

UiT

THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty Bioscience, Fisheries and Economics
Department of Arctic and Marine Biology

UNDERSTANDING WINTER PATTERNS OF ZOOPLANKTON DIEL VERTICAL MIGRATION (DVM) IN A HIGH ARCTIC FJORD (KONGSFJORDEN, SVALBARD)

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Julie Cornelius Grenvald

A dissertation for the degree of Philosophiae Doctor – January 2017



Thysanoessa inermis
Hopcroft/UAf/NOAA/CoML

5000 µm



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The University Centre in Svalbard



The Arctic University of Norway



SUPERVISORS

Prof. Jørgen Berge (main supervisor)
The Arctic University of Norway (UiT)
Department of Arctic and Marine Biology

Assoc. prof. Tove M. Gabrielsen (co-supervisor)
The University Centre in Svalbard (UNIS)
Department of Arctic Biology

Prof. Paul E. Renaud (co-supervisor)
Akvaplan Niva (APN)
Department of Arctic R&D

Dr. Finlo Cottier (co-supervisor)
The Scottish Association for Marine Science (SAMS)
Environmental Physics and Autonomous Technology Group

Prof. Torkel Gissel Nielsen (co-supervisor)
DTU AQUA, National Institute of Aquatic Resources
Section for Marine Ecology and Oceanography

PREFACE

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To study the literature on DVM in the marine environment is like a "mer à boire"
(Joop Ringelberg)

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SUMMARY

Recent Arctic studies contradict the long-held paradigm of winter quiescence and document activity levels in the marine food web even during the darkest months of winter. Acoustic studies in the high Arctic Kongsfjorden (Svalbard) show that some species within the zooplankton community exhibit vertical migrations throughout the dark winter, a phenomenon that has attained considerable attention. However, previous studies have failed to unequivocally identify which species are actually performing these winter migrations. It is furthermore unclear why zooplankton organisms migrate in a seemingly constant dark environment. Despite that the sun is below the horizon for several months during polar night, there is evidence that some zooplankton species are able to perceive low levels of solar background irradiance. It remains unknown whether the species responsible for the acoustic patterns are able to detect light, and thus drive mid-winter migrations and/or if biological rhythms are involved. Finally, the ecological implications of polar night migrations in functioning of the biological pump are less studied during winter. It is essential to gather baseline data during the polar night period to understand the global significance (if any) of winter migrations relative to the biochemical cycle in a warming Arctic.

Acoustic data derived from Acoustic Doppler Current Profilers (ADCPs) and Acoustic Zooplankton and Fish Profilers (AZFPs), supplemented by extensive plankton net sampling during winter, were used to examine patterns of Diel Vertical Migration (DVM) throughout the polar night period in Kongsfjorden (Svalbard) and to characterize responsible zooplankton species behind winter migrations (**Paper I, IV**). Light measurements were conducted to derive atmospheric light levels present during polar night (**Paper III**). Spectral sensitivity tests were performed to investigate zooplankton visual systems and capability to detect low light levels underwater during mid-winter (**Paper III**). Expression levels of a circadian clock gene identified from the Antarctic krill were examined in *Thysanoessa* spp. krill both during a laboratory experiment and in wild-caught krill individuals to evaluate if circadian rhythms in the clock gene were involved in observed polar night migration patterns (**Paper II**). Vertical fluxes of carbon and nitrogen of dominant polar night migrants (respiration and excretion) were studied to investigate ecological impacts of zooplankton migrations over the transition from winter to autumn (**Paper IV**).

The results showed that polar night DVM is a complex phenomenon that involves different migration patterns (**Paper I, IV**). It is evident, despite extreme low light levels, that light is the proximate cue for polar night migrations. Classical DVM was present during civil twilight and zooplankton performed vertical migration throughout a large part of the water column. The DVM signal became gradually stronger with the establishment of more distinct day-night cycles (**Paper I, IV**). During mid-winter (astronomical and nautical twilight) classical DVM was mostly absent. Instead, migration patterns were restricted to surface waters, of unsynchronized character, and not strictly coupled to the diel cycle (**Paper I**). Mid-winter migrations became periodically of more synchronized character (**Paper I**) possible due to enhanced light sources other than solar (i.e. lunar light). Zooplankton net sampling, in parallel with acoustics revealed that copepods, chaetognaths and krill were dominant species (**Paper I, IV**) but krill dominated the zooplankton biomass (**Paper IV**) and were

responsible for the observed acoustic patterns during polar night. Spectral sensitivity tests showed that krill (*T. inermis*) were able to perceive solar background illumination down to 20 m depth during winter (**Paper III**). The lack of rhythmicity in the clock gene of krill caught during polar night (**Paper II**) implied that the available light sources (**Paper III**) were not sufficient to entrain endogenous circadian rhythms in krill. Thus the molecular and electrophysiological experiments (**Paper II, III**) suggested that polar night migration was exogenously governed by ambient light levels, when these were at a certain threshold, rather than endogenously controlled. Predation pressure was not evaluated in the present study but it may be that the ultimate driver for the periodically more synchronized migration patterns was a response by zooplankton to avoid predators that would have higher prey-catch success in more light influenced phases. The fact that classical DVM occurred outside of the short season of high photosynthesis, and that DVM was discontinuous over the seasonal cycle (**Paper IV**), likely limits its function in the biological pump of Arctic ecosystems if an annual budget is to be estimated. A tighter coupling between DVM and bloom periods is expected as a consequence of warming in Arctic. Stronger stratification and northward advection of krill are factual effects of climate warming. It is therefore anticipated that the role of DVM, in the functioning of the biological pump, particularly where krill are or will be prevalent in the zooplankton community, will be enlarged.

The combined use of acoustic data and zooplankton net sampling served to identify responsible migrants behind the winter acoustic patterns. This study presents a very first glimpse into a biosphere in which the dominant light source appears to trigger a response in zooplankton species, which does not always follow a diurnal pattern. A thorough investigation of the proximate and ultimate drivers, such as a better knowledge of the absolute light climate during polar night and the physiological-behavioral mechanisms of polar night active species is needed to fully understand how polar night migrational patterns are governed and guided.

List of papers

- I. **Grenvald JC**, Callesen TA, Daase M, Hobbs L, Darnis G, Renaud P, Cottier F, Nielsen TG, Berge J (2016) Plankton community composition and vertical migration during polar night in Kongsfjorden. *Polar Biology* 39(10): 1879–1895. doi:10.1007/s00300-016-2015-x
- II. **Grenvald JC**, Vader A, Reinardy H, Berge J, Renaud P, Gabrielsen TM (manuscript) Does the cryptochrome 2 gene (*cry2*) act as a circadian clock gene in krill (*Thysanoessa* spp.) during polar night in Kongsfjorden (Svalbard)?
- III. Cohen JH, Berge J, Moline MA, Sørensen AJ, Last K, Falk-Petersen S, Renaud PE, Leu ES, **Grenvald JC**, Cottier F, Cronin H, Menze S, Norgren P, Varpe Ø, Daase M, Darnis G, Johnsen G (2015) Is ambient light during the high Arctic polar night sufficient to act as a visual cue for zooplankton? *PLoS ONE* 10 (6): e0126247. doi: 10.1371/journal.pone.0126247
- IV. Darnis G, Hobbs L, Geoffroy M, **Grenvald JC**, Renaud P, Berge J, Cottier F, Kristiansen S, Daase M, Søreide J, Wold A, Morata N, Gabrielsen TM (accepted) From polar night to midnight sun: diel vertical migration, metabolism and biogeochemical role of zooplankton in a high Arctic fjord (Kongsfjorden, Svalbard). *Limnology and Oceanography*

Abbreviations

ADCP	Acoustic Doppler Current Profiler
AZFP	Acoustic Zooplankton Fish Profiler
Chl <i>a</i>	Chlorophyll <i>a</i>
<i>cry2</i>	Cryptochrome 2
CTD	Sensor that measure conductivity (salinity), temperature, density
DVM	Diel vertical migration
ERG	Electroretinogram
MIK	Methot-Isaac-Kidd plankton net (mesh size 1500 μm , opening 3.15 m^2)
MPS	Multi Plankton Sampler (mesh size 64/180 μm , opening 0.25 m^2)
<i>pep-ck</i>	Phosphoenolpyruvate carboxykinase
VPR	Video Plankton Recorder
qRT-PCR	Quantitative reverse transcription PCR
WP2	Plankton net (mesh size 50/180/200 μm , opening 0.25 m^2)
WP3	Plankton net (mesh size 1000 μm , opening 1 m^2)

INTRODUCTION

The behavioral pattern when zooplankton, key species in marine pelagic food webs, move from the upper part of the water column to a deeper part, and vice versa, in a synchronous manner within the 24 h cycle, is termed Diel Vertical Migration (DVM). Most zooplankton taxa employ DVM as part of a survival strategy, and it is a widespread behavioral pattern throughout the world's oceans (Cushing 1951, Tarling & Johnson 2006). A classical DVM pattern consists of an ascent at dusk from depth to surface waters followed by a descent at dawn to daytime depths (Cushing 1951). Many marine predators search for zooplankton prey visually and their success in finding prey is linked to ambient light intensity (Hays 2003). By adopting DVM the phenomenon enables a refuge for zooplankton from visual predators at depth during daylight and allows feeding in food-rich surface waters under the cover of darkness (Bollens & Frost 1989, Frost & Bollens 1992, Fortier et al. 2001, Hays 2003, Ringelberg 2010). On rare occasions, zooplankton employ reverse migrations in an attempt to avoid predation by larger predators that perform classical DVM (Ohman et al. 1983). While there are several reasons why zooplankton undertake DVM, there is consensus that avoidance of light-mediated mortality (visual predation) explains evolution of this widespread behavior (Gliwicz 1986, Lampert 1989, 1993, Bollens & Frost 1991, Hays 2003).

Polar regions are characterized by strong seasonality in light regime, primary production and sea ice cover. High-latitude species show great adaptation to the large seasonal changes. Adaptations include diapause (Hirche 1996, Fiksen 2000), seasonal vertical migrations (Conover 1988), slow growth and low metabolism (McLaren 1963), and extensive energy storage (Lee & Hagen 2006, Falk-Petersen et al. 2009). These traits maximize the ability of polar organisms to take advantage of seasonal pulses of food while minimizing mortality and metabolism during the non-feeding season (Ji et al. 2010). In polar regions, the high seasonality in the light regime makes zooplankton DVM more complex than at lower latitudes (Ringelberg 2010, Last et al. 2016). Large seasonal changes in DVM patterns are characteristic of high-latitude ecosystems with strong, classical, and synchronized DVM patterns around the spring and autumn equinox, where daytime and nighttime are of similar length. DVM patterns are generally weaker and unsynchronized during summer and winter when polar regions experience constant conditions of midnight sun or darkness, respectively (Fischer & Visbeck 1993, Blachowiak-Samolyk et al. 2006, Cottier et al. 2006, Cisewski et al. 2010, Wallace et al. 2010, Berge et al. 2014). Despite that the body of DVM literature is extensive and covers studies from throughout the world's oceans, DVM knowledge from high-latitudes is scarce. Knowledge from winter seasons in polar regions is, particularly, limited possibly due to a long-held paradigm declaring that most biological processes are at a minimum during winter (Piepenburg 2005, Smetacek & Nicol 2005) and due to logistical constraints of sampling high-latitude regions.

The fundamental mechanisms that underlie DVM are physiological-behavioral responses to exogenous factors and endogenous changes. Exogenous factors include changes in light intensity, gravity, temperature, salinity, oxygen, hydrostatic pressure and fish-mediated exudates (Gliwicz 1986, Forward 1988). Sex, ontogeny, state of feeding, and biological rhythms are examples of endogenous parameters also involved in migration behavior

(Lampert 1989, Bollens & Frost 1991, Hays 1995, Hays et al. 2001). Biological or circadian rhythms are physiological and behavioral patterns that oscillate with a 24 h periodicity (such as DVM, feeding and reproduction). Circadian 'clocks' are the central control mechanisms that drive circadian rhythms and have evolved to allow organisms to time coordination with the diel cycle between endogenous biological processes and the external environment to ensure fitness and survival (Dunlap et al. 2004, Strauss & Dirksen 2010).

DVM is often considered from proximate and/or ultimate aspects. Proximate aspects are concerned with exogenous cues that control migration behavior, whereas ultimate aspects relate to evolutionary or adaptive benefits such as why zooplankton migrate and what the fitness gains are for migrating individuals (Lampert 1993, Ringelberg 1999, Cohen et al. 2009). It is widely accepted that light is the proximate trigger for migration (Forward 1988, Ringelberg 1995, 2010, Fortier et al. 2001). Thus, many zooplankton species show negative phototactic responses to light intensity, and swim away from the light source (Forward 1988). Three hypotheses suggest to explain the proximate role of light for DVM (Cohen and Forward (2005) and references therein): the preferendum (isolume) hypothesis, the rate of change hypothesis, and the endogenous rhythm hypothesis. The preferendum hypothesis states that migrations follow specific isolumes (i.e. levels of constant irradiance) as zooplankton ascend with a preferred light level (isolume) at sunset and descend with this isolume at sunrise. Little vertical movement occurs during the day as surface irradiance and depth of isolumes remain relatively constant. In the rate of change hypothesis, relative rate of change in light intensity initiates migration, and zooplankton are most responsive to rates that occur at sunrise and sunset. During the day, the rate of change in light intensity is too low to cause vertical movement (Clarke 1934, Ringelberg 1964). The endogenous rhythm hypothesis, unlike the preferendum and rate of change hypotheses, suggests that light indirectly acts as a cue ('Zeitgeber') to synchronize or entrain vertical migration with the diel light cycle (Rudjakov 1970, Dunlap et al. 2004).

Several hypotheses have been tested to explain the adaptive value or ultimate causes of vertical migration. Commonly, these hypotheses are associated with the energetic and demographic costs/benefits related to migration behavior (Lampert 1989, Ringelberg & Van Gool 2003). While the benefit of downward migration at dawn is escape from light-dependent mortality (predator avoidance), upward migration at dusk reflects evasion of the costs present at depth (e.g. low phytoplankton abundance). Upward and downward migrations are, moreover, energetic costly due to swimming activity and potential crossing of temperature gradients in the water column (McLaren 1963, Lampert 1989). The fact that zooplankton individuals respond to exogenous factors, (such as light, predator kairomones, food concentration), and are able to choose the best trade-off solution to balance food requirements and predation risk demonstrates a 'decision-making' mechanism (Gliwicz 1986, Ringelberg 1991, 1995, 1999, Lampert 1993, Pearre 2003).

In polar regions, seasonality becomes more apparent with increased latitude (North or South) leading to longer duration of the extreme photoperiods (midnight sun and polar night). The extreme photoperiods are typical found at $>70^{\circ}\text{N}$ in the Arctic marine systems (Fig. 1). In the Antarctic, $>70^{\circ}\text{S}$ is on the land, thus the Antarctic marine ecosystems do not experience similar 'extreme' variations as in the Arctic. The Arctic light regime ranges, at its

extreme, from one day to six months of constant darkness during winter (polar night) and one day to six months of midnight sun during summer (Fig. 1, Berge et al. (2015b)). The sun does not rise above the horizon during the polar night period, which makes light conditions atypical in polar regions compared to lower latitude regions. With increasing latitude, irradiance levels gradually decline, thus the light regime during polar night is highly heterogeneous (Fig. 1). The Arctic region is divided into three polar night regions; civil, nautical, and astronomical, to differentiate between levels of darkness (Fig. 1). Astronomical polar night (when the sun is between 12° and 18° below the horizon) covers the Arctic Ocean, whereas the surrounding seas fall within either nautical polar night (when the sun is between 6° and 12° below the horizon) or civil polar night (when the sun is less than 6° below the horizon) zones. This characterization is not solely a geographic separation, as a single location within the region experiences several twilight periods (Fig. 1) due to the angle of sun below the horizon that alters during the winter period (Sakshaug et al. 2009). Moonlight, starlight, and auroral light, available for some parts of the polar night period are irradiance sources other than solar background irradiance (Båtnes et al. 2013, Cohen et al. 2015, Last et al. 2016).

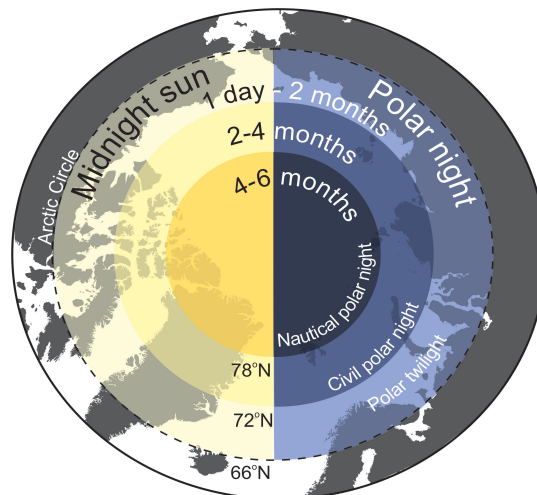


Figure 1: Area of the Arctic region where the sun remains above (midnight sun) or below (polar night period) the horizon throughout the 24 h cycle. Duration of polar night conditions and degree of darkness depend on latitude and lasts at its extreme over 180 days at the North Pole (Berge et al. 2015b). Within the region of civil polar night, the sun lies below the horizon for 2-4 months but a faint glow of light (twilight) remains around midday. In the region of nautical polar night, no twilight is present, although, there is a faint glow of light around midday due to solar background illumination. In the astronomical polar night region (not visualized on the map) all visible twilight is absent, and the state of darkness lasts for up to 11 weeks (Map: Berge et al. 2015, unpublished).

Baseline biological knowledge during winter is important to gather as such knowledge contributes to the sparse literature and improves comprehension of Arctic ecosystem patterns and processes. Recent findings from Svalbard challenge the previous concept of biological quiescence during winter at high-latitude (Piepenburg 2005, Smetacek & Nicol 2005) and provide evidence for biological activity at many trophic levels (see Berge et al. (2015a) and references therein). Literature on winter biology from high-latitude regions has, therefore, started to increase, although there are still many gaps to fill. Despite no measurable pelagic primary production, herbivorous and omnivorous mesozooplankton species are present throughout the water column, although in lower abundance than during summer and autumn (Kwasniewski et al. 2003, Walkusz et al. 2009, Seuthe et al. 2011).

Diversity, abundance, growth, and reproduction in benthic communities are also recorded at levels comparable to the productive season (see Berge et al. (2015a) for a review). The presence of juvenile stages of herbivorous, omnivorous, and carnivorous zooplankton taxa, shows evidence for reproduction during winter (Willis et al. 2006, Falk-Petersen et al. 2009, Walkusz et al. 2009, Buchholz et al. 2012). Occurrence of krill and appendicularian fecal pellets in sediment traps indicates feeding activity in surface waters. Active foraging by amphipods (*Themisto* spp.), seabirds (e.g. little auks, *Alle alle*), and several fish species (e.g. polar cod, *Boreogadus saida*, haddock, *Melanogrammus aeglefinus*, and Atlantic cod, *Gadus morhua*) has also been observed during the dark winter in Svalbard (Kraft et al. 2013, Berge et al. 2015a). Predation during the polar night is less studied but based on the fact that light structures predator-prey interactions (Forward 1988, Bollens & Frost 1991, Cohen et al. 2009), the threat of visual predation may be reduced as predators likely have reduced success to catch prey during the darkest months of winter in the Arctic.

Further evidence of polar night activity patterns are acoustic observations of DVM with weak patterns in mid-winter that gradually becomes more pronounced with the gradual increases of conventional day-night cycles in end of the winter period (Berge et al. 2009, Wallace et al. 2010). Knowledge of which species are responsible for these acoustic polar night migration patterns does not exist at present despite recent polar night studies. It furthermore complicates the understanding of which cues that drive DVM behavior in polar environments when conventional day-night cycles are absent. In consideration of the hypotheses (preferendum, rate of change, and endogenous rhythm) that explain the role of light as the proximate cause for DVM (Cohen and Forward, 2005), relative changes in light intensity may be too small to entrain DVM throughout several months of constant darkness. A study shows that some zooplankton species (*Calanus* spp.) are able to perceive low light intensity equal to the levels of solar background illumination available during polar night (Båtnes et al. 2013). It has previously been proposed that endogenous rhythms may explain activity patterns during the dark winter (Berge et al. 2009, Last et al. 2016, Tran et al. 2016). It remains unclear whether the unidentified species behind acoustic migration patterns are able to perceive low light levels during polar night and/or if circadian clock gene(s) may be involved in these migration patterns. It is, therefore, relevant to investigate behavioral-physiological parameters of polar night migrants to resolve whether exogenous and/or endogenous cues explain activity patterns to identify proximate and ultimate causes of winter migrations.

Migratory zooplankton play an important role in global biogeochemical cycles by their modification and transport of inorganic and organic material throughout the water column (Longhurst & Glen Harrison 1988, Zhang & Dam 1997, Steinberg et al. 2002, Turner 2002). Zooplankton thus make a major contribution to the 'biological pump' by transport of sequestered atmospheric carbon dioxide to the deep sea (Arrigo 2005, Steinberg et al. 2008, Darnis & Fortier 2012). Increased air temperatures, stronger stratification and reductions in sea ice cover are factual consequences of warming in the Arctic region (Cottier et al. 2007, Comiso 2012) and has large impacts on many trophic levels in the marine ecosystem (Wassmann et al. 2011, Meier et al. 2014, Leu et al. 2015). The question arises as to how these environmental changes will affect the annual carbon budget, primary production and

higher trophic levels in the future (Arrigo & van Dijken 2011). The role of winter DVM by active zooplankton in mediating vertical transport of organic and inorganic material remains poorly resolved in the Arctic. A better understanding of the fundamental mechanisms of migration patterns in polar night active species will help determine the global significance of winter DVM (if any) in the high Arctic. Finally, data from the present study may be used to develop models of life-history strategies of zooplankton, food-web interactions, and zooplankton-mediated carbon fluxes during extreme photoperiods to elucidate ecosystem impacts caused by vertical migration in a warming Arctic.

Objectives

The overall aim of this study was to investigate whether diel vertical migration (DVM) persists throughout the polar night in a high Arctic fjord (Kongsfjorden, Svalbard). The following objectives were addressed to answer the main research question:

1. To describe the community composition of marine plankton organisms in a high Arctic fjord system during polar night and to characterize responsible zooplankton species behind the observed acoustic migration patterns (**Papers I, IV**).
2. To investigate molecular and electrophysiological mechanisms in species responsible for polar night migration patterns in Kongsfjorden (**Papers II, III**).
3. To assess the importance of zooplankton vertical migrations by measurements of carbon via respiration and excretion of ammonium over the transition from polar night to autumn equinox in Svalbard waters (**Paper IV**).

APPROACH

Study site

The Svalbard archipelago consists of several islands where Spitsbergen is the largest one (Fig. 2) that borders Fram Strait to the west. Svalbard is situated in the region of the civil and nautical polar night zone (Fig. 1). In Kongsfjorden, where sampling for the present study was conducted, polar night lasts from the end of October until mid-February (Berge et al. 2015b). During the polar night period, Kongsfjorden experiences three different twilight periods (Tran et al. 2016). Civil twilight lasts from October 24 to November 9 and from February 1 to 17. Nautical twilight lasts from November 10 to December 11 and from December 31 to January 31, whereas astronomical twilight lasts from December 12 to 30.

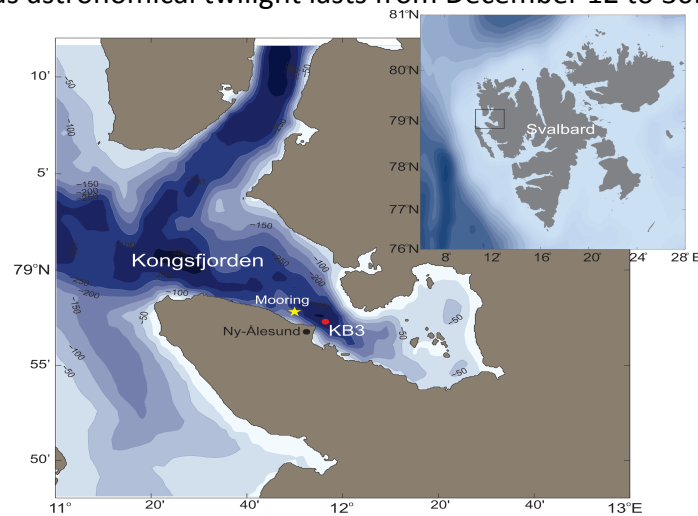


Figure 2: Study site (Kongsfjorden, Svalbard), KB3 sampling station, and position of mooring (map: Malin Daase).

Kongsfjorden (78°N) on the west coast of Svalbard (Fig. 2) is a wide glacial fjord that consists of two main basins separated by a 30 m deep sill in the inner part of the fjord (Svendsen et al. 2002). The fjord is open to the West Spitsbergen Shelf and is, therefore, largely influenced by advection of warm, saline Atlantic Water from the West Spitsbergen Current and colder, fresher Arctic water with origin from the East Spitsbergen Current (Svendsen et al. 2002, Cottier et al. 2005). As mixing of warmer Atlantic and colder Arctic water occurs along the coast of Svalbard (Svendsen et al. 2002, Cottier et al. 2005), water masses carry associated Arctic and Atlantic fauna into the fjords of Svalbard (Basedow 2004, Willis et al. 2006). Kongsfjorden is well suited to study temporal variability in zooplankton vertical migration, as the fjord experiences nearly four months of continuous darkness, light, and conventional day–night cycles, respectively, during the annual cycle (Cottier et al. 2006, Berge et al. 2015b).

In Kongsfjorden the zooplankton assemblage (Fig. 3), distribution and abundance of species vary with seasonal and annual changes in oceanographic conditions (Kwasniewski et al. 2003, Basedow 2004, Willis et al. 2006). Calanoid copepods, known to exhibit vertical migration, are abundant species in the zooplankton community of Kongsfjorden (Kwasniewski et al. 2003, Willis et al. 2006, Walkusz et al. 2009, Berge et al. 2014). Other migrant species such as *Metridia* spp. copepods and *Themisto* spp. amphipods are also

common species in the fjord system (Hays 1995, Willis et al. 2006, Berge et al. 2009, Kraft et al. 2013, Dalpadado et al. 2016). Several krill species, known to perform migrations for most parts of the year, are also found in the Kongsfjorden ecosystem (Buchholz et al. 2010, 2012, Huenerlage et al. 2015, Dalpadado et al. 2016).

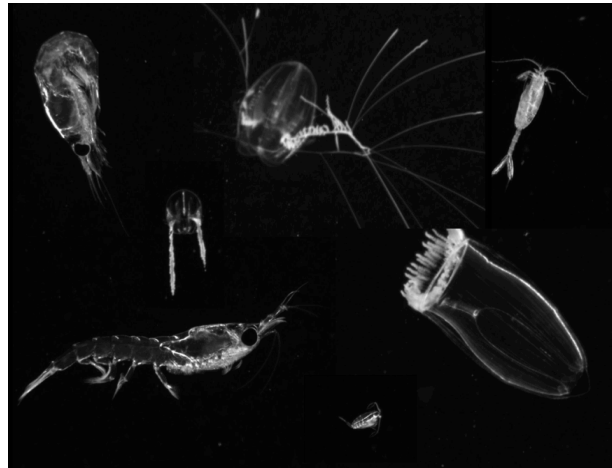


Figure 3: Zooplankton images collected by a video plankton recorder (VPR) in Kongsfjorden (Svalbard) January 2014 at the KB3 sampling station (photo: Fredrika Norrbin).

Data collection

Sampling for this study was conducted in Kongsfjorden during consecutive winters (2013-2014) in vicinity of the 330 m deep station KB3 (78°540 N, 12°000 E, Fig. 2, Table 1). Zooplankton net sampling (**Paper I-IV**) was carried out mainly from R/V *Helmer Hanssen* (see Table 1 for further information on sampling). Acoustic instruments (ADCP and AZFP) attached to moorings (**Paper I, IV**) were deployed over the course of 2013-2014, at a short distance from the station KB3 (Fig. 2) to supplement sampling by plankton nets. Vertical profiles of salinity, temperature, and fluorescence (**Paper I, IV**) were obtained with a CTD (Seabird SBE 911) equipped with a fluorometer (Seapoint Sensors, Inc.) in parallel with net deployments. Water samples to study community composition of protists and measure chlorophyll *a* (Chl. *a*) levels were sampled at 5, 15, 35, 75, 150 and 330 m (**Paper I**). Sampling of zooplankton was conducted with various plankton nets deployed in horizontal or vertical tows (Table 1). To identify DVM migrants (**Paper I, IV**) zooplankton nets were deployed within one hour of local midnight and noon. Gene expression patterns of the circadian clock gene (*cry2*) were studied in individual krill head RNA, evaluated by qRT-PCR, and quantified as fold-change (Pfaffl 2001) relative to the reference gene *pep-ck* (**Paper II**). An irradiance sensor and an all-sky camera measured atmospheric light intensities and differences in skylight scenarios in Ny-Ålesund (Fig. 2) adjacent to Kongsfjorden. Spectral irradiance from the atmosphere was used as an input into a radiative transfer model to characterize the underwater light field (**Paper III**).

Sampling	January (2013)	February (2013)	January (2014)	May (2014)	September (2014)
Chlorophyll <i>a</i> (chl. <i>a</i>)	-	-	(16-19th) a	-	-
Protists	-	-	(17-18th) a	-	-
Zooplankton net	(15-17th) f, g	(10-11th) f, g	(16-20th) b, c, d, e, f, g	(12-14th) c, f	(23-27th) f, g
ADCP (300 kHz)	✓	✓	✓	-	✓
AZFP	-	-	✓	-	✓
Light measurements	-	-	(21-22th)	✓	✓
Genetics	(16-17th) g	(10th) g	(18-19th) g	-	-
Respiration measurements	-	-	✓	✓	✓

Table 1: Overview of gear and sampling time. a) Niskin bottles, b) WP2 net (50 μm), c) WP2 net (180/200 μm), d) WP3 net (1000 μm), e) MPS net (64 μm), f) MPS net (180 μm), g) MIK net (1500 μm).

Interpretation of polar night migration patterns

Zooplankton net sampling and hydro-acoustics reveal vertical distributions of zooplankton and are useful to study behavioral patterns such as DVM. Sampling limitations exist for both approaches. With conventional zooplankton net sampling, data may suffer from underestimation due to avoidance of nets by fast swimming species, e.g. krill and amphipods (Wiebe et al. 2004). Another disadvantage of vertical net tows is individuals within depth intervals are pooled thus precision of depth distribution between day and night is weakened (Pearre 2003). In addition, single use of nets provides a coarse depth resolution that may not detect depth-specific behaviors. Sampling during harsh weather conditions, such as during polar night, may complicate sampling due to heavy gear and that nets may freeze upon retrieval. Fragile gelatinous planktonic organisms are likely extruded and destroyed in nets. Several sampling biases exist and successful sampling, nevertheless, depend upon species present, as zooplankton net sampling may seem suitable to target some species over others (Wiebe & Benfield 2003, Wiebe et al. 2004). In recent years it has become increasingly common to employ acoustic instruments (**see box 1**) to rapidly map vertical distributions and migration patterns of zooplankton (Cottier et al. 2006, Falk-Petersen et al. 2008, Berge et al. 2009, 2014, van Haren & Compton 2013). In comparison to conventional net sampling, acoustic methods prove useful to assess distribution of plankton at high spatial or temporal resolution (depending on whether the instruments are mounted on a vessel or mooring) without disturbing the observed organisms. Acoustic instruments are able to record data continuously, and thus contribute to datasets covering extensive periods of time with a high sampling frequency. In the case of acoustic devices being deployed on moorings, long-term acoustic data can therefore demonstrate both diel and seasonal migration patterns of zooplankton. This has shown to be useful in the Arctic (Cottier et al. 2006, Berge et al. 2009, 2014, Wallace et al. 2010, Hamilton et al. 2013), where sampling from ships may be hindered during the winter period. The application of acoustic data is, however, limited as no direct taxonomical information is provided as compared to sampling with nets. In the case of acoustics deployed on moorings, spatial resolution (i.e. horizontal) apart from water column depth (i.e. vertical) is poor. To overcome sampling biases, a combined approach with zooplankton net sampling and acoustics enables the most detailed investigation of biological and physical processes in the water column.

BOX 1 BIOACOUSTICS

Bioacoustics provide useful information on distribution, migrations, abundance and patchiness of zooplankton and pelagic fish. Acoustic instruments detect any organism or 'scatterer' in the water column that reflects sound and data are processed as backscatter (S_v , dB). With knowledge of morphological and behavioral features of zooplankton functional groups present in the water column, additional use of scattering models allows an estimation of contribution of each functional zooplankton group (derived from supplementary plankton net sampling) to the overall backscatter signal (Stanton et al. 1996, 1998), and therefore biomass.

ADCPs (Acoustic Doppler Current Profilers) record data at a single frequency (e.g. 300 kHz, **Paper I, IV**). The frequency setting of these ADCPs is optimal to detect scatterers of a few millimeters in size (> 2.4 mm) thus suitable in the study of small mesozooplankton species (Berge et al. 2014). As ADCPs are difficult to calibrate, the ability to reliably discriminate species acoustically is limited. This limitation can be partly overcome by using them in conjunction with single frequency echo-sounders to create a multi-frequency system, which allows for target strength calibration and abundance estimates (Brierley et al. 1998, 2006, Fielding et al. 2004). The four beam configuration of an ADCP also allows for derivation of vertical velocity data (i.e. speed of scatterers).

AZFPs (Acoustic Zooplankton Fish Profilers) are able to record data at four frequencies (125, 200, 455, and 769 kHz, **Paper IV**). In contrast to ADCPs, AZFPs provide more taxonomical information (such as classification and discrimination of species and sizes) of the zooplankton community due to the multi-frequency settings (Anderson et al. 2007, Lavery et al. 2007, Tarling et al. 2014). As AZFPs are calibrated echo-sounders they can be used for quantitative measurements by conversion of backscatter to biomass.

Biomass derivations (AZFP method) Acoustic data are sorted with a software tool (e.g. EchoView), divided into depth layers (bins), and backscatter from each bin is exported. Lower and higher frequencies are compared to discriminate fish from zooplankton with known acoustic properties of fish populations. Density of zooplankton in each cell (ind. m^{-3}) is calculated with predicted backscatter of individual organisms (target strength (TS, dB) for each zooplankton functional group (Stanton et al. 1994). Next, water column abundance (ind. m^{-2}) is calculated. Average dry weight (W) of zooplankton individuals from net samples is used in biomass derivation of scatterers. Mean dry biomass (mg m^{-3}) within each cell associated with the various functional zooplankton groups is calculated according to (Parker-Stetter & Horne 2009, **Paper IV**). Dry biomass of each functional group is then converted to carbon content based on C:W values from literature.

To study circadian activity patterns by molecular approaches

Several techniques exist for studying and quantifying gene expression and its regulation. Most of these techniques, such as microarray analysis and quantitative reverse transcription-PCR (qRT-PCR), measure levels of mRNA. It is also possible to measure protein levels directly by Western blot. Other more advanced molecular techniques make large-scale studies of gene expression possible such as metatranscriptomics (Leininger et al. 2006). These methods allow for measuring expression patterns of multiple genes simultaneously and enables determination of which genes that are active and which genes that are turned off. Little is known about the molecular and cellular basis of photoperiodism (Bradshaw & Holzapfel 2007), and by applying a molecular approach to examine expression levels of clock genes involved in circadian rhythms may prove useful to resolve the molecular mechanisms of polar night migrations.

The underlying principle of circadian clocks is successive cyclic gene activation, which makes up an auto-regulatory feedback loop for which one cycle takes 24 h. Circadian clock systems include three major mechanisms (Fig. 4): 1) the input pathway, an oscillator with a period of 24 h that keeps time, and perceives environmental cues such as light and temperature; 2) the central oscillator, based on transcriptional/translational feedback loops comprised of positive and negative elements (Roenneberg & Merrow 2005) that allow entrainment of the clock. Positive elements activate expression of clock genes, and negative elements inhibit expression of the positive elements (Bell-Pedersen et al. 2005); and 3) the output pathway, which governs expression of genes that regulate rhythms in physiology, and behavior throughout an organism. Although clock genes involved in this feedback loop mechanism can differ in various organisms, the principle is common to all of them (Young & Kay 2001).

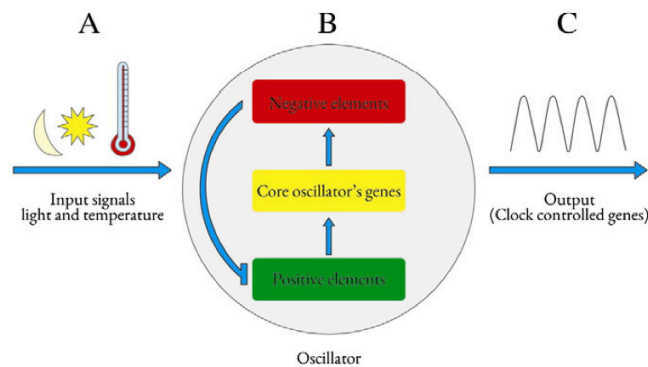


Figure 4: Simplistic overview of a circadian clock system. The clock includes three components: (A) input pathways, that regulate the oscillator response to external stimuli (e.g. light and temperature); (B) an oscillator, which generates and sustains rhythms, the oscillator — the molecular core architecture compose positive and negative elements, which form the feedback loop and maintain a 24 h cycle. The oscillator is responsible for control of expression of genes that participate in a variety of circadian-controlled processes known as clock-controlled genes (CCGs). (C) Output pathways, which convey rhythmic information from the oscillator to other physiological systems (Figure by Sorek et al., 2014) $B_{0 < 3}$.

In the present study RNA was extracted from individual krill collected *in situ* or from a laboratory experiment (**Paper II**). RNA was reverse transcribed into complementary DNA (cDNA) with the enzyme reverse transcriptase. The amount of cDNA in a specific clock gene was quantified fluorescently by qRT-PCR. Gene expression changes of clock genes were next quantified as fold-change values (Pfaffl, 2001) relative to reference gene(s). Finally, gene expression data were modelled (e.g. CircWaveBatch v3.3) to check for significant circadian rhythmicity patterns in investigated clock genes.

Spectral sensitivity of krill

Any physiological experiment that utilizes visual stimulation techniques requires determination of spectral sensitivity $S(\lambda)$ of the visual system (i.e. photoreceptors in the eye of study organism). The identification of $S(\lambda)$ in organisms is necessary to comprehend behavioral patterns and physiological mechanisms; if wavelengths of light are not perceived by an organism, then they are not relevant for these processes. As for the polar night, $S(\lambda)$ of active migrants is relevant to determine if polar night migrants are able to perceive subtle irradiance levels and if such potential detection can explain activity patterns during the dark winter. Classically, $S(\lambda)$ is determined directly from measurements performed in the eye (i.e. electrophysiological recording from photoreceptors outer segment of the photoreceptor) (Cohen & Frank 2006, Cohen et al. 2010, 2015) or indirectly by behavioral methods (Detto et

al. 2006, Båtnes et al. 2013). For electrophysiological measurement of $S(\lambda)$ functions of visual neurons, flash methods are often used, in which the test animal is exposed to monochromatic light (i.e. single wavelength) flashes, while electrical responses are recorded extra- or intracellularly.

Electroretinograms (ERGs) measure extracellular electrical activity generated in the retina in response to a light stimulus when electrodes are embedded subcorneally in the eye of the test animal. It is thus possible to measure at which wavelengths (i.e. colors) the eye of a certain animal is able to detect. The most usual approach to determine $S(\lambda)$ using ERGs is by the criterion response method (Cohen & Frank 2006). Light intensity at a given wavelength is adjusted until a peak amplitude response above background noise (usually at 25 or 50 μV) is achieved. This is repeated across the spectrum (350nm to 650nm) (see box 2).

BOX 2 KRILL EYE ELECTROPHYSIOLOGY

Methodology The test animal is glued to a small tap on the stand, submerged in a temperature-controlled water bath within a light-tight Faraday cage, and a microelectrode is positioned subcorneally under dim red light (680nm). A flash test is carried out to ensure the electrode is properly placed in the eye and that the animal responds to light. Before initiation of the experiment intensities of the test flashes are adjusted so that the response from the eye is approximately the same at each wavelength according to the criterion response method (Cohen & Frank 2006) to ensure full dark acclimation of the eye. A custom software tool is used to control instruments and adjust required wavelengths and to record responses of the eye at respective wavelengths. Light intensities (flashes) are obtained at each wavelength to get a constant response with intervals of 10-20 nm between flash wavelengths. Spectral sensitivity $S(\lambda)$ is estimated as the reciprocal of the irradiance needed to invoke the criterion response at different wavelengths across the spectrum. $S(\lambda)$ is then normalized to the wavelength of maximum sensitivity (highest peak) thus it is revealed at which wavelengths the animal is most sensitive to (Fig. 5).

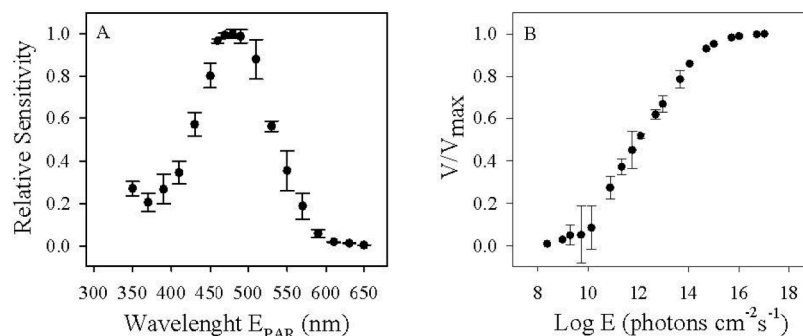


Figure 5: Example of A) a spectral sensitivity curve and B) a response-irradiance curve ($V/\text{Log}I$). Both curves are measured on ventral lobe of the krill species *Thysanoessa longicaudata*, ($n = 2$) in January 2014 during an UNIS student course (AB 334).

Underwater light modelling Radiative transfer models such as HydroLight computes radiance flow and related quantities (irradiances, reflectances, diffuse attenuation functions, etc.) in any water body. Radiation transfer is energy transfer in the form of electromagnetic radiation. Propagation of radiation through a medium (e.g. water) is affected by absorption, emission, and scattering processes. Spectral irradiance from the atmosphere was used as input into the HydroLight model to characterize the underwater light field and to derive an underwater light field that could be related to krill vision (*T. inermis*) (Paper III). The light field throughout the water column was computed as a function of depth, direction, and wavelength. Modelled underwater light fields were transformed into "krill utilized photons" (E_{krill}) by weighting the modelled scalar irradiance (E_0 ; units of $\mu\text{mol photons m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) at each wavelength (nm) by the ability of *T. inermis* to detect light at that wavelength according to its normalized visual spectral sensitivity absorbance spectrum determined electrophysiologically.

Ecological implication of vertical carbon and nitrogen fluxes in polar marine systems

Vertical carbon (C) and nitrogen (N) fluxes from surface waters to the deep ocean are fundamental to study in order to understand the global cycling of C and N (Bollens et al. 2011). In Arctic waters, the role of zooplankton respiration and excretion in mediating vertical transport of carbon and nitrogen remains poorly resolved, especially for the winter months. It is thus important to gather such winter data so knowledge of vertical fluxes is covered on full annual scale. All processes involved in C fixation from surface waters to the deep ocean, such as fixation of inorganic C during photosynthesis, transformation of C by food-web processes (trophic interactions), physical mixing, transport, and gravitational settling are referred collectively as the 'biological pump' (**see box 3**). To assess the role of the biological pump in transfer of C and N from surface waters to the deep ocean requires quantification of processes that contribute to export production.

BOX 3 THE BIOLOGICAL PUMP

The biological pump (Fig. 6) is the ocean's biologically driven sequestration of C from the atmosphere to the deep sea, where components of the pump are responsible for transformation of dissolved inorganic carbon (DIC) into organic biomass and 'pump' it in particulate or dissolved form into the deep ocean. Inorganic nutrients and carbon dioxide (CO₂) are fixed during photosynthesis by phytoplankton and released as dissolved organic matter (DOM), and available for consumption by herbivorous zooplankton (e.g. copepods and krill) that produce fast sinking fecal pellets. Biotic (microbial degradation and zooplankton fragmentation) and abiotic processes (turbulence and decreased particle flux with depth) influence the amount of particulate organic carbon (POC) exported from surface. A fraction of the POC that leaves the surface ocean reaches the seabed and are consumed, respired, or buried in sediments, where C is stored there for millions of years until the thermohaline circulation returns deep-ocean DIC to the atmosphere on millennial timescales. This export production drives the biological pump.

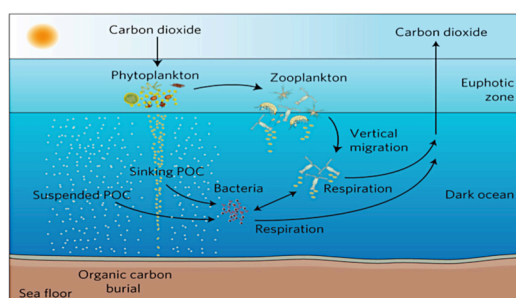


Figure 6: The biological pump. Carbon is fixed by phytoplankton during photosynthesis in euphotic surface waters as they use carbon dioxide (CO₂), nitrogen (N), among others, to produce particulate organic carbon (POC). POC is grazed by herbivorous zooplankton, or consumed directly or indirectly by heterotrophic microbes (such as bacteria and viruses). Between 1-40% of the primary production is exported out of the euphotic zone. Remineralization of organic matter in the water column converts organic carbon back to CO₂. Only a small fraction of the surface production reaches the sea floor (Illustration from (Herndl & Reinthaler 2013)).

C and N export occur both passively by sinking of detrital material but also actively by zooplankton DVM (Dam et al. 1995, Zhang & Dam 1997, Brierley 2014). At depth zooplankton release CO₂ and NH₄⁺ through respiration and excretion (Longhurst & Glen Harrison 1988, Steinberg et al. 2008). Respiration of carbon and excretion of nitrogen continue throughout day and night because excretory products are continuously produced as a result of basic metabolism (Hays et al. 1997).

Measurements of C and N export fluxes Quantification of passive and active export processes is necessary to assess the role of the biological pump in transfer of C and N to depth. Sediment traps deployed at a certain depth is an useful method to study gravitational passive export fluxes (see Ducklow et al. 2001 and references therein). Vertical fluxes of C and N by migratory zooplankton are often calculated by comparisons of day- and nighttime profiles of biomass estimates from certain depth layers often in parallel with sediment trap data (Longhurst & Glen Harrison 1988). Such combined studies allow for comparison of active and passive transport fluxes. Respiration rates of migratory zooplankton are calculated by the decrease of oxygen in bottle experiments over time compared to controls without animals. Oxygen consumption rates are then transformed to respiratory carbon usually with published relationships of zooplankton respiratory quotients, body weights and assimilation efficiencies. As for excretion, rates are often estimated from same bottle incubations used for respiration as the differences in NH₄⁺ concentration between incubation bottles and animal-free control bottles at the end of the experiment.

SUMMARY OF RESULTS

Paper I: Polar night in Kongsfjorden – plankton composition and patterns of vertical migration

Depth-stratified sampling (water samples, zooplankton nets and acoustics) was conducted during two consecutive winter field campaigns in Kongsfjorden to describe the plankton community and characterize species responsible for acoustic migration patterns. Acoustics revealed that depths of the vertical migrations varied throughout the polar night period (Fig. 7). Classical and synchronized DVM throughout most of the water column was observed during the first and final parts (civil twilight) of the winter period consistent with presence of more distinct day-night cycles. Zooplankton net sampling during civil twilight (February) detected DVM in agreement with acoustic observations (Fig. 7). Unsynchronized migration patterns, not of a classical DVM character, and restricted to surface waters (20-30 m), was observed in acoustics and in plankton nets during the darkest months (astronomical and nautical twilight).

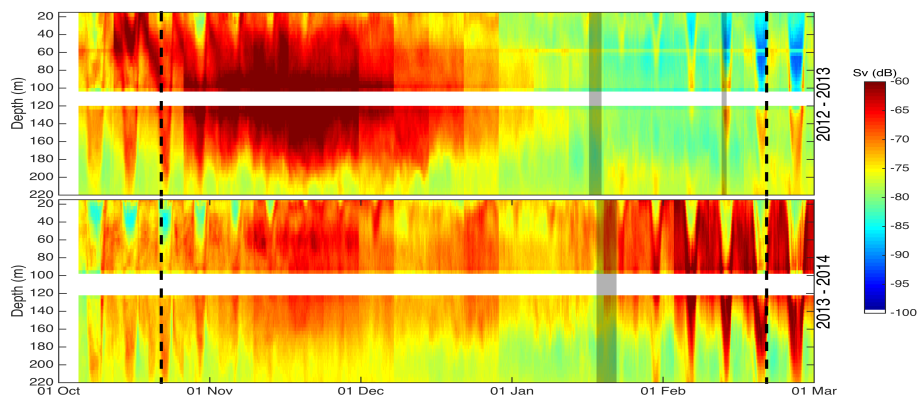


Figure 7: Two consecutive years of winter ADCP data (five months from 1 October to 1 March 2012/13 and 2013/14) presented as backscatter values (dB, Sv). The ADCP data are weekly averages compiled to one 24 h dataset representing 7 days of data. The vertical dotted lines represent the polar night period in Kongsfjorden (Svalbard) when the sun is below the horizon (>24 h). The color scale (right hand side) indicates backscatter values (dB). The upper panel is 2012/2013 data, and the lower panel is 2013/2014 data. The vertical gray shaded areas indicate concurrent sampling time with zooplankton nets. The white bar (~ 100 m depth) illustrates position of the sediment trap. No data exists from this depth layer

Ciliates of the order Strombidiida were most dominant (> 50 % of the total biomass) in the community composition of protists. Copepod nauplii (mainly cyclopoids) and small copepods (*Oithona similis*, *Pseudocalanus* spp., *Microcalanus* spp.) were dominant species and mainly distributed in the upper 50 m in the smallest zooplankton net fractions (50 and 64 μm). Small copepods were also numerous in the larger zooplankton fraction (200 μm) followed by *Calanus* spp. and *Metridia longa* copepods. These species were distributed throughout the water column with no evidence of classical DVM. Chaetognaths (*Parasagitta elegans*) and krill (*Thysanoessa inermis*) were dominant species within the macrozooplankton community (1500 μm) (Fig. 8). Classical DVM behavior was detected for krill and chaetognaths in the civil twilight period (February) but not during nautical twilight (January). Larger individuals were, moreover, observed at night compared to day during civil twilight. Such diel size class difference was not evident during nautical twilight, where krill and chaetognaths were of similar lengths at both night and daytime.

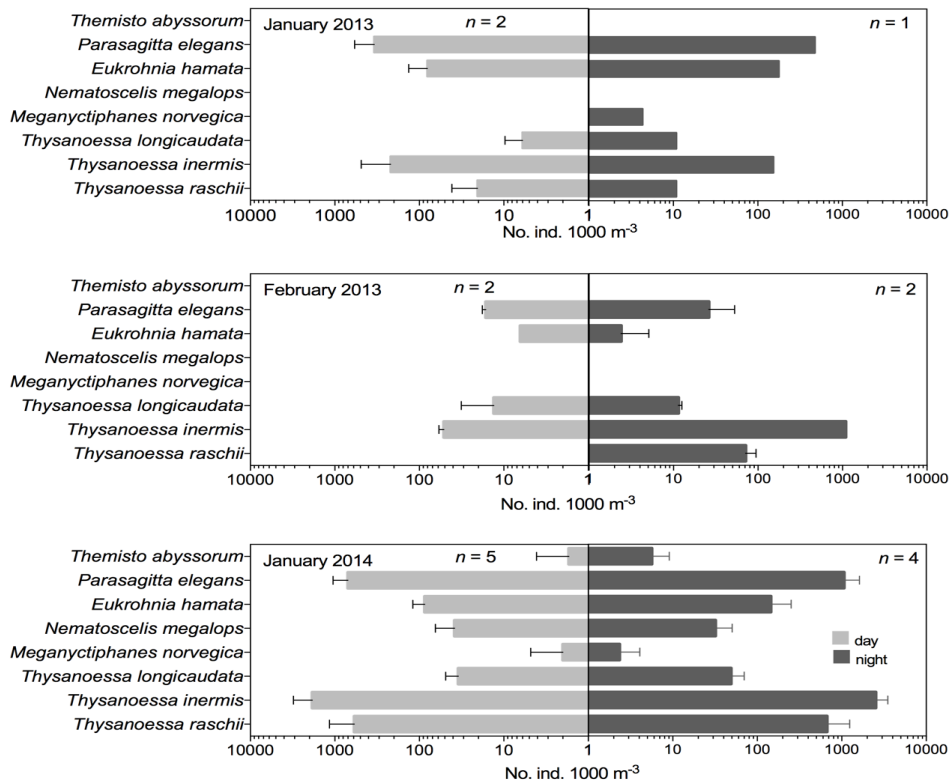


Figure 8: Average of night (dark gray, right side) and day (light gray, left side) MIK net samples of most abundant macrozooplankton species (ind. 1000 m⁻³) at 0–30 m depth in Kongsfjorden January 2013, February 2013 and January 2014. n = numbers of zooplankton net samples. Error bars show standard deviations.

Paper II: Evidence of a circadian clock gene in krill (*Thysanoessa* spp.)

Gene expression levels of the circadian clock gene cryptochrome 2 (*cry2*) were investigated in wild-caught krill collected over 24 h during nautical twilight (January 2013 and 2014) and over 48 h in experimental manipulated krill. Krill were acclimated in the laboratory to LD conditions 8 h of light and 16 h of darkness (equivalent to light conditions during spring). *Cry2* (target gene) and *pep-ck* (reference gene) primers developed against the Antarctic krill species *Euphausia superba* were used to amplify *Thysanoessa* spp. cDNA. Sequenced PCR products showed that both *cry2* and *pep-ck* had high sequence identity with *cry2* (NCBI FM200054) and *pep-ck* (NCBI FJ441178) genes of *E. superba*. No circadian expression patterns in *cry2* were detected in wild-caught *Thysanoessa* spp. (both years). As for acclimated krill, a diurnal light-response with higher gene expression levels in the light phase than during dark hours was shown (Fig. 9). The cyclic pattern in *cry2* expression observed during the LD treatment was not found under experimental conditions of constant darkness (DD).

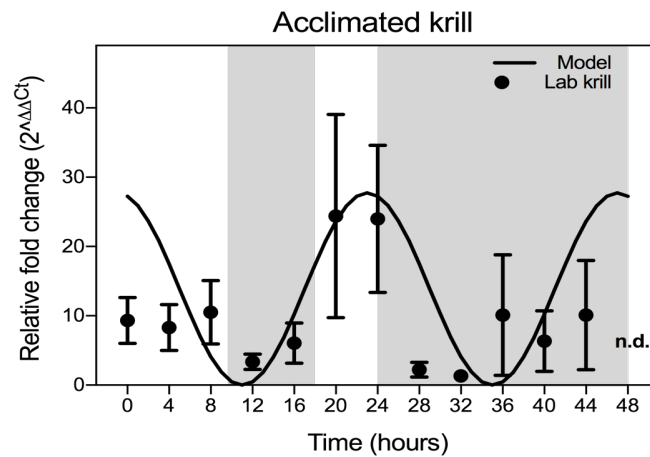


Figure 9: Gene expression of the cryptochrome 2 gene (*cry2*), expressed as fold change, in *Thysanoessa* spp. during a 48 h lab experiment relative to a circadian model. First 24 h (LD) = 16 h of light, 8 h of darkness (lights-off at 10PM, lights-on at 6AM). Last 24 h (DD) = 24 h of darkness. Shaded areas indicate when light was switched off and white areas represent times when light was on. Krill were sampled every 4 hours throughout the lab experiment (48 h). Data are mean of fold change values \pm SEM ($n = 10$). nd = no data.

Paper III: Spectral sensitivity of krill eye during polar night

Light measurements from high-sensitivity sensors were used in parallel with *in vivo* spectral sensitivity measurements of *T. inermis*. Radiative transfer modelling was used to evaluate if krill were able to detect available underwater light during polar night. The all-sky camera and E_{PAR} sensor showed that ambient irradiance during nautical twilight was in the range $1\text{--}1.5 \times 10^{-5} \mu\text{mol photons m}^{-2} \text{s}^{-1}$ with highest intensity at solar noon when the sun was at its highest elevation below the horizon. The electrophysiological experiments demonstrated visual spectral sensitivity of *T. inermis* eye in the blue spectrum (492 nm) (Fig. 10). Skylight spectral-irradiance data ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) propagated through the water column by radiative transfer modelling revealed a major spectral peak in ambient light transmission at blue-green wavelengths (465-485 nm) at depth. This indicated that photon capture of atmospherically derived light could be perceived by krill at those wavelengths. Modelled underwater light fields transformed into ‘krill utilized photons’ were compared to light-mediated behavior in other krill species (Myslinski et al. 2005) and revealed that light perception of *T. inermis* krill extended to over 20 m depth during polar night.

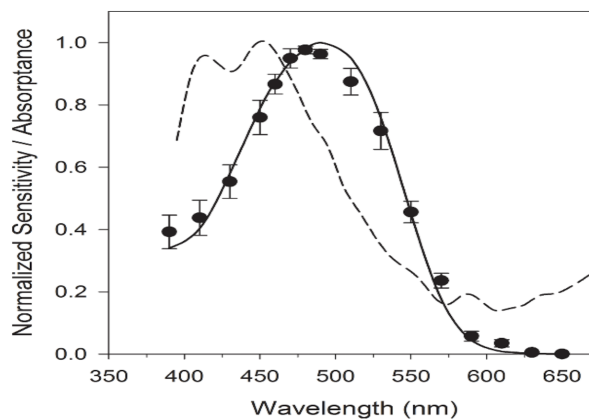


Figure 10: *Thysanoessa inermis* visual spectral sensitivity and spectral composition of skylight in the polar night (dashed line). Spectral sensitivity data are means (\pm standard error, $n = 5$) with the best-fit rhodopsin absorbance (solid line, $\lambda_{max} = 492$ nm). Spectral irradiance measured in air at noon on January 21 has been normalized to its peak; integrated absolute irradiance as PAR for this measurement was $1.3 \times 10^{-5} \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Paper IV: Metabolism and biogeochemical role of zooplankton DVM

The effect of classical and synchronized DVM on export of carbon and nitrogen to depth was studied over a seven-month time series of acoustic data collected with a moored Acoustic Zooplankton Fish Profiler (AZFP) in parallel with plankton net sampling and laboratory experiments. Remineralization of carbon via zooplankton respiration and excretion of ammonium at depth was measured to assess importance of active flux transport. Multi-frequency analysis from the moored AZFP showed that patterns of classical and synchronized DVM started at the end of January and persisted until beginning of April (Fig. 11). Migration patterns were unsynchronized prior to classical DVM and during the midnight sun period. Periods with classical DVM behavior occurred outside of the main season of primary production (late May to late June). Classical DVM resumed and reached a regular 24 h period in September (Fig. 11). DVM signals were strongest during spring and autumn equinox when day-night cycles were distinct. Averaged over the entire study period, the AZFP data analysis in parallel with taxonomical information from plankton nets, demonstrated that krill contributed >90% of the total diel migrant biomass (krill, copepods and chaetognaths). Large copepods (dominated by *Calanus* spp.) and chaetognaths (mainly *Parasagitta elegans*) also performed diel migrations throughout the study period. Active transport of carbon and nitrogen to depth through synchronous DVM was discontinuous over the annual cycle due to suspension of DVM during parts of the polar night and midnight sun period.

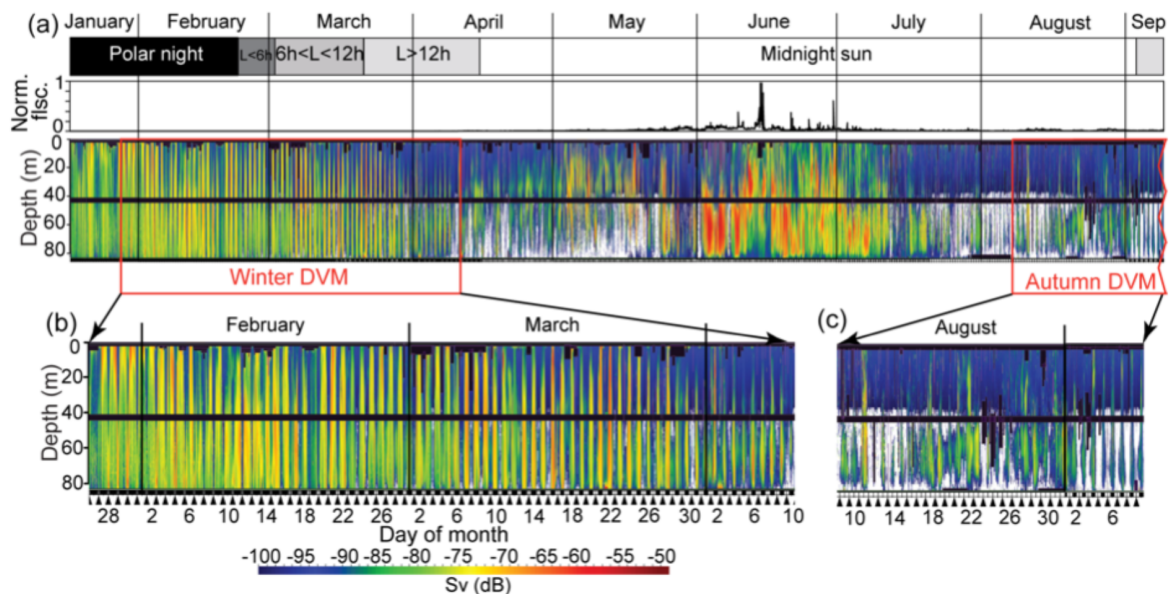


Figure 6: (a) Time series of relative fluorescence (normalized) at 37 m depth and backscatter for the 125 kHz frequency of the AZFP in Kongsfjorden from 17 January to 9 September 2014, and expanded views of (a) winter DVM period (28-January-10 April) and (c) onset of autumn DVM (10 August-9 September) defined qualitatively by visual analysis of the echogram.

DISCUSSION

One of the main observations from this study is the fact that polar night DVM is a complex phenomenon and involves several migration patterns. Light appears to trigger a migratory response in zooplankton species but this does not always follow a diurnal pattern. Although the observations imply that patterns of mid-winter migrations tend to be more synchronized in periods when solar light is supplemented by illumination sources (e.g. lunar light), the possibility of endogenous rhythms contributing to the increased migration behavior cannot be discounted. The synchronized migration signals in periods with more distinct day-night cycles suggest that zooplankton employ migrations to reduce predation risk.

Polar night migratory patterns - zooplankton species involved, consequences and causes

The zooplankton assemblage during winter and over the transition to spring and autumn was generally similar to previous records from Kongsfjorden (Kwasniewski et al. 2003, Willis et al. 2006, Walkusz et al. 2009, Buchholz et al. 2010, 2012, Dalpadado et al. 2016) with krill, chaetognaths, and copepods as the most abundant taxa (**Paper I, IV**). Despite that most *Calanus* spp. enter diapause during winter (Conover 1988), previous acoustic-based studies speculated that *Calanus* spp. and krill were the likely candidates behind winter migration patterns (Berge et al. 2009, 2014, Båtnes et al. 2013). The findings presented here, however, demonstrate that *Thysanoessa* spp. krill, and not *Calanus* spp. copepods, are mainly responsible for the acoustic patterns of polar night migration (**Paper I, IV**). The dominance of *T. inermis* krill in Kongsfjorden (**Paper I, IV**) corresponds well with previous observations that the distribution of *Thysanoessa* species has extended further north (Dalpadado & Skjoldal 1996).

The patterns of vertical migration show different characteristics throughout the polar night period (**Paper I, IV**), which is best visualized in the acoustic data due to higher temporal resolution compared to the zooplankton net samples. Classical DVM during the civil twilight periods exhibits a weaker signal compared to during spring/autumn equinox (**Paper I, IV**) but demonstrates the proximate role of light in triggering classical DVM when day-night cycles are present (Forward 1988, Cohen et al. 2009). During mid-winter (astronomical and nautical twilight), migration patterns are, in contrast to the civil twilight period, not of classical DVM character (**Paper I, IV**). Rather, migration patterns appear to be unsynchronized and restricted to surface waters, although, patterns tend to become of more synchronized character periodically (**Paper I**). The findings that classical DVM does not persist throughout the polar night period (**Paper I, IV**) conflict with previous winter observations in Kongsfjorden (Berge et al. 2009, Wallace et al. 2010) and in the Antarctic region (Cisewski & Strass 2016). Conversely, absence of DVM during mid-winter has, in correspondence to this study (**Paper I, IV**), been reported in other polar regions (Fischer & Visbeck 1993, Gaten et al. 2008, Cisewski et al. 2010). The general understanding that the polar night is a continuously and completely dark period is contradicted by the observation that solar background illumination is elevated at noon (**Paper III**), and periodically supplemented by other atmospheric light sources such as starlight, lunar, and auroral light (Berge et al. 2009, 2012, Båtnes et al. 2013, Last et al. 2016). It seems, despite krill eye detectability of low light intensities (**Paper III**) that the solar background irradiance was simply not strong enough to

trigger classical DVM during mid-winter possibly as light intensity (isolumens or rate of change) were too low, i.e. the preferendum and rate of change hypotheses (Cohen & Forward 2005 and references therein). The presence of light sources other than solar background illumination was not evaluated in the present study but recently an acoustic-based study demonstrated that migration signals during mid-winter were stronger during full-moon phases in the Arctic (Last et al. 2016). These findings are in line with previous records of krill (*Meganyctiphanes norvegica*) that show altered migration behavior under lunar cycles as a response to predator avoidance (Tarling et al. 1999), and confirm that lunar light affect migration behavior of krill. In consideration of the capability of krill to perceive low light levels (**Paper III**), it is reasonable to believe, that if solar background irradiance is supplemented with lunar and/or auroral light, the enhanced light levels will trigger migration. This may possibly explain why mid-winter migrations periodically tend to become more synchronized (**Paper I**). Migration patterns may, on the contrary, be unsynchronized during mid-winter for several reasons. First of all, migration of zooplankton at the individual level during periods of midnight sun explains unsynchronized patterns as there is no optimal time for organisms to migrate due to the constant conditions of light (Blachowiak-Samolyk et al. 2006, Cottier et al. 2006). An equivalent situation may occur during the darkest parts of the polar night, if the changes in absolute light levels are not sufficiently strong to trigger or drive a classical DVM pattern. Monthly and annual variations in irradiance sources (solar, lunar, auroral) and cloud cover may explain differences in mid-winter migration patterns observed in this study (**Paper I, IV**) and previous observations (Berge et al. 2009, Wallace et al. 2010). Other likely explanations for the unsynchronized migrations include a shift towards the lunar cycle (Last et al. 2016) or migration patterns related to solar illumination that may, for various reasons, such as clouds, sea ice cover and variable optical properties in the water column, be changing in a way not easily characterized as a diurnal pattern. The results that both krill (**Paper III**) and *Calanus* spp. copepods (Båtnes et al. 2013) are able to perceive solar background illumination, moreover, suggests that other polar night active zooplankton species, such as chaetognaths and *M. longa* copepods (**Paper I, IV**), may also detect such low light levels. The findings presented here show evidence for zooplankton responses to even slight changes in light (**Paper I, IV**), although, it is unclear at which threshold light intensities the various polar night vertical migration patterns are triggered.

Despite that predator-prey interactions were not examined in the present study, it is logical that predation success by predators that rely on vision may be lower during mid-winter. Since atmospheric light vary during the polar night (Båtnes et al. 2013, Last et al. 2016) indicates that the threat of predation may be elevated in periods with enhance illumination, at least by predators that use vision to find prey. While light in its various forms (e.g. solar, lunar, auroral) has a proximate role for polar night migration patterns, it may be that predation pressure is the ultimate driver of the migratory behavior. Size-dependent migration where larger zooplankton perform more pronounced DVM than smaller and less conspicuous zooplankton (Lampert 1993, De Robertis 2002) was observed for krill and chaetognaths during civil twilight but not during nautical twilight (**Paper I**). Conversely, as diel size classes of krill and chaetognaths were similar during nautical twilight (**Paper I**) implies that the need to employ migration was reduced at this time possibly as a consequence of the darker light regime compared to during civil twilight. A larger predation

pressure during civil twilight may explain the diel differences in length size class data (**Paper I**) as the presence of short day-night cycles imposes a larger need for zooplankton to adopt migration to reduce potential predation compared to the nautical twilight. Studies show that zooplankton are able to evaluate predation pressure by presence of chemical exudates (kairomones) from fish predators (Gliwicz 1986, Bollens & Frost 1991, Ringelberg 1991, 1999, Lampert 1993). The ability by zooplankton to sense predation risk may explain differences in migration behavior between civil and nautical twilight (**Paper I**). Thus if this is valid it reveals how zooplankton 'decide' when to employ migration to increase survival. The threat of predation may, however, not be completely absent during the astronomical and nautical twilight periods as a recent study suggests that visual predators may be capable to switch to mechanical, tactile or olfactory stimuli during polar night (Kraft et al. 2013).

Zooplankton searching for food may also explain mid-winter migration patterns. Phytoplankton concentration was low during polar night due to lack of primary production but food sources, available for zooplankton consumption, were small-sized zooplankton and nauplii but also detritus, marine snow particles, and sediments (**Paper I**). Although feeding data of polar night migrants (e.g. krill and chaetognaths) were not available, previous studies have observed that chaetognaths and krill feed during winter to some extent (Cleary et al. 2012, Grigor et al. 2014, Berge et al. 2015a). Under conditions of low predation risk, individual zooplankton may search for food particles throughout the entire water column in a 'random' manner with no limitations to undertake migration behavior at specific time intervals, which may explain unsynchronized migration patterns (**Paper I**). On the other hand, it may be more beneficial to remain in surface waters throughout the diel cycle than to spend energy on migration. This strategy would also explain the surface distribution of smaller zooplankton individuals (krill and chaetognaths), and lack of diel differences in size classes during nautical twilight (**Paper I**). It is important to stress that absence of larger size classes could be due to feeding near the bottom where food requirements may be more sufficient during this time of winter. The questions, however, remain, of whether it is beneficial to wait in an inactive mode to reduce energy risking starvation, or if it is more beneficial to actively search for food despite low food availability? The findings presented here (**Paper I, IV**) suggest that polar night migrants adopt behavioral strategies or 'decision making' mechanisms that enable zooplankton individuals to 'choose' best possible migration amplitude to maximize fitness in highly seasonal environments.

A light-dependent clock gene in polar night krill

The finding that the *Thysanoessa* spp. *cry2* gene required exogenous light to show rhythmic patterns (**Paper II**) is in agreement with the endogenous rhythm hypothesis (Rudjakov 1970, Dunlap et al. 2004, Cohen & Forward 2005) and confirm that circadian rhythms are dependent on distinct day-night cycles for entrainment. It is reasonable to believe that the gene was not involved in polar night migration, a finding that matches the lack of classical DVM during mid-winter (**Paper I**), and suggests that endogenous control of migration is not actual during darkest months of winter. The arrhythmic expression patterns found in the *Thysanoessa* spp. clock gene (*cry2*) during experimental conditions of constant darkness and in wild-caught krill (**Paper II**), however, contrast observations in Antarctic krill (Teschke et al. 2011). Maintained rhythmicity, although, only observed over two days of constant darkness

was demonstrated in the same clock gene (*cry2*) in Antarctic krill (Teschke et al. 2011). The authors postulated based on their results that the *cry2* gene was endogenously regulated and possibly involved in DVM behavior of Antarctic krill. Despite that the results were presented for Antarctic krill, it was anticipated in the present study (**Paper II**) that the *cry2* gene would be similarly endogenous governed in *Thysanoessa* spp. due to the close phylogenetic relationship of krill (Richter 2002). It is, however, reasonable that *cry2* cyclicity did not persist in wild-caught *Thysanoessa* spp. as the wild-caught krill had experienced several months of constant darkness (**Paper II**), and *cry2* rhythms are generally lost after a few days of experimental conditions of constant conditions of darkness or light (Levy et al. 2007, Peres et al. 2014). It would be interesting to see whether the *cry2* gene in Antarctic krill is able to maintain rhythmicity throughout the winter period.

As the clock gene shows rhythmicity under light conditions equivalent to spring and autumn equinox (**Paper II**, under experimental conditions) confirm that light is essential to initiate circadian rhythms in Arctic zooplankton (see Fig. 12 for further explanation). Despite the ability of krill to detect light even during the darkest part of the polar night period (**Paper III**) imply that the exogenous light levels during mid-winter are not strong enough to entrain the *cry2* gene (**Paper II**) or migration patterns (**Paper I**) to a 24 h cycle, thus krill migrations become of unsynchronized character. It is unclear whether enhanced illumination that seems to trigger periodically synchronized migration during mid-winter (**Paper I**), may also be strong enough to initiate cycling in the *cry2* gene and thus entrain migratory behavior. The central role of light for entrainment of migration is evident in the acoustic data (**Paper I, IV**) and shows the establishment of short day-night cycles are sufficient to drive classical DVM (**Paper I, IV**), thus it remains unknown whether light conditions during civil twilight are strong enough to entrain circadian rhythms in the *Thysanoessa* spp. *cry2* gene as samples do not exist (**Paper II**).

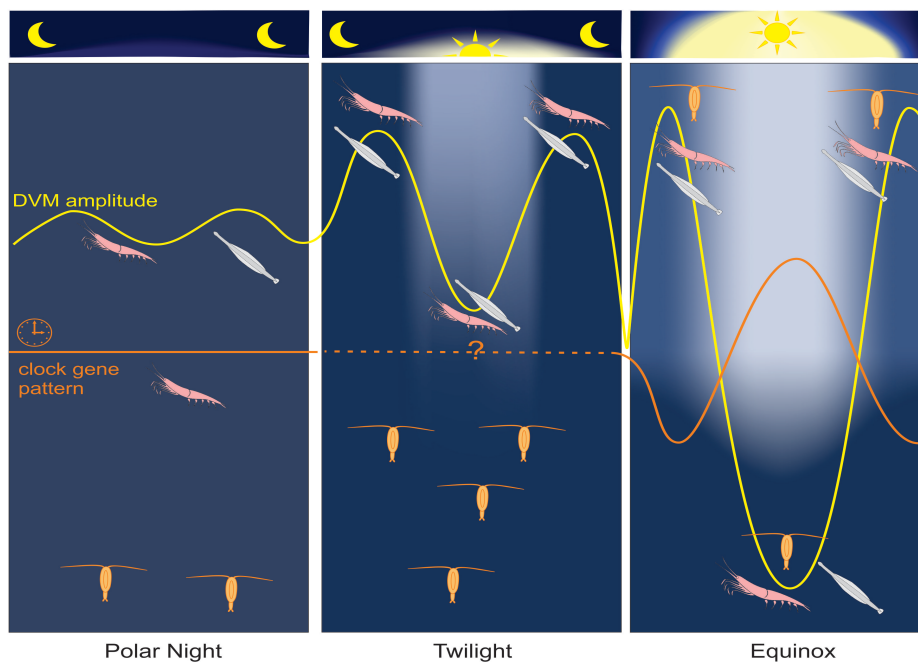


Figure 12: Schematic overview of polar night activity patterns in Kongsfjorden (Svalbard) as observed in present study. 'Polar night' (in the figure) represents findings from the astronomical and nautical twilight periods (mid-winter), whereas

'Twilight' signifies findings from the civil twilight period. Equinox refers to spring and autumn where day-night cycles are distinct, and daytime and nighttime are of equal lengths. The seasonal variations in light regime conditions demonstrate the proximate role of light to cause variable migration patterns. Classical DVM was not detected during the darkest parts of the polar night. Instead migration patterns were restricted to surface waters and of unsynchronized character. In contrast, classical DVM was evident during civil twilight but DVM signals were strongest during equinox periods. Krill, chaetognaths and copepods were abundant species in the Kongsfjorden zooplankton assemblage, although, krill were main responsible for acoustic winter migration patterns and dominated in terms of biomass. The investigated krill clock gene did not show circadian rhythms during nautical twilight, and laboratory experiments demonstrated that the clock gene required light to show diurnal patterns. No clock gene data exist from civil twilight. Despite that krill were able to perceive low levels of solar light during polar night, the background solar light intensities were not sufficient to entrain the clock gene to the diel cycle. Absence of circadian rhythmicity in clock gene and classical DVM during mid-winter suggests that the available light sources were too low to entrain circadian rhythms. Periodically, migration patterns tend to become synchronized during mid-winter, which may be due to lunar light. It is unknown whether lunar light is sufficient to entrain clock gene rhythms in *Thysanoessa* spp. krill. The potential ultimate causes for polar night migration behavior were increased threat of predation during periods with enhanced irradiance sources but also migration of individuals (i.e. feeding need), and endogenous control by other clock genes (see text for further explanation, Image: Malin Daase).

As some Arctic species are able to maintain circadian rhythms, whereas other loses circadian rhythmicity during extreme photoperiods may relate to different coping strategies for survival. A number of Arctic studies demonstrate contrasting observations during the extreme photoperiods with persistence (i.e. for migratory songbirds, scallops) of circadian rhythmicity (Silverin et al. 2009, Ashley et al. 2013, Tran et al. 2016) and absence (i.e. reindeer) of circadian rhythms (Stokkan et al. 2007, van Oort et al. 2007, Lu et al. 2010), equivalent to lack of rhythms in the present study (**Paper II**). The lack of a selective advantage of driving reindeer through diel cycles in an environment without 24 h periodicity (i.e. during extreme photoperiods) was proposed to explain arrhythmic patterns found in Arctic reindeer (Lu et al. 2010). The arrhythmic expression of the *cry2* gene in krill (**Paper II**) is in line with the suggestions for loss of rhythms in Arctic reindeer (Lu et al. 2010). The circadian rhythms in scallops were recorded at 5 m depth (Tran et al. 2016), whereas wild-caught krill were collected down to 30 m depth (**Paper II**). It therefore implies that krill experienced less light, which may explain the arrhythmic patterns. In the study of scallops (Tran et al. 2016), the authors suggested that circadian rhythmicity during polar night was maintained either by 1) a robust circadian clock, independent of light for synchronization, 2) that scallops were able to maintain synchronization with the available low light irradiance cues, or 3) lastly, that the circadian clock was not endogenous controlled. It is unclear how the circadian clock in krill acts since only a single clock gene was examined (**Paper II**) but, compared to the suggestions for scallops, krill did not seem equipped with a robust clock or able to align circadian rhythms with the low light intensity. Thus the findings presented here suggest that the circadian clock was simply not endogenous controlled in *Thysanoessa* spp. It is possible that the spectral sensitivity of the krill eye (**Paper III**) and the light-mediated characteristics of the *cry2* gene (**Paper II**) are finely tuned adaptations that have evolved to allow krill to employ the best survival strategy when exogenous factors in the environment signal that there is a need to employ migration behavior. Thus it may be of higher advantage to align behavior to exogenous cues rather than rely on endogenous rhythms in an environment where exogenous factors (e.g. phytoplankton bloom) change rapidly across seasons. It is important to stress that whereas the reindeer (Lu et al. 2010) and krill (**Paper II**) studies investigated circadian patterns in a single parameter (gene or hormone), behavioral patterns are complex and depend on the interaction of many genes (Dunlap et al. 2004). Thus despite that endogenous rhythms were not observed in the krill clock gene (**Paper II**), it

may be that other clock genes than *cry2* are able to maintain circadian rhythmicity throughout the polar night.

Ecosystem implications of zooplankton vertical migration – past, present and future

Findings presented here add new insights into the limited knowledge of respiration and ammonium excretion rates of Arctic zooplankton migrants during transition from polar night to autumn equinox (**Paper IV**). The fact that classical and synchronized DVM (spring and autumn) occurs outside of the phytoplankton bloom period (**Paper IV**) corresponds with past observations of DVM in Kongsfjorden (Wallace et al. 2010). As active transport of carbon and nitrogen to depth through classical DVM was discontinuous over the annual cycle (**Paper IV**) due to suspension of DVM during parts of the seasonal cycle (**Paper I, IV**), coupling of polar night migrations to the biological pump is probably limited in high Arctic marine ecosystem during winter. It is, however, likely that zooplankton-mediated processes other than vertical migration such as production of fecal pellets, high winter mortality, and sinking of molted exoskeletons (Daase et al. 2014), may have important contributions to carbon and nitrogen export during periods of the annual cycle when DVM is suspended. These biological processes should be investigated during less studied periods (winter) outside of the short productive season, to achieve an accurate description of the role of zooplankton activity and involvement in the biological pump of Arctic marine ecosystems.

The ecological consequences of how a warmer climate in Arctic regions will modify the nature and extent of DVM remain uncertain. It is anticipated that the role of zooplankton vertical migrations will have larger impact in the functioning of the biological pump if DVM periods become longer (Arrigo 2005, Pemberton & Nilsson 2016). As a consequence of warming, sea ice reductions have large implications in regions of the Arctic, where sea ice is widespread. Enhanced phytoplankton blooms due to longer growth seasons (Arrigo & van Dijken 2015) may increase zooplankton consumption and vertical fluxes of organic material. As a consequence of a thinner sea ice cover, the duration of DVM periods may extend, and increased down-welling irradiance has the potential to trigger stronger migrations patterns as zooplankton may employ DVM to greater depths to avoid more well-lit surface. On the other hand, it is expected that nutrient limitation due to stratification, may limit primary production and zooplankton consumption thus lessening vertical fluxes of organic material between surface and bottom waters (Pemberton & Nilsson 2016).

In recent years, krill abundances have been high in areas of the Arctic region and demonstrates that species associated with warmer water are entering high Arctic fjords (Buchholz et al. 2010, Johannessen et al. 2012, Dalpadado et al. 2016). These observations compare well with the finding that krill dominate the zooplankton biomass in Kongsfjorden (**Paper I, IV**). The consequences of warming in Arctic ecosystems and enhanced primary production (Drinkwater 2011) possibly favors feeding conditions and improved growth conditions for herbivorous zooplankton species. It is anticipated that ecologically and economically fish species such as herring, cod and capelin will benefit from the higher abundances of krill and other mesozooplankton species (Dalpadado et al. 2012). Boreal zooplankton may continue to expand into Arctic areas with increasing warming trends, whereas species associated with the Arctic food web, such as *Themisto libellula*, may

experience population declines (Dalpadado et al. 2012). This may ultimately result in changes in structure and energy flow of marine food webs in Kongsfjorden and other Arctic areas influenced by warmer water masses (Dalpadado et al. 2016). It is uncertain whether top-down (i.e. increased predation) or bottom-up (i.e. nutrient supply) control, or a combination of both, will dominate Arctic ecosystem structure with the continued tendencies of warming. To understand how changes in climate will affect population dynamics, productivity, and function of the biological pump, migratory behavioral parameters (i.e. swimming speed, feeding patterns) and fluxes of organic material over the entire annual cycle of both resident and 'new' zooplankton taxa need to be addressed both locally and on a pan-Arctic scale. Finally, this data can show useful in modelling to address these questions, as it is practically impossible to measure all physical and biological processes that affect vertical migration and associated ecological impacts.

CONCLUSIONS

The results showed that polar night DVM is a complex phenomenon involving several migration patterns throughout the winter period. Classical DVM was present only during civil twilight, with the occurrence of short day-night cycles, and zooplankton performed vertical migration throughout most parts of the water column. Migration patterns gradually became of a stronger DVM character and occurred over greater depths with the establishment of more conventional day-night cycles, which demonstrates the proximate role of light to trigger synchronized migration behavior. Throughout the astronomical and nautical twilight periods, classical DVM was absent and the mid-winter migration patterns were unsynchronized, restricted to surface waters, and not strictly coupled to solar illumination (the diel cycle). Periodically, mid-winter migrations became of more synchronized character possibly due to enhanced illumination of other light sources than solar. Predation pressure was not evaluated in the present study but the diel differences in size classes of chaetognaths and krill suggested that threat of predation was higher during civil twilight compared to nautical twilight. Thus the ultimate driver for classical DVM during civil twilight and the periodically synchronized migration patterns during mid-winter was possibly a response by zooplankton to avoid visual seeking predators.

Zooplankton net sampling, in parallel with acoustics revealed that copepods, chaetognaths and krill were dominant species but krill (*Thysanoessa* spp.) dominated the zooplankton biomass, and were largely responsible for the observed acoustic patterns during polar night in the high Arctic Kongfjorden. The spectral sensitivity tests showed that krill were able to perceive levels of low solar light intensities during the darkest part of the polar night. The *cry2* gene in krill required light to express a circadian rhythm, but despite the detectability of krill eyes to perceive solar background illumination, these levels were not strong enough to entrain endogenous circadian rhythms in krill that had experienced several months of exposure to darkness (Fig. 12). Thus the molecular and electrophysiological experiments suggested that polar night migration was exogenous governed by ambient light levels, when these were strong enough, rather than endogenous controlled.

Krill migration contributed significantly to carbon sequestration during the spring and autumn equinox when classical DVM patterns were strong. The role of krill migration in the functioning of the biological pump during winter was limited as classical and synchronized migration was fundamentally absent during the darkest parts of the polar night period.

FUTURE DIRECTIONS

This study contributes with novel insights into polar night zooplankton vertical migration, and species involved in such behavioral patterns in Kongsfjorden (Svalbard). There are, however, still knowledge gaps to fill. First of all, light appears to trigger a migratory response in zooplankton species but this does not always follow a diurnal pattern. In order to fully understand how patterns of migrations are governed and guided by a variety of light sources (solar, lunar, auroral illumination), a better knowledge of the absolute light climate during the polar night, and not the least, how various organisms are able to utilize the available light levels, is needed.

Despite that the data presented here add to the sparse literature on Arctic winter biology, it is essential to increase the understanding of biological activity patterns during the polar night period. In addition to the question 'who is there' a further look into 'why and how' zooplankton perform vertical migrations (ultimate and proximate causes) during parts of mid-winter should be focused. To fully understand the adaptive significance of polar night migration patterns, ultimate causes such as predation risk and feeding patterns that may be involved in 'decision-making' should be investigated to comprehend why and how zooplankton species decide to employ DVM behavior periodically. To measure levels of fish kairomones *in situ* at multiple sampling points throughout the whole polar night period, and compare fish kairomone concentrations with acoustic migration patterns is a relevant study that could resolve predation-cued migration in further detail. Laboratory experiments could also be designed to study behavioral responses of zooplankton at different kairomone concentrations.

The combination of acoustics and zooplankton net sampling seems a suitable approach to answer the 'who is there' question and to demonstrate vertical migration at high resolution scale. It may, however, be useful to improve the dataset by more frequent plankton net sampling to cover the entire polar night period. Zooplankton net sampling would also benefit from more frequent deployments across the diel cycle as simple 'day' and 'night' samples may not track migratory movements of zooplankton at a high enough resolution. It is also relevant to apply sampling protocols comparable to those used in present study in other areas of the Arctic to investigate if winter migration patterns are similar throughout the region. It is furthermore relevant to examine migration patterns of individual organisms to resolve whether individuals within zooplankton populations adopt different migration strategies, which may explain patterns of unsynchronized migration during polar night. The approaches used in the present study do not detect migration patterns of individual zooplankton organisms. Video plankton recorder (VPR) techniques could be an advantage as VPR deployments allow small-scale input data (such as precise depth distribution and individual size) that are hard to obtain with traditional sampling methods (plankton net sampling and acoustic surveys).

As several biological and physical processes are involved in DVM, molecular analyses are highly relevant to employ in the field of DVM research to study this complex phenomenon. Multi-gene approaches such as metatranscriptomics may be beneficial to conduct in future

experiments as these techniques have the capability to reveal regulation of multiple genes involved in physiological and behavioral activity patterns. Further investigation of other timekeeping genes (e.g. seasonal, annual, lunar) is also relevant to understand how Arctic organisms time-coordinate biological rhythms with the external environment. It is essential to understand physiological-behavioral mechanisms involved in temporal synchronization to predict responses of key Arctic species that thrive in an environment influenced by climatic changes. A combined approach of molecular techniques, conventional (plankton net sampling) and descriptive methods (acoustics) may seem a suitable methodology to resolve complex polar night activity patterns in future DVM research.

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