Pseudocalanus mimus

Frost's (1989) taxonomic analysis of *Pseudocalanus* established the existence of three previously unrecognized species, including *Pseudocalanus mimus*, which he named *mimus* (Latin, imitator) in reference to the strong resemblance of this species to *P. minutus*. According to Frost (1989), *P. mimus* is essentially endemic to waters of the North Pacific Ocean. The range of the species extends from Oregon to a few previously reported occurrences just north of Bering Strait. Our results suggest that its distribution extends much further north, into the slope region of the Chukchi Sea, and east into the

Beaufort Sea. Populations of *P. mimus* may have been gone undetected in previous work in the region due to the uncertainties concerning the description of *Pseudocalanus* species before the taxonomic revision by Frost (1989). For example, Springer et al. (1989) classified all *Pseudocalanus* individuals collected over the southern Chukchi Shelf as a single group (*Pseudocalanus* sp.). Our observations are possible evidence of a northward range expansion of this species in response to global warming since Johnson (1956, 1958) reported only *P. minutus* in the Chukchi and Beaufort Seas. At present, I cannot determine which explanation is correct because knowledge on the life cycle of *P. mimus* is lacking.

The distribution of *P. mimus* nauplii could provide some insights into its life cycle in the western Arctic. Nauplii of *P. mimus* were common in surface waters over the shelf and slope of the Chukchi and Beaufort Seas, suggesting *P. mimus* was actively breeding and reproducing over the shelf during summer 2004. In the Gulf of Alaska, *P. mimus* was the dominant *Pseudocalanus* species over the shelf during spring and summer 2001 (Napp et al., 2005). There, egg production rates for the species increased from minima in March to maxima in May during the spring bloom of phytoplankton (Hopcroft et al., 2004) and were highest over the middle shelf (Napp et al., 2005). The elevated abundances of *P. mimus* nauplii in summer 2004 in the Chukchi and Beaufort seas suggest a similar reproductive strategy over the shelves north of Bering Strait. Furthermore, Frost (1989) indicated that the life history of *P. mimus* resembles that of *P. minutus* in coastal waters (McLaren et al., 1989). In this respect, *P. mimus* may occur predominantly as copepodid stage 5 (C5) during late spring and summer, and reproduce annually (Frost, 1989; McLaren et al., 1989). Samples collected from the Oregon coastal

upwelling system suggest that *P. mimus* produces multiple generations there during summer (Frost, 1989). The Chukchi and Beaufort seas are the northernmost locality records of *P. mimus* and thus this species most likely produces only one generation during summer in these arctic continental shelves.

CONCLUSIONS

Molecular analyses of a subset of samples showed that four copepod species dominated the community of nauplii in the Chukchi and Beaufort seas and Canada Basin during summer 2004. These copepod species, *O. similis C. glacialis*, *P. minutus*, and *P. mimus*, comprised 82% of the nauplii sequenced. Abundance and distribution patterns of the nauplii of these species suggested that each species has a distinct life history despite some being nearly indistinguishable based on morphological characters (e.g. congeneric species, *P. mimus* and *P. minutus*). The physical properties and circulations patterns of the surface waters influenced the composition of the community of copepod nauplii, with warm surface temperatures likely having detrimental effects on the number of *C. glacialis* nauplii. Knowledge of the physical and environmental parameters that shape the life cycle strategies and reproduction of each species are necessary to develop bio-physical models and predict how climate warming will affect the abundance and distribution copepods in this rapidly changing environment.

Table 3.1 Station name, date, location, depth, number of nauplii enumerated, and number of nauplii sequenced.

Station name	Date collected	Latitude (N)	Longitude (W)	Depth (m)	Volume filtered (m^3)	No. of nauplii enumerated	No. of nauplii sequenced
BC1	22-Jul-04	71.077	159.431	88	0.026	1028	92
BC3	24-Jul-04	71.638	155.904	171	0.033	850	92
BC5	26-Jul-04	71.992	154.719	960	0.029	289	92
EB1	29-Jul-04	71.291	152.541	52	0.074	925	92
EB3	30-Jul-04	71.573	152.469	156	0.065	570	92
EB8	1-Aug-04	72.614	151.844	3850	0.063	87	87
EB6	3-Aug-04	71.962	152.142	2124	0.071	645	92
BC8	9-Aug-04	72.691	152.955	3761	0.027	76	76
EHS1	10-Aug-04	72.217	159.201	49	0.064	312	92
EHS4	11-Aug-04	72.612	158.728	91	0.031	133	92
EHS7	14-Aug-04	72.865	158.317	1077	0.056	476	92
EHS11	16-Aug-04	73.388	157.415	3153	0.066	420	92
EHS12	16-Aug-04	73.795	156.728	3668	0.067	71	71
WHS8	18-Aug-04	73.901	157.854	3760	0.064	104	92
WHS6	19-Aug-04	73.48	159.61	2110	0.065	294	92
WHS3	22-Aug-04	73.102	160.506	217	0.055	335	92
WHS1	24-Aug-04	72.737	161.301	52	0.086	401	92

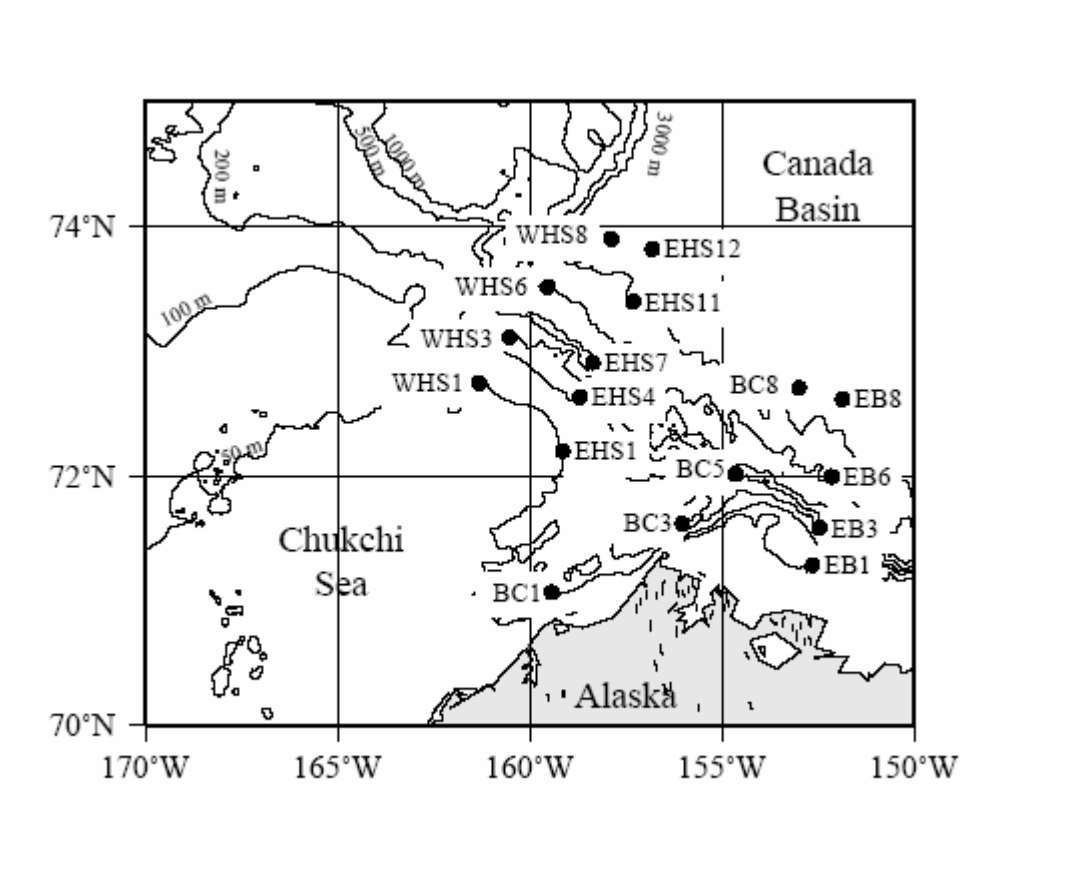


Figure 3.1 View of the study area in the western Arctic occupied by the USCGC Healy in summer 2004. Sections are denoted (in chronologic order of sampling) by region as Barrow Canyon (BC); East Barrow (EB); East Hanna Shoal (EHS); and West Hanna Shoal (WHS). Black circles indicate the station locations of the samples selected for molecular analysis.

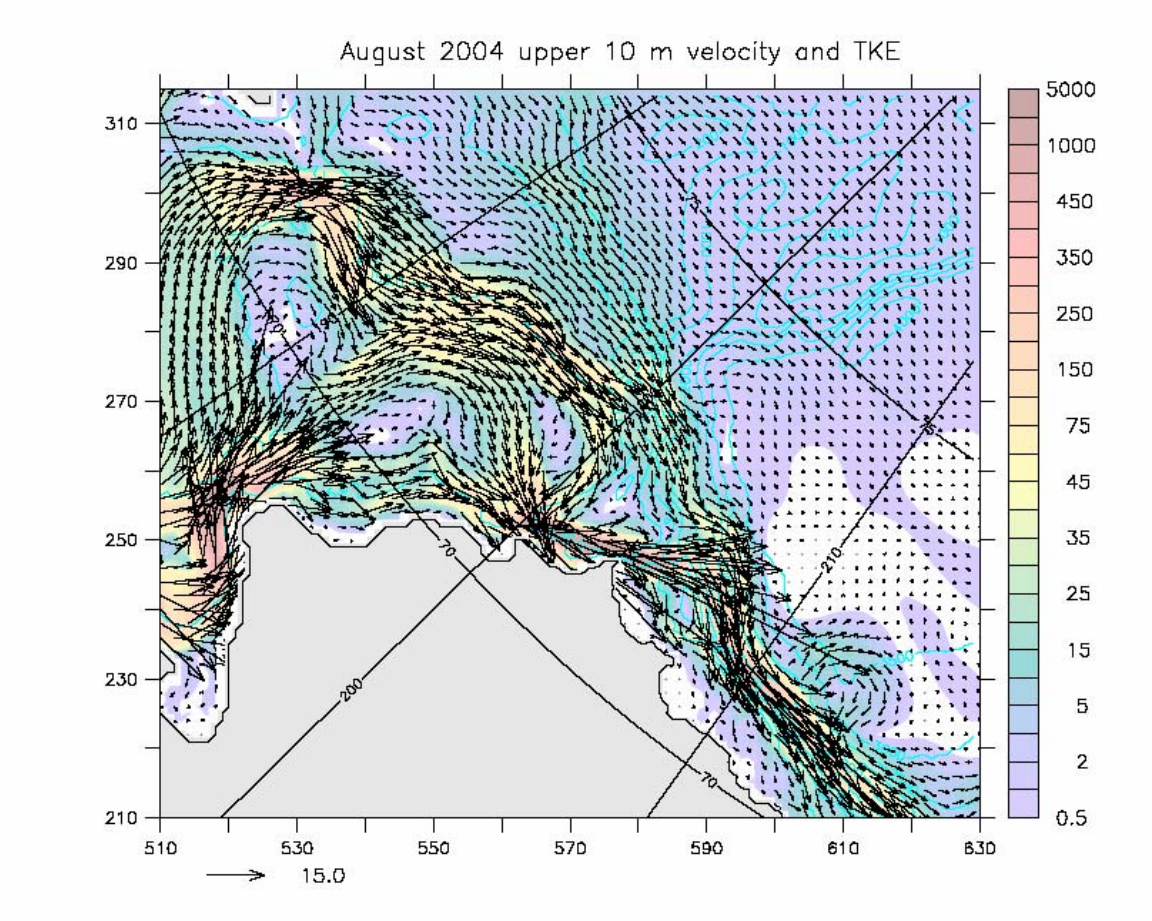


Figure 3.2 Figure of modeled mean upper 10 m velocity (v) and total kinetic energy (TKE) for August 2004. TKE ($v^2/2$) is the shading in the figure and is a way to easily identify the faster/slower velocities. Every other vector is shown in the figure. (Courtesy of Dr. Maslowski and Dr. Clement).

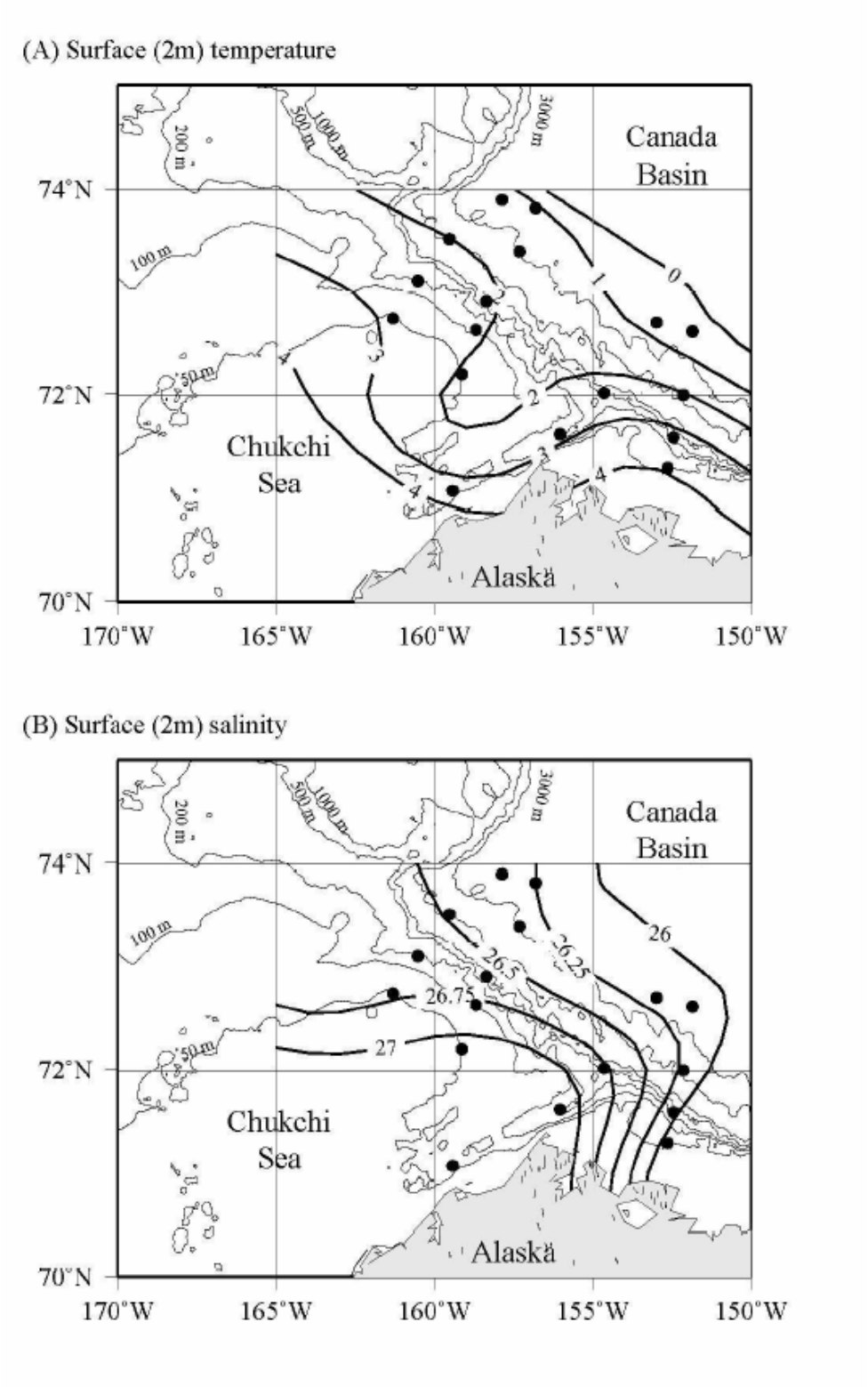


Figure 3.3 Contours maps of the western Arctic depicting the temperature (A) and salinity (B) at 2 m depth during summer 2004. The contours were plotted using data collected with the continuous-flow seawater system. Black circles indicate the station locations of the samples selected for molecular analysis.

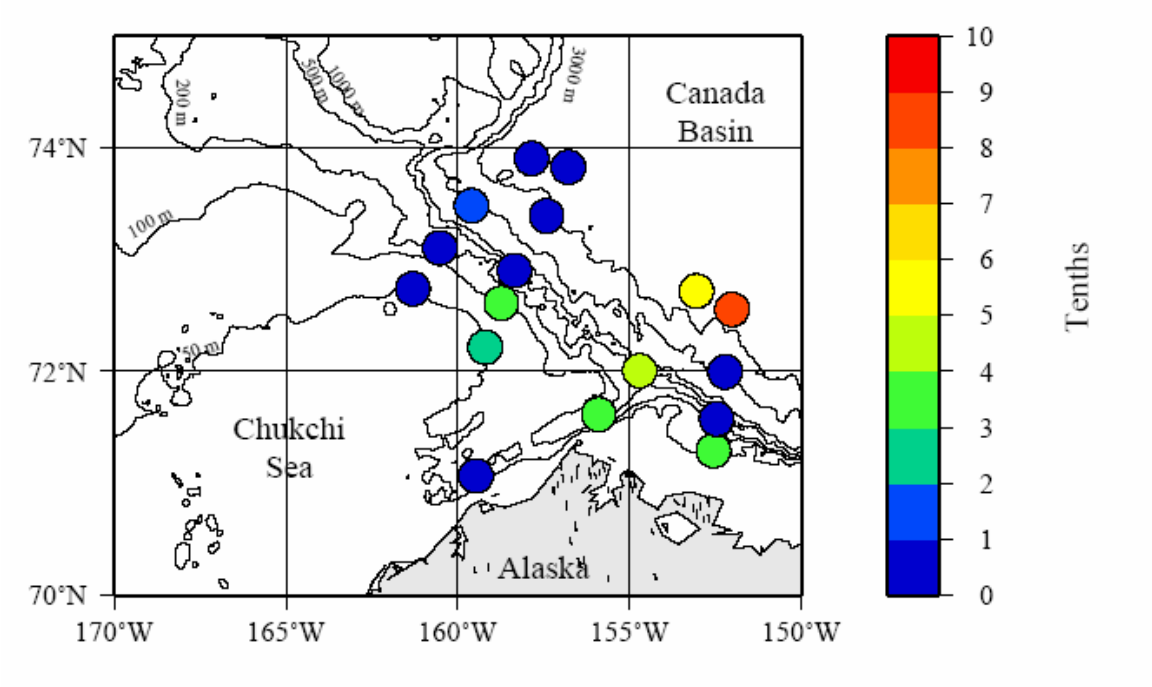


Figure 3.4 Summer 2004, ice cover at stations where samples were selected for molecular analysis.

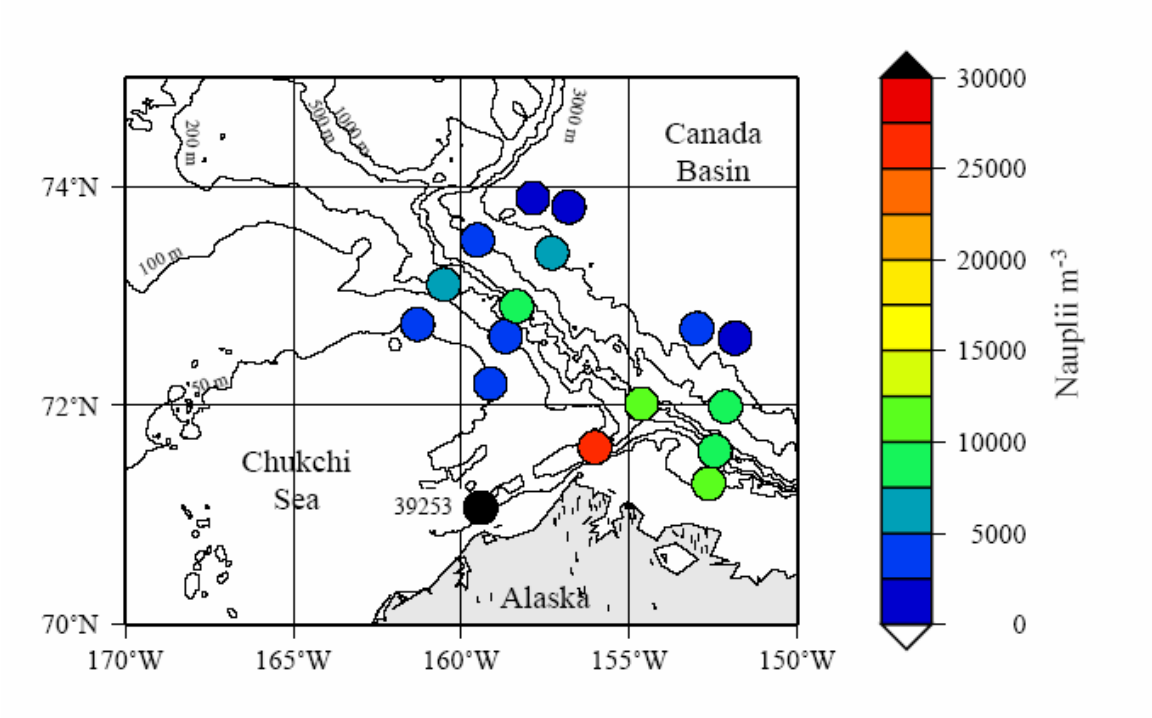
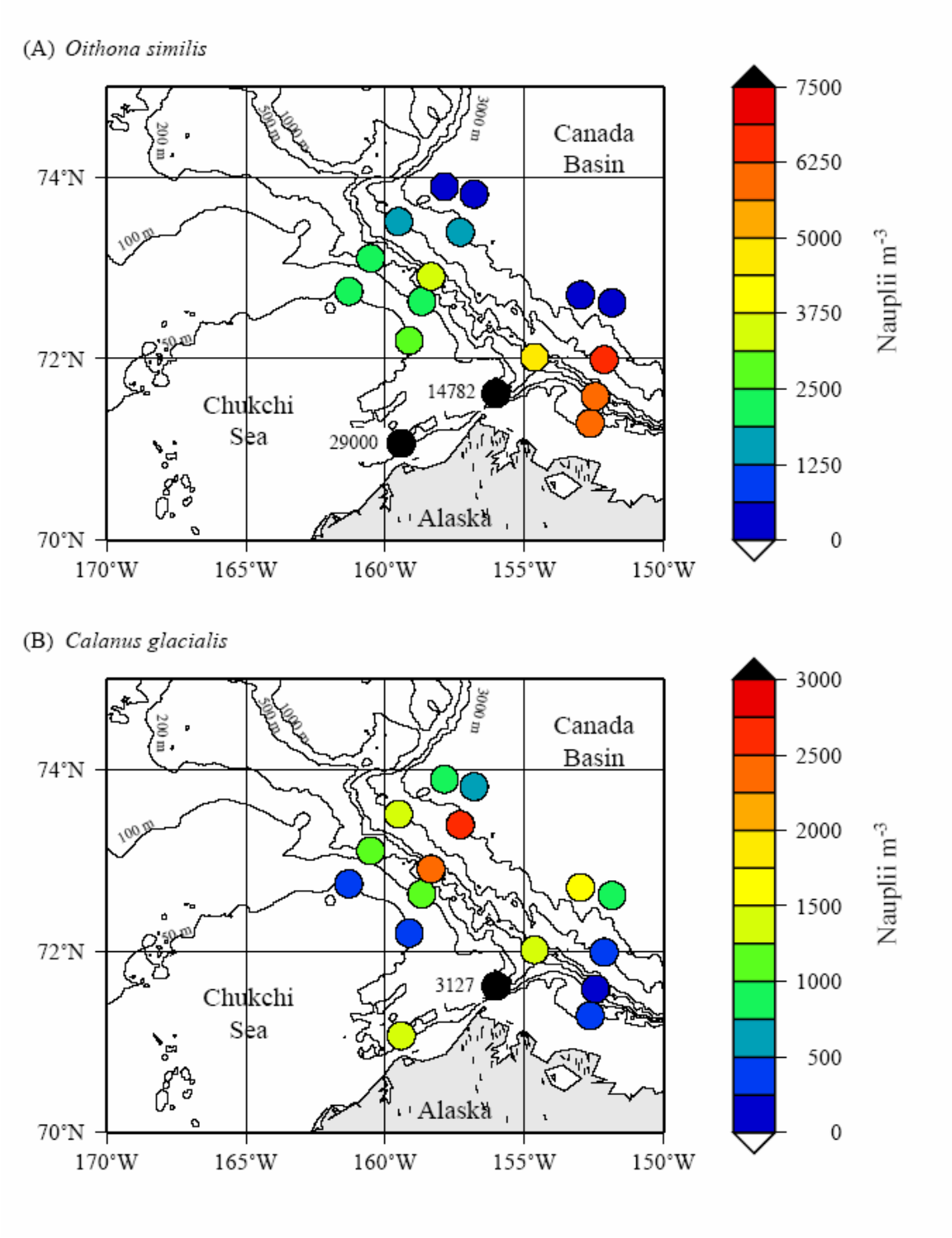


Figure 3.5 Map showing the distribution and abundance of copepod nauplii during summer 2004. High abundances are plotted in black.


(A) Oithona similis
Canada Basin
Chukchi Sea
Alaska
14782
29000
200 m
500 m
1000 m
3000 m
100 m
50 m
74°N
72°N
70°N
170°W
165°W
160°W
155°W
150°W
7500
6250
5000
3750
2500
1250
0
Nauplii m-3
(B) Calanus glacialis
Canada Basin
Chukchi Sea
Alaska
3127
3000
2500
2000
1500
1000
500
0
Nauplii m-3

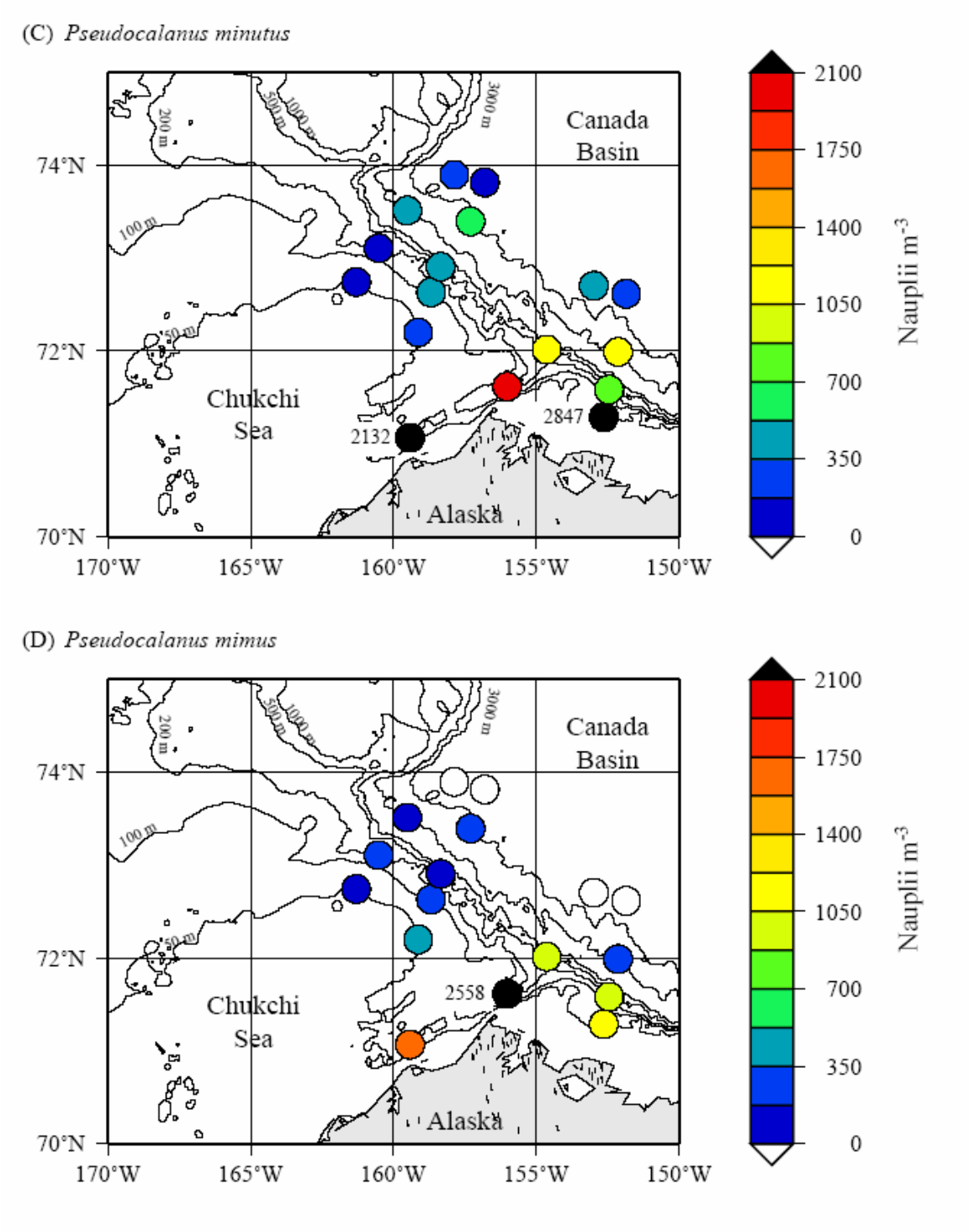


Figure 3.6 Maps showing the species-specific distribution and abundance of copepod nauplii during summer 2004 as determined using a molecular identification method. The dominant species are: (A) *O. similis*, (B) *C. glacialis*, (C) *P. minutus*, and (D) *P. mimus*. High abundances are plotted in black, white circle indicate that no nauplii of the species were found.

CHAPTER 4: EGG PRODUCTION OF *CALANUS GLACIALIS* IN THE CHUKCHI AND BEAUFORT SEAS

INTRODUCTION

The Chukchi and Beaufort seas are regions of elevated primary production (Codispoti et al., 1991; Hill and Cota, 2005), which derives from the input of nutrients from the Pacific Ocean through Bering Strait (Walsh et al., 1989), from rivers (Carmack and Macdonald, 2002; Carmack et al., 2004), and from upwelling of deep arctic water at the shelfbreak (Münchow and Carmarck, 1997; Nikolopoulos and Pickart, 2007; Llinás et al., 2007). Of particular interest are the production, transformation and fate of carbon in this rapidly changing ecosystem. Planktonic processes that can modify the vertical flux of carbon, such as grazing and secondary production, are critical to understand and model the carbon cycle. Previous work on food limitation for the mesozooplankton community in the western Arctic was inconclusive due to the inability to differentiate between the similar copepod species *Calanus glacialis* and *C. marshallae* (Plourde et al., 2005). In the Chukchi and Beaufort seas, *C. glacialis* is the dominant copepod species in terms of biomass while *C. marshallae* might be found in the area, transported there in Pacific water. The objectives of this work are to: 1) validate previous reproductive studies with *C. glacialis* in the western Arctic, 2) understand the physical and biological factors controlling the life cycle adaptations of this species in the western Arctic ecosystem, and 3) provide some insights into the potential effects of climate warming on the reproduction and population dynamics of *C. glacialis*.

The biology of Calanus glacialis

The calanoid copepod *C. glacialis* is found over most of the Arctic Ocean, its surrounding seas, and adjacent subarctic areas. Due to its large size and abundance, this arctic species dominates the zooplankton biomass of many arctic ecosystems. In the Laptev Sea, *C. glacialis* can account for up to 61% of the total zooplankton biomass (Kosobokova et al., 1998), 45% in the White Sea (Kosobokova, 1999) and 21% in the central Arctic Ocean (Auel and Hagen, 2002). Flexible life cycle strategies and reproductive behaviors are partly responsible for the success of this species. North of the polar front in the Barents Sea and adjacent Arctic Ocean, the species completes its development in two years, while over the rest of its distribution annual cycles are suggested (Conover, 1988). Life cycles of at least two years have been observed off the west coast of Greenland (Madsen et al., 2001). During the feeding season (roughly May to August), *C. glacialis* stores lipid reserves in the form of wax esters (Sargent and Falk-Petersen, 1988) to survive during winter when food is less abundant and to molt to the adult stage in spring when ice breakup begins. Overwintering stages are generally late stage copepodites C4 and C5, which enter a period of reduced metabolism after their ontogenetic migration to deeper water in fall (Tande et al., 1985, Conover and Huntley, 1991). In addition, a reproductive strategy combining food-dependent and food-independent egg production has allowed *C. glacialis* to maximize recruitment levels under low and high food concentrations, respectively (Hirche and Kattner, 1993). Life cycles vary among populations and from environment to environment (Conover, 1988; Madsen et al., 2001).

Life cycle in the western Arctic

The distribution and abundance of *C. glacialis* in the Chukchi and Beaufort seas indicate that the species is well adapted to the seasonality of the environment and to the timing of increased primary production. Here, this copepod is considered a shelf-associated species whose reproductive success depends on the input of nutrient-rich Pacific water to the northern Chukchi Sea and the Chukchi and Beaufort shelf-break regions (Plourde et al., 2005). From spring to fall, *C. glacialis* is the most abundant large-bodied copepod found in the Chukchi and Beaufort shelves. It accounts for a large portion of the mesozooplankton total biomass, and is an important component of the local food web (Lane et al., 2007). This omnivorous copepod is preyed upon by most planktivores in the ecosystem and plays key roles in the regional pelagic ecosystem and the fate of primary production. For example, egg production experiments with *C. glacialis* have been used to analyze food limitation for the mesozooplankton community (Plourde et al., 2005). In recent years however, ecological studies with *C. glacialis* have been compromised by difficulty in morphologically discriminating between *C. glacialis* and *C. marshallae,* a sibling species transported from the Bering Sea. Both species may co-occur in the Chukchi and Beaufort seas so molecular identification is necessary to validate such studies.

Arctic change and C. glacialis

In recent decades, the Arctic has experienced unprecedented changes (ACIA, 2004). The influence of these climatic changes on zooplankton populations has been the subject of numerous studies (Heath et al., 1999; MERCINA, 2003; Pershing et al., 2004), which focused on the eastern Arctic ecosystem and the economically important copepod

Calanus finmarchicus. These studies revealed that circulation changes and climatic variability played an important role in determining the abundance of *C. finmarchicus* in the North Atlantic and in reorganizing the zooplankton assemblages in the ecosystem. Although similar processes are expected to occur over the entire Arctic Ocean, little information is available on the plausible regime shifts exhibited by zooplankton associated with other Arctic ecosystems. For example, despite the important ecological role of *C. glacialis* in the western Arctic, it is not known exactly how this species is maintained in this changing ecosystem. Its central role and association with arctic waters (Grainger, 1963) makes it a good species to directly study the consequences of climate change in the Arctic, yet little attention has been placed on either the role of *C. glacialis* as plankton indicator of ecosystem state or its response to global warming.

The objective of this study is to describe the physical and biogeochemical processes that affect egg production of the dominant copepod *C. glacialis* (including molecular identification of the species) in the western Arctic. I begin with a description of the physical and chemical properties of the water column across transects occupied in summer 2002 and spring and summer 2004 proceeding from the Chukchi and Beaufort shelves to the Canada Basin to provide a view of the hydrographic conditions in the region. This is followed by a description of the spatial and seasonal patterns of egg production in *C. glacialis*. I use mitochondrial cytochrome oxidase I sequences, in conjunction with a recently developed high-throughput protocol (Llinás et al., submitted), to distinguish *C. glacialis* from *C. marshallae*. Finally, I assess the responses of reproduction in *C. glacialis* to rising temperatures, with an eye towards anticipating future food web changes in this arctic ecosystem.

MATERIALS AND METHODS

Field sampling

As part of the SBI project, physical, chemical and biological measurements were collected from the USCGC *Healy* during cruises in summer 2002, spring 2004, and summer 2004 to the western Arctic. Hydrographic stations concentrated along four shelf-basin lines extending across the Chukchi and Beaufort outer shelves into the Canada Basin (Figure 4.1): West Hanna Shoal (WHS), East Hanna Shoal (EHS), Barrow Canyon (BC) and East Barrow (EB). Depending on ice conditions, the stations were repeated during all three SBI Process Cruises. Depending on time constraints and female copepod abundances, egg production experiments were carried out at a variety of stations. The differences in station locations among spring 2002 and spring and summer 2004 can complicate comparisons and will be discussed later.

During the summer 2002 cruise (17 July – 26 August), 45 stations were occupied, with egg production experiments conducted at 14 of the stations (Fig 1A). In summer 2002, the ice cover was at a record low and the four shelf-basin lines were occupied completely. Relatively heavy sea ice limited sampling only at the northernmost stations of the lines.

During the spring 2004 cruise (15 May – 23 June), 35 stations were occupied and egg production rates were measured at 15 stations (Figure 4.1B). Portions of the BC and EHS sections were re-occupied and additional outer shelf stations were also occupied. Ice cover in spring 2004 was > 90%. Because of ice conditions, observations during spring 2004 did not include the WHS and EB sections.

During the summer 2004 cruise (17 July – 26 August), sampling was done under ice-free conditions that almost matched the 2002 record low level of ice cover. A total of 60 stations were occupied and egg production rates were measured at 14 stations (Figure 4.1C). The four shelf-basin lines were re-occupied and extended further into the Canada Basin.

Hydrographic data

The SBI Service Group provided the hydrographic data from conductivity-temperature-depth (CTD) casts and water samples at all stations. The hydrographic sampling protocols are described elsewhere (Codispoti et al. 2005; Codispoti et al., 2007) and are only summarized here. The physical (temperature and salinity), chemical (nutrients) and biological (chlorophyll *a*) data were collected using modified WOCE/JGOFS protocols (Codispoti et al. 2005). The hydrographic sampling system comprised a Sea Bird Electronics (SBE) CTD mounted on a 24-botle rosette with 10-L Niskin bottles. All instruments were calibrated according to WOCE methods and samples were analyzed using quality control protocols that meet WOCE standards.

Egg production experiments

For egg production experiments with *C. glacialis*, copepods were captured in slow vertical tows of a ring net (150 μm with non-filtering cod ends) in the upper 100 m or from near-bottom to the surface when bottom depth was less than 100 m. Immediately after capture, single females were identified with a Wild M5 microscope and placed in plexiglass cylinders with 360 μm mesh false bottoms inside 60-ml bottles filled with 0.2 μm filtered sea water. The first females encountered were used for the experiments and,

whenever possible, 12 individuals were used per station. At some stations during the summer 2002 and 2004 cruises, experiments were carried out with fewer animals due to the scarcity of females. All microscope work was performed so that water temperature remained near 0°C at all times. After sorting, bottles with single females were placed in a cold room with approximate ambient temperature (-1°C) at all times in dim light.

Following the 24 h incubation, eggs were collected and counted for each female. The number of fecal pellets produced during the incubation was also counted and used as an indicator of feeding conditions. This approach assumes that the amount of fecal material produced by a copepod is related to the rate of ingestion (Corner et al., 1972), which in turn is dependent on the available food concentration (Mullin et al., 1975). For all females the prosome length was measured and the reproductive state of female gonadal development was scored as described by Smith (1990). Females were then preserved in ethyl alcohol. After enumeration, eggs were resuspended in 22 ml vials with 0.2 μm filtered sea water and kept at ambient conditions in the cold room. Eggs were checked after one week for hatching success. After the experiment was completed, the unhatched eggs and nauplii were preserved in ethyl alcohol.

Molecular identification

After the 24 h incubation, females were transferred individually into cryogenic vials with 95% ethyl alcohol for preservation before molecular identification. To distinguish between the morphologically similar species *Calanus glacialis* and *C. marshallae,* DNA was extracted from each female collected in spring and summer 2004. No females were preserved for molecular identification during the summer 2002 egg

production experiments. The extraction, amplification, sequencing and identification protocols are described elsewhere (Llinás et al., submitted) and are summarized here.

The DNA extraction, isolation, amplification and sequencing reactions were performed in 96-well plates, with four wells used as positive and negative controls and as secondary identifiers of the orientation and identification of the plates for sequencing. To clean the females of contaminants, each individual was rinsed three times in sterilized nano-filtered water and transferred individually into wells filled with a proteinase K-based extraction solution consisting of 60µl of 1X TE (pH 7.4), 10 µl of cholic acid (sodium salt; 30% solution in water), 10 µl of buffer (1.6M Tris HCl, 0.2M EDTA, both at pH 8.0), 19 µl of proteinase K (20 mg/ml), and 1 µl of 0.1% sodium dodecyl sulfate (SDS). After extraction, the DNA was isolated using a magnetic beads protocol adapted from the Genfind kit (Agencourt, Beverly, MA, USA).

To prevent the amplification of pseudogenes, I used the internal primers 44F (5' - CAG GGC TGA GTA TGA TTA TTC - 3') and 575R (5' - GTC AGA GAA GGT CGT ATT TAG -3') to amplify the mitochondrial cytochrome oxidase I gene (mtCOI). Polymerase chain reactions had a final volume of 50 µL and contained 10 µL of the DNA template, 2 mM dNTP, 40 pmoles of each primer, 4µL of Taq, and a reaction buffer (final concentrations: 50 mM tris HCl pH 9.2, 16mM (NH4) 2SO4, 2.25 mM MgCl2, 2% DMSO, 0.1% Tween 20) (Paschall et al., 2004). Reaction conditions were 30 s hot start at 80°C followed by 40 cycles of 15 s at 94°C, 1 min at 50°C and 1 min at 72°C. A final extension for 8 min at 72°C was carried out after the PCR cycles. A negative (no template) and a positive control (validated standard) were included on every plate. PCR products were purified using the Ampure magnetic bead purification kit (Agencourt,

Beverly, MA, USA), on the liquid-handling robot. Following cleanup, PCR products were sequenced using 3.2 pmoles of primer, 1μL of BigDye Terminator (Applied Biosystems), 1.9 μL of reaction buffer, and 1μL of template in a 10μL reaction. The sequencing thermocycling protocol consisted of 40 cycles at 94°C for 15 s, 50°C for 20 s and 60°C for 4 min.

For identification of the 342 females collected in 2004, I sequenced unidirectionally using the primer 44F, and used a species identifier script designed to compare unidentified sequences to consensus sequences. The script, developed by Richardson et al. (2006), uses MATLAB (Mathworks, Natick, MA, USA) and the bioinformatics toolbox MBEToolbox (Cai et al., 2005). Details of the program can be found elsewhere (Richardson et al., 2006; Llinás et al., submitted).

Statistical analyses

Before analysis, estimates of reproduction, growth, and associated parameters were normalized using log-transformation to satisfy assumptions required by the t-test. Egg viability, expressed as a percentage, was arcsin-transformed. I corrected for sampling bias by removing all locations that were not re-occupied from the analysis. For inter-annual comparisons, I used only data from stations that were occupied in both 2002 and 2004 summers. For seasonal analysis, I compared data from stations re-occupied in spring and summer 2004. The objective of these analyses was not to test the statistical significance of the experimental data, but to examine the trends in the data. In consequence, I chose a moderate level of significance (5%) that overstates the biological differences of the analysis and understates its statistical signficance.

RESULTS

This study investigates egg production rates of the dominant copepod *C. glacialis* during expeditions in summer 2002 and spring and summer 2004. During the SBI cruises, hydrographic sampling was concentrated along four shelf-basin lines extending from the outer shelves into the basin. I use the data from the sections to describe the hydrographic processes occurring on the shelf (<200 m), over the slope (200-2000 m) and in the basin (>2000 m) regions. Inter-annual and seasonal biogeochemical comparisons provide a background to interpret the data from the egg production experiments.

Experimental and environmental variables may contribute to the variability in our results. The location of the egg production experiments is far from ideal with respect to providing a spatial description of the reproductive biology of *C. glacialis* in the Chukchi and Beaufort seas. Abnormally ice free conditions and warm surface water in the region could create significant differences in the biological processes observed. In addition, the spatial patterns may result from temporal changes to some extent since western transects (EHS and WHS) were occupied later in the summer season than eastern sections (BC and EB).

Hydrographic conditions during 2002 and 2004

Barrow Canyon

Previous work in the region in 2002 and 2004 demonstrated that dissolved inorganic nitrogen limited phytoplankton growth and that the head of Barrow Canyon was a region of enhanced biological production (Codispoti et al., 2007). Dissolved inorganic nitrogen comprises ammonium + nitrate + nitrite. Nitrate sections are used here to describe the distribution of the limiting nutrient at Barrow Canyon in summer 2002

(Figure 4.2A), spring 2004 (Figure 4.3A) and summer 2004 (Figure 4.4A). During both summers, nitrate depletion in the upper 20 m was ubiquitous, particularly at stations over the slope and basin. In spring 2004, surface nitrate concentrations were low at shelfbreak and slope stations.

The distributions of nitrate displayed plume-like features in summer 2004 (Figure 4.4A). These features are generated by the advection of nutrient-rich Pacific waters entering the Chukchi Sea through Bering Strait. Pacific waters flowing northward over the shelf are modified by interaction with the sediments, sinking of biogenic material and cooling and freshening due to ice melt. Temperature sections in summer 2002 (Figure 4.2B) and 2004 (Figure 4.4B) showed similar plume-like features. Sections of silicate, light transmission and ammonium were also indicative with the presence of these features.

In the Chukchi and Beaufort seas, low temperatures prevail year round and the effect of temperature on density is small here so that stratification is effectively determined by salinity. I show salinity sections to describe the stratification in summer 2002 (Figure 4.2C), spring 2004 (Figure 4.3C) and summer 2004 (Figure 4.4C).

The springtime nitrate depletion occurred in surface waters associated with elevated chlorophyll *a* values (Figure 4.3D). In spring, chlorophyll *a* at the surface and subsurface reached 13 and 29 μg/L, respectively. By summer, a distinct subsurface chlorophyll maximum had developed just below the polar surface layer at a depth of 10-30 m with similar high values (30 μg/L; Figure 4.4D). The location of the chlorophyll maximum corresponded to a sharp vertical gradient in nutrient concentrations. During

both seasons, the subsurface maximum was confined to the shelf and shelfbreak areas, and rapidly decreased with distance offshore.

Chukchi and Beaufort shelfbreaks

Our observations cover two distinct shelf environments: the Chukchi and Beaufort shelves. The EHS section provides a background for the processes occurring in the Chukchi shelfbreak region west of Barrow Canyon (Fig 4.5). The section east of the canyon, the EB line, characterizes the hydrography of the Alaskan Beaufort Shelf (Figs. 4.6 and 4.7). A comparison between the biogeochemical distributions in both environments revealed some similarities and some differences. For the EHS section, I show only data from 2002 because the conditions were very similar both years. In contrast, pronounced differences in nitrate concentrations and temperatures were observed along the EB line. In consequence, I present both 2002 and 2004 EB sections.

Nitrate depletion at the surface (0-20m) was ubiquitous in summer 2002 (Figs. 4.5A and 4.6A). Maximum nitrate concentrations (>14 μM) occurred at ~150 m. In 2002, the nitrate maxima were associated with temperatures of ~-1.7°C (Figs. 4.5B and 4.6B) and salinities of ~33 (Figs. 4.5C and 4.6C). The nitrate maxima and temperature minima usually designate the Upper Halocline Layer (UHL) whose dominant contributors are Pacific waters flowing along the shelf break of Chukchi and Beaufort seas (Rudels et al., 1994; Weingartner et al., 1998). The UHL at the EHS section (>15 μM, Figure 4.5A) had higher nitrate maxima compared to the EB line (<15 μM, Figure 4.6A), possibly reflecting their distinct origins. The nitrate rich fraction that flows through Bering Strait is carried onto the Chukchi shelfbreak through Herald Valley in the western Chukchi,

whereas the Alaskan Coastal Water flowing into Barrow Canyon tends to have much lower nitrate concentrations (Walsh et al., 1989).

In summer 2004, the nitrate signal of the UHL was muted along the EB line due, in part, to higher primary production in 2004 (Kirchman et al., 2007). The EB section was occupied on the same calendar days both years, but in 2004 nitrate depletion occurred from the surface to ~50 m (Figure 4.7A), the surface layer was 5°C warmer (Figure 4.7B), and Chl *a* concentrations were located farther offshore (Figure 4.7D). In 2004, the maximum Chl a concentrations were associated with the warm surface water.

Away from the influence of the warm surface waters, the location of elevated Chl *a* concentrations over the Beaufort shelf break and slope regions was similar between years. Along the EHS line, high Chl *a* concentrations were also observed at the same location in 2002 and 2004. Despite the consistent patterns, different processes may explain the occurrence of elevated Chl *a* values because on the EHS section high values were observed over the shelf (Figure 4.5C), while on the EB section high concentrations occurred over the shelf break and slope in 2002 (Figure 4.6C) and only over the slope in 2004 (Figure 4.7C).

Patterns in sea ice coverage

Our results must be considered in light of the Arctic-wide trend of reduction in ice coverage. In summer 2002 and 2004, sampling was carried under unprecedented ice-free conditions (Figs. 4.8A and 4.8C). In September 2002, sea ice in the Arctic reached a record minimum (Serreze et al., 2003). In the past, a low ice year was generally followed by a rebound to near-normal conditions; however, 2002 was followed by two more low-ice years, both of which almost matched the 2002 record (Meier et al., 2005). These

unprecedented summer lows were coupled with weak wintertime recoveries and rapid ice melting in spring. Ice coverage was still heavy during spring 2004 (Figure 4.8B), but a rapid ice retreat in late June – early July displaced the ice-edge further northward into the basin in summer 2004 than in 2002 (Figs. 4.8A and 4.8C). In the study area, ice coverage was significantly lower in summer 2004 than in 2002 (t-test, $p<0.05$).

Molecular identification

Genetic analyses verified that the 342 individuals used in the egg production experiments during spring and summer 2004 were *C. glacialis*. No molecular identification was possible for the 2002 samples, however based on the 2004 results it can be assumed that only *C. glacialis* females were used in the egg production experiments during both years. The mtCO1 sequences revealed that different haplotypes (alternative forms of the mtCO1 gene) existed within the western Arctic. Haplotype diversity has been successfully used in other studies with copepods to investigate the connectivity of populations and the barriers to gene flow between these populations (Bucklin et al., 1996; Goetze, 2005). In the Chukchi Sea however, there were no clear spatial or seasonal patterns in haplotype distribution, suggesting that the *C. glacialis* individuals were part of the same population. This might be an artifact of the small scale at which the study was done; population differences might be observed over broader spatial and temporal scales (Goetze, 2005).

Calanus glacialis

Body size and feeding index

Excluding one station on the EB line in summer 2002 with unusually small individuals, the range of prosome length was similar for all cruises (Figure 4.9). There was no significant difference in prosome length between stations re-occupied in summer 2002 and 2004 (t_{171}, p>0.05). At stations re-occupied during both seasons in 2004, females were significantly larger in spring than in summer (t_{116}, p<0.05).

I observed high spatial variability in body size. In general, body size decreased along shelf-to-basin and west-to-east axes. Smaller animals dominated shelf regions while the large females (> 3.8 mm) were more abundant in the Canada Basin (see BC and EB lines, Figure 4.9C). Females collected at the shelfbreak and slope regions usually had intermediate prosome lengths. In summer 2002, larger females dominated the populations in stations influenced by waters from the central and western Chukhi shelf along the WHS line compared with populations with smaller females on the EB line influenced by Alaskan Coastal Water (Figure 4.9A).

The rate of fecal pellet production is closely related to feeding and ingestion rates of copepods, and therefore the amount of fecal material produced by a copepod will be dependent on the available food concentration (Corner et al., 1972; Dagg and Walser, 1986). However, pellet production is dependent on the food source and the efficiency of assimilation, which were not quantified. This complication will be discussed later.

I counted fecal pellets as an indicator of the gut fullness, and possibly feeding rate, of experimental *C. glacialis*. Feeding rates were similar in summer 2002 and 2004, judging from the number of fecal pellets produced (t_{171}, p>0.05; Figure 4.10A and 4.10C). In 2004, the number of fecal pellets produced was considerably higher in spring than in summer (t_{116}, p<0.05; Figure 4.10A and 4.10B). This suggests that either food

availability was lower in summer, females were using a different food source, or both. The highest rate of pellet production was observed at the head of Barrow Canyon in spring (~ 10 fecal pellets f^{-1} d^{-1}; Figure 4.10B).

Gonad maturation, clutch size and egg production

Egg production experiments with *C. glacialis* were carried out in summer 2002, and spring and summer 2004 at different locations along the shelf-basin sections. Spawning occurred at all stations on the shelf, slope and basin in the Chukchi and Beaufort seas, with one important exception. In 2004, at a station over the 100 m isobath on the EHS line, only stage 1 and 2 females were collected and egg production was not observed. The observation of a summer population of immature females is surprising, but the lack of spawning is not since immature females do not reproduce. In our experiments, stage 3 and 4 females reproduced. This is inconsistent with previous work, which showed that only stage 4 females reproduce (Smith, 1990). For the purposes of this study, I defined mature females as females with gonad maturity states 3 and 4.

Mature females occurred at all stations where egg production was observed (Figure 4.11), with only one station in summer 2004 where mature females were absent. In summer 2004, I observed a second station with low percentages of mature females (17%, Figure 4.11C). Despite these low percentages, when stations re-occupied during both years were compared, no appreciable inter-annual difference was detected (t_{171}, p>0.05). While during both summers immature and spent females represented a significant fraction of the female population, few immature females were present in

spring (< 2%). In 2004, the number of mature females was significantly higher in spring when compared to summer (t_{116}, p<0.05).

Clutch size in *C. glacialis* ranged between 1 and 158 eggs clutch^{-1}, with > 75% of the observations between 25 and 75 eggs clutch^{-1}. The largest clutch was counted during spring 2004, at an outer shelf station in the vicinity of Barrow Canyon (Figure 4.12B). In summer 2002 and 2004 (Figure 4.12A and 4.12C), the largest clutch sizes were 98 eggs clutch^{-1} and 85 eggs clutch^{-1}, respectively. The high variability and small number of clutches restricted our ability to detect significant inter-annual and seasonal differences in the distribution of clutch size between re-occupied locations. However, in summer 2004, predominantly small clutch sizes were found at the BC and EB lines, which were under the influence of the warm Alaskan Coastal Water.

A comparison of the summer 2002 and 2004 egg production data reveals several similarities, but also some differences. During both summers, the highest rates of egg production were observed at the head of Barrow Canyon. In 2002 however, the production rate at that location was higher (62.5 eggs f^{-1} d^{-1}; Figure 4.13A) than in 2004 (13.3 eggs f^{-1} d^{-1}; Figure 4.13C). In summer 2002, egg production rates averaged 17.1 eggs f^{-1} d^{-1}, while in 2004 the mean was 11.4 eggs f^{-1} d^{-1}. The difference in the location of the ice edge and the stations occupied may complicate the comparison. In summer 2004, the shelf-basin lines were extended deeper into the Canada Basin and particularly low rates were observed at these basin stations along the EB and BC lines (Figure 4.13C). To correct for sampling bias, I removed all stations that were not re-occupied in 2004. A comparison between stations occupied both years indicated that there was no significant

inter-annual difference in egg production rates (t_{171}, p>0.05), despite the lower ice cover in summer 2004.

Our observations suggest that egg production in *C. glacialis* may be higher in spring than in summer. In spring 2004, 10 out of 14 sampling locations had egg production rates > 24 eggs f^{-1} d^{-1} (Figure 4.13B). In contrast, in summer 2002, 5 out of 14 stations showed egg production rates higher than 24 eggs f^{-1} d^{-1} (Figure 4.13A), and in summer 2004 only one station had higher rates (Figure 4.13C). As before, the differences in station location may complicate the comparison, but the overall trend indicates that higher rates of egg production occur in spring, when the ice edge is over the shelf.

Hatching success

Hatching success for *C. glacialis* nauplii was determined at most stations in spring and summer 2004 (Figure 4.14). A comparison of hatching success between seasons suggests that egg viability was higher in spring than summer 2004. Egg viability for both seasons ranged between 0 and 100 %. On average, in spring 72% of the eggs laid successfully hatched into nauplii while in summer only 45% of the eggs were viable. I observed two stations with remarkably low hatching success. In spring, the outer shelf station in the vicinity of Barrow Canyon with the highest rate of fecal pellet production (Figure 4.10B) had the lowest average hatching success for the entire experiment (1%, Figure 4.14A). In summer, the station over the 100 m isobath on the EB section showed the lowest egg viability for the season (16%, Figure 4.14B).

Spatial patterns were similar in spring and summer. A general shelf-to-basin positive gradient in hatching success was observed, particularly in summer (Figure 4.14B). In spring, the gradient was observed along the EHS line but not along the BC line

(Figure 4.14A), where elevated rates of primary production (Codispoti et al., 2005; Codispoti et al., 2007) and upwelling events (Münchow and Carmarck, 1997) at the canyon influence egg viability.

Shelf, slope and basin patterns

I observed several trends in the data on a shelf-to-basin gradient. Regional and temporal variations however complicated the analysis of such patterns. To study variations from the shelf into the basin, I divided the stations into three bathymetric regions: the shelf (<200 m), slope (200-2000 m) and basin (>2000 m) regions. This approach ignores variability not due to water column depth, yet it permits a broad analysis of the shelf-to-basin gradients of *C. glacialis* growth and reproduction, and has been successfully used to study variations in primary production and mesozooplankton grazing in the Chukchi and Beaufort seas (Kirchman et al., 2007; Campbell et al., 2007).

Physiological processes showed different responses to the distinct hydrographic conditions over the three bathymetric regions. Feeding activity decreased with the drop in Chl *a* concentrations over the slope and basin regions, and in summer 2004 hatching success was higher offshore (Table 4.1). Plourde et al. (2005) reported that egg production rates in *C. glacialis* decrease along the same shelf-to-basin sections. However, in our experiments summer egg production rates were slightly lower in the shelf region, but not significantly different than rates in the slope region (Table 4.1). Hatching success was higher over the basin (Table 4.1). The difference between Plourde et al. (2005) and our observations arises from the classification of stations into oceanographic regions. Plourde et al. (2005) grouped stations on the basis of environmental or water-mass characteristics rather than topography. In their study, stations were classified based on 0–

40 m physical and chemical water mass properties using mean values of physical and chemical data to determine four distinct hydrographic regions. As a result, only two stations clustered into their Region 1 (inner shelf) category in summer 2002. The stations correspond to two sampling locations in Barrow Canyon. Barrow Canyon is a site of elevated rates of primary productivity and biomass (Codispoti et al., 2005; Codispoti et al., 2007), which would explain why Plourde et al. (2005) reported high egg production rates at the inner shelf. Our results also show high feeding and egg production rates at the mouth of Barrow Canyon. Different circulation processes at the canyon separate this biological "hot spot" from other shelf areas and merits special attention.

DISCUSSION

Integrating molecular markers and ecology

Although both *C. glacialis* and *C. marshallae* may occur in the Chukchi and Beaufort seas, the molecular data indicate that only *C. glacialis* was present in significant numbers. This is not surprising given the proximity of the Arctic Ocean and the remoteness of the Pacific Ocean, where *C. glacialis* and *C. marshallae* are endemic, respectively. In the case of *C. marshallae*, the flow of Pacific-origin water to the Chukchi and Beaufort seas provides a mechanism whereby the species may be transported into the area. When present, *C. marshallae* has historically occurred in low numbers (Johnson, 1958; English and Horner, 1977). Furthermore, molecular identification of copepod nauplii collected in the region found no *C. marshallae* nauplii (Chapter 3), suggesting that the species was not reproducing over the Chukchi and Beaufort outer shelves nor in the Canada Basin.

I used molecular identification to validate our experiments because previous ecological studies with *C. glacialis* in the western Arctic were compromised by the difficulty in morphologically discriminating between *C. glacialis* and *C. marshallae.* Our results suggest that past studies did not involve a species-complex (Plourde et al., 2005), but were instead carried out with *C. glacialis*. This validation of our and previous results has important implications for understanding the reproductive biology of *C. glacialis*. Our results show that egg production rates were subject to strong spatial and seasonal variability, which may be related to ice cover, feeding conditions, and changes in circulation processes. In contrast, secondary production by the species was similar during both summer in 2002 and 2004.

The effects of physical and biological processes on egg production

Egg production rates of *C. glacialis* at some stations over the shelf and over Barrow Canyon were among the highest measured for this species. Rates at these locations were comparable to those reported for the Northeast Water Polynya (Ashjian et al., 1995). These high rates of egg production were the result of the advection of nutrient-rich Pacific water, upwelling of slope waters, and the subsequent increase in primary production. The upwelling of deep Arctic water is particularly important at Barrow Canyon where wind forcing and canyon dynamics generate frequent reversals of flow (Mountain et al., 1976; Münchow and Carmarck, 1997; Weingartner et al., 1998), which transport nutrient-rich waters to the surface and carry *C. glacialis* onto the slope and shelf regions. These highly productive areas contrast with surrounding regions, such as the Canada Basin and the Beaufort Sea, where the levels of chlorophyll were low. Previous studies demonstrated that egg production by *C. glacialis* is dependent on food supply

during summer (Hirche, 1989; Tourangeau and Runge, 1991). I observed that feeding activity and egg production rates decreased with the drop in Chl *a* concentrations over the slope and basin regions.

Seasonal patterns in egg production

Although no direct measurements of feeding activity were made, our results support the hypothesis that in spring *C. glacialis* were feeding under the ice before the onset of the phytoplankton bloom. I did not examine gut contents and thus cannot rule out the possibility that these copepods were also feeding on phytoplankton present at low concentrations in the water column and on microzooplankton. However, our data are consistent with observations that *C. glacialis* readily consumes ice algae in spring in the Chukchi and Beaufort seas (Campbell et al., 2007). During spring sampling, ice coverage was > 90% and the springtime nutrient depletion in some of the surface waters may be largely due to consumption by the early ice algae bloom (Codispoti et al., 2007). Under-ice grazing by this calanoid copepod has been reported for other Arctic regions such as Hudson Bay (Runge and Ingram, 1988; Runge and Ingram, 1991) and confirmed by the presence of ice algal species in the guts of *C. glacialis* (Runge and Ingram, 1991). Also, egg production for *C. glacialis* supported by ice algae has been reported for the Hudson Bay (Tourangeau and Runge, 1991) and the Barents Sea (Hirche and Kosobokova; 2003).

I have considered an alternative explanation for our observations of spawning under ice during spring 2004. It is possible that food-independent reproduction, fuelled by internal energy resources, occurred at some locations since during our study *C. glacialis* reproduced under thick ice where chlorophyll levels were low. It has been shown that *C. glacialis* uses stored lipids to lay eggs at low ambient food concentrations

(Smith, 1990; Hirche and Kattner, 1993). In spring, food availability was sufficient for egg production and elevated chlorophyll concentrations indicated that algae biomass was high, particularly at the shelf and shelfbreak regions. Our spring observations however did not extend into the basin, where ambient food supplies are generally low and lipids may be partly responsible for egg production (Kosobokova and Hirche, 2001). Lipid content may play an important role in egg production over the shelves prior to the spring bloom and over the basin at low ambient food concentrations. In late spring and throughout summer in the Greenland Sea, lipids are exhausted and egg production is closely related to feeding (Hirche, 1989).

Information on ice algae, phytoplankton and microzooplankton community composition in the Chukchi and Beaufort seas is limited, but recent studies indicate that different assemblages occur in spring and summer (Sherr et al., 2003; Hill et al., 2005). In spring, the phytoplankton population is characterized by a mixture of prasinophytes, chrysophytes, diatoms, haptophytes and dinoflagellates (Hill et al., 2005). After ice retreat in the summer, the major contributors to the shelf community are diatoms that are the principal group in high-chlorophyll, bloom populations (Booth and Horner, 1997; Hill et al., 2005). The distribution of microzooplankton in Arctic water indicates that nano- and microplankton size classes are evenly abundant during winter and nanoplanktonic heterotrophic protists dominate during summer (Sherr et al., 2003). From these observations, it can be inferred that the food sources for larger zooplankton such as copepods are seasonally variable. Our results do not directly suggest that egg production or hatching success of *C. glacialis* were reduced in summer by distinct feeding conditions. The high egg production rates observed in spring could result from females

initiating gonad maturation before the onset of the ice algae bloom. The lower rates in summer could be due to aging individuals and exhausted reproductive apparatus. However, under the current trends of declining ice cover (McPhee et al., 1998; Serreze et al., 2003; Stroeve et al., 2005) and rapid ice retreat (Cooper et al., 2006), I can expect extended summer conditions to prevail in the future (Comiso and Parkinson, 2004). As a result, the relatively high rates of egg production and elevated egg viability observed in spring will be replaced by the moderate rates of egg production and low hatching success found in summer.

Ecological responses to warming

Average temperatures in the Arctic are predicted to increase by 4-7°C over the next century (Corell, 2006), and most studies indicate that rising temperatures will have negative impacts on Arctic species (ACIA, 2005). Arctic species are specially adapted to cold temperatures that serve as an effective barrier to colonization by sub-polar species. Global warming is reducing this barrier, bringing exogenous species into the Arctic ecosystem and stressing native species. In this changing environment, the behavioral and physiological adaptations of the polar and sub-polar species could determine future ecosystem responses.

Warming of the Arctic Ocean occurs mainly in the upper ocean owing to changes in net surface heat flux with the atmosphere (Barnett et al., 2005; Levitus et al., 2005) and increased inflow of warm Pacific and Atlantic water (Woodgate and Aagaard, 2005; Zhang et al., 1998). In the eastern Arctic, Atlantic water could bring more *C. finmarchicus* into the southern part of the Barents Sea while, in the western Arctic, Pacific water could transport more *C. marshallae* into the southern region of the Chukchi

Sea. Previous studies have shown that *C. finmarchicus* is unable to persist under arctic conditions (Grainger, 1961; Tande et al., 1985). Stage five copepodites of this species in Arctic waters do not undergo sexual differentiation nor molt into adults, and females do not reproduce successfully (Tande et al., 1985). In the North Atlantic, water temperatures of ~ 5°C are a regular feature for the growth of *C. finmarchicus*, and growth and development rates are significantly slowed in near freezing water (Campbell et al., 2001). The cold water may hinder reproduction of *C. finmarchicus* since in the Arctic temperature during most of the growth season is < 0°C (Tande et al., 1985). I speculate that physiological processes in *C. marshallae* may be hindered by cold temperatures in a similar way. In the Bering Sea, water temperature is ~ 3°C during the *C. marshallae* spawning season (Smith and Vidal, 1986), and in the Oregon upwelling zone where *C. marshallae* thrives temperatures range from 8 to 10°C (Peterson, 1979). These observations suggest that *C. marshallae*, like *C. finmarchicus*, may be an expatriate in the cold Arctic waters. A slight increase in surface temperatures of 2 to 3°C may benefit these sub-polar species, but could negatively affect species with behavioral and physiological adaptations to colder habitats.

In July and August 2004, I observed a stronger inflow of warm (7°C) Alaskan Coastal Water and an increase in primary production relative to 2002 (Codispoti et al., 2007; Kirchman et al., 2007). Our results indicate that the increase in primary production did not result in an increase in secondary production (egg production rates). The lack of response may reflect negative thermal effects on *C. glacialis*, as I did see high percentages of immature females at stations with abnormally warm surface temperatures. Temperature has been shown to stop spawning of *C. glacialis*, with no egg production

when the seawater temperature at the surface (1 m) reaches 9.5°C (Kosobokova, 1993). In the White Sea, the increase in temperature in the surface layer appears to be the key factor causing the termination of the spawning period of *C. glacialis* (Kosobokova, 1998). When surface temperatures reached 5°C, females left the surface and stopped spawning. Similar behavioral responses were reported from western Norway (Niehoff and Hirche, 2005). However, it is not clear that higher temperatures in 2004 restricted secondary production. Experiments on grazing estimates for *C. glacialis* and other copepods found low grazing impacts on primary production (15% of the daily primary production) in the western Arctic (Campbell at al., 2007). Hence, primary production in the Chukchi and Beaufort seas exceeds the demands for egg production (secondary production) and a large fraction of the primary production is exported locally to the benthos or transported offshore (Campbell at al., 2007). This decoupling between primary and secondary production in the region could also explain why the increase in primary production in the Chukchi and Beaufort seas did not entail an increase in secondary production by pelagic copepods.

Our observations raise questions about the physiological responses of *C. glacialis* to climate warming. In the Greenland Sea, *C. glacialis* females reproduced only when they had reached reproductive state 4 (Smith, 1990). Our results indicate that spawning can occur at a younger state, since stage 3 females successfully reproduced in 2002 and 2004. Furthermore, eggs from state 3 and 4 females had similar hatching rates. Is *C. glacialis* reproducing at an earlier state due in part to climate change? There is insufficient evidence to answer this question but this environmental effect should be studied more closely.

CONCLUSIONS

Molecular analyses validate previous reproductive studies with *C. glacialis* in the western Arctic and confirm that the life cycle of *C. glacialis* is synchronized to the strong seasonality of the Arctic environment. There was no evidence that the closely related species *C. marshallae* was present in significant numbers or laying eggs in the region. Changes in the composition of ice algae and phytoplankton blooms can potentially impact the capacity of *C. glacialis* for reproduction and survival. At present, it is difficult to predict how the mesozooplankton community in the western Arctic will respond to climate change, but previous studies and the data herein suggest that significant warming will negatively impact egg production in *C. glacialis* and perhaps benefit the sub-polar species *C. marshallae*.

ACKNOWLEDGEMENTS

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Table 4.1 Physiological and reproductive characteristics of female copepods collected in the Chukchi and Beaufort seas in summer 2002 and spring and summer 2004.

	Bathymetric Region	Prosome length (mm ± SD, N)	No. eggs per female (eggs f^{-1} d^{-1} ± SD,	Clutch size (eggs $clutch^{-1}$ ± SD,	Gonad stage (stage ± SD, N)	No. of fecal pellets (pellets f^{-1} d^{-1} ± SD,	Hatching success (% ± SD, N)
Summer, 2002	Shelf	3.51 ± 0.28, 69	16 ± 25, 69	50 ± 17, 22	2.7 ± 1.1, 69	1 ± 1, 69	n/a
	Slope	3.57 ± 0.24, 71	18 ± 30, 71	44 ± 30, 29	2.9 ± 0.9, 71	1 ± 1, 71	n/a
	Basin	3.80 ± 0.21, 3	23 ± 39, 3	68 ± n/a, 1	4.0 ± 0.0, 3	2 ± 1, 3	n/a
Spring, 2004	Shelf	3.65 ± 0.26, 95	27 ± 42, 107	67 ± 41, 43	3.7 ± 0.5, 93	4 ± 4, 107	77 ± 27, 27
	Slope	3.82 ± 0.26, 60	27 ± 42, 60	44 ± 46, 37	3.7 ± 0.5, 48	5 ± 5, 60	63 ± 43, 19
	Basin	3.99 ± 0.22, 12	2 ± 8, 12	29 ± n/a, 1	3.3 ± 0.6, 12	2 ± 1, 12	92 ± n/a, 1
Summer, 2004	Shelf	3.59 ± 0.30, 57	13 ± 22, 57	34 ± 23, 22	3.0 ± 1.0, 55	1 ± 1, 57	29 ± 39, 20
	Slope	3.82 ± 0.27, 60	16 ± 24, 54	47 ± 18, 18	3.6 ± 0.5, 54	4 ± 5, 60	58 ± 43, 17
	Basin	3.81 ± 0.21, 52	5 ± 13, 52	32 ± 15, 8	2.7 ± 1.0, 52	1 ± 1, 52	57 ± 43, 7

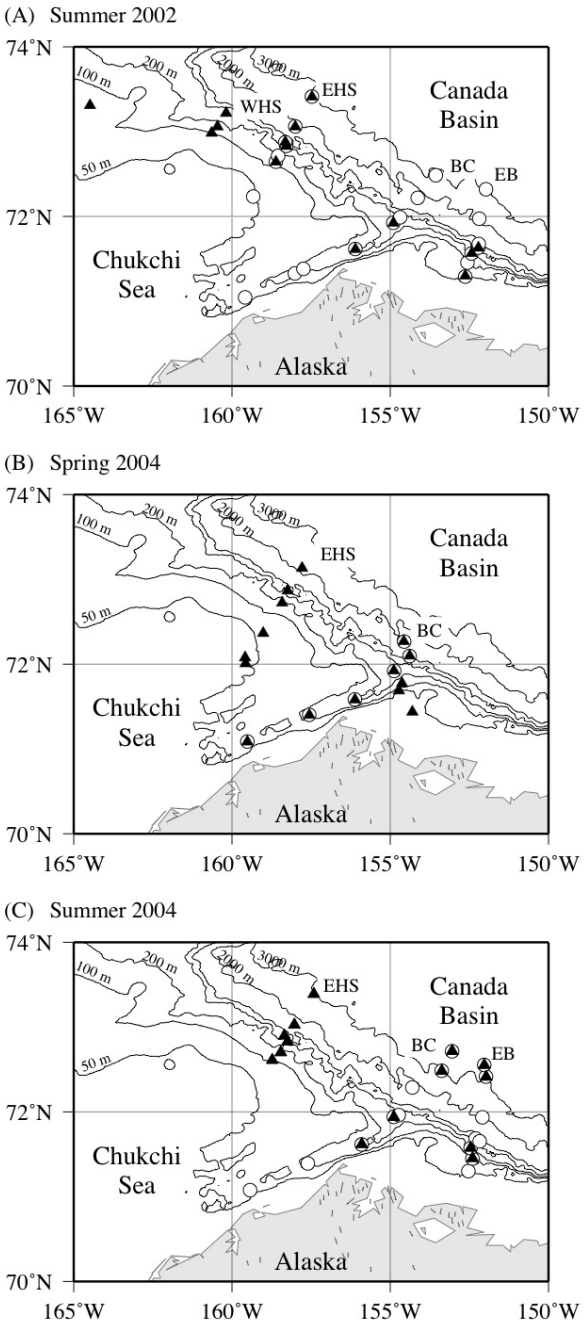


Figure 4.1 View of the study area in the Chukchi and Beaufort seas, showing the hydrographic and egg production stations occupied by the USCGC *Healy* in (A) summer 2002, (B) spring 2004, and (C) summer 2004. Sections are denoted (in chronologic order of sampling) by region as Barrow Canyon (BC); East Barrow (EB); East Hanna Shoal (EHS); and West Hanna Shoal (WHS). The CTD stations used in the vertical sections are plotted as large white circles. The stations where egg production experiments were carried are plotted as black triangles.

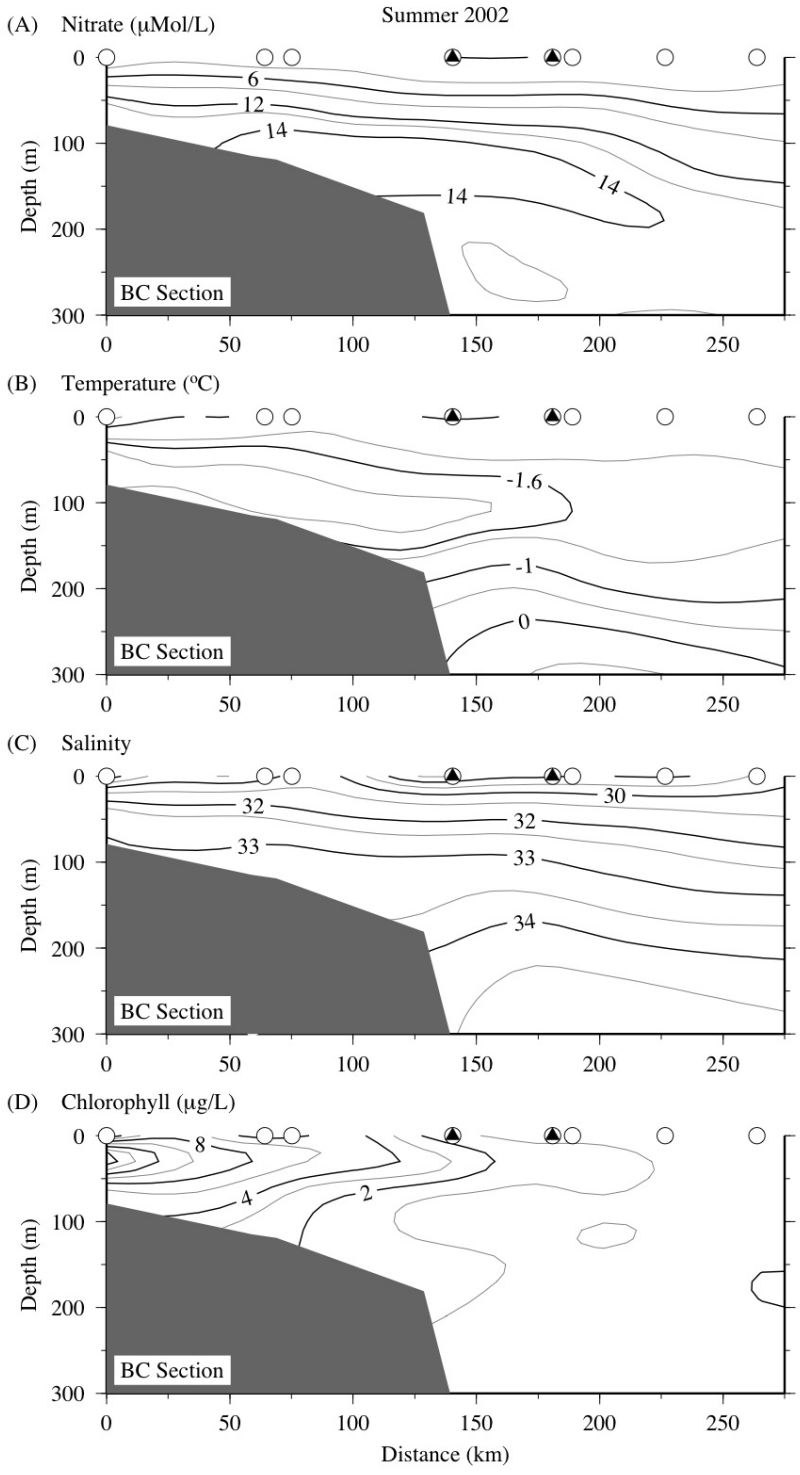


Figure 4.2 Vertical sections along the summer 2002 BC transect (see Figure 1 for location). (A) Nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

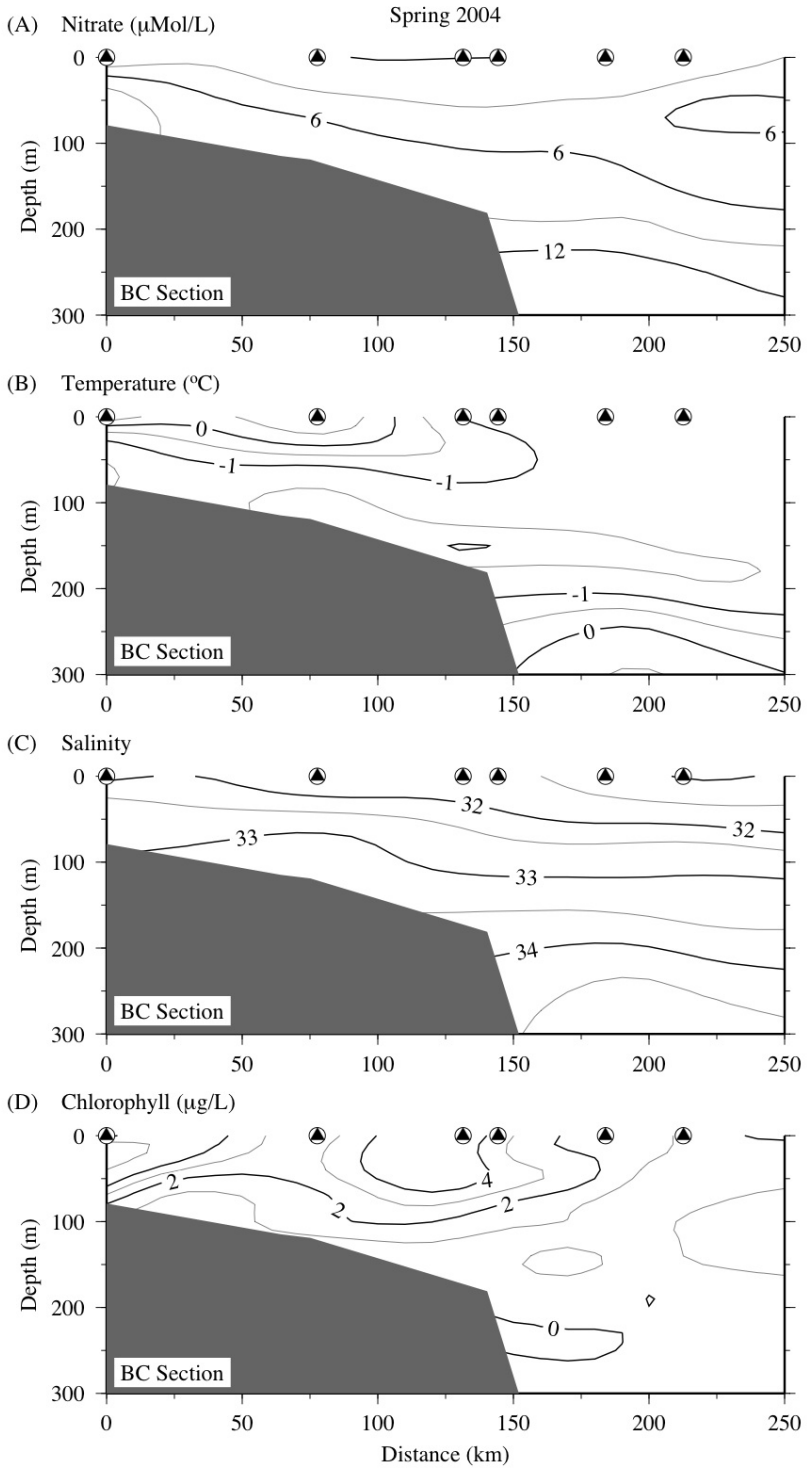


Figure 4.3 Vertical sections along the spring 2004 BC transect (see Figure 1 for location). (A) nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

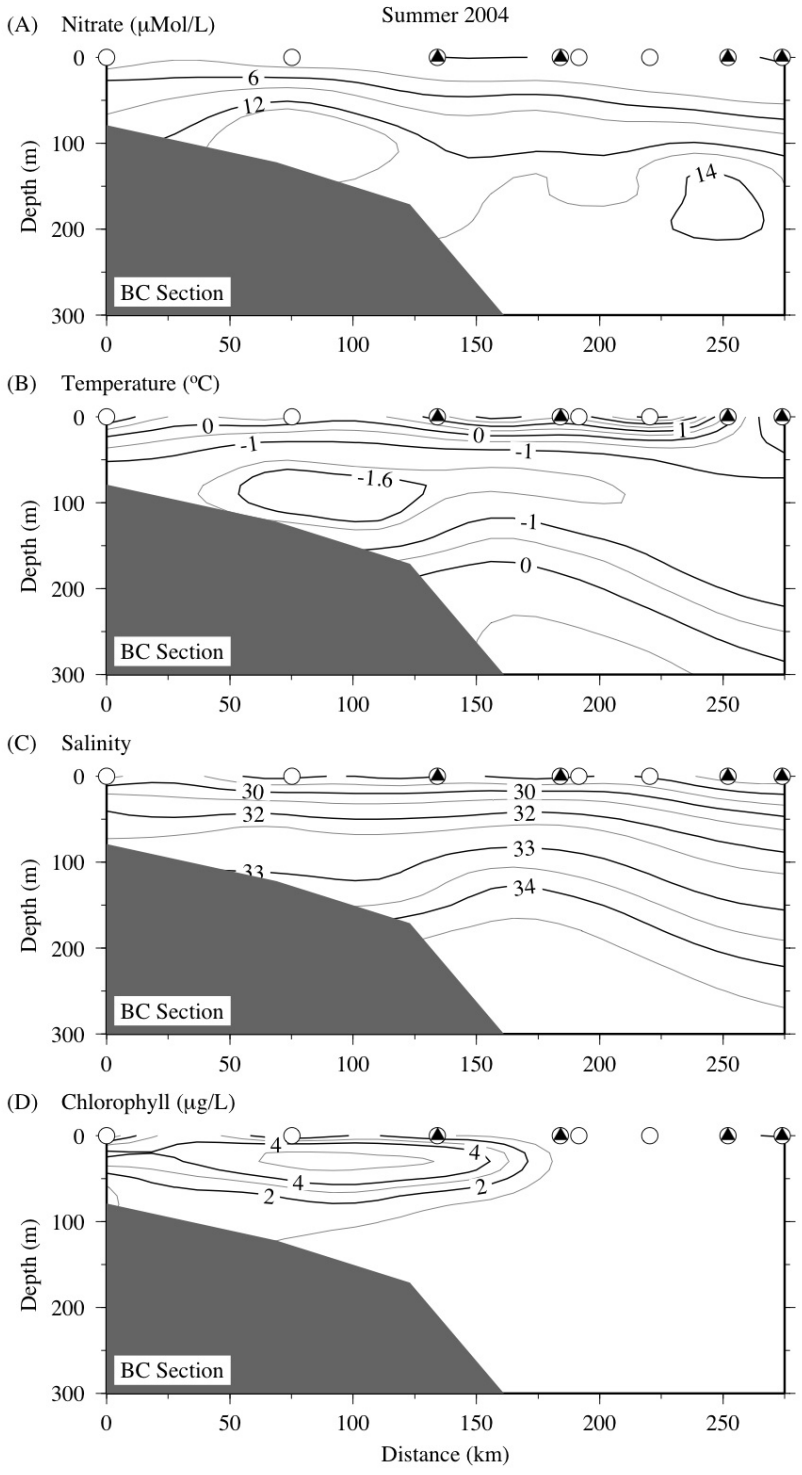


Figure 4.4 Vertical sections along the summer 2004 BC transect (see Figure 1 for location). (A) nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

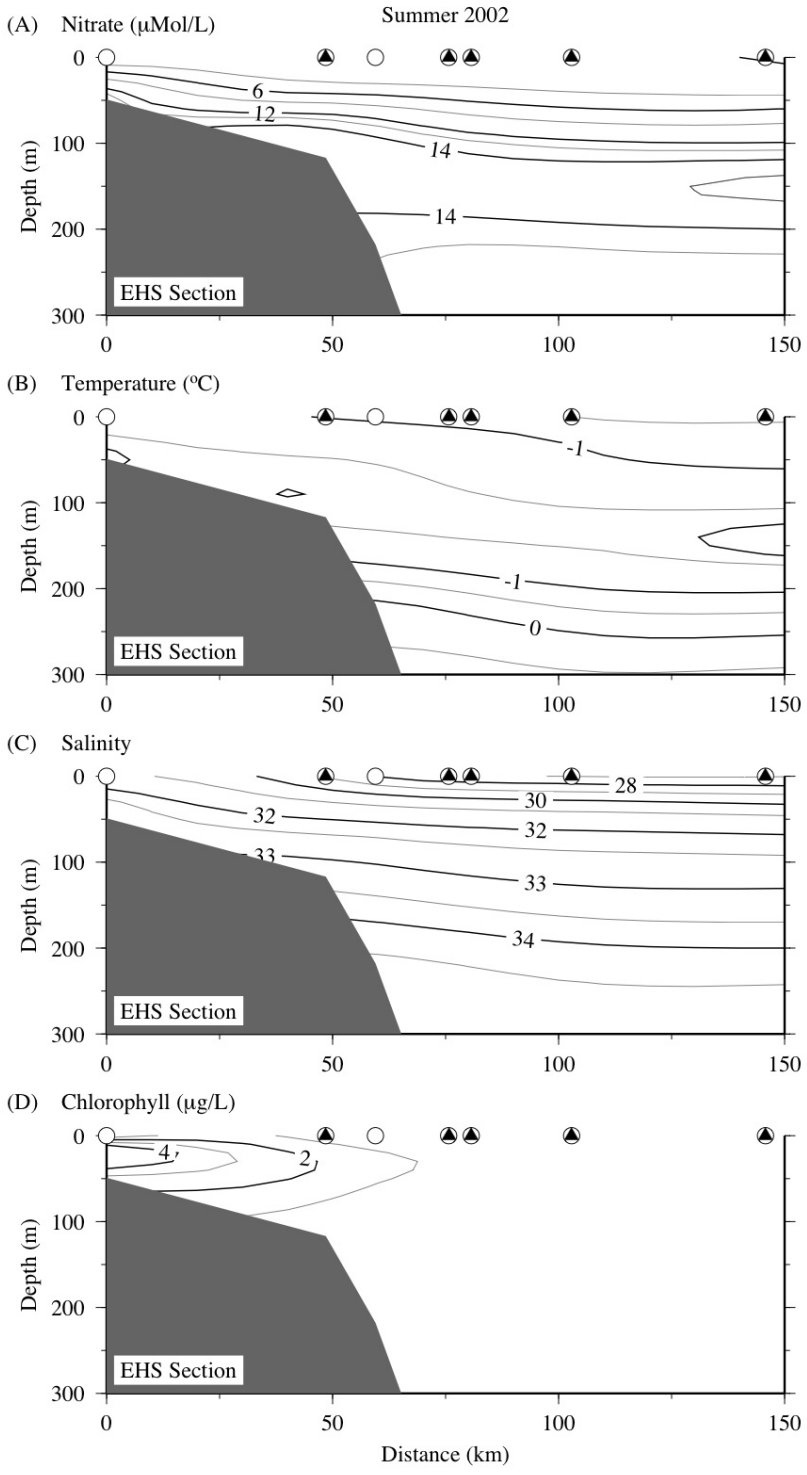


Figure 4.5 Vertical sections along the summer 2002 EHS transect (see Figure 1 for location). (A) nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

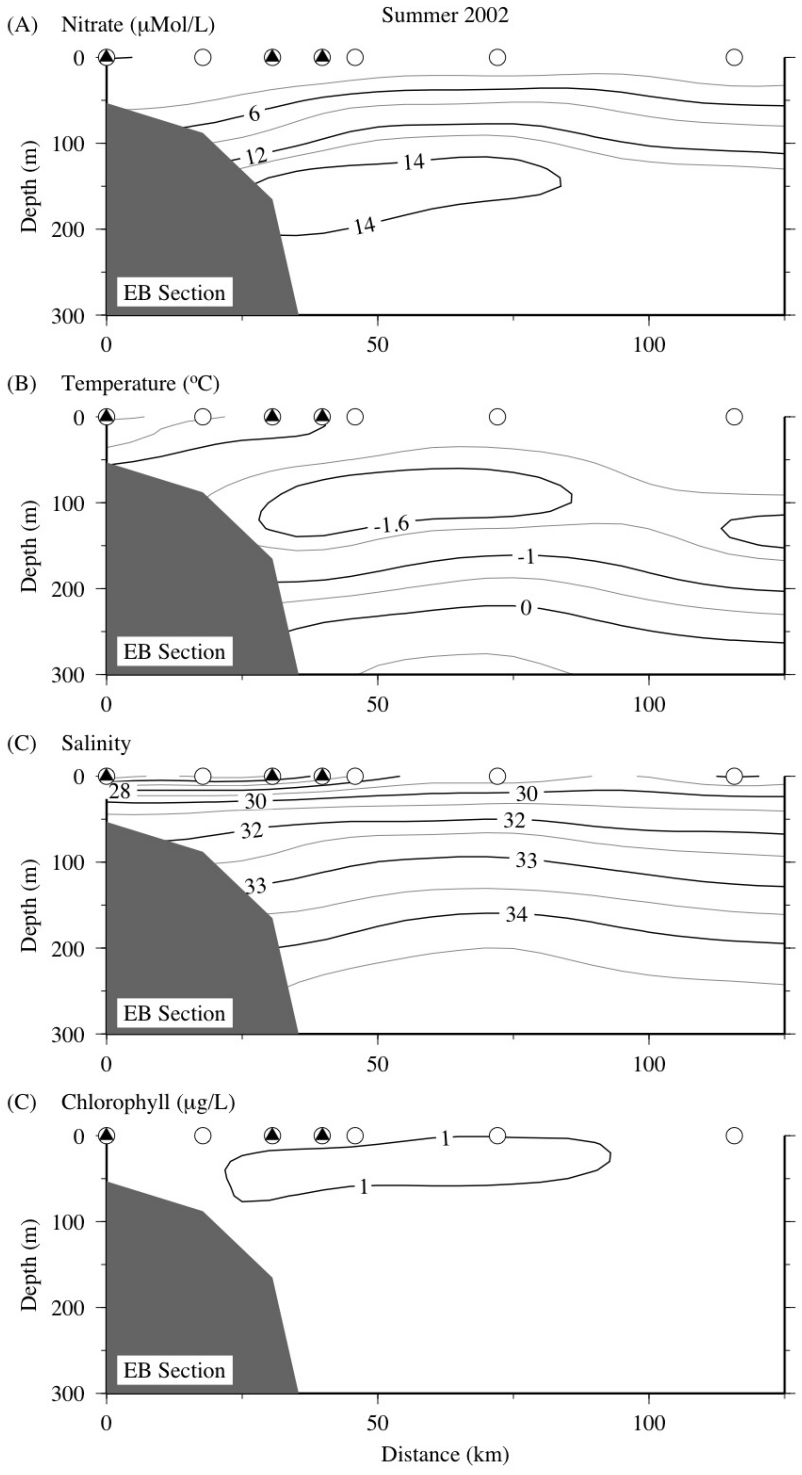


Figure 4.6 Vertical sections along the summer 2002 EB transect (see Figure 1 for location). (A) nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

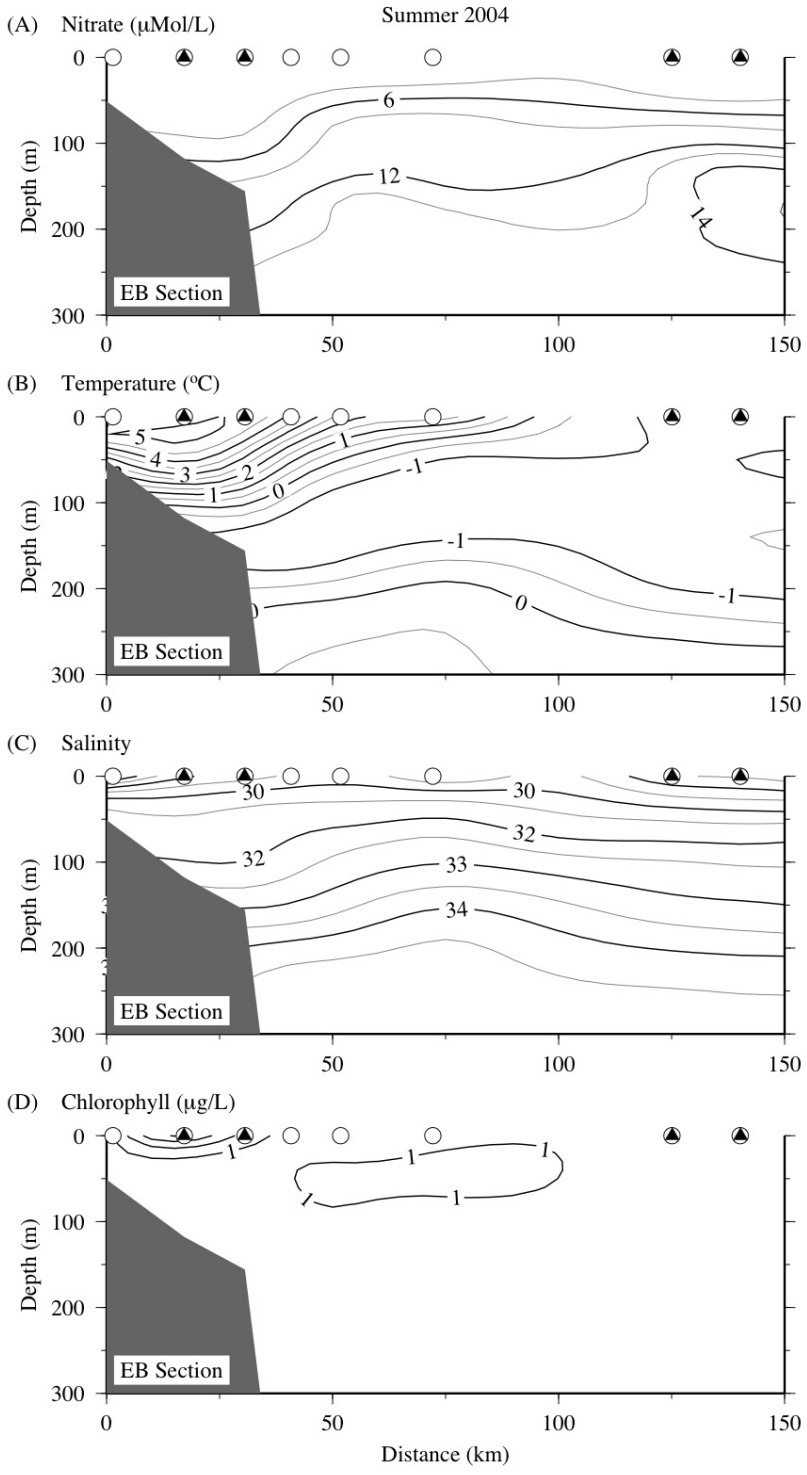


Figure 4.7 Vertical sections along the summer 2004 EB transect (see Figure 1 for location). (A) nitrate (µMol/L), (B) temperature (°C), (C) salinity, and (D) chlorophyll (µg/L). The hydrographic stations are plotted as large white circles, stations where egg production experiments were carried plotted black triangles.

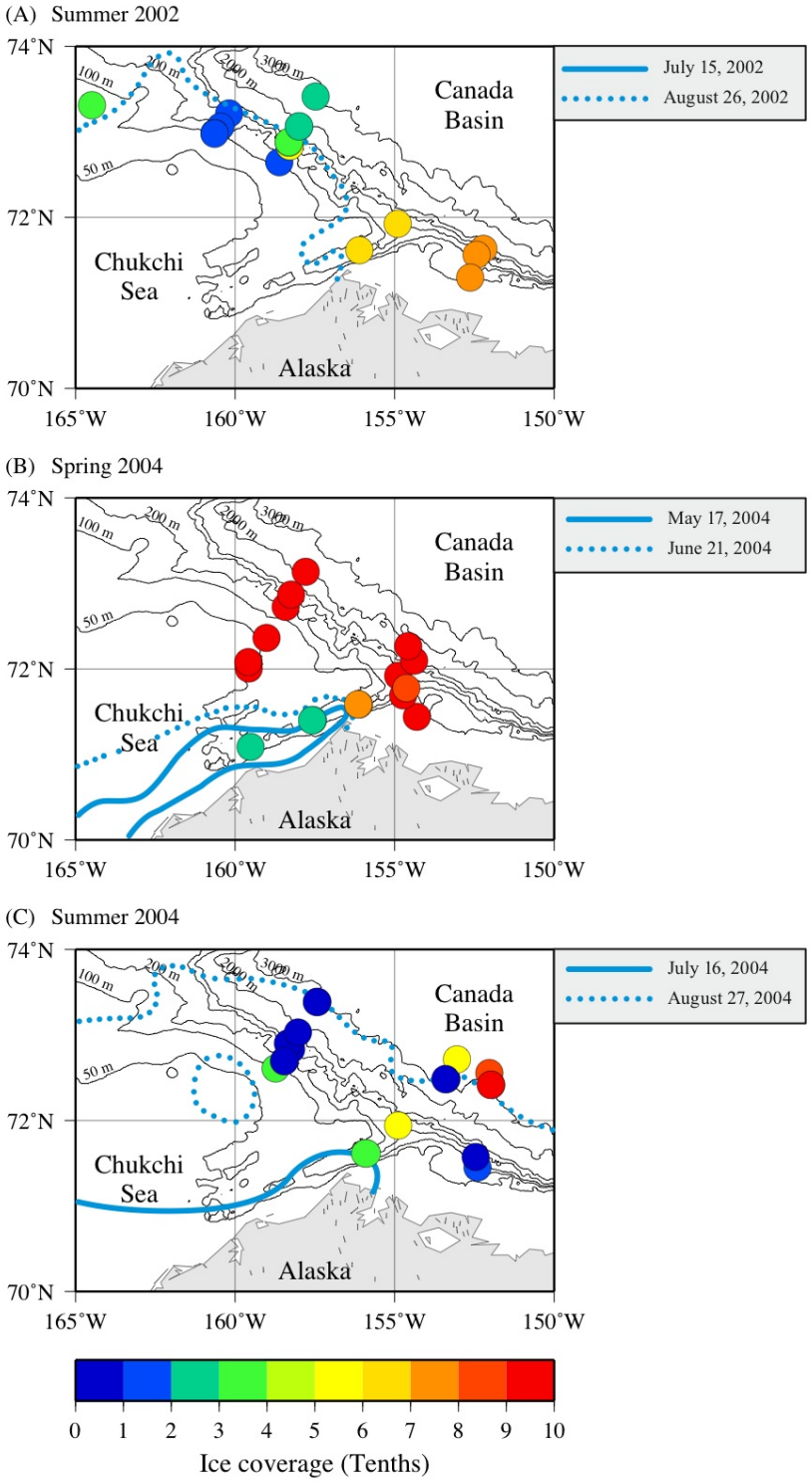


Figure 4.8 Ice cover at stations where egg production experiments were carried in (A) summer 2002, (B) spring 2004, and (C) summer 2004. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

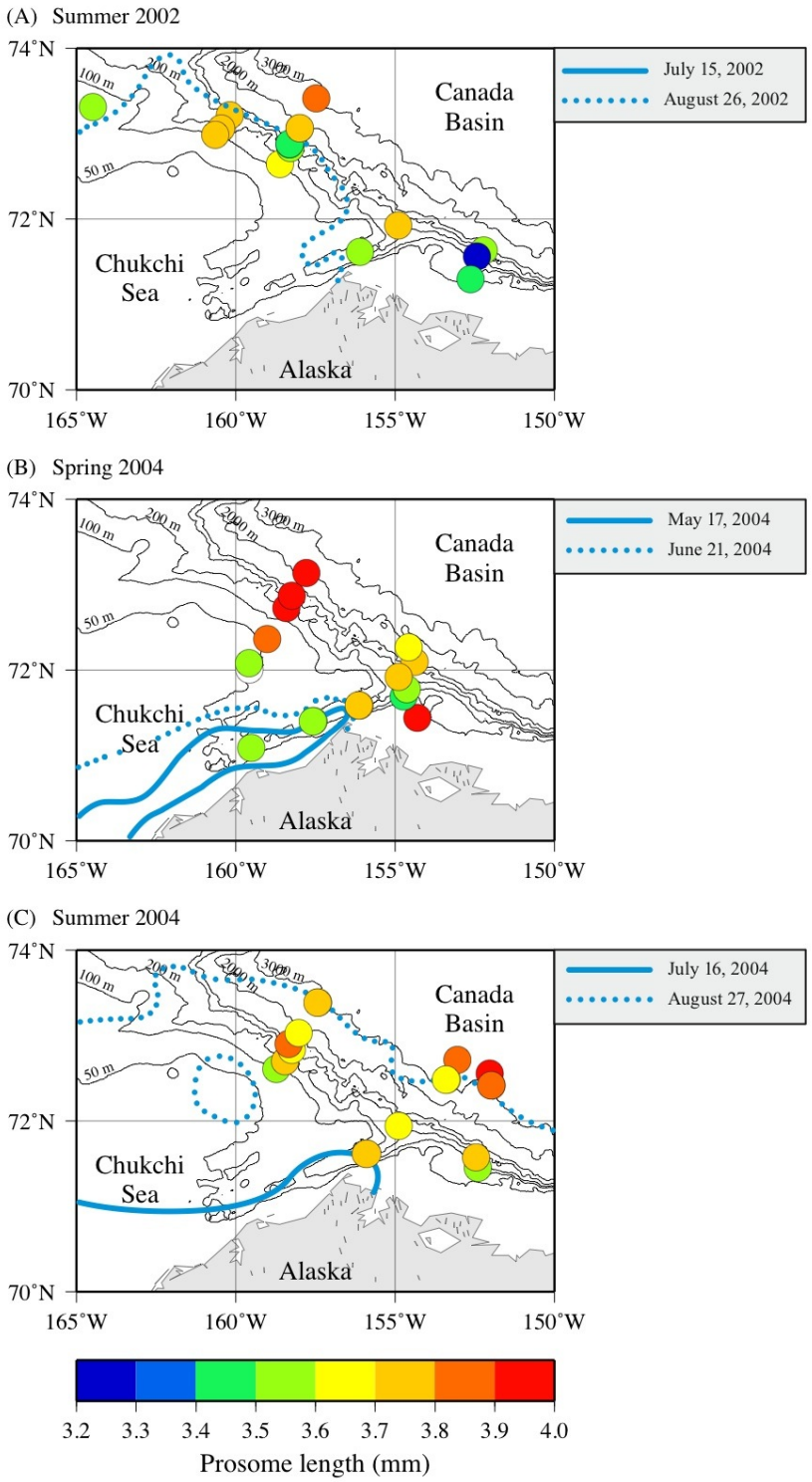


Figure 4.9 Patterns in prosome length distribution for *Calanus glacialis* adult females during (A) summer 2002, (B) spring 2004, and (C) summer 2004. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

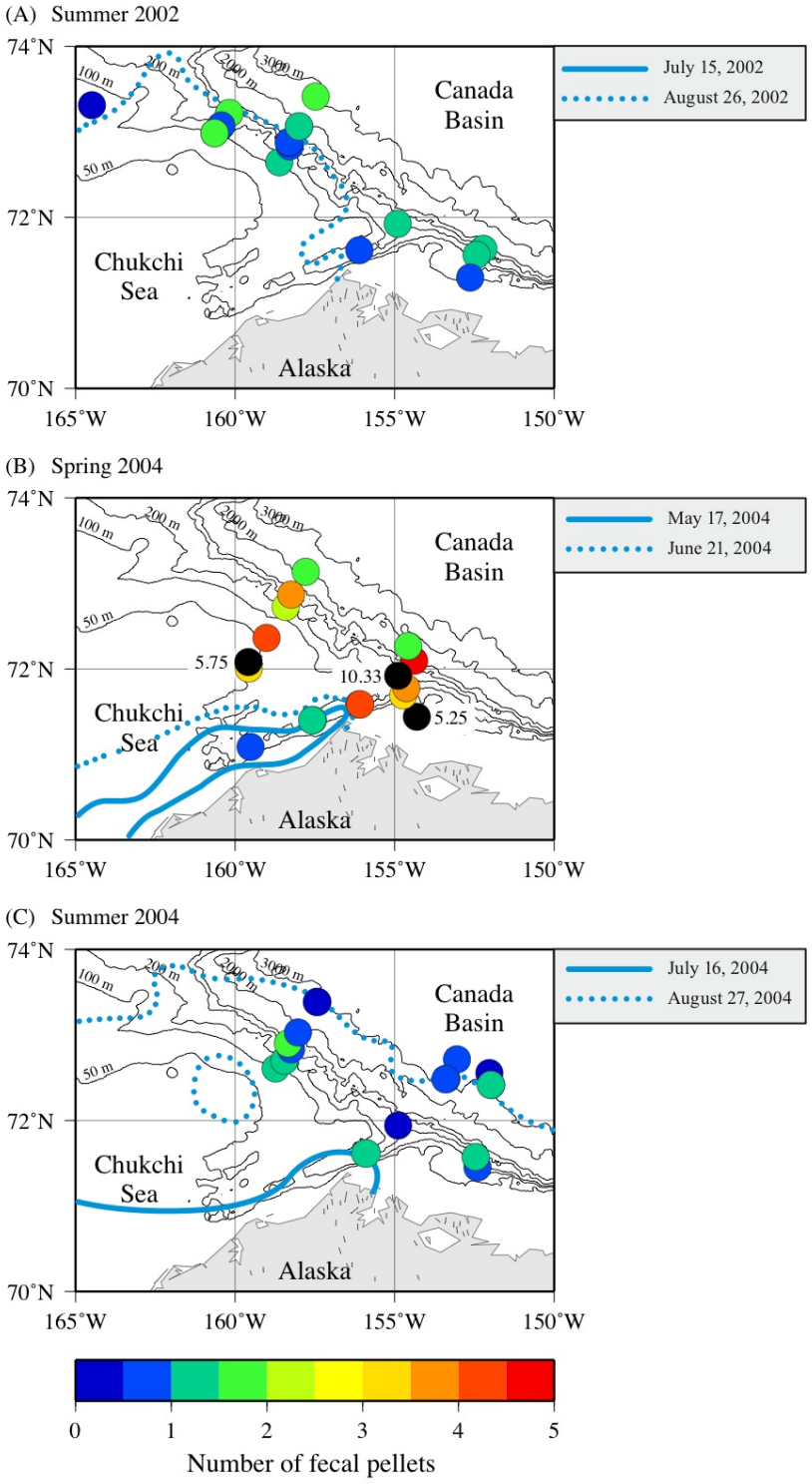


Figure 4.10 Patterns in fecal pellet production rates for *Calanus glacialis* adult females during (A) summer 2002, (B) spring 2004, and (C) summer 2004. High production rates are plotted in black. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

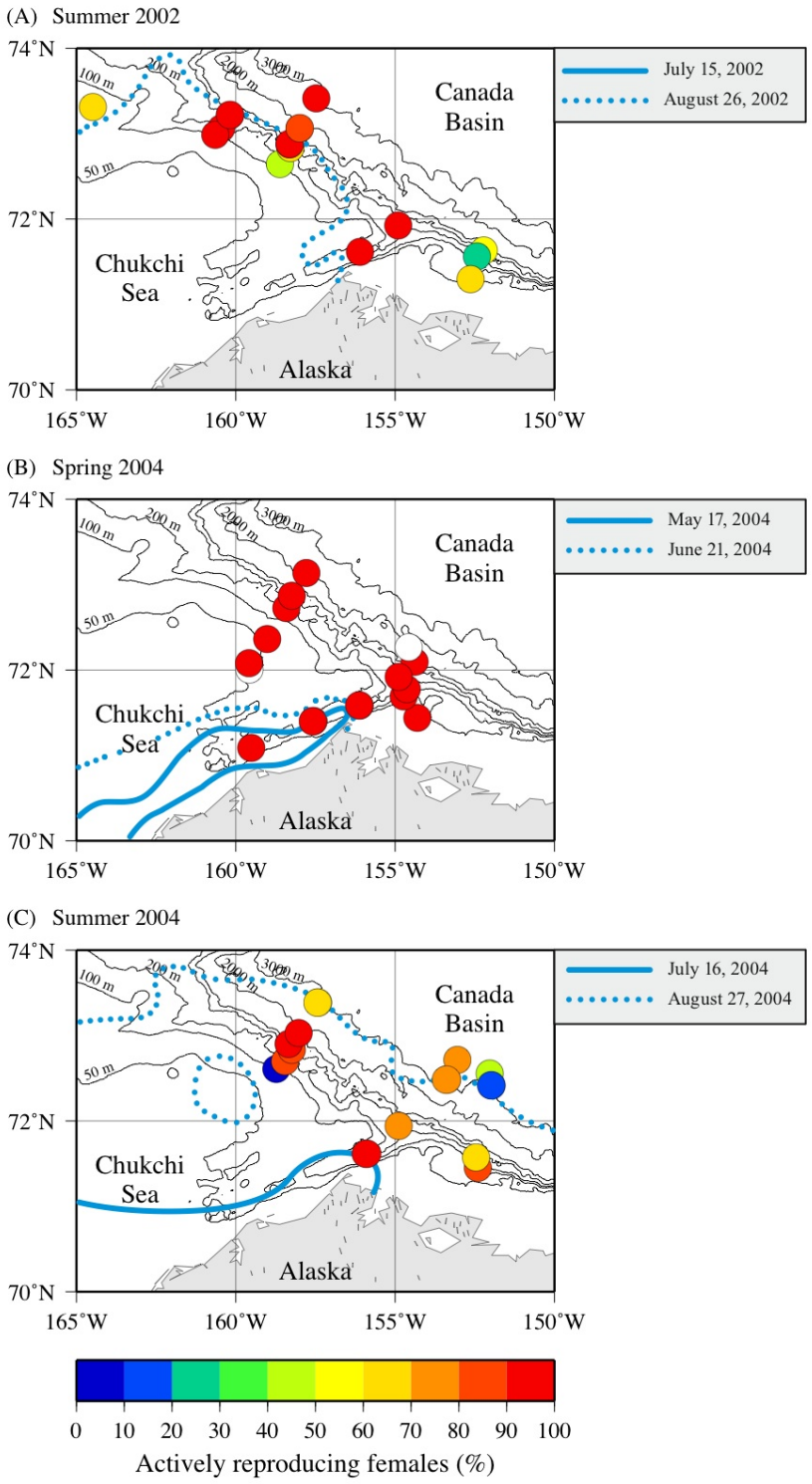


Figure 4.11 Percentage of actively reproducing *Calanus glacialis* females during (A) summer 2002, (B) spring 2004, and (C) summer 2004. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

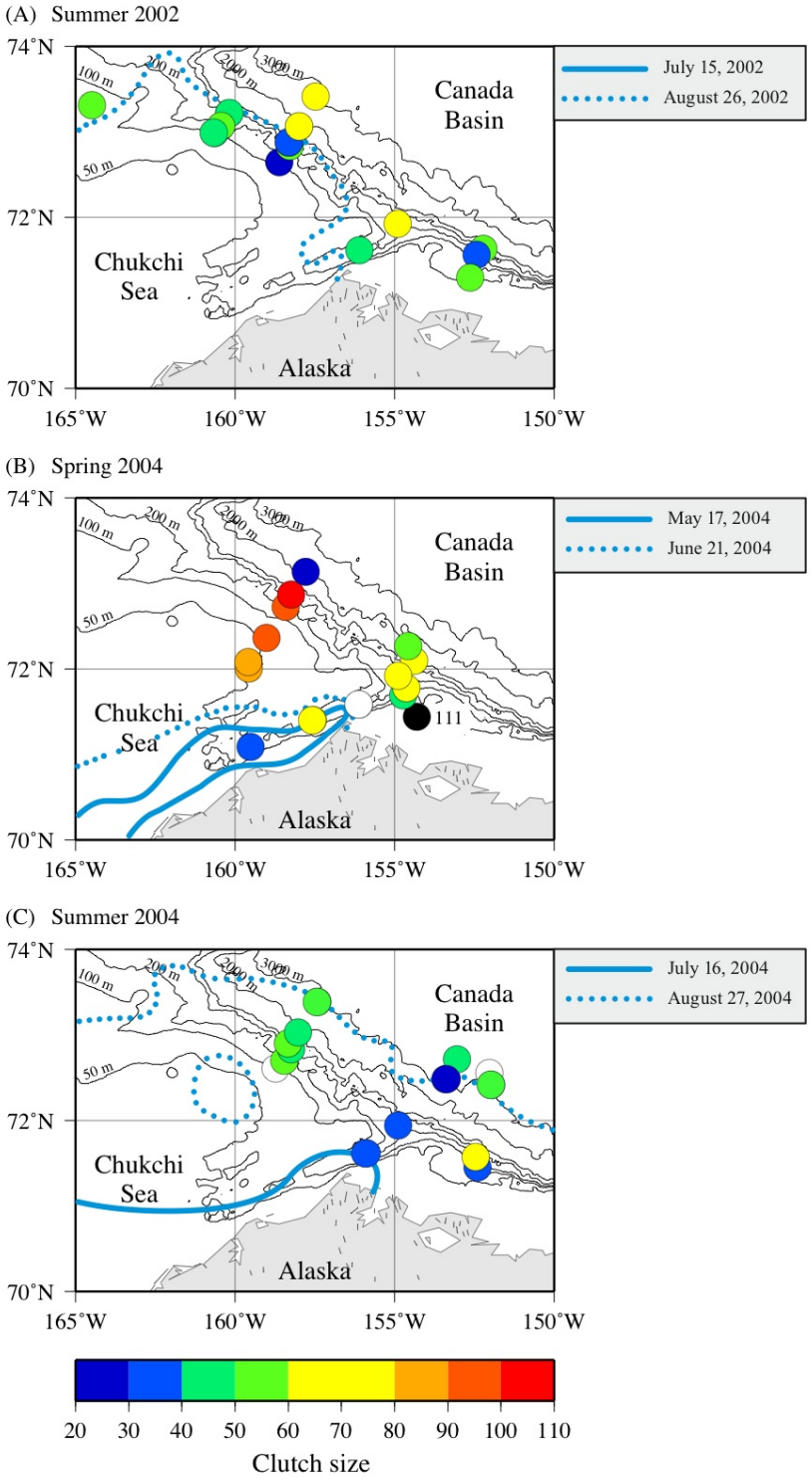


Figure 4.12 Patterns in clutch size for *Calanus glacialis* adult females during (A) summer 2002, (B) spring 2004, and (C) summer 2004. Large clutches are plotted in black, no clutch data are plotted in white. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

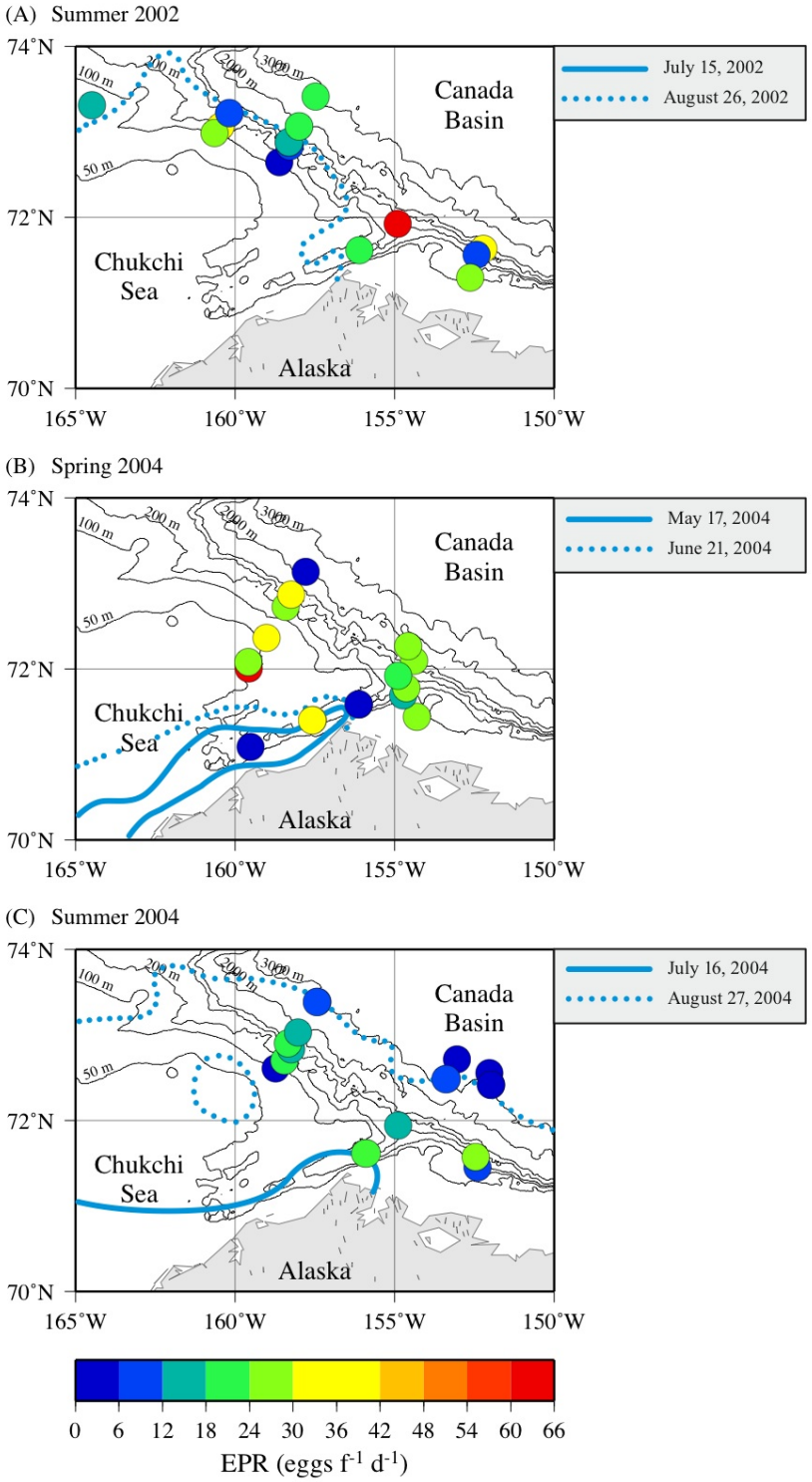


Figure 4.13 Patterns in egg production rates for *Calanus glacialis* adult females during (A) summer 2002, (B) spring 2004, and (C) summer 2004. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

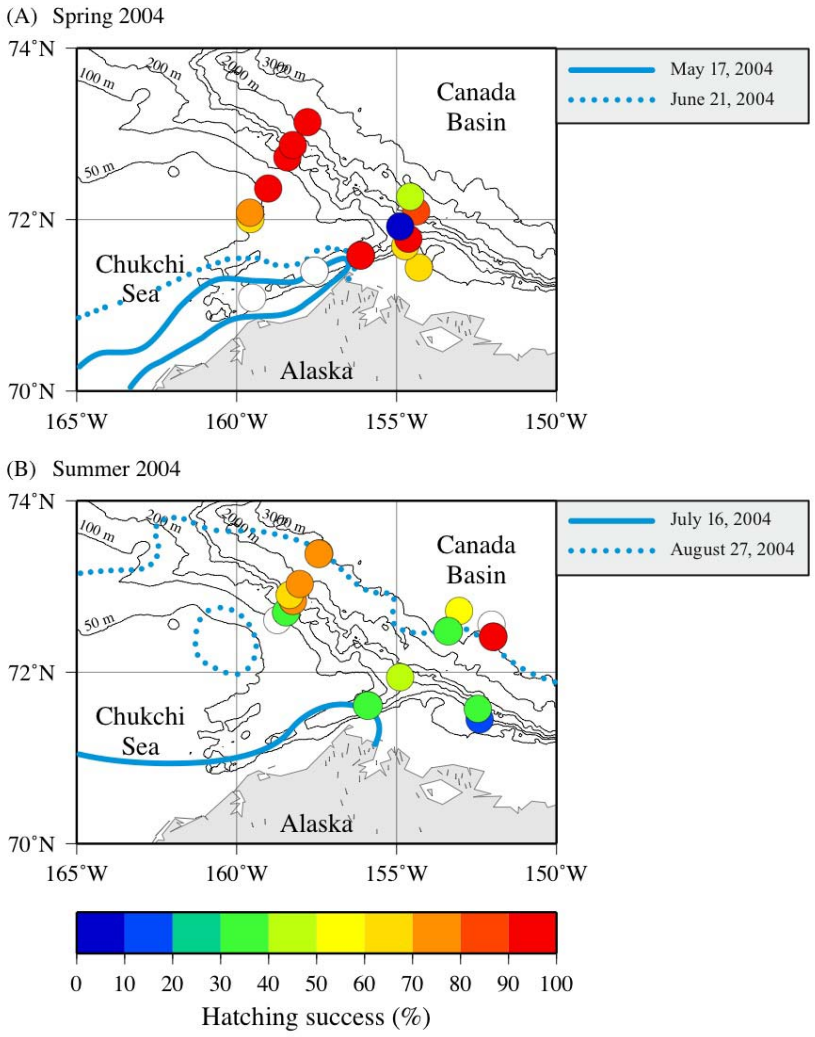


Figure 4.14 Pattern in egg viability for *Calanus glacialis* adult females during (A) spring 2004 and (B) summer 2004. No hatching success data are plotted in white. A solid blue line indicates the approximated location of the ice edge at the beginning of each cruise and a dotted blue represents the ice edge location at the end of the cruise (adapted from www.natice.noaa.gov).

CHAPTER 5: THE EFFECTS OF EDDY TRANSPORT ON ZOOPLANKTON BIOMASS AND COMMUNITY COMPOSITION IN THE WESTERN ARCTIC

INTRODUCTION

The extensive continental shelves surrounding the Arctic Ocean occupy one third of its area, connect it to subarctic regions, and can impact the deeper regions of the basin. The Chukchi shelf, for example, links the Pacific and the Arctic oceans. Waters flowing through the Chukchi Sea are significantly modified during their transit, via atmospheric forcing and interaction with the sediments, and play a major role in the stratification and circulation of the Arctic Ocean. The Chukchi Sea is also a region of intensive biological productivity, which provides nutrients and biota important to the Arctic ecosystem (Ashjian et al., 2005; Codispoti et al., 2005). Despite the key role of shelf-basin interactions, the mechanisms and rates of the water mass exchanges across the shelf and slope remain poorly understood. Possible mechanisms of off-shelf transport include wind-forced upwelling and downwelling (e.g. Melling, 1993), outflow through canyons (e.g. Garrison and Becker, 1976), and instability of boundary currents along the shelfedge, leading to eddy formation (e.g. Manley and Hunkins, 1985; Pickart et al., 2005). In this paper, I investigate the role that such boundary current eddies might play in the transport of zooplankton into the Arctic Basin, and the effects that this process can have on the shelf and basin ecosystems and their respective food webs.

Hydrography of the Chukchi and Beaufort seas and Canada Basin

The productivity of the pelagic ecosystem of the western Arctic is regulated by 1) physical forcing mechanisms and hydrographic characteristics of the waters transiting through Bering Strait and over the Chukchi and Beaufort shelves to the Arctic Ocean (Springer et al., 1987; Grebmeier and Harvey, 2005); 2) shelf-basin exchange processes between the Chukchi and Beaufort seas and the adjacent Canada Basin (Ashjian et al., 2005; Kadko and Muench, 2005); and 3) changes in sea ice including ice extent and thickness, timing of ice melt/thaw cycles, and location of the ice edge (Hansen et al., 2003; Eicken et al., 2005).

Water from the Pacific Ocean enters the Chukchi Sea through Bering Strait, with an average transport of 0.8 Sv (Roach et al., 1995). Proceeding from west to east across the strait, three distinct northward flowing water masses are identified: Anadyr, Bering Shelf and Alaskan Coastal waters (Figure 5.1). On the western side, the inflow is dominated by Anadyr water that has the lowest temperatures, highest densities and highest nutrient concentrations (Walsh et al., 1989). The Bering Shelf water is a mixture of Bering Sea water with less saline, cold water resident on the northern Bering shelf (Coachman et al., 1975). Adjacent to Alaska, the Alaskan Coastal water is easily identified by its warm summer temperature and relatively low salinities and nutrient concentrations (Coachman et al., 1975). Although there is large variability on a variety of timescales, most of the inflowing waters continue northward over the Chukchi shelf where they undergo modifications through physical, chemical and biological processes. Recent models (Winsor and Chapman, 2004) and observations (Woodgate et al., 2005; Weingartner et al, 2005) indicate that Pacific-origin waters follow three general pathways

across the shelf that are determined by the bathymetry. The two main branches are channeled by Herald Canyon on the western shelf and by Barrow Canyon on the eastern shelf, while a third branch flows northward through a gap between Herald and Hanna Shoals that is known as the Central Channel. The modified Pacific waters reach the shelfbreak of the Canada Basin, and, according to modeling studies (Winsor and Chapman, 2004; Spall, 2007), the flow then turns eastward to form a shelfbreak current along the upper slope of the Chukchi and Beaufort Seas. Observational evidence supports the existence of such an eastward-flowing shelfbreak jet (Pickart 2004; Mathis et al., 2007; Nikolopoulos and Pickart, 2007); however, the jet readily reverses due to easterly, upwelling-favorable winds (Muenchow et al., 2006; Nikolopoulos and Pickart, 2007).

Shelf-basin exchange mechansisms

The lateral exchange of biological, chemical and physical properties between shelf and basin can impact ecosystem characteristics and biogeochemical processes of both regions (Walsh, 1995). That such exchange takes place is readily apparent by the significant amount of Pacific water observed seaward of the Chukchi and Beaufort Shelves in the interior Canada Basin (e.g. Shimada et al., 2001; Steele et al., 2006). However, the mechanisms mediating cross-slope transport in the western Arctic are unresolved. Early studies suggested that some portions of the dense, Pacific winter water can be directed down the canyons and directly enter the Arctic Basin (Coachman and Barnes, 1961). The recent studies mentioned above describe the presence of an eastward-flowing Pacific water jet that may contribute to the transfer of shelf production and organisms to the interior of the Canada Basin through different transport processes.

One plausible mechanism for transport is *via* hydrodynamic instability of the shelfbreak current leading to eddy formation (Manley and Hunkins, 1985; Spall et al., 2007). Pickart (2004) showed that the potential vorticity structure of the eastward-flowing shelfbreak jet satisfies the necessary conditions for baroclinic instability, and observations exist when eddies have been formed from this current west of Barrow Canyon along the edge of the Chukchi Sea (Pickart et al., 2005; Mathis et al., 2007). A similar process is believed to occur along the edge of the Beaufort Sea (Pickart et al., 2005). Other eddy formation mechanisms have been put forth as well, most of them involving the flow of water through Barrow Canyon. This includes frictional effects of the canyon wall (D'Asaro, 1988), the sharp bend in topography at the mouth of the canyon (Cenedese and Whitehead, 2000), and the sinking of dense water as it flows through the canyon (Chao and Shaw, 2003; Pickart et al., 2005). Regardless of the formation process, the eddies formed by the boundary current are able to carry plankton off the shelf (Ashjian et al., 2005).

Another cross-slope exchange mechanism involving the shelfbreak jet is that due to wind-forced upwelling (e.g. Aagaard and Roach, 1990; Münchow and Carmarck, 1997). Pacific-origin storms can lead to a strong Ekman circulation with offshore flow in the upper layer and onshore flow of Arctic basin water onto the shelf (Pickart et al., 2006). During these storms, which are characterized by easterly winds, the shelfbreak jet is reversed and can reach peak speeds exceeding 50 cm/s. These events are most prevalent in the fall and winter months (Pickart and Nikolopoulos, 2007) during the active storm season (Wilson and Overland, 1986). However, summertime upwelling events do occur (as discussed later in the chapter).

A final key mechanism influencing water mass exchange and productivity in the region is ice cover. Annual cycles of freezing and melting sea ice alter the physical properties of waters flowing over the shelves and can impact the biota of the region by reducing the quantity of photosynthetically active radiation at the surface of the water column. Ice formation begins in shallow areas in October (Arrigo and Dijken, 2004), and after that the ice-edge advances rapidly, covering the Chukchi Sea. By late February it is at the shelfbreak of the Bering Sea (Grebmeier et al., 1995). During the coldest months, the seasonal flow of Pacific water through Bering Strait and across the Chukchi Shelf is reduced (Coachman and Aagaard, 1988; Roach et al., 1995; Woodgate et al., 2005a), and over the shelves, brine formation and cooling modify the winter resident water (Weingartner et al., 1998; Woodgate et al., 2005b). Ice melt begins in April and continues until it reaches a seasonal minimum in September. During this time, phytoplankton blooms follow the receding ice-edge and rapidly deplete the nutrients in shelf waters recently exposed to light, and, at the ice-edge, productivity increases (Smith and Sakshaug, 1990; Hill and Cota, 2005). High rates of primary production extend from May to August and results in increased abundance of most zooplanktonic taxa in summer and fall (Smith and Schnack-Schiel, 1990). These highly productive areas formed during the summer are important habitats for birds, fish and mammals in the Arctic (Dickson and Gilchrist, 2002; Bengtson et al., 2005). Given the role of ice cover in the Arctic system, a disappearance of the sea-ice (Parkinson et al., 1999; Comiso, 2002; Comiso and Parkinson, 2004) would change the stratification of the Arctic Ocean and marginal seas, which in turn would affect the polar ecosystem dramatically.

Zooplankton and food webs of the study area

The zooplankton community on the Chukchi and Beaufort shelves reflects the mixture of water masses of Pacific and Arctic origin. Small-sized copepods such as *Oithona similis* and *Pseudocalanus* spp. numerically dominate the zooplankton community of these continental shelves. The prevalence of small copepod species subsequently leads to proliferation of benthic organisms because most of the primary production sinks to the bottom, as is the case in the southern Chukchi Sea where benthic communities of high biomass occur (Grebmeier and Dunton, 2000). In contrast, the presence of large-bodied copepods over the shelf enhances the pelagic ecosystem. From the south, Pacific waters transport large copepods such as *Neocalanus* spp. and *Calanus marshallae* (Springer et al., 1989), and the flow from the Arctic Ocean onto the shelves carries other large copepods such as *Calanus hyperboreus*, *Calanus glacialis* and *Metridia longa* (Johnson, 1958; Thibault et al., 1999). By virtue of their abundance and high lipid content (Sargent and Falk-Petersen, 1988) these large copepods are of major importance in Arctic food webs, and although there are still some uncertainties about their effects on shelf ecosystems (Lane et al., 2007), it is clear that some of these copepod species feed on the shelf-derived primary production (Plourde et al., 2005) and require mechanisms to return to the basin to undergo diapause (Conover, 1988; Conover and Huntley, 1991). Offshore transport is necessary for some Arctic copepod species to complete their life cycle, and variability in offshore transport can play a significant role in modifying the shelf and basin ecosystems.

Eddies in the western Arctic

The first observation of an arctic eddy was made in 1937, but it was not until the completion of the Arctic Ice Dynamics Joint Experiment (AIDJEX) in 1972 that mesoscale eddies were thoroughly documented. Based on the AIDJEX ice camp data, and from more recently collected drifting buoy data, the eddy encounter rate is 1-2 per 100 km in the Canada Basin (Manley and Hunkins, 1985; Plueddemann and Krishfield, 2007). This indicates that the Canada basin is populated with a large number of subsurface eddies. Most of the eddies in the southern portion of the basin are anti-cyclones embedded in the halocline (Plueddemann and Krishfield, 2007) and are comprised of Pacific-origin water (Muench et al., 2000). This indicates that they are not formed locally and likely originate from the edges of the Chukchi and Beaufort shelves as discussed above. Isotope half-lives (Kadko et al., 2006) and tracer distributions (Muench et al., 2000) indicate that the ages of eddies range from weeks to over a year.

Several different types of eddies, as distinguished by their structure and core properties, have been observed in the western Arctic. As noted above, most of the features measured during AIDJEX, and more recently by drifting buoys, were subsurface anti-cyclones. Both cold-core and warm-core (relative to the ambient surrounding water) eddies have been observed. This may be due to the seasonal differences of the water being advected by the shelfbreak jet: cold, winter-transformed Bering/Chukchi water in late-spring and summer, and warm Bering/Chukchi water in late-summer and fall (see Mathis et al., 2007). Another type of eddy observed in the southern Canada basin is a warm-core, surface-intensified anti-cyclone (Pickart et al., 2005). These eddies likely emanate from the shelfbreak jet in the vicinity of Barrow Canyon during the late-

summer/early-fall time period when the jet is advecting very warm and buoyant Alaskan Coastal Water. Satellite ice maps reveal these features being spawned from the outflow through Barrow Canyon (G. Stossmeister, pers. comm., 2003). Recently, another class of eddy has been observed in the northern Canada Basin by ice-tethered profilers (Timmermanns et al., 2007). These are sub-surface anti-cyclones similar to the features observed during AIDJEX, but shallower and less dense. Timmermanns et al. (2007) argue that they are formed along a hydrographic front in the northern part of the basin. Because of insufficient measurements, it is difficult to say at this point which type of eddy is most prevalent in the western Arctic. However, it is clear that sub-surface anti-cyclones are commonly found in the southern Canada Basin, and that the shelfbreak of the Chukchi and Beaufort seas is a major source of these features.

The objectives of this paper are to determine the mechanisms and rates of shelf-basin exchanges of zooplankton associated with eddy formation from the shelfbreak jet – in particular, due to cold-core anti-cyclones – and to understand how on-shelf and off-shelf transport shape the Arctic ecosystem. I begin with a description of the physical and chemical properties of the water column and the vertical distribution of zooplankton across a shelf-basin transect in the Chukchi Sea occupied in summer 2004. Among other things, this provides a view of the shelfbreak jet. This is followed by a description of a cold-core, anticyclonic eddy observed later that year. I interpret the water mass properties and species composition in the eddy using the summer boundary current transect as a proxy of the conditions present at the time the eddy was generated. Finally, I estimate the grams of carbon of zooplankton transported annually into the Arctic Ocean

via eddies, and discuss the effects shelf-basin exchange processes can have on the shelf and basin food webs.

METHODS

The data were collected on two cruises to the Chukchi/Beaufort seas and the adjacent Canada Basin on the USCGC *Healy*, in summer from July 17 to August 26, and in early fall from September 2 to October 1, 2004. The cruises were part of the Western Arctic Shelf Basin Interactions (SBI) program. In summer, stations were located along transects from the Chukchi or Beaufort shelves to the slope waters of the Canada Basin. I focus on the West Hanna Shoal (WHS) transect occupied between August 18 and 24, 2004 (Figure 5.2). The WHS section is provided as background on the physical structure and zooplankton distribution of the water column across the shelf-basin boundary. In early fall, a subsurface, cold-core, anti-cyclonic eddy was sampled over the slope of the Chukchi Sea between September 25 and 26, 2004 (Figure 5.2). The sampling in both seasons of 2004 was carried out under unusually ice-free conditions.

Collection and analysis of hydrographic data

The SBI Service Group provided the hydrographic data from conductivity-temperature-depth (CTD) casts and water samples at all stations. The hydrographic sampling protocols are described by Codispoti et al. (2005) and are only briefly summarized here. The physical (temperature and salinity), chemical (nutrients) and biological (chlorophyll *a*) data were collected using modified WOCE/JGOFS protocols. The hydrographic sampling system was comprised of a Sea Bird Electronics (SBE) 911+ CTD mounted on a 24-place rosette with 10-l Niskin bottles. All instruments were

calibrated according to WOCE methods and samples were analyzed using quality control protocols that meet WOCE standards. The estimated accuracy for temperature is .001^{o}C. For salinity the accuracy ranges from .002 (deep water) to .01 (mid-depth). Prior to the eddy CTD section, the feature was mapped using expendable CTDs (Figure 5.2B), with an accuracy of .02^{o}C and .04 for temperature and salinity, respectively (see Kadko et al., 2007). Vertical sections were constructed for various properties, including potential temperature and density (referenced to the sea surface), transmissivity, and nutrients.

In order to analyze the boundary current at the WHS line I made use of the shipboard ADCP data collected during the cruise. In particular I used the Healy's 75 KHz narrow band data for the WHS section. The data were first de-tided using the Oregon State University 5 km resolution Arctic tidal model (Padman and Erofeeva, 2004), in an effort to remove the dominant barotropic tidal signals. Encouragingly the predicted tides were very small (< 2 cm/s) at this location and time. After subtracting out the tides, the latitude and longitude of the ADCP ensembles were projected along a regression line that included the positions of the CTD stations (this was necessary because some of the ADCP data were collected while the ship drifted on station). Then the component of velocity normal to the regression line was gridded using Laplacian-Spline interpolation (cross-stream resolution of 2 km, vertical resolution of 5 m).

Since I am interested in the geostrophic flow of the boundary current, and since there were no ADCP data shallower than 20 m or near the bottom (due to the normal blanking associated with the surface and bottom), I computed the absolute geostrophic velocity for the WHS line by referencing the thermal wind shear to the rotated ADCP velocity. Unfortunately the coarse CTD spacing – in particular the fact that

there was only one CTD station on the shelf – made this problematic. To get around this I objectively interpolated the CTD temperature (T) and salinity (S) data to fill in the gaps (cross-stream resolution of 10 km, vertical resolution of 10 m), then computed a vertical section of thermal wind shear from the gridded T and S fields. Finally, the thermal wind velocities were referenced using the laterally averaged (gridded) ADCP data between each 10 km point. (At each location along the section the reference velocity was computed over the common depth range of the thermal wind and ADCP velocities.) As a consistency check on this approach I note that the absolute geostrophic velocity section so computed, and the original ADCP velocity section, are very similar in structure.

Zooplankton sampling

I obtained distributions of zooplankton using a MultiNet® fitted with 150 μm mesh nets at four of the eight stations of the WHS shelf-basin transect in summer and at six of the eleven stations across the eddy in fall (see Figure 5.2B). In summer, zooplankton sampling intervals varied according to bottom depth. In fall sampling intervals were 0-50, 50-100, 100-150, 150-200 and 200-300m. After collection, the sample was poured through a 150 μm mesh sieve to remove seawater and then preserved in 95% ethyl alcohol for enumeration and identification in the laboratory.

Laboratory analysis

Plankton identification and enumeration

Zooplankton samples were enumerated at the University of Miami's Rosenstiel School of Marine and Atmospheric Science. Net samples for taxonomic enumeration were split several times in a Folsom splitter, and three aliquots were counted for each

taxonomic category identified. Organisms were identified to the lowest possible taxonomic level. For the copepods *Calanus glacialis* and *Metridia longa* each developmental stage was counted individually. For *Oithona similis* adult females, adult males and all copepodid stages were counted. For *Pseudocalanus* spp. and *Oncaea* spp. adult females, adult males and copepodid stages C5-C4 were counted, whereas copepodid stages C3-C1 were grouped with unidentified calanoid copepodites. The identification of chaetognaths, appendicularians, gastropods and other planktonic taxa was carried out only to broad taxonomic levels. Abundance in terms of individuals per cubic meter ($ind.m^{-3}$) was estimated for each category by dividing the number counted by the fraction of the sample counted for that category and dividing the result by the volume of water filtered.

Zooplankton dry weight and carbon

I used wet displacement volumes (Ahlstrom and Thrailkill, 1963) to estimate zooplankton dry weight and carbon (Wiebe et al., 1975; Wiebe, 1988). First, the total volume of the plankton with its preserving ethanol was measured in a 100-ml graduated cylinder. The plankton was then separated from its preserving ethanol using a 150 μm sieve. I poured the sample into the cone-shaped sieve where the plankton was retained and the ethanol was collected in another 100-ml graduated cylinder. Each sample was drained for 5 minutes before reading the volume of ethanol alone. The plankton volume was calculated based on the difference between the total volume of the plankton with its preserving ethanol and the volume of ethanol alone. The displacement volume was then divided by the volume filtered for each net to obtain displacement volumes (DV) in terms of ml/m^3. Finally, I calculated zooplankton carbon (C) using the empirical equation:

Log (DV) = -1.434 + 0.820 Log (C) (Wiebe et al., 1975; Wiebe, 1988)

Wet displacement volumes were run after 2 years of preservation hence problems associated with plankton shrinkage with time of preservation may have occurred (Ahlstrom and Thrailkill, 1963). Our samples, however, were dominated by copepods which show the least volume loss with time (Ahlstrom and Thrailkill, 1963; Wiebe et al., 1975).

RESULTS

Summer shelf-basin section

The 2004 SBI summer cruise consisted of relatively wide station spacing that did not adequately describe temporally and spatially small-scale features such as eddies. As this study was not designated to determine the time and location of eddy formation, I cannot definitely answer the important question, what conditions existed at the formation site of the eddy? Nevertheless, I provide the WHS sections as background on the physical structure and zooplankton distribution of the shelfbreak current, which I believe are very similar to the source of the core water of the eddy observed in fall. The WHS section was occupied approximately one month before the eddy survey; I note that the estimated age of the eddy (Kadko et al., 2006) was on the order of months. The timing of the WHS section is thus appropriate to capture the water mass and plankton community likely to be contained in the eddy.

Hydrography and state of the shelfbreak jet

During summer the undisturbed shelfbreak tends to flow eastward along the edge of the Chukchi Sea (Pickart et al., 2005; Mathis et al., 2007). Depending on the precise time of season, and probably the severity of the preceding winter, the current advects

either cold winter-transformed Chukchi/Bering Water (temperature < -1.65^{o}C) or summertime Chukchi/Bering Water (temperature near 0^{o}C). During the SBI summer 2004 cruise the current was advecting primarily the former. However, the WHS line was occupied during an easterly wind event. The event consisted of two separate wind peaks on the order of 5-10 m/s, with a lull in between (Figure 5.3). The influence of this easterly wind is reflected in the structure of the shelfbreak jet at the time of the transect.

The vertical sections of potential temperature and absolute geostrophic velocity for the WHS transect are shown in Figure 5.4, overlaid on potential density. One sees that the boundary current has enhanced eastward flow on the outer shelf and shelfbreak, with a second peak near 200 m on the upper slope. Offshore of this there is a westward-flowing surface-intensified jet of similar magnitude (centered at stations 56-57, Figure 5.4B). In light of the wind forcing at the time, this structure is completely as expected based on mooring array data obtained from the shelfbreak jet farther to the east (in the Beaufort Sea) during SBI. Using an empirical orthogonal function (EOF) analysis, Nikolopoulos and Pickart (2007) showed that during periods of upwelling (easterly winds) the shelfbreak jet alternates between a reversed state (during the height of the storm) and a "recovered" state (after the storm ends). These two states are shown in Figures 5.5A and 5.5B respectively. Interestingly, the recovered state contains a secondary maximum (or deep tail, as Nikolopoulos and Pickart (2007) called it) similar in structure to the enhanced eastward flow in the WHS velocity section near 200 m.

To investigate this further, I regressed the zonal 10 m wind speed record from the meteorological station at Point Barrow, AK with the EOF modal amplitude timeseries from Nikolopoulos and Pickart (2007). Not surprisingly, the two timeseries are

significantly correlated at the 95% confidence level. Based on this relationship, it implies that the reversed boundary current in Figure 5A corresponds to strong easterly winds (order 15 m/s) and the recovered boundary current (Figure 5.5B) occurs when the winds are weakly out of the west (3-5 m/s). The EOF configuration that corresponds most closely with the structure of the boundary current observed during the WHS transect is shown in Figure 5.5C. Keeping in mind that the mooring array was located on the Beaufort slope some 300 km downstream of the WHS line, and that the mooring data are from 2002-3, the similarity is striking (compare Figures 5C and 4B). Using the above regression, the corresponding value of the EOF modal amplitude in Figure 5C implies a wind speed of 5 m/s out of the east. This compares favorably with the mean easterly wind speed computed over the duration of the WHS velocity section (the shaded area in Figure 3) of 5.6 m/s.

To summarize, the WHS transect apparently captured the shelfbreak jet in a partially wind-driven state. One must keep in mind, however, that the transect took approximately 4 days to occupy, and hence the velocity and hydrographic fields represent a mixture in space in time. It is possible that the entire shelfbreak jet reversed at some point, with significant upwelling (although the lull in the wind speed during the middle of the occupation probably limited this to some extent). The occurrence of upwelling would explain why the coldest temperatures (associated with the winter-transformed water) do not coincide with the eastward shelfbreak flow (compare Figures 5.4A and 5.4B); the shoreward advection of warmer water from the lower slope to the shelfbreak would moderate the temperatures there (see Pickart, 2004). I note that the hydrographic section occupied prior to the WHS line on the summer cruise (under variable westerly winds)

showed a large amount of winter-transformed water on the outer-shelf and shelfbreak. The distributions of nutrients along the WHS transect show enhanced concentrations near the shelfbreak (Figure 5.6). This is typical of the eastward-flowing shelfbreak jet in the Chukchi Sea as it advects winter-transformed Pacific-origin water (e.g. Weingartner et al., 1998; Pickart et al., 2005), and is consistent with the idea from models that the origin of the jet is the outflow from Herald Canyon. A hydrographic/velocity survey of Herald canyon, carried out during the same time period of the 2004 SBI summer cruise, supports this notion (R. Pickart, pers. comm., 2006). I surmise that the upwelling during the WHS occupation did not impact the nutrient distributions near the shelfbreak as much as the temperature, because of higher ambient nutrient concentrations at depth and due to stirring up of regenerated nutrients from the sediments during the storm (consistent with the transmissometer data, not shown).

Zooplankton distribution

I identified several mesozooplankton categories on the WHS section, including 20 copepod species, 5 copepod genera, and 20 other categories where identification was made to the lowest taxonomic rank possible. Copepods were by far the most abundant group during the summer and fall periods. I will focus on two large Arctic copepods, *Calanus glacialis* and *Metridia longa*, one ubiquitous species, *Oithona similis,* and two copepod genera *Pseudocalanus* and *Oncaea* that are predominantly neritic and oceanic taxa respectively. Other species of interest are *Acartia longiremis*, whose presence is indicative of coastal waters, and the Pacific-origin copepods *Metridia pacifica* and *Neocalanus flemingeri*.

I obtained distributions of zooplankton at four of the eight stations of the WHS shelf-basin transect in summer, 2004. Total zooplankton abundance decreased with depth with the highest abundance observed in surface waters (0-50 m) over the shelfbreak (8,316 ind.m^{-3}, Figure 5.7A). For waters of the same density, zooplankton abundance decreased with distance from the shelf (Figure 5.7A). The cyclopoid copepod *Oithona similis* was the most abundant organism, especially over the shelf and in surface waters (Figure 5.4B), and its distribution established the total zooplankton trend. The distributions of *Pseudocalanus* spp. and *Oncaea* spp. followed opposite patterns to each other. In the WHS section *Pseudocalanus* spp. were numerous over the shelf and present in relatively low numbers at the most offshore station (Figure 5.7C). An important feature of the distribution of this copepod genus was the high abundance in subsurface waters (50-100 m) associated with the shelfbreak current (Figure 5.7C). In the same subsurface waters, *Oncaea* spp. were present in low numbers, and were only numerically important in surface waters (0-100 m) over the slope and basin (Figure 5.7D), an expected distribution since *Oncaea* spp. are characteristic of polar waters and are commonly found at bottom depths up to 2000 m in the Arctic Basin (Heron et al., 1984).

Interestingly, the Arctic copepods *Calanus glacialis* and *Metridia longa* were found in significant numbers in the region of the shelfbreak and even on the shelf (Figs. 7E-F). This is another indication that upwelling occurred during the easterly wind event as the section was being occupied, consistent with the velocity results discussed above. *Metridia longa* is a basin species, capable of pronounced diel vertical migrations, and intrinsic to the Arctic Ocean and its surrounding seas (Brodskii, 1967). The high concentration of this copepod on the upper slope at station 58 is where the upslope flow

would be strong during upwelling (Pickart et al., 2006), and it is consistent with the upward tilt of the Atlantic water isotherms (warmer than 0^{o}C) at this location (Figure 5.4A).

The enhanced concentration of *Metridia longa* at the shoreward-most station implies that the upwelled water penetrated onto the shelf (Figure 5.7E). Arctic water was also indicated by the presence of *C. glacialis* in subsurface waters (50-100 m) at station 58. I suspect that the concentration decreases near the bottom of this station because this species is found at shallower ambient depths in the interior basin (Figure 5.7F). Copepodids of another Arctic copepod, *C. hyperboreus,* were also abundant in subsurface waters over the shelfbreak (8 ind.m^{-3}, data not shown). It is expected that these Arctic species would not normally be found in significant quantities in the undisturbed, eastward-flowing shelfbreak jet (i.e. under non-upwelling conditions).

Eddy Section

Roughly a month after the summer section was occupied, I sampled a subsurface, anticyclonic eddy in the vicinity of the WHS section (Figure 5.2B). The core of the feature contained cold, winter-transformed Bering/Chukchi water, similar to that being advected by the shelfbreak jet during the earlier process cruise. Isotope half-lives, oxygen concentrations and respiration rates within the eddy indicated an age on the order of months (Kadko et al., 2006). I infer, therefore, that the eddy was spawned from the shelfbreak jet sometime during spring/summer 2004. While I am unable to determine the precise area and time of formation, it is reasonable to use the WHS section, in particular station 58 (in the depth range of 50-100 m) as a source function to interpret the physical and chemical structure of the eddy and its zooplankton distribution.

Eddy-core properties

The eddy survey is described in Mathis et al. (2007) and Kadko et al. (2007) and is only briefly summarized here. After locating the feature using expendable bathythermographs (XBTs), a rapid high-resolution survey was carried out using expendable CTDs (XCTDs) along with the shipboard ADCP. The XCTD grid was approximately 30 km on a side with 5 km resolution and took roughly 24 hours to complete (Figure 5.2B). This provided a three-dimensional snapshot of the eddy (for a lateral view of the feature see Figure 9 of Mathis et al., 2007). Immediately following this, a transect was occupied through the center of the eddy using the shipboard CTD package, including water samples and net tows. From this survey, it was determined that the eddy had a radius of ~ 8 km and its core was located at an approximate water depth of 160 m (Figure 5.8) on the continental slope, centered over the 1000 m isobath. Inside the eddy core, silicate (> 40 μMol/L, Figure 5.8A), nitrate (> 15 μMol/L, Figure 5.8B) and phosphate (> 2 μMol/L, Figure 5.8C) concentrations were the highest for the section. Also, the eddy core had low temperatures (< -1.7 °C, Figure 5.9A). The eddy core had very similar physical (temperature and density) and chemical (silicate, nitrate, phosphate) properties to the shelfbreak jet observed in summer (Figs. 4 and 6), charactersitic of Pacific-origin water (see also Pickart et al., 2005).

The ADCP data showed relatively high echo intensities at the eddy core when compared with waters of similar density outside the eddy (not shown). This observation and the low transmissivity found at the eddy core (84%, Figure 5.9B) suggest that different sized particles were confined inside the eddy and actively transported by it. Low chlorophyll *a* (Figure 5.9C) and phaeopigment concentrations (data not shown) at

the eddy core suggest that phytoplankton cells were not transported in significant numbers to the Arctic Basin by this subsurface eddy. It is possible that phytoplankton cells were not present at the time and place of formation of the eddy or that grazing by copepods within the eddy subsequently reduced cell concentration prior to our sampling.

Advection of zooplankton

The sections (Figs. 10A-F) represent the first observations of the vertical distribution of mesozooplankton across an eddy in the western Arctic. The abundances are overlaid on potential density to facilitate the analysis of the effects of the physical and chemical structure of the water column on the vertical distributions of zooplankton across the eddy (Figure 5.10A-F) and along the shelf-basin line (Figure 5.7A-F). The zooplankton distribution across the shelf-basin summer section (Figure 5.7A-F) is used as reference.

While the bulk of the zooplankton was above 50 m (Figure 5.10A), there was a clear signature in the eddy: within the central density range of the feature (26.5–26.8 kgm^{-3}) the zooplankton abundance was higher at the eddy core (180 $ind.m^{-3}$, Figure 5.10A) than outside the influence of the eddy (108±23 $ind.m^{-3}$, N=5). As observed earlier in the summer section, *O. similis* was the most abundant organism in surface waters and its distribution dictated the total zooplankton distribution. I observed a sharp decline in *O. similis* abundance with depth and slightly elevated numbers at the eddy core (23 $ind.m^{-3}$, Figure 5.10B) in comparison to waters of the same density outside the eddy (12±3 $ind.m^{-3}$, N=5). Similar vertical distributions for *Pseudocalanus* spp. and *Oncaea* spp. were found. Inside the eddy core, *Pseudocalanus* spp. (37 $ind.m^{-3}$, Figure 5.10C) and *Oncaea* spp. (33 $ind.m^{-3}$, Figure 5.10D) abundances were higher than they were in

waters of the same density outside the eddy (18±6 ind.m^{-3}, N=5 and 14±4 ind.m^{-3}, N=5 respectively). In the upper 100 m, the abundances of the two genera were associated with elevated chlorophyll *a* concentrations (Figure 5.9C); although not a perfect match, abundances were generally higher at stations with elevated chlorophyll *a* values.

The vertical distributions of the Arctic copepods *M. longa* and *C. glacialis* were particularly interesting. As discussed above, their presence at the shelfbreak during the summer WHS section was likely the result of wind-driven upwelling (Figs. 7E and 7F). The high abundance of *M. longa* at the eddy core (46 ind.m^{-3}, Figure 5.6E) compared to its average abundance in waters of the same density outside the eddy (29±9 ind.m^{-3}, N=5) implies that the particular formation event that spawned the eddy occurred near a time of enhanced easterly winds. I note that the hydrodynamic instability hypothesis for the formation of the eddies does not require the presence of wind (e.g. Spall et al., 2007). Hence, a storm might have occurred prior to the eddy being formed, resulting in Arctic-origin water being entrained into the feature. Another possibility is that the eddy formed as a result of the wind forcing—a hypothesis yet to be explored. The vertical distribution of *C. glacialis* in the eddy was also similar to its distribution in the shelfbreak summer section. In summer, *C. glacialis* was highly abundant in subsurface waters (50-100 m) on the upper slope (Figure 5.7F), and in fall it was also abundant in the top part of the eddy (Figure 5.10F). In the upper part of the water column above this, its distribution seems to be related to chlorophyll *a* concentrations (Figure 5.9C). The other Arctic copepod, *C. hyperboreus,* was observed in low numbers (~ 1 ind.m^{-3}, data not shown) with higher numbers at depths below 200 m. Its vertical distribution was not associated with the physical structure of the eddy.

Metridia pacifica and *A. longiremis* were found in the eddy core, although in very low abundances. Their presence is consistent with a shelfbreak origin of the subsurface eddy. *Metridia pacifica* is a expatriate from the Bering Sea Basin transported by Pacific water, and *A. longiremis* is a coastal taxon of the marginal seas, probably entrained in the winter-transformed water.

Biomass

Zooplankton biomass was most elevated in the upper 50 m at stations closer to the shelf (stations 129 and 131), and in subsurface waters (50-100 m) at the farthest offshore station (station 121). In surface water, the high abundance of zooplankton was responsible for the elevated biomass. In subsurface waters at station 121, the large-bodied copepod *C. glacialis* probably accounts for much of the increase in biomass of zooplankton observed. Deeper in the water column, zooplankton biomass was higher near the eddy core (6.31 mg C m^{-3}, Figure 5.11) when compared with similar density water outside the eddy (3.50±1.83 mg C m^{-3}, N=5). The eddy center contained elevated numbers of the large copepods *M. longa* and *Paraeuchaeta glacialis* and other large zooplankton including amphipods and chaetognaths. Despite its low abundance in general, *C. hyperboreus* possibly contributes to the observed biomass values, in particular for deep samples.

DISCUSSION

Distinct zooplankton communities populate the continental shelf and the basin of the Arctic Ocean. The community composition varies seasonally. In summer over the

Chukchi shelf, the zooplankton assemblage is a mixture of species that have neritic affinities (e.g., *Acartia longiremis*, *Pseudocalanus* spp.) and basin affinities (e.g., *Calanus glacialis*). Offshore of the shelf, the assemblage is generally dominated by basin species. Our observation of a shelf zooplankton assemblage inside the surveyed eddy located over the continental slope suggests that eddies could be one important mechanism for zooplankton transport from the Arctic Ocean periphery into the interior basin. The zooplankton assemblage inside the eddy was readily differentiable from the basin assemblage supporting the idea that eddies can effectively transport and maintain shelf zooplankton communities in the basin. This is indirectly supported by reports of bowhead whales congregating in dense zooplankton patches offshore of the Beaufort Sea (Moore, pers. comm.) to forage on their key prey, copepods (Lowry et al., 2004). As the eddy ages, species typical of the Arctic Basin will progressively replace the shelf zooplankton in the eddy. It appears that eddy transport can substantially affect zooplankton biomass and community composition and that, in combination with other shelf-basin exchange processes, it can regulate Arctic food webs.

Copepod distribution

The copepod distributions seen in summer and fall were influenced by distance offshore and the water masses. During summer, elevated abundances of copepods were observed in subsurface waters on the upper slope, associated with either the shelfbreak jet or upwelled basin water due to the easterly winds at the time. In fall, zooplankton in the center of the eddy were more abundant and had higher biomass than zooplankton from similar density water outside the eddy. These patterns are consistent with advection of secondary production from the shelf to the basin by such features (see also Ashjian et al.,

2005). The shelfbreak and the eddy center were particularly interesting with elevated abundances of several taxa. The high abundances of zooplankton inside the eddy are explained by their high numbers in subsurface waters at the shelfbreak from where the eddy likely originated.

Pseudocalanus spp. were present in high numbers in surface waters over the shelf, in subsurface waters at the shelfbreak, and inside the eddy; an expected distribution for a genus that is predominantly coastal with an unrefined shelf-basin gradient (Corkett and McLaren, 1978). *Pseudocalanus* spp. are scarce and patchy in the Arctic basin where it is generally considered an expatriate from the surrounding neritic waters, especially the Chukchi Sea, where it is common (Johnson, 1958; Brodskii, 1950; Corkett and McLaren, 1978; Horner and Murphy, 1985; Lane et al., 2007). Records of *Pseudocalanus* spp. from the central parts of the basin probably represent populations carried offshore by surface currents, filaments and eddies of the type discussed here. These populations may survive temporarily by feeding under ice (Conover et al., 1986; Runge and Ingram, 1991), but it is unlikely that they are sustained indefinitely in basin waters. The absence of the genus in some surveys, and its scarcity and patchiness in the more central parts of the basin may have resulted from variability in the mesoscale processes responsible for its transport from the continental shelves.

As mentioned above, the significant number of Arctic-origin Calanoid copepods observed over the Chukchi Shelf and upper slope during the summer cruise was likely the result of upwelling. The persistent occurrence of these species over the Chukchi Shelf (Johnson, 1958; English and Horner, 1977; Plourde et al., 2005) suggests that on-shelf intrusions of Arctic Ocean water are common events. Frequent reversals of flow have

been observed at Barrow Canyon (Mountain et al., 1976; Münchow and Carmarck, 1997; Weingartner et al., 1998), though this may be partly related to canyon dynamics. On the other hand, the mooring data from the Beaufort SBI array (Figure 5.2) reveal that the shelfbreak jet reverses quite often at that location: during the first year alone there were 27 major upwelling events over the course of the year (R. Pickart, pers. comm.). It is likely that similar wind-driven processes occur to the west of Barrow Canyon along the shelf edge of the Chukchi Sea, consistent with the results presented here. The transport of Arctic biota onto the shelves should be modulated seasonally. The majority of the upwelling storms in the Beaufort Sea (and likely the Chukchi Sea) seem to be Pacific-origin cyclones (Pickart et al., 2006), and the frequency of these disturbances increases significantly in the fall and winter months (Terada and Hanzawa, 1984). Such seasonal variability is particularly important in the Arctic where zooplankton life cycles and distributions are also strongly seasonal, and alterations in the physical transport processes of zooplankton can potentially drive large match-mismatch variations in distribution and community structure of both zooplankton and their predators (Mackas and Coyle, 2005).

Eddy mediated offshore transport

The boundary region between the Chukchi and Beaufort seas and the adjacent Canada Basin is an exceptionally dynamic zone, defined by sharp gradients of chemical, physical and biological properties. At the shelfbreak, large aggregations of plankton and particles have been observed (Ashjian et al., 2005; Moran et al; 2005). I observed high abundances of *M. longa* on the upper slope, under the influence of the shelfbreak jet, and elevated numbers of *C. glacialis* and *C. hyperboreus* in subsurface waters above it. In addition to advection by the currents, such high concentrations may be due in part to a

combination of aggregation behaviors and retention mechanisms. The vertical distribution of *M. longa* may be explained by its foraging behavior as this copepod feeds on marine snow and diatom particles, which are observed in high concentrations in the shelfbreak current (Ashjian et al., 2005). The proposed behavior is plausible as *M. longa* has an omnivorous diet (Haq, 1967), is characterized by extensive diel vertical migrations (Brodskii, 1950) and its predominantly found below 100 m (Smith, 1988). In this sense, its life cycle strategy is different from that of *C. glacialis*. The latter occupies the upper 100 m where it feeds on large diatoms cells (Mullin, 1963) despite the presence of other potential food sources.

As discussed earlier, numerous mechanisms may be responsible for shelf-basin exchange in the western Arctic. Based on the large number of eddies observed in the Canada Basin (Manley and Hunkins (1985); Plueddemann and Krishfield, 2007), the potential vorticity structure of the shelfbreak jet (Pickart, 2004), and direct observations of eddies emanating from the current (Pickart et al., 2005), it is likely that hydrodynamic instability of the shelfbreak jet and subsequent eddy formation is a major contributor to the cross-slope exchange. To get an idea of the flux of zooplankton due to this process I did the following simple calculation. Following Mathis et al. (2007) I assume that roughly 100 cold-core eddies are formed each year, which is consistent with measured eddy populations in the Canada Basin. . To calculate the average zooplankton biomass I used nine biomass estimates and obtained an approximation of the grams of carbon (g C) of zooplankton transported inside this small eddy. Taking an average eddy volume to be 25 km^3 (Pickart et al, 2005), based on the average zooplankton biomass inside the observed eddy (4.52 mg $C.m^{-3}$), the corresponding total carbon export into the basin

equals 1.1 x 10^8 g C per year. This represents less than 0.01% of the 1.8 x10^{12} g C of zooplankton transported annually into the Chukchi Sea through Bering Strait (Springer et al., 1989). This large discrepancy suggests that other mechanisms such as surface and canyon currents are the dominant mechanisms of off-shelf flux of secondary production (Ashjian et al., 2005). Alternately, it can be inferred that much of the influx of zooplankton biomass from the Pacific is either consumed or deposited into the sediments before it reaches the northern Chukchi shelfbreak.

Climate change and food webs

The implications of the occurrence of Pacific-origin copepods in the Arctic Ocean are interesting because of the potential impact they might have on polar ecosystems. Zooplankton surveys have demonstrated the incidence of large, numerically important Pacific species on Arctic shelves (Johnson, 1956; Springer et al., 1989), and in the Arctic Ocean interior. The subarctic species are carried into the Arctic through Bering Strait, the only communication passage between the Pacific and Arctic oceans. The flow of water through the strait plays a significant role in transporting large copepods because of the correlation of volume transport with zooplankton biomass. Future changes in general circulation patterns could have profound effects on shelf food webs. Over the last 30 years, the cyclonic flow regimes associated with positive Arctic Oscillation values may have increased the northward transport of Pacific waters and their constituents (Walsh et al., 2004). Under this scenario, the advection of sub-arctic copepods will be enhanced. The large, sub-arctic copepods have life cycles that include diapause and therefore need a deep-water environment to survive. Overwintering at depth may become possible in the Canada Basin if the season of ice-cover was shorter. If subarctic copepods were able to

survive and reproduce in the Arctic marginal seas and basins, the food web and carbon cycling in the region will be altered.

Variability in the advection of warm water through Bering Strait also impacts the position of the ice edge (Goose et al., 1997). The location of the ice edge in the Chukchi varies from year to year, receding either slightly shoreward or slightly seaward of the shelfbreak (Gloersen et al., 1992). Recent model studies of ice cover and upwelling suggest that the overall circulation might be dramatically altered by the position of the ice-edge with respect to the shelfbreak (Carmack and Chapman, 2003). In the first scenario investigated by Carmack and Chapman (2003), the ice edge is located over the shelf where it restricts wind-driven upwelling and virtually no exchange occurs across the shelfbreak. In the second scenario, the ice edge is located over the slope and the upwelling circulation brings deep basin water well onto the shelf. This idealized study assumes, however, that the windstress is not transmitted through the pack-ice, which may limit the relevance of the results to the Chukchi and Beaufort Seas. For example, many of the upwelling events measured by the SBI Beaufort slope mooring array occurred during extensive ice cover. Nonetheless, receding ice coverage and enhanced winds suggest that Carmack and Chapman's (2003) latter scenario may become more widespread (Serreze et al., 2003; Stroeve et al., 2005; Meier et al., 2005). Enhanced upwelling would likely result in an increased number of large Arctic-origin copepods on the shelves and would have dramatic impacts on the shelf food webs.

From the perspective of global warming, our observations are especially important because climate models predict an enhanced northward transport of Pacific waters and a reduced ice extent (Vinnikov et al., 1999; Walsh et al., 2004). If these

trends persist, large Pacific-origin copepods would become more common on the Chukchi and Beaufort shelves, and alter shelf food chains. Small copepods are less effective at grazing spring blooms, in particular the pulses of large diatoms characteristic of Polar Regions (Smith and Sakshaug, 1990). Sinking aggregates of phytoplankton fuel the benthic food web. In contrast, elevated numbers of basin copepods on the shelf could graze down the phytoplankton bloom and drive the food web towards a more productive pelagic environment. Furthermore, large copepods have longer life cycles, often including pelagic carbon storage in the form of lipid in diapausing individuals (Conover, 1988; Miller and Clemons, 1988; Smith, 1990), altering the biogeochemistry of the shelves.

CONCLUSIONS

In the western Arctic, the sustainability of some key copepod populations is dependent on transport to the shelf to grow and develop while feeding on shelf phytoplankton, and rely on offshore transport to return to deep water and complete their life cycle. These zooplankton populations are important reservoirs of organic matter and can have a significant role in regulating the fate of shelf primary production. Our data suggest that eddy transport may be important for certain species to complete their life cycles, and in defining their geographic distributions. Indirectly, it can be inferred that other mesoscale phenomena associated with frontal systems can retain and transport zooplankton as well. For example, high abundances of zooplankton and elevated nutrient concentrations were associated with the shelfbreak current. Our results also support the hypothesis that alterations in the extent and timing of physical transport processes of zooplankton can drive large ecosystem changes since zooplankton life cycles and

distributions are strongly seasonal and closely coupled to mesoscale phenomena (eddies. fronts, shelfbreak currents, sea ice edges).

Our data reveal the important role of mesoscale features, but at present the frequency and progression of these events remains uncertain. Despite the implications of this work, our survey represents only a snap-shot of a shelf-basin line and an eddy in the western Arctic. I suggest that future work include time-series surveys of mesoscale phenomena to examine the evolution of the chemical and biological properties of eddies as they become incorporated into the central basin.

ACKNOWLEDGEMENTS

I thank the officers and crew of the USCGC *Healy* for their support during these hydrographic surveys and cruises. I am indebted to my SBI colleagues: Drs. L. Codispoti and J. Swift for their help in collecting and analyzing the hydrographic data, and Dr. D. Kadko for his comments. Dora Pilz helped with the identification of some copepod species and counted the summer samples, and Peter Lane provided guidance and advice during the entire project. I thank Paula Fratantoni and Daniel Torres for their assistance using the shipboard ADCP data. This work was supported by the National Science Foundation under Grant Nos. OPP-9815682 (LL and SS) and OPP-0125004 (JM), and the Office of Naval Research under grant N00014-02-1-0317 (RP).

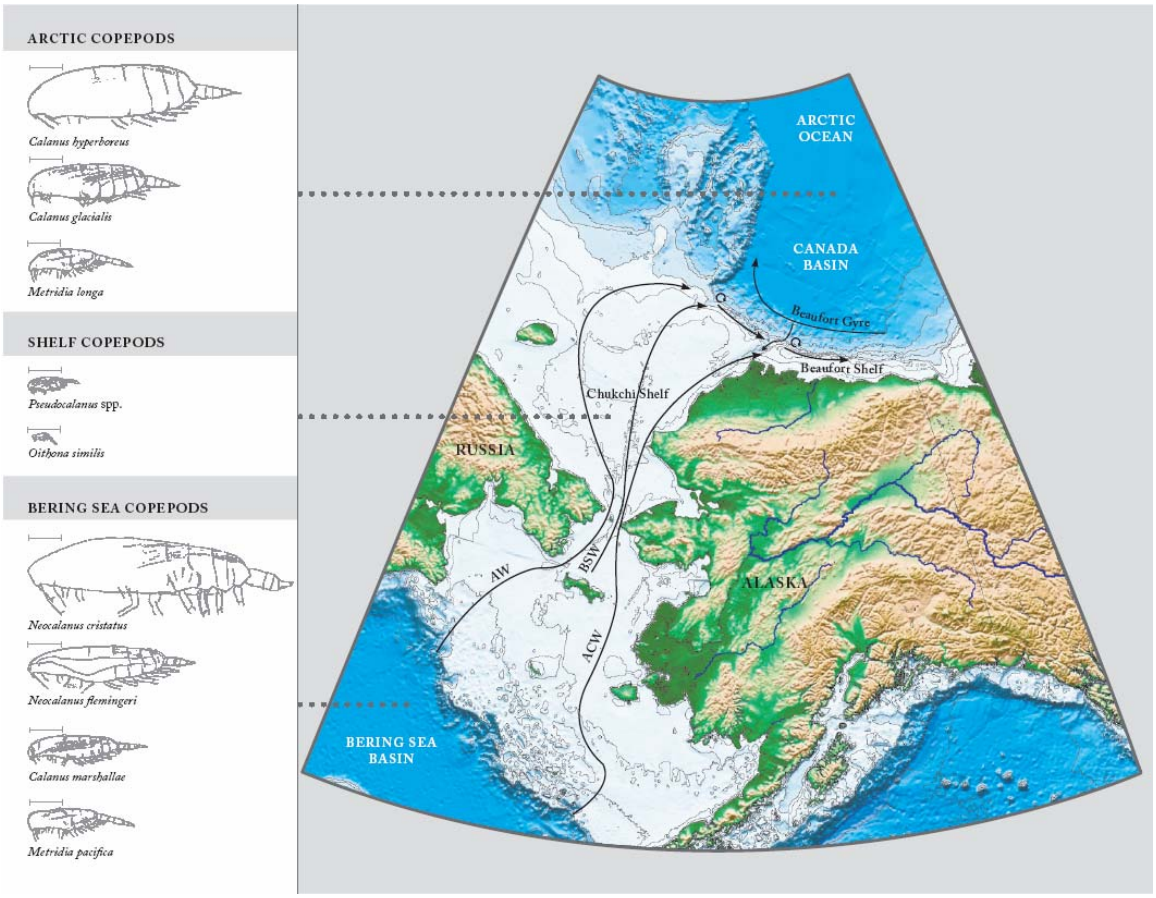


Figure 5.1 Representation of the dominant Arctic and sub-arctic copepods (at same magnification) and the mean circulation over the Chukchi and Beaufort Seas. The three main branches of Pacific-origin water from west to east are Anadyr Water (AW), Bering Shelf Water (BSW) and Alaskan Coastal Water (ACW).

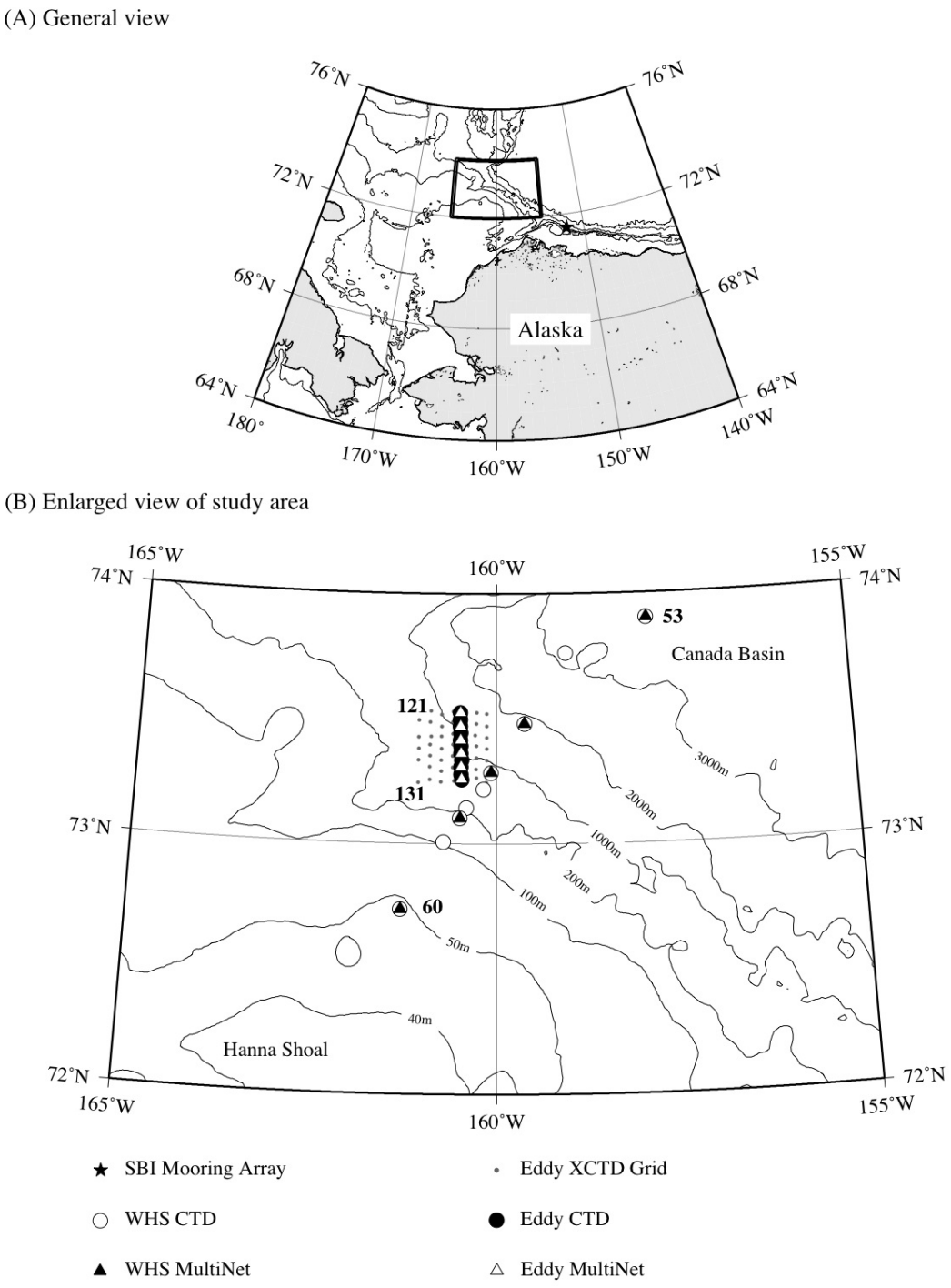


Figure 5.2 (A) Study area in the Chukchi and Beaufort seas. (B) Enlarged view of study area, showing the hydrographic and biological stations occupied by the USCGC *Healy* in summer and fall 2004. The summer line consisted of 8 hydrographic stations of which 5 had concurrent zooplankton sampling. The eddy survey in fall consisted of 11 hydrographic stations with zooplankton sampling at every other station.

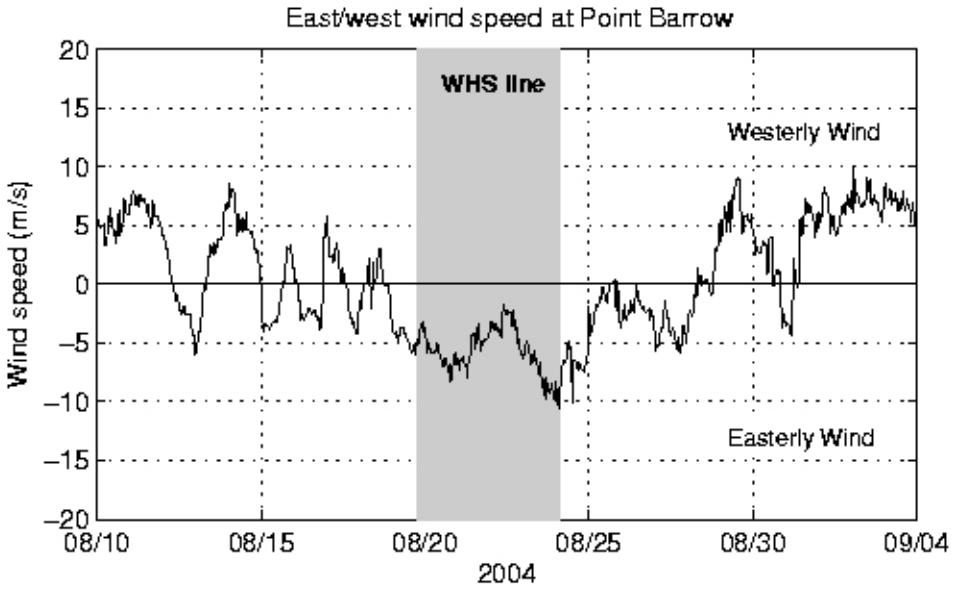


Figure 5.3 Timeseries of zonal 10 m windspeed from the Pt. Barrow, AK weather station. The time period of the WHS transect is indicated by the gray shading. Easterly winds are upwelling favorable.

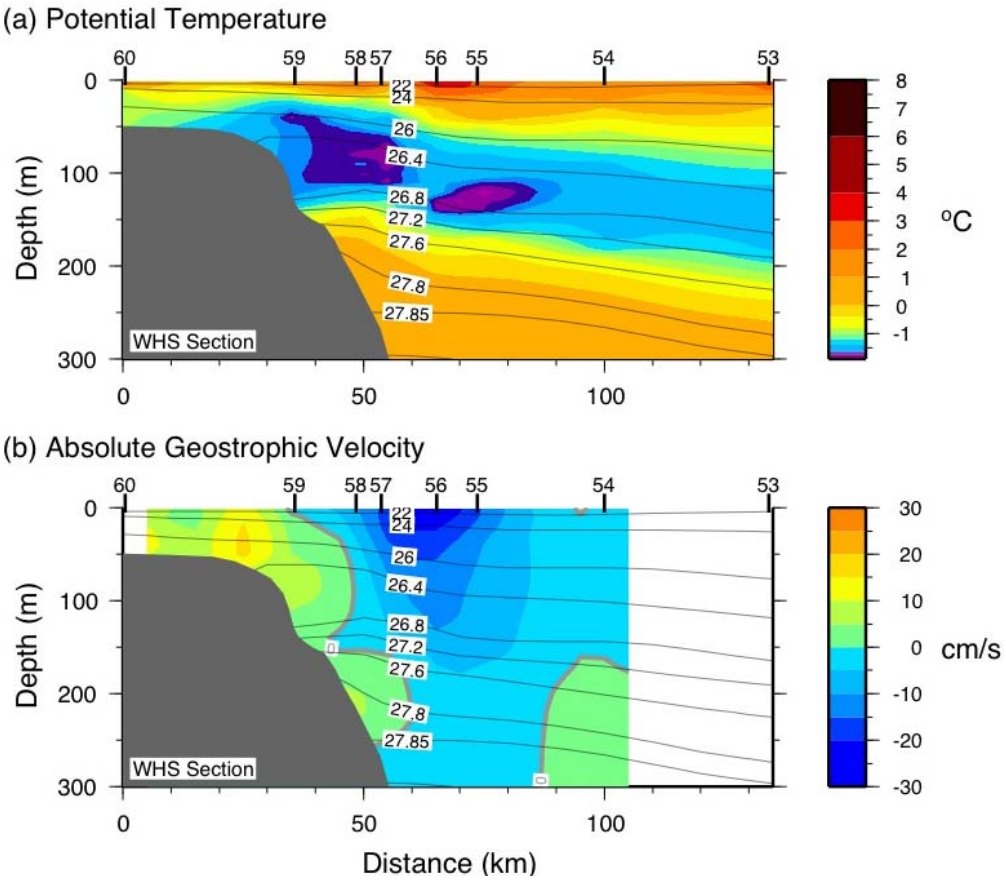


Figure 5.4 Vertical sections along the summer 2004 WHS transect (see Figure 2b for location). The potential density contours (kg/m^3) are overlaid on the colored (a) potential temperature and (b) absolute alongstream geosrophic velocity. Positive flow is eastward. The CTD station positions are indicated along the top.

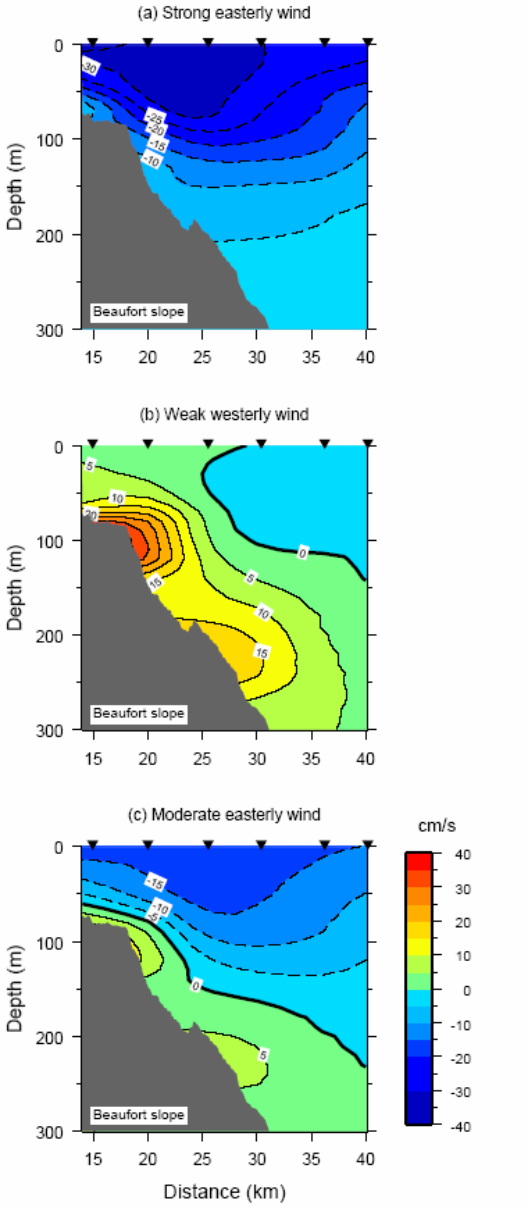


Figure 5.5 Reconstructed alongstream velocity from the SBI Beaufort slope mooring array (see Figure 2a for location) using the upwelling EOF results of Nikolopoulos and Pickart (2007). In each case a particular value of the mode has been added back into the mean field to produce a realization. Positive flow is eastward, and the mooring locations are indicated along the top. (a) Realization corresponding to a strong easterly wind (modal amplitude equal to +1 standard deviation, see Nikolopoulos and Pickart (2007)). (b) Realization corresponding to a weak westerly wind (-1 standard deviation modal amplitude). (c) Realization that most closely matches the calculated WHS absolute geostrophic velocity section. This corresponds to a moderate easterly wind (see text).

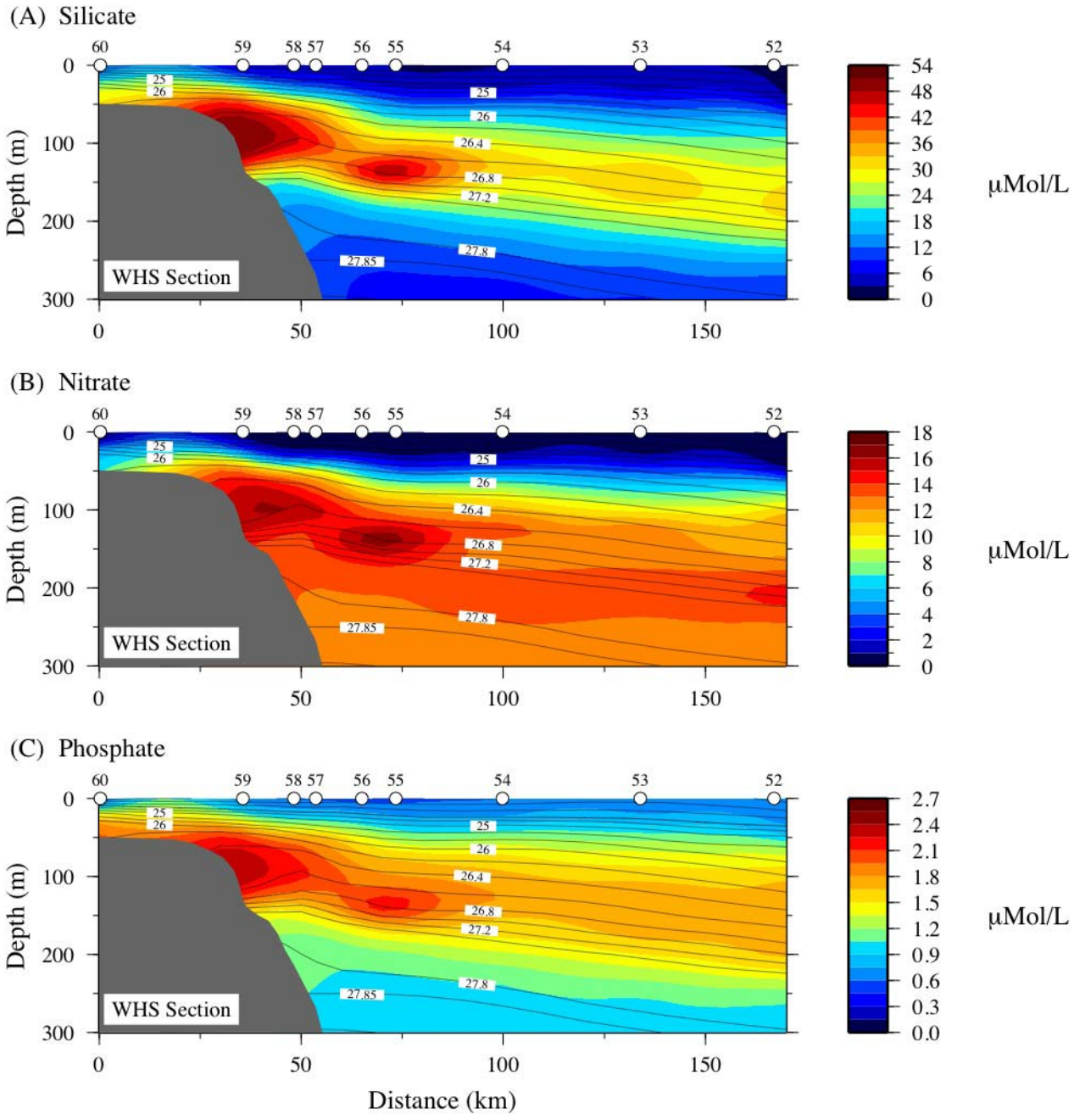
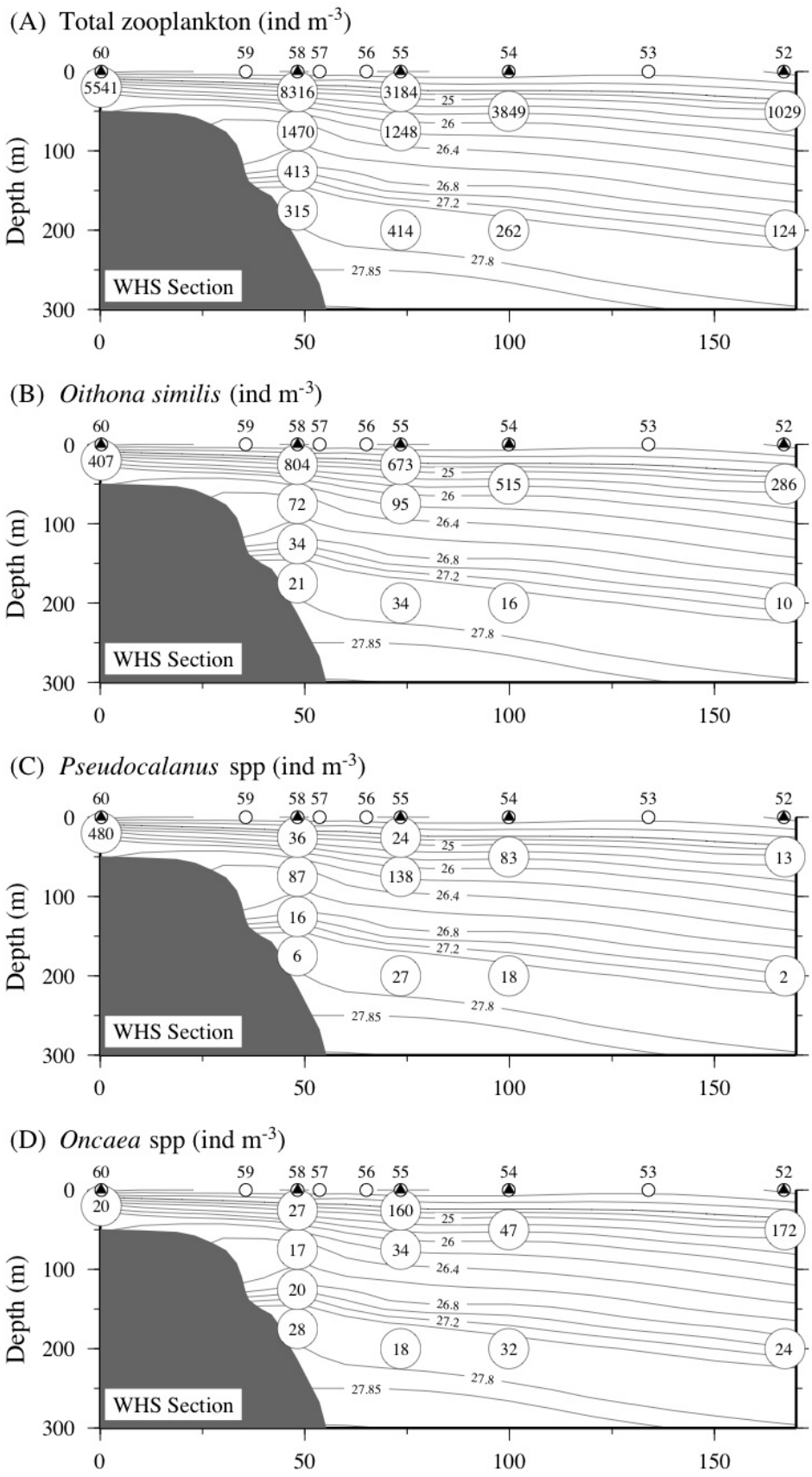


Figure 5.6 Summer 2004, West Hanna Shoal vertical section. The potential density contours (kg/m^3) are overlaid on the colored vertical sections of (A) silicate (µMol/L), (B) nitrate (µMol/L), and (C) phosphate (µMol/L). Hydrographic stations are plotted as white circles (see Figure 2B).


(A) Total zooplankton (ind m-3)
(B) Oithona similis (ind m-3)
(C) Pseudocalanus spp (ind m-3)
(D) Oncaea spp (ind m-3)
60 59 58 57 56 55 54 53 52
Depth (m)
0 100 200 300
0 50 100 150
WHS Section
25
26
26.4
26.8
27.2
27.8
27.85
5541 8316 3184 3849 1029
1470 1248
413
315 414 262 124
407 804 673 515 286
72 95
34
21 34 16 10
480 36 24 83 13
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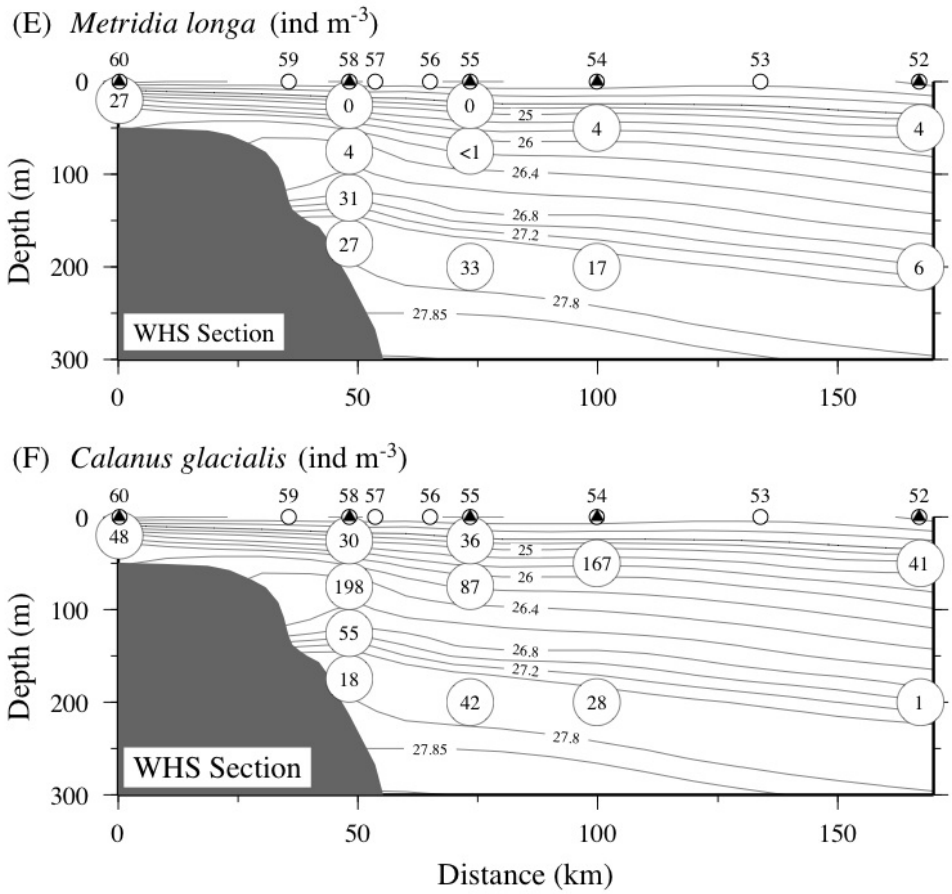


Figure 5.7 Summer 2004, West Hanna Shoal vertical distribution of zooplankton abundances (ind m^{-3}). The potential density contours (kg/m^3) are overlaid on the vertical sections of (A) total zooplankton, (B) *Oithona similis*, (C) *Pseudocalanus* spp., (D) *Oncaea* spp., (E) *Metridia longa*, and (F) *Calanus glacialis*. Numbers indicate abundance values. Stations where zooplankton samples were collected are plotted as black triangles (see Figure 2B).

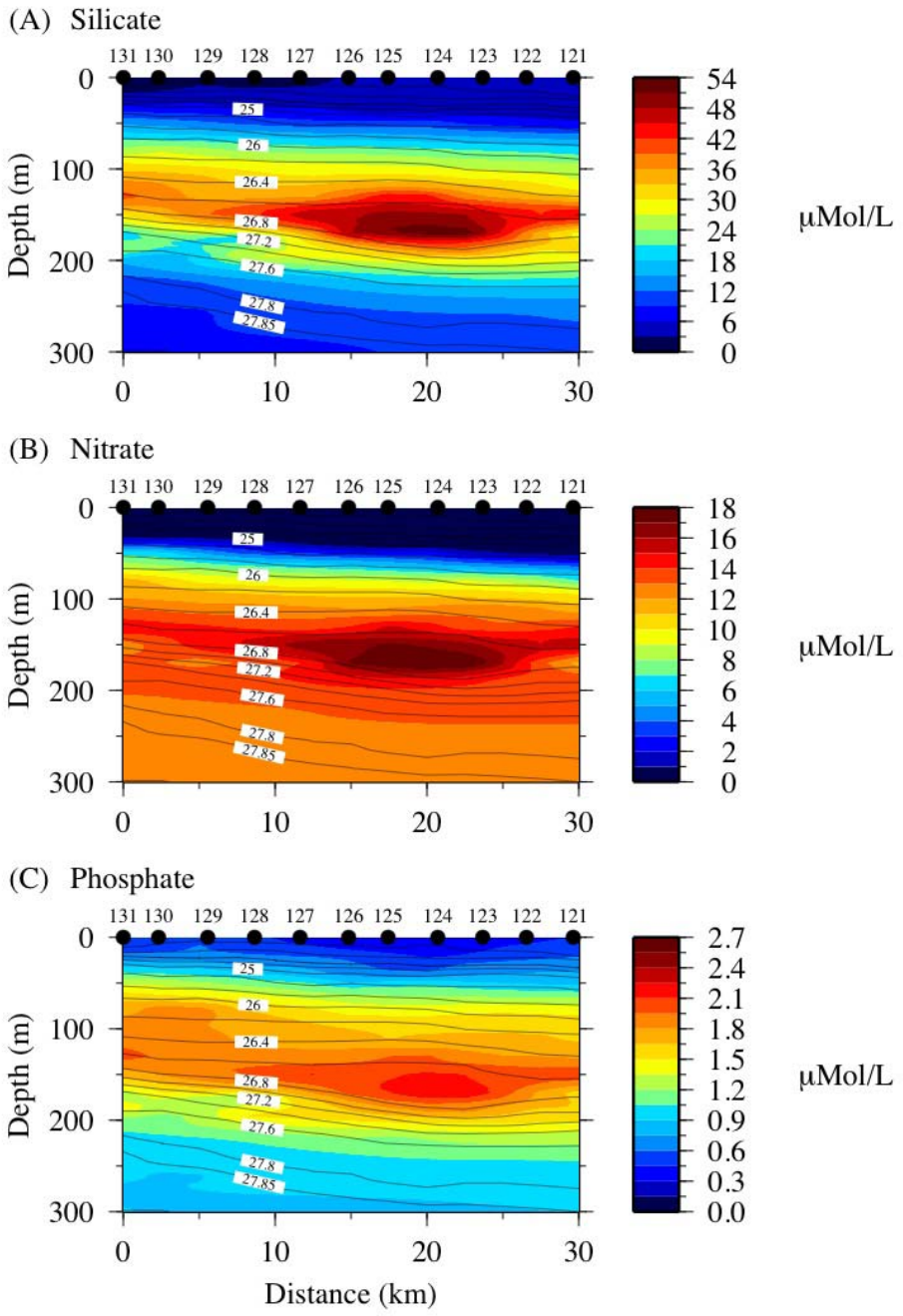


Figure 5.8 Fall 2004, eddy vertical section of chemical properties. The potential density contours (kg/m^3) are overlaid on the colored vertical sections of (A) silicate (µMol/L), (B) nitrate (µMol/L), and (C) phosphate (µMol/L). Hydrographic stations are plotted as black circles (see Figure 2B).

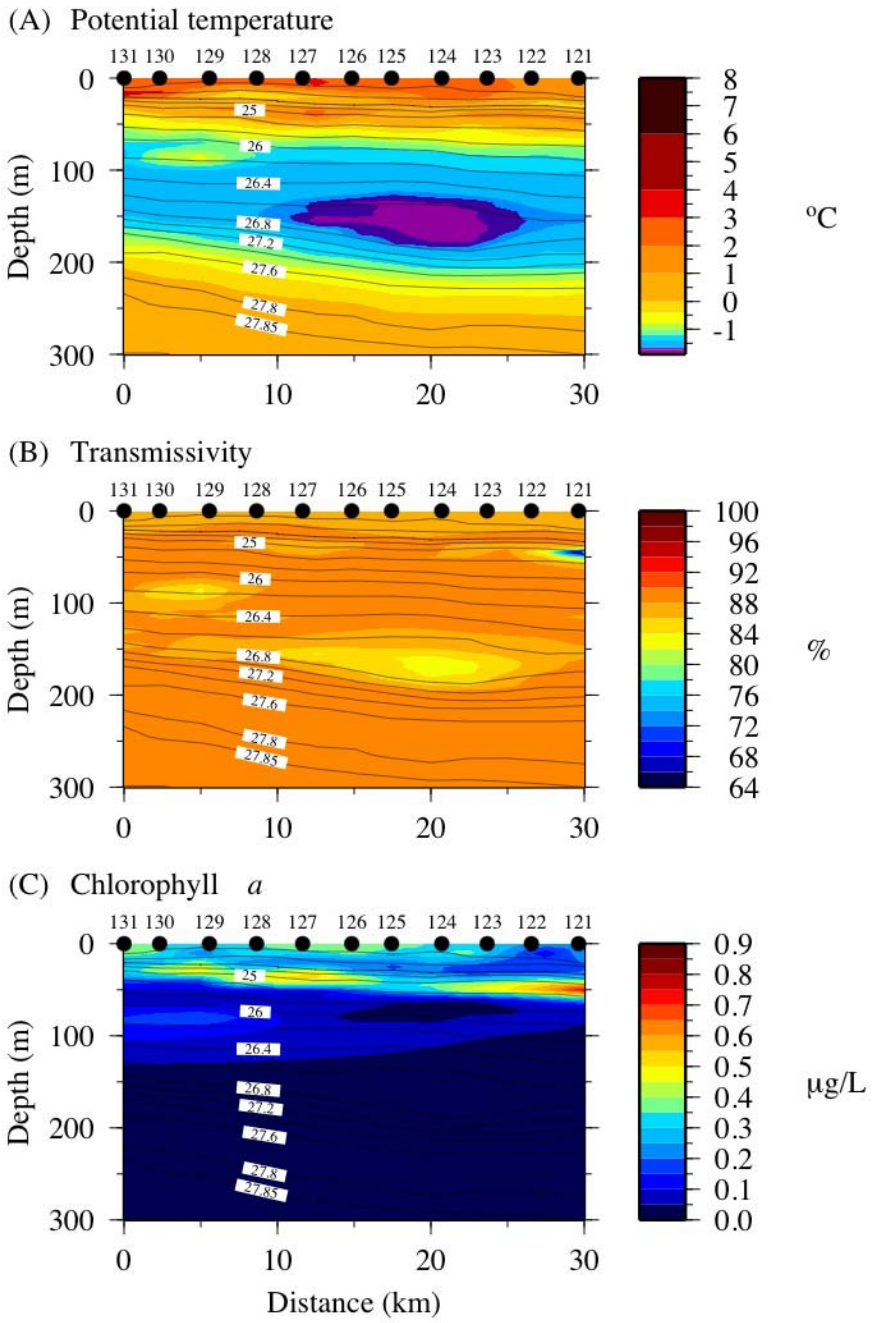


Figure 5.9 Fall 2004, eddy vertical section of physical and biological properties. The potential density contours (kg/m^3) are overlaid on the colored vertical sections of (A) temperature (°C), (B) transmissivity (%), and (C) chlorophyll *a* (µg/L). Hydrographic stations are plotted as black circles (see Figure 2B).

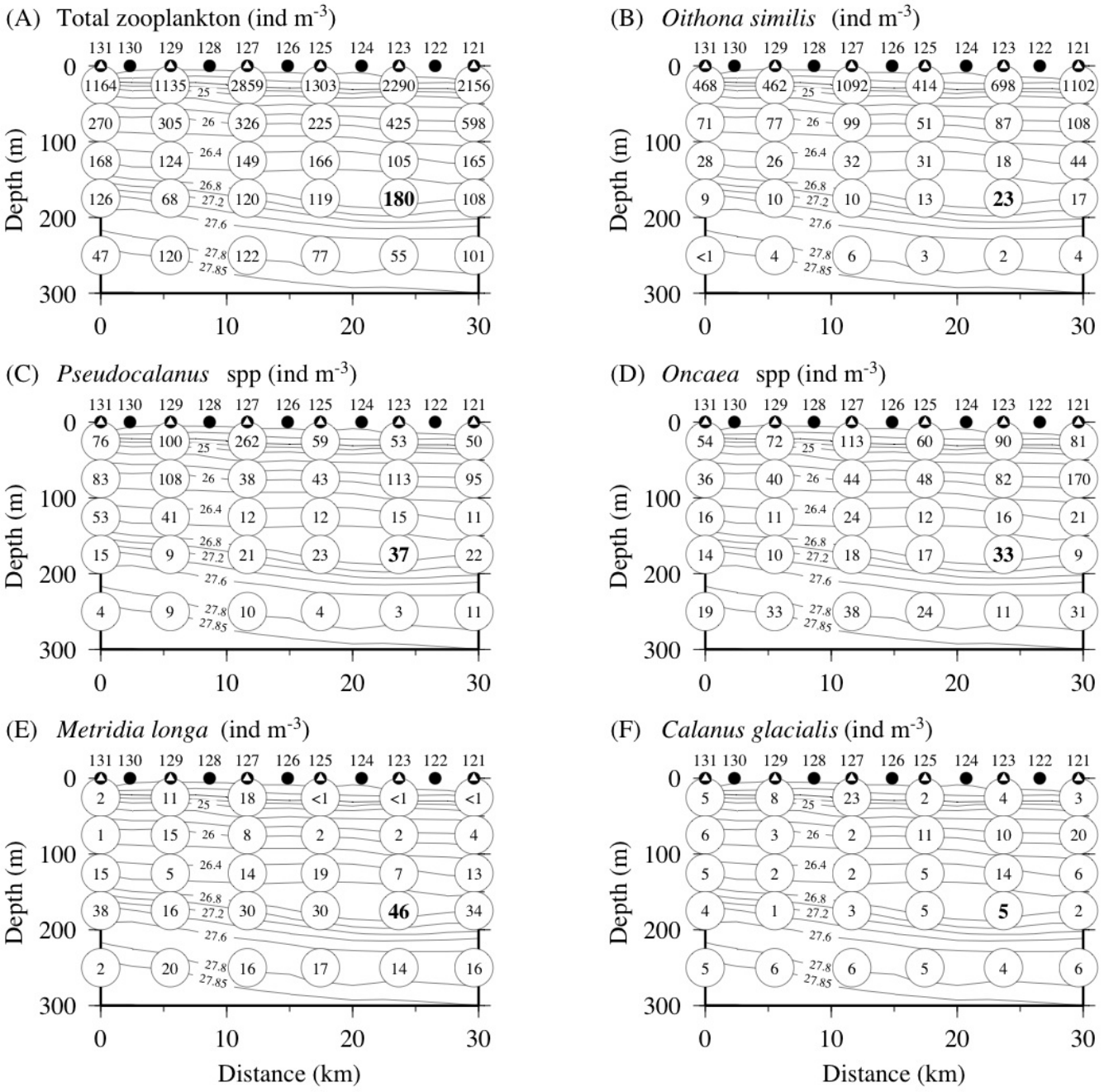


Figure 5.10 Fall 2004, eddy vertical section of zooplankton abundances (ind m^{-3}). The potential density contours (kg/m^3) are overlaid on the vertical sections of (A) total zooplankton, (B) *Oithona similis*, (C) *Pseudocalanus* spp., (D) *Oncaea* spp., (E) *Metridia longa*, and (F) *Calanus glacialis*. Numbers indicate abundance values and the abundance inside the eddy is highlighted in bold. Stations where zooplankton samples were collected are plotted as white triangles (see Figure 2B).

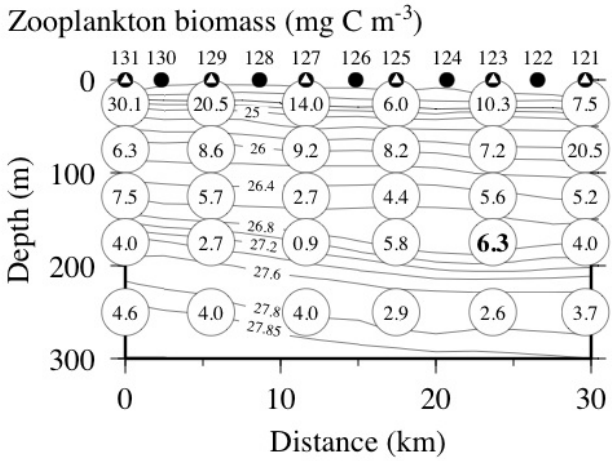


Figure 5.11 Fall 2004, eddy vertical section of zooplankton biomass (mg C.m^{-3}). The potential density contours (kg/m^3) are overlaid on the vertical section zooplankton biomass. Numbers indicate biomass estimates and the biomass inside the eddy is highlighted in bold. Stations where zooplankton samples were collected are plotted as white triangles (see Figure 2B).

CHAPTER 6: SUMMARY AND CONCLUDING REMARKS

In the preceding chapters new insights into the ecology and structure of zooplankton communities in the Chukchi and Beaufort seas and Canada Basin are provided as is a synthesis of what is currently known about this Arctic ecosystem. This final chapter presents a synopsis of the work and places the insights within a pan-arctic perspective. Although the work focuses on the zooplankton community of the western Arctic, many of the findings presented apply to other regions of the Arctic Ocean and the world. This chapter also discusses the challenges ahead with respect to achieving further understanding of how climate variation affects the functioning of marine ecosystem in the Arctic.

EXTRAPOLATION TO OTHER ARCTIC REGIONS

It is now widely accepted that global warming is occurring and that rising temperatures have substantial implications for high-latitude ecosystems. Sea ice and ocean circulation help control water column stratification, marine productivity, and ocean biology. Warming will alter the sea ice distribution and so change the stratification and circulation, influence biological productivity of polar seas, and possibly affect the many people dependent upon arctic resources. Our ability to forecast these changes is limited by our knowledge of how biological cycles are affected by physical forcing. Extrapolation of the results from this work to other Arctic ecosystems will provide a better understanding of Arctic zooplankton necessary to model and predict possible responses to future climatic change. The discussion below is centered on the transport of zooplankton by eddies, currents and other mesoscale processes common to frontal

systems in the western and eastern Arctic. First, the biogeographic distributions of *Pseudocalanus* species in the western Arctic and in the North Atlantic Ocean are compared. Then, the life cycle of *Calanus finmarchicus* in the Norwegian Sea is contrasted with the life cycle of *C. glacialis* in the Chukchi Sea in relation to the circulation pattern of each region. Lastly, the Barents Sea is used as a guide to understand the Chukchi and Beaufort seas.

Western vs. eastern Arctic

The results obtained are relevant to other Arctic frontal systems, despite the fact that frontal systems in the western Arctic have considerably different characteristics than those in the eastern Arctic. Fronts in the eastern Arctic involve southward flow of Arctic water and an adjacent northward flow of Atlantic water confined to the same channel (e.g. Fram Strait). In the western Arctic there is no significant southward flow, yet the periodic southward flows associated with upwelling events are important for the transport of arctic zooplankton onto adjacent shelves. Another difference is the strong association of marginal ice zones with fronts and current jets in the eastern Arctic, whereas in the western Arctic the ice-edges migrate meridionally over a far greater distance, so ice-edge related fronts are correspondingly impermanent (Muench, 1990). Ice-edges on both Arctic regions are physical boundaries and their location can have considerable effects on the ecosystem.

Pseudocalanus distributions

The patterns of abundance and distribution of *P. minutus* and *P. mimus* nauplii in the western Arctic suggest that these two species are ecologically distinct despite being

nearly indistinguishable based on morphological characters. Although both species were important components of the community of nauplii, their distributions differed significantly. *Pseudocalanus minutus* nauplii were found in all shelf, slope, and basin samples and declined in numbers with increased water column depth. In contrast, the spatial extent of *P. mimus* was limited to the shelf and slope regions. Similar biogeographic distributions have been observed between the sibling species *Pseudocalanus moultoni* and *P. newmani* on Georges Bank in the North Atlantic Ocean (Bucklin et al., 2001). This raises important questions about the distribution and abundance of *Pseudocalanus* in high-latitude ecosystems that require molecular examination of larval and juvenile life stages of each *Pseudocalanus* species. What is the seasonal timing and spatial location of reproduction and recruitment of each species? What physical, chemical, and environmental parameters shape the life cycle strategies of each species? What *Pseudocalanus* species is better adapted to survive in a warming Arctic? Answers to these questions are necessary to develop physical-biological models that simulate the interaction between circulation patterns and each species life history. These models can then be used to study the extent to which climate change will affect the abundance and distribution of different copepod species.

The elevated numbers of *P. minutus* nauplii in summer and the presence of *P. minutus* nauplii in surface waters over the Canada Basin, indicate that reproduction in this species is timed to take advantage of the spring bloom and that the species is also able to exploit primary production derived from the sea-ice. *Pseudocalanus minutus* has thus flexible strategies and is well adapted to the Arctic ecosystem. A warming of the Arctic and a higher inflow of Pacific water as predicted by most climate models (IPCC, 2007)

however will favor the subpolar species *P. mimus*. This hypothesis is supported by studies showing that *P. mimus* is the dominant *Pseudocalanus* species in warmer shelf ecosystems (Napp et al., 2005) and data presented herein. Chapter 3 shows evidence of a northward range expansion of this species during 2004, a warm year with large inflow of Alaskan Coastal Water (Codispoti et al., submitted).

Similar life cycles

In the Norwegian Sea, the abundance and distribution of *C. finmarchicus* is influenced by the creation and persistence of mesoscale features. As overwintering individuals ascend in spring, they are directed by surface currents to the shelf areas (Bryant et al., 1998). During spring and summer, *C. finmarchicus* feeds and reproduces in the upper layer of the water column, and in July stage five copepodids (CV) move to deep water and enter diapause (Marshall and Orr, 1955). Some areas of the Norwegian Sea are capable of supporting self-sustaining populations of *C. finmarchicus* through the interaction between the seasonal ontogenetic migration and the shallow and deep circulation of the eastern North Atlantic and Norwegian Sea (Bryant et al., 1998; Halvorsen et al., 2003). Similarly, in the outer shelf and slope areas of the Chukchi Sea, the abundance and distribution of *C. glacialis* is influenced by mesoscale processes. On-shelf flows transport late copepodids onto the Chukchi shelf from the Canada Basin, where they grow and reproduce (Plourde et al., 2005). By late summer/early fall, canyon currents, filaments and eddies carry the C5 copepodids back to their overwintering habitat, the deep Canada Basin. Results presented here and the elevated numbers of *C. glacialis* in the Chukchi and Beaufort seas (Lane et al., 2007) suggest that the population of *C. glacialis* in the Chukchi and Beaufort seas is also self-sustained; however, this

conclusion should be now explored through a particle-tracking model to determine the fate of local populations.

As depicted in Figure 6.1, circulation processes in the Chukchi Sea can return individual copepods to their original location. During late spring/early summer, the bulk of the egg production in *C. glacialis* occurs over the slope region (Chapter 4; Figure 6.1A). Hatching occurs shortly after and individuals rapidly go through the usual stages of the *C. glacialis* life cycle (nauplia N1-N6 and copepodid C1-C5) fueled by the elevated shelf-derived primary production. This is supported by the elevated numbers of *C. glacialis* nauplii and copepodid stages found over the slope (Chapter 3; Lane et al., 2007; Smith, unpublished data). By late summer/early fall, canyon currents, filaments and eddies carry the C5 copepodids to the Canada Basin (Chapter 5; Figure 6.1B), where the population migrates to deep waters to overwinter. The clockwise circulation of the Beaufort Gyre retains overwintering individuals in the Canada Basin during the winter season (Figure 6.1C). The Beaufort Gyre can potentially play an equivalent retention role to the Norwegian Sea gyres (Bryant et al., 1998) since water mass movement within the Beaufort Sea should conform to the mean clockwise circulation pattern of the gyre (Manley and Hunkins, 1985). By spring/ early summer next year, upwelling of deeper basin water onto the shelf in response to easterly winds transports some populations of C5 copepodids back to their original location, the slope region (Chapter 5; Figure 6.1D).

At the same time, another hypothesis can be formulated stating that any production of this species in the Chukchi Sea can be exported to adjacent regions (Figure 6.1D). Populations retained by the Beaufort Gyre will be exported to the Arctic Basin. These populations can survive by feeding under ice (Chapter 4 and references therein).

The exported populations can potentially return to the Chukchi Sea after completing 2 or 3 generations in the Arctic Basin because the Beaufort Gyre makes one complete rotation about every 4 years (Steele et al., 2004). This is highly speculative and the connectivity of *C. glacialis* populations in the Arctic Ocean remains unstudied. Circulation patterns (Plueddemann et al., 1998; Macdonald et al., 2004) suggest that adjacent regions such as the Canadian archipelago and the Beaufort Sea or the Beaufort Sea and the Chukchi Sea allow individual organisms to easily move between these regions and that boundary currents can potentially advect organisms from the eastern Arctic to the western Arctic and vice versa.

Copepods of the Barents Sea in the context of the Arctic Ocean

The Barents Sea is connected to the Norwegian Sea as well as the Arctic Ocean. The Atlantic water inflow into the Barents Sea between Norway and Bear Island is about 2 Sv (Ingvaldsen et al., 2002), more than twice the mean transport of Pacific water through Bering Strait. The bathymetry of the Barents Sea guides water mass transport, and the Atlantic water flows along the Norwegian continental slope as the North Cape Current. Similarly, Pacific-origin water is steered by shelf canyons, and flows eastwards along the slope of the Chukchi and Beaufort seas. Atlantic water is separated from colder and fresher Arctic water from the north by the Polar Front (Pfirman et al., 1994). In the region of the Polar Front in the eastern Arctic, Atlantic water transports the sub-arctic Atlantic copepod species *C. finmarhicus* while Arctic water carries *C. glacialis* (Grainger, 1963; Unstad and Tande, 1991). In the western Arctic, Arctic water also carries *C. glacialis* and Pacific water transports *C. marshallae* (Frost, 1974). In both the eastern and western Arctic, sub-arctic water is generally the most plankton-rich water

mass in terms of biomass. Mixed waters generally have intermediate amounts of plankton, and Arctic water has the lowest plankton biomass. The Barents Sea, which has been continuously studied over the last thirty years, provides an analogue that can be used as a guide for understanding the Chukchi and Beaufort seas. In the Barents Sea, zooplankton biomass has been shown to vary in relation to climatic conditions (Sakshaug et al., 1994; Dalpadado et al., 2003). The zooplankton biomass in warm years is generally higher than in cold years when lower Atlantic inflow transports fewer planktonic organisms a situation that in turn affects abundance of capelin, cod, seabirds and marine mammals. Similar effects in higher trophic levels in the pelagic ecosystem of the Chukchi and Beaufort seas may occur in response to variability of the Pacific inflow.

CHALLENGES AND SUGGESTIONS

A fundamental conclusion of this work is that changes associated with temperature, circulation patterns, and ice cover will likely affect the composition of the zooplankton community in the western Arctic. Alterations in the zooplankton community structure could disrupt the functioning of the contemporary food webs on these Arctic shelves (Hirche et al., 2006) and adversely impact the native populations that depend on these marine resources. Given that the real value of climate research is to prepare society for possible futures, and that such research must be based both on an understanding of the past and present as well as an ability to reliably predict future scenarios (Carmarck et al., 2006), three recommendations emerge from this dissertation. First, the role of mesoscale processes in the functioning of the Arctic ecosystem is one of the largest gaps in our knowledge and needs further investigation. Second, validation data for western Arctic marine ecosystems is required to properly model key forcing processes at the shelves,

slope and basin regions. Third, to move forward Arctic research must work across disciplines.

Study the role of mesoscale processes

Two important mesoscale processes were discussed in the previous chapters: upwelling along continental margins and eddies. Chapter 5 substantiates the notion that upwelling events and eddies provide mechanisms for exchange of zooplankton between the Arctic shelf and basin regions, but this needs further investigation. Upwelling-favorable winds (Muenchow et al., 2006) in the Chukchi and Beaufort seas can also bring nutrient-rich water from the deep Canada Basin onto the adjacent shelves and alter the regional biogeochemistry. In spring, strong stratification in the Arctic causes rapid phytoplankton bloom development and nutrient depletion. Secondary small blooms can arise depending on the episodic replenishment of nutrients in shelf waters by upwelling events and other physical processes (Sakshaug, 2004).

The generation and occurrence of eddies also requires future investigation. Eddies in the Arctic have the potential to transport significant amounts of carbon and nutrients (Mathis et al., 2006) and could affect food webs in the less productive basin by transporting expatriates (Olli et al., 2007). However, there is still considerable work to be done to understand 1) the prominence of eddies in the western and eastern Arctic, 2) their role in the hydrographic structure of the Arctic Ocean, and 3) the effects of eddy transport in regional food webs. This study provided only a snapshot of the distribution of zooplankton inside one cold core eddy in the Arctic Ocean. To examine the evolution of the chemical and biological properties of eddies as they become incorporated into the central basin, time-series surveys should be conducted in the future. Long term

monitoring instrumentation will be required to elucidate the effects of the formation and subsequent migration of eddies on the Arctic system.

Develop biological-physical models

The complexity of marine ecosystems in the Arctic can cause intricate responses to changes in external forcing. These ecosystem readjustments may be large and perhaps have irreversible ecological consequences. In the future, the goal must be to understand and ultimately to predict how the Arctic ecosystem responds to natural and human-induced changes. The development of precise biological-physical models will only be realized if observational projects and model studies are designed and developed together with the explicit objective of predicting marine ecosystem responses to physical forcing over a range of times scales.

Regional aspects will influence the choice of key variables and relevant target species. This work pointed out the importance of temperature, ice coverage, and circulation patterns in modifying biological processes, and suggested using the calanoid copepod *C. glacialis* as an indicator species of ecosystem health in the western Arctic. This approach has been particularly effective in the North Atlantic Ocean using the target species *Calanus finmarchicus* (Tande and Miller, 2000; Beaugrand et al., 2003) to predict fisheries stocks (Wiebe et al., 2001). In the western Arctic however, much remains to be done before I can deliver long-range predictions of the responses of its populations and communities to climate change. The majority of data sets for the region are limited both temporally and spatially when compared with the general problem of the effect of climate variability on the whole ecosystem. The challenge for the future is to obtain validation

data for the biological-physical models in the western Arctic and use these models to anticipate ecosystem changes.

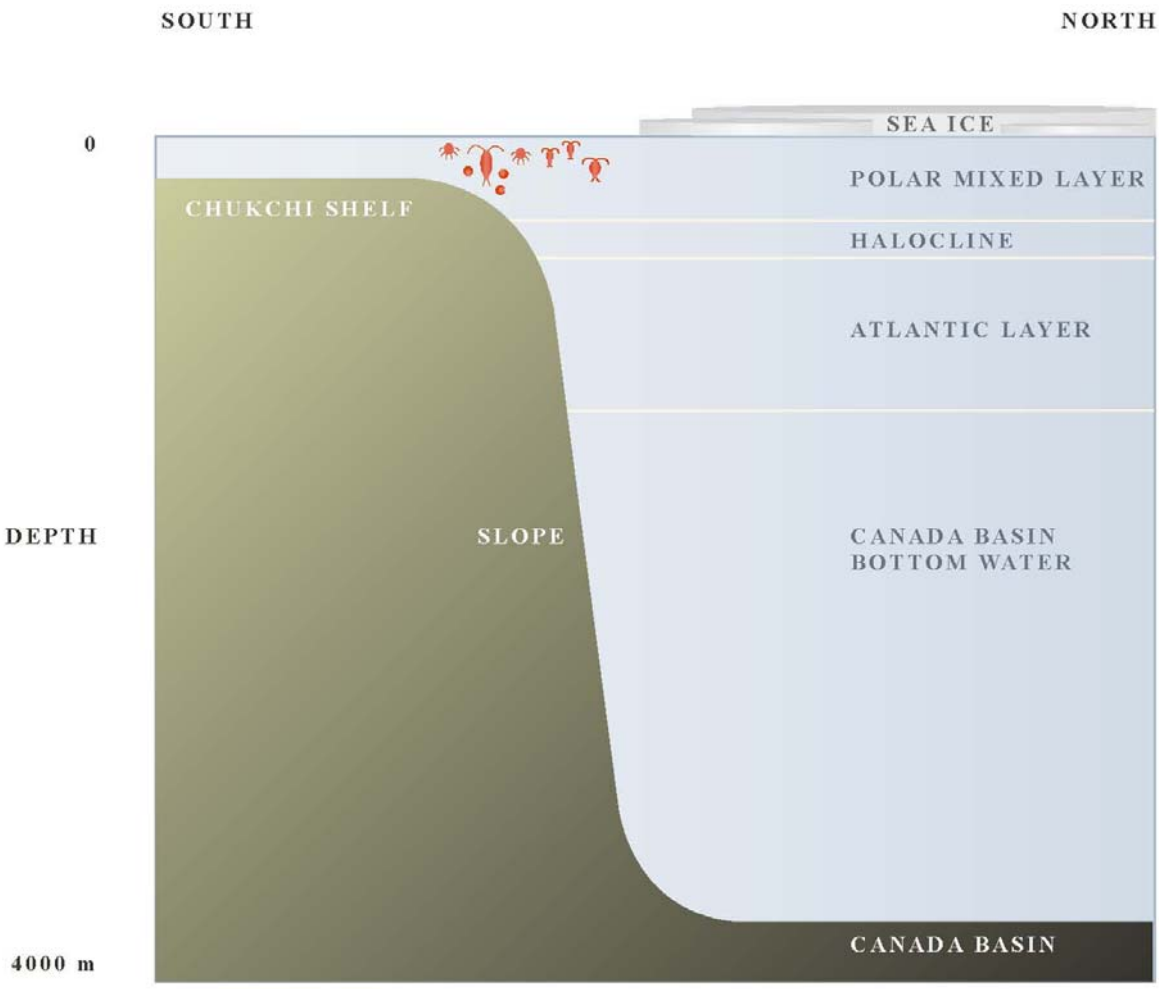
Work across disciplines

Adopting innovative and integrative approaches will be required to advance our understanding of the ecological effects of present and future climatic variations in the Arctic Ocean. While some the chapters in this dissertation describe how environmental variables can affect zooplankton, the problem of understanding the effects of climate change really requires a highly multidisciplinary approach (Ottersen et al., 2004). Future programs need to promote collaboration across the marine science communities and promote the application of new technologies and methods from the areas of remote sensing, genomics, physics, molecular biology, chemistry, and microbiology. One example is the development of biological-physical models to predict ecosystem changes.

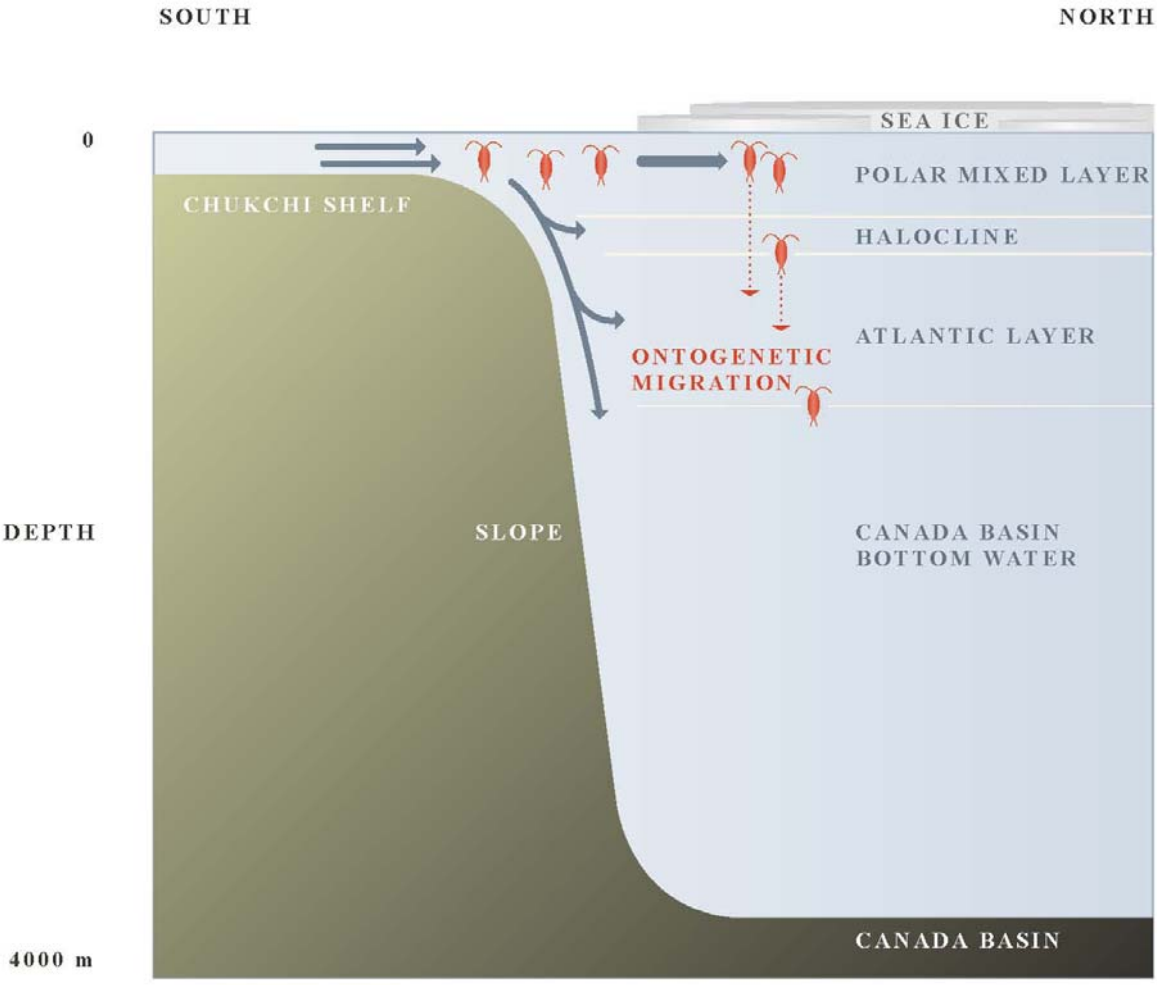
This approach is already transforming our understanding of the Arctic ecosystem. Recently, a new form of microalgae was discovered in the Arctic Ocean while analyzing DNA sequences in samples of seawater (Not et al. 2007). Analyses of 18S risomal RNA sequences reveal that the unknown organisms formed an independent phylogenetic group among major eukaryotic taxa. This discovery had profound impacts in the fields of microbial ecology and evolution and in our estimates of biodiverisity in the Arctic (Not et al. 2007). This dissertation also incorporated analyses of DNA sequences and demonstrated that the integration of molecular and microscopic data can advance our understanding of ecosystem diversity and function. This study also integrated physical and chemical properties with biological sampling to elucidate the effects of mesoscale processes on regional food webs. Such approaches represent major improvements in the

way Arctic research is conducted, and are ready to be applied to high-priority research questions that can benefit from these new tools (National Research Council, 2003).

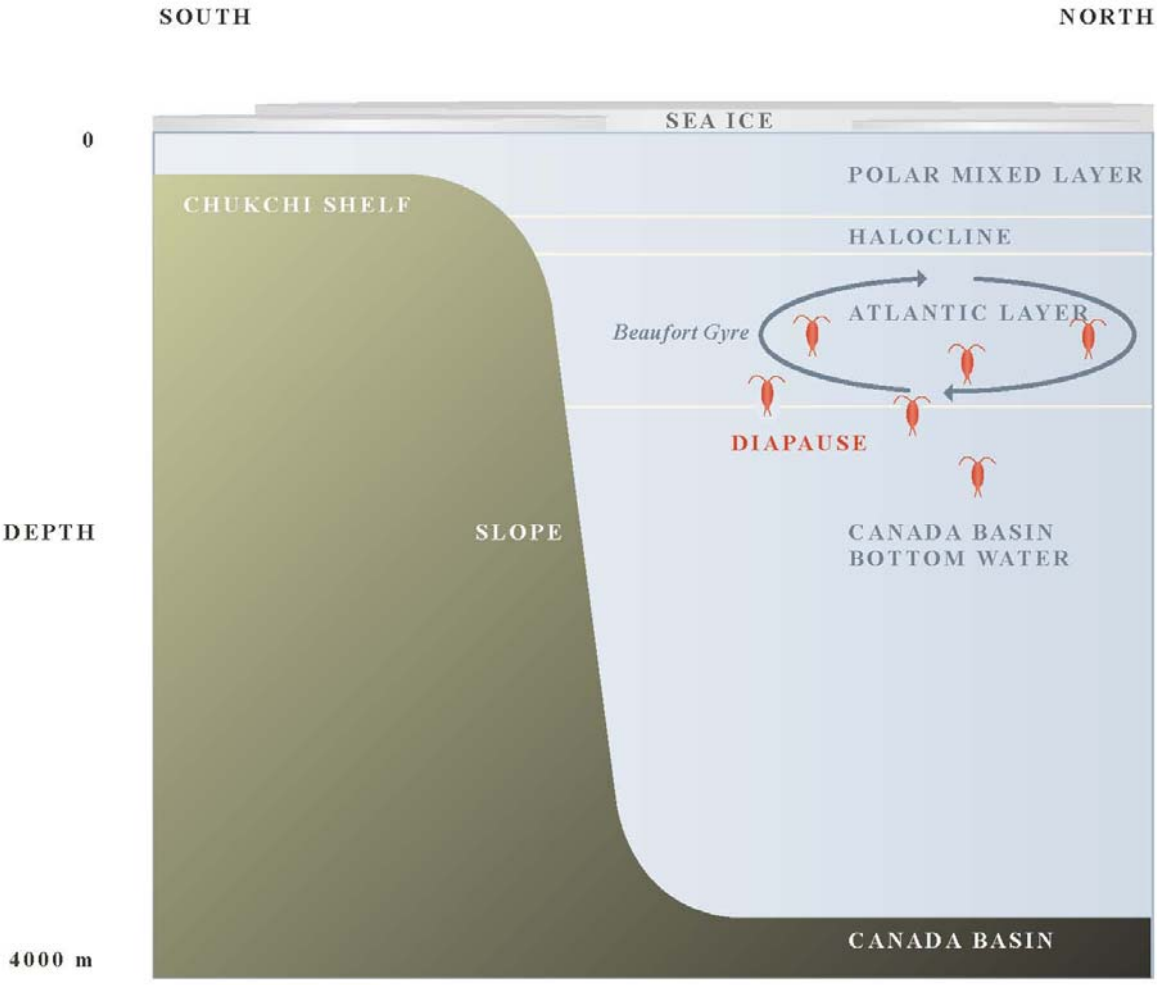
A) Late spring/early summer



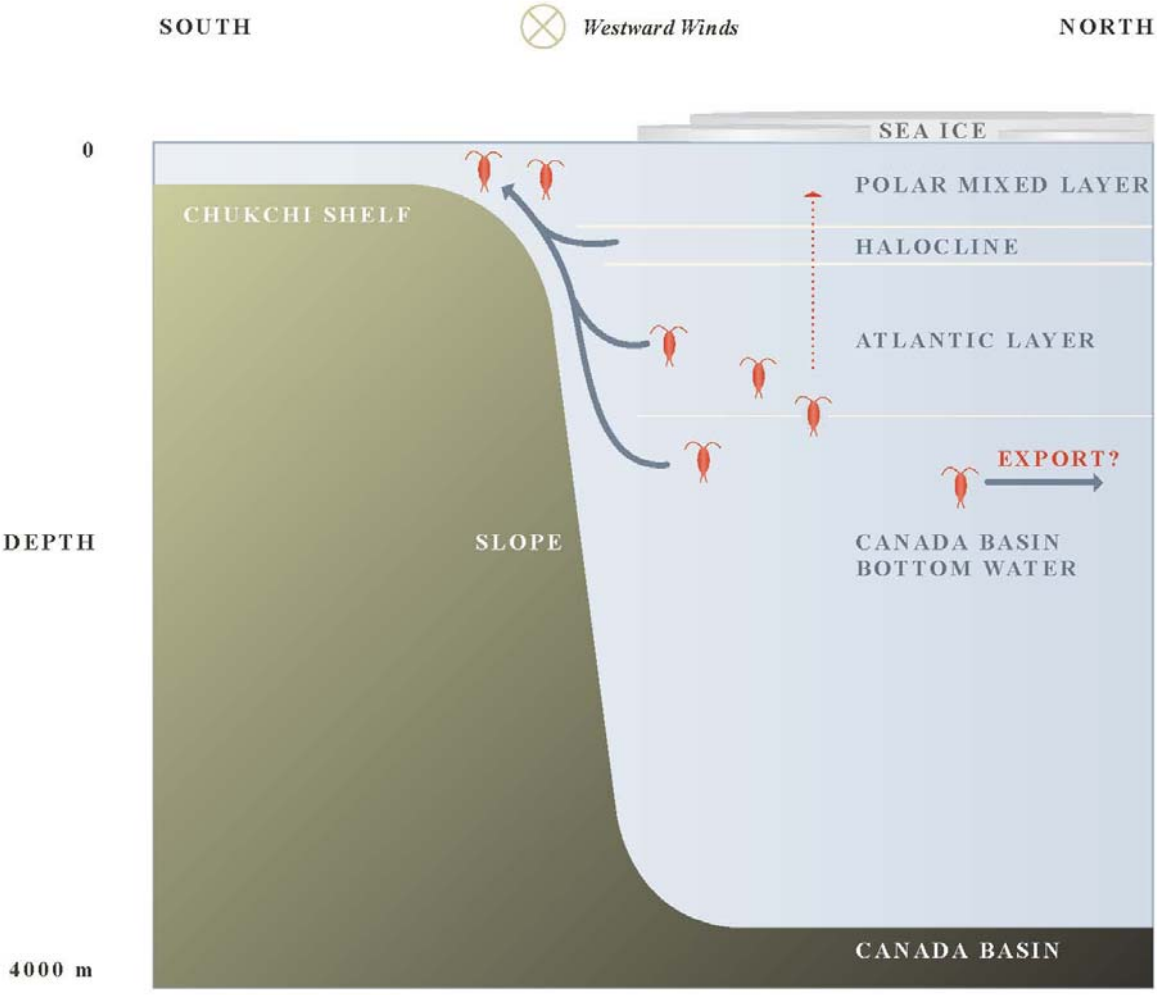
B) Late summer/early fall



C) Late fall/winter



D) Spring


SOUTH
Westward Winds
NORTH
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DEPTH
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SEA ICE
POLAR MIXED LAYER
CHUKCHI SHELF
HALOCLINE
ATLANTIC LAYER
EXPORT?
SLOPE
CANADA BASIN
BOTTOM WATER
CANADA BASIN

D) Overview

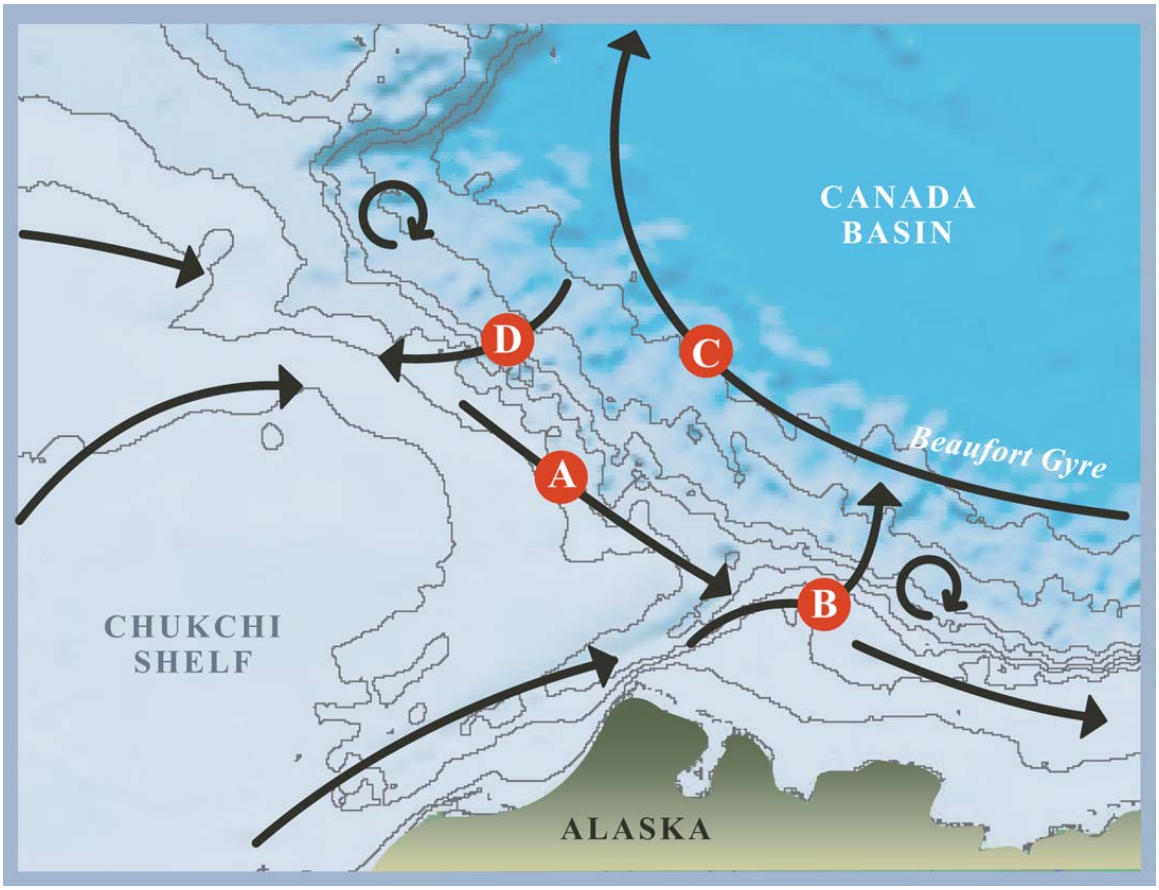

Figure 6.1 Depiction of the life cycle of the copepod *C. glacialis* in the Chukchi Sea in relation to oceanic circulation processes. A) During late spring/ early summer, the population reproduces, grows (stage egg-C4), and accumulates lipid reserves (C5). B) During late summer/ early fall, the population is advected offshore and migrates to the deep basin (ontogenetic migration). C) During late fall/ winter, the population of C5s overwinters in the deep basin (diapause). D) During spring, upwelling of deeper basin water, in response to westward winds, transports copepods back onto the shelf. E) Overview of the depicted life cycle.

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VITA

Leopoldo Llinás was born in Bogotá, Colombia on January 30, 1980, to Guillermo Llinás and Cecilia Rocha. He has two younger siblings, his sister Andrea and his brother Guillermo. After graduating from the Lycée Français Louis Pasteur in 1998, he attended the Universidad de los Andes for two years. In 2000, he transferred to the University of Miami, USA, to fulfill his long time dream of studying Marine Biology. He spent the last year of his undergraduate working at the Rosenstiel School of Marine and Atmospheric Sciences.

In June 2002, he was admitted to the Rosenstiel School and shortly after he departed on his first research cruise to the Arctic. He was granted the degree of Doctor of Philosophy from the Graduate School of the University of Miami in December 2007. Leopoldo is currently employed as a science teacher at Palmer Trinity High School in Miami, Florida.