B.Sc. Umweltwissenschaften

Bachelorarbeit

Circadian rhythms in oxygen uptake rates and swimming behaviour of the copepod *Calanus finmarchicus*

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Abstract

In the marine environment herbivorous copepods represent an important link between the level of primary production and higher predators. In its main distribution areas of the North Atlantic and subarctic waters, the herbivorous copepod *Calanus finmarchicus* (Gunnerus, 1765) dominates the zooplankton communities in biomass. As a main prey species for commercially important fish stocks, *C. finmarchicus* links energy from the basis of marine pelagic food webs to higher trophic levels. *C. finmarchicus* performs diel vertical migration (DVM), highly synchronized to the diel fluctuations in light (day and night). The normal DVM pattern is characterized by an ascend to the surface at dusk and a descent to deeper layers at dawn. Yet, researchers have not found what the migration directly triggers. Light is supposed to be the most important cue. However, a few studies suggested that a biological clock is involved in DVM. The recent identification of clock genes in *C. finmarchicus* support the suggestion that an endogenous timing system may be involved in rhythms like DVM in *C. finmarchicus*. Thus, the aim of this work was to assess the role of light (photoperiod) on DVM and diel metabolic processes in *C. finmarchicus* and to detect the possible involvement of endogenous rhythmicity in these processes. To test this, laboratory experiments to the diel swimming behaviour and oxygen uptake rates were performed under light/dark (16 h L:8 h D) and constant darkness (DD) conditions, using the CV stage of *Calanus finmarchicus*. Copepods were sampled from an isolated population in Loch Etive, Scotland. In the laboratory experiments copepods showed a migration behaviour that is highly synchronized to the LD cycle, whereas a damped migration continued under DD conditions. Significant 24-hour oscillations in the vertical distribution were found in the migration experiment during the first day under LD conditions and during all three days under constant darkness. Also an oscillation in oxygen uptake rates was found under DD conditions. Overall, the results stress the importance of light for DVM of *C. finmarchicus* and suggest the involvement of an endogenous rhythm in diel patterns of vertical migration and metabolic processes. Regarding some limitations especially in respiration measurements, this work may provide a basis for further investigations on the true cause zooplankton DVM, implicating the role of biological clocks.

Zusammenfassung

In marinen Lebensräumen stellen herbivore Copepoden eine wichtige Verbindung zwischen den Ebenen der Primärproduktion und den höheren Prädatoren dar. Der herbivore Copepod *Calanus finmarchicus* dominiert die Zooplanktongemeinschaften in seinen Hauptverbreitungsgebieten im Nordatlantik und in subarktischen Gewässern. *C. finmarchicus* ist die Hauptbeute für wirtschaftlich bedeutende Fischbestände und leitet Energie von Primärproduzenten an höhere trophische Ebenen weiter. Wie viele andere Zooplanktonorganismen führt *C. finmarchicus* eine tägliche Vertikalwanderung in der Wassersäule durch, welche den täglichen Schwankungen des Lichts folgt. Die normale Vertikalwanderung ist von einem Aufstieg in der Abenddämmerung an die Oberfläche und einem Absinken in tiefere Wasserschichten in der Morgendämmerung gekennzeichnet. Forscher konnten bis heute nicht herausfinden, was die tägliche Vertikalwanderung direkt auslöst, Licht gilt jedoch als wichtigster Faktor. Dennoch lassen einige Forschungen vermuten, dass eine innere Uhr an dem Timing der Vertikalwanderung beteiligt ist. Die Identifikation von ‚Uhr-Genen' in *C. finmarchicus* unterstützen die Vermutung, dass endogene Rhythmen an der Steuerung der Vertikalwanderung von *C. finmarchicus* beteiligt sind. Vor diesem Hintergrund war das Ziel dieser Arbeit, die Bedeutung von Licht (Photoperiode) für die Vertikalwanderung sowie für metabolische Prozesse zu untersuchen und die potentielle Beteiligung von endogenen Rhythmen an diesen Prozessen festzustellen. Dafür wurden Laborexperimente zum täglichen Schwimmverhalten und Sauerstoffverbrauch vom CV Stadium von *C. finmarchicus* unter Licht/Dunkel (16 h L: 8 h D) und konstanter Dunkelheit (DD) durchgeführt. Die Copepoden wurden aus einer isolierten Population in Loch Etive in Schottland entnommen. Die Experimente zeigten ein Migrationsverhalten, welches stark mit dem vorgegebenen Licht/Dunkel-Zyklus synchronisiert ist. Ein in der Amplitude gedämpftes Migrationsverhalten war unter konstanter Dunkelheit zu erkennen. Statistische Analysen zeigten signifikante 24 h Oszillationen der Vertikal-verteilung der Copepoden während des ersten Tages des LD-Zyklus und während aller drei Tage konstanter Dunkelheit. Auch eine rhythmische Sauerstoffaufnahme wurde in konstanter Dunkelheit gefunden. Die Ergebnisse zeigen die hohe Bedeutung von Licht für die Migration sowie für metabolische Prozesse von *C. finmarchicus* und zeigen klare Hinweise auf die Beteiligung einer inneren Uhr an diesen Prozessen. Auch wenn Ungenauigkeiten, vor allem während der Sauerstoffmessungen, berücksichtigt werden müssen, stellt diese Arbeit eine wichtige Grundlage für weitere Forschungen zur täglichen Vertikalwanderung dar und zeigt, dass die innere Uhr bei diesen Forschungen berücksichtigt werden muss.

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1 Introduction

Calanus finmarchicus is a pelagic living, mostly herbivorous copepod. It is distributed throughout the North Atlantic and subarctic waters (Conover, 1988; Falk-Petersen et al., 2009). The centres of distribution are found at the east coast of the U.S. (Maine), and along the Norwegian west coast, where it dominates the zooplankton communities (Conover, 1988; Hirche et al., 1997; Hirche & Kosobokova, 2007; Falk-Petersen et al., 2009;). As a primary consumer the copepod feeds on phytoplankton blooms in surface waters. In this course, low-energy carbohydrates and proteins in phytoplankton and ice-algae are converted into high energy wax-esters (Falk-Petersen et al., 2009). Its occurrence in high numbers as well as its high fat content makes *C. finmarchicus* an energy rich and important prey for large fish stocks like herring *Clupea harengus* (Dalpadado et al., 2000) and atlantic cod (*Gadus morhua*, Drinkwater, 2005; Sundby, 2000), as well as for seabirds and whales (Dalpadado et al., 2000; Falk-Petersen et al., 2007; Falk-Petersen et al., 2009;). Its important position in the food web, linking energy to higher trophic levels, its high lipid content and the occurrence in high abundances make *Calanus finmarchicus* a key species of the pelagic ecosystems of the North Atlantic (Niehoff & Hirche, 2000). Over the last decades, scientists observed a northward shift of Atlantic species leading to an introduction of *C. finmarchicus* into arctic waters around Spitsbergen (Svalbard) (Beaugrand et al., 2002). A shift in the spatial distribution of key species like *C. finmarchicus* may have severe effects on local food webs and the structure of zooplankton communities (Hirche & Kosobokova, 2007).

The life of C. *finmarchicus* is highly determined by rhythms, on both diel and seasonal scales. Over its active period from spring to late summer especially late stage and female *Calanus finmarchicus* display distinct diel vertical migration (DVM; Marshall & Orr, 1960; Dale & Kaartvedt, 2000). A recent work studying DVM in the arctic found diel migration patterns also in smaller copepodites (CI-CIV) of *C. finmarchicus* (Daase et al., 2015). Besides a diel pattern of locomotor activity, several zooplankton species exhibit a diel activity cycle on both behavioural and physiological levels. A diel rhythm in feeding activity is reported for *Calanus* and other Zooplankton species (Mackas & Bohrer, 1976; Baars & Oosterhuis, 1984; Mayzaud & Conover, 1984; Haney, 1988), showing higher feeding activities during the night time, that are closely related to diel migration patterns. However, diel feeding patterns were also found in non-migrating species, suggesting that also other factors might be involved in the control of feeding rhythms. On the physiological level Tande & Slagstad (1982) were able to identify diel rhythms in digestive enzyme activity. Additionally, Mayzaud & Conover (1984) found diel changes in diet ingestion and amylase activities for different neritic zooplankton species (for example

C. helgolandicus and *Clausocalanus arcuicornis*) and related their findings to an endogenous control of cyclic feeding and digestive enzyme activity.

In winter, the diel activity cycle is interrupted by a period of hibernation (diapause). During diapause, animals spent the cold months in deep water layers in a state of reduced activity (Falk-Petersen et al., 2007). This behaviour is closely related to the reproduction strategy of *C. finmarchicus*: in spring, *C. finmarchicus* hatch from eggs and run through 6 naupliar (NI-NVI) and 5 copepodite stages (CI – CV) during the following months. In late summer, CV stage copepodites accumulate in surface waters and build up big internal lipid reserves in preparation of diapause (Falk-Petersen et al., 2009). Additionally, development stops and lipid rich CV *C. finmarchicus* descent to deep layers for diapause, to survive the harsh and unfavourable winter months. During this period, copepods cease DVM and stay in deep water layers of 500 to 2000 m depth, dependent on the region (Hirche, 1996; Falk-Petersen et al., 2009). Moreover, diapausing animals stop feeding and reduce their metabolic activities to a minimum, living solely from their internal fat depots by respiring at low rates (Tande, 1982; Hirche, 1983; Ingvarsdóttir et al., 1999;). Overwintering copepods are also characterised by reduced digestive enzyme activity as well as low transcription rates, visible in low RNA/DNA ratios (Hirche, 1983; Hirche, 1996; Wagner et al., 1998). In late winter, CV stage Copepods terminate diapause, develop to adults and ascent to the surface to feed on phytoplankton (Marshall & Orr, 1955; Falk-Petersen et al., 2009). These processes are mainly build on internal reserves (Tande, 1982). Fuelled by direct food uptake, females release eggs into the water column as soon as primary production increases at the surface to start a new generation (Marshall & Orr, 1955; Hirche, 1996). In the course of climate induced warming, especially higher latitudes are thought to face major shifts in the onset of phytoplankton blooms (Hansen et al., 2003; Leu et al., 2011). Due to increasing temperatures and thus, an earlier retreat of sea ice in spring (Stroeve et al., 2007), phytoplankton blooms are thought to peak earlier in the season (Hansen et al., 2003; Leu et al., 2011). Since *C. finmarchicus* is dependent on food to reproduce, the timing of terminating diapause to the occurrence of phytoplankton blooms is of great importance. A timing mismatch at this stage may have a severe effect on the copepod population and associated predators (Søreide et al., 2010; Leu et al., 2011). Thus it is of great importance to understand the mechanisms that underlie the timing of seasonal events in *C. finmarchicus*.

While the life cycle strategy is well observed and described (Conover, 1988; Hirche, 1996; Falk-Petersen et al., 2009), the underlying processes for initiation and termination of DVM and diapause remain poorly understood and the current state of the art is outlined below.

The strong rhythmicity on both diel and seasonal timescales in *Calanus finmarchicus* shows strong synchronicity to the fluctuations in light intensity and photoperiod (Marcus, 1986; Falk-Petersen et al., 2009). Besides other factors like temperature, food supply or fat content, day length (Photoperiod) is mentioned as a possible cue triggering daily (DVM, Marshall & Orr, 1960; Falkenhaug et al., 1997; Dale & Kaartvedt, 2000b; Tarling et al., 2002) and seasonal (diapause) events (Miller et al., 1991; Hirche, 1996; Irigoien, 2004; Johnson et al., 2008). Recent transcriptome studies of *C. finmarchicus* were able to identify several core clock genes of the *D. melanogaster* timing system in *Calanus finmarchicus* (Christie et al. 2013).
In this respect the aim of this study was to identify the impact of photoperiod on diel vertical migration and daily metabolic functions and to identify, if an endogenous rhythm in *C. finmarchicus* is responsible for DVM in this species.

1.1 Diel vertical migration in zooplankton

DVM is a periodical movement of mainly zooplankton organisms occurring in both freshwater and marine environments. The migration behaviour is a wide-spread phenomenon amongst a broad range of zooplankton taxa and known to be the biggest animal migration in terms of biomass on earth (Hays, 2003). The normal DVM pattern is well known for more than a century. It is characterized by an ascend to the surface at dusk and a return to deep layers by descending at dawn (Marcus & Scheef, 2010). The depth difference between the deep and the surface depth may range from a few meters in lakes to several tens to hundreds of meters in marine environments and is termed the *amplitude* of DVM (Ringelberg, 1999). When discussing the reasons for such a behaviour, we need to distinguish between two categories:

1. How did the behavioural patterns evolve in the history of life, regarding the processes of evolution? (ultimate factor)
2. What directly initiates the migration on a diel basis? (proximate factors; Dunlap et al., 2004)

Researchers widely agree that the ultimate reason for DVM in herbivorous zooplankton species is predator evasion (Zaret & Suffern, 1976; Marcus & Scheef, 2010; Joop Ringelberg & van Gool, 2003). The predator evasion hypothesis suggests that DVM evolved from a trade-off between predation pressure and feeding. While residing in deep water layers during the day, the animals avoid visually hunting predators (for example predatory zooplankton and fish). During the dark night hours visual predators may be absent or predation success may be limited due to poor

light conditions and the migrators ascend to feed on phytoplankton concentrated in the surface layers.

Since predation pressure as a cue is hard to sense, it needs an alternative cue to time the migration. The discussion of these proximate factors is more divers and includes a variety of environmental parameters and hypotheses (Marshall & Orr, 1960; Ringelberg & van Gool, 2003; Cohen & Forward Jr, 2005). Although, temperature and food availability are known to alter the migration pattern, most studies suggest light to be the most important proximate factor (Stearns & Forward, 1984; Ringelberg, 1999; Cohen & Forward Jr, 2002; Marcus & Scheef, 2010). Particularly in copepods, the relatively high rate of change in light intensity during dusk and dawn are thought to trigger the migration during these times (Stearns & Forward Jr, 1984b; Ringelberg, 1999; Cohen & Forward, 2005b; Cohen & Forward, 2002).

But not only sunlight is able to cause a synchronized vertical migration. Only recently, a work by Last et al. (2016) revealed that moonlight as another proximate factor is able to drive zooplankton migration behaviour. Last et al. analysed a multitude of acoustic profiling data to the migration behaviour of zooplankton during the arctic polar night. They found that zooplankton organisms across the arctic switch from a solar (24 h) to a lunar (24.8 h) rhythm in their migration behaviour when the sun diminishes during polar night. Furthermore, a mass sinking of zooplankton to a depth of ~50 m occurs in synchronicity with the monthly period of full moon (every 29.5 days).

Besides regarding DVM as a direct response to light, other studies suggested that light functions as a synchronizer for endogenous rhythms that trigger DVM (Harris, 1963; Enright & Hamner, 1967). A study by Cohen & Forward (2005b) included investigations on the rate of change hypothesis as proximate cue for the DVM of *Calanopia americana* under laboratory conditions. The results revealed light (rate of change) as a factor able to influence descend or ascend movements of the copepods but not being the only reason. Under constant darkness conditions *C. americana* showed significant DVM, clearly pointing towards the involvement of an endogenous rhythm in the control of DVM. Earlier studies on e.g. *C. finmarchicus* and *Daphnia magna* (Harris, 1963; Enright & Hamner, 1967) also found ongoing migration, as well as diurnal patterns of locomotor activity in constant darkness conditions in the laboratory.

These studies support our assumption that endogenous timing mechanisms are involved in *C. finmarchicus* DVM.

1.2 A Biological clock as a timer for diel and seasonal rhythms

Geophysical cycles like the rotating system of sun earth and moon have fundamental influence on life on earth (Kuhlman et al., 2007; Foster & Roenneberg, 2008). Seasons, caused by the earth's tilt and orbit around the sun, as well as tides (the result of gravitational forces of sun and moon) and the diurnal light cycle (due to earth's rotation around its own axis) determine the life of most organisms on earth (Strauss & Dircksen, 2010; Tessmar-Raible et al., 2011). Consequently, many organisms react to these cyclic changes of their environment with periodic fluctuations of physiological and behavioural processes (Aschoff, 1954).

In the course of evolution a broad range of organisms ranging from bacteria to humans have evolved endogenous *biological clocks* that help to match diel fluctuations of the environment (Foster & Roenneberg, 2008; Allada & Chung, 2010; Teschke et al., 2011; Shimmura & Yoshimura, 2013). Without any external time cue these biological clocks run with a period of close to but not exactly 24 hours and are therefore called *circadian* (Latin: circa = about; dies = a day) (Kuhlman et al., 2007). To synchronize to environmental fluctuations, in e.g. light, temperature or food availability, the clocks are entrained by a so called *Zeitgeber* (German for *time giver*), which resets the clock on a diel basis and thus creates a 24 h rhythm. Since the light/dark cycle is created by the earth's rotation and therefore not changing, the most reliable and thus most widely used *Zeitgeber* is light (Aschoff, 1954). Especially in terrestrial organisms, changes in ambient temperature may be able to reset internal clocks and thereby act as an additional *Zeitgeber* (Aschoff, 1954; Liu et al., 1998). Furthermore, other environmental factors like fluctuations of food availability or social cues may alternatively play a role in the timing of rhythms (Aschoff, 1954).

While the origins of endogenous biological timing systems are assumed to be numerous, the molecular structure of them is similar (Dunlap, 1999). In general, oscillating systems are based on transcriptional/posttranslational negative feedback loops which create a rhythm of approximately 24 hours (Dunlap, 1999; Kuhlman et al., 2007). While the cyclic activation and inhibition of so called *clock genes* is a single cell property without any actual need for intercellular communication, in most biological clocks groups of autonomous cellular clocks are connected to central *pacemakers,* generating coherent timing information to time physiological and behavioural processes (Strauss & Dircksen, 2010; Kronfeld-Schor et al., 2013). Vice versa, the pacemaker is reset by environmental stimuli like light and therefore is able to synchronize outputs to the day/night cycle. Besides keeping time with diel fluctuations, a circadian clock may also provide information about the progression of the season e.g. by sensing the day length

(photoperiod) (Oster et al., 2002) and thereby may be important to time seasonal events like the initiation or termination of diapause (Davis, 2002).

Research on circadian clock systems is of big importance and experienced huge improvements and new insights in recent years. Nevertheless, works mainly focussed on land based model organisms such as mouse, fruit fly or thale cress, while knowledge about clocks in marine organisms is scarce (Tessmar-Raible et al., 2011; Kronfeld-Schor et al., 2013). The similar molecular makeup, as well as the evolution of biological clocks in different species again and again, stresses the adaptive advantage of synchronizing the own body functions and behaviour to the geophysical cycle of day and night (Yerushalmi & Green, 2009).

Marine organisms have always been living in a highly rhythmic environment. besides daily fluctuations of light due to the day/night cycle, the world's oceans are dominated by a variety of rhythms with different periods (Tessmar-Raible et al., 2011). Coastal environments are strongly influenced by tides, creating a highly dynamic ecosystem. Major changes in a range of environmental parameters like salinity, temperature, humidity, exposure to predators etc. require a high adaptive capacity by its inhabitants (Wilcockson & Zhang, 2008; Tessmar-Raible et al., 2011). Many of these coastal living organisms, like the flatworm *Symsagitiffera roscoffensis* (former *Convoluta roscoffensis*; Bohn, 1903) or the shore crab *Carcinus maenas* (e.g. Naylor, 1958), adapted to these fluctuations by evolving circatidal rhythmicity. Biological clocks that run with a period of 12.4 h (semidiurnal tides) or 24.8 h (diurnal tides), to match or even anticipate the changes (Wilcockson & Zhang, 2008; Naylor, 2010; Tessmar-Raible et al., 2011;). Besides coastal regions, open waters around the world are affected by rhythms as well: a broad range of planktonic organisms perform synchronized DVM, tuned to the day-night cycle and occur in a large latitudinal range from tropic regions to the poles (Hays, 2003; Naylor, 2010). Light is known to play a major role in achieving the rhythmic migration and in combination with the pervasive utilization of endogenous timing systems across a broad range of organisms, it supports our hypothesis of a biological clock triggering DVM. Regarding the importance of *C. finmarchicus* to a number of marine food webs it is of fundamental scientific interest to understand the underlying causes that affect DVM and the timing of annual diapause, to predict changes in population structure and trophic interactions in the course of a changing climate.

According to the current knowledge of DVM in zooplankton and the impact of biological clocks as potential driver for daily and seasonal events in marine pelagic organism, we hypothesized that

- *Calanus finmarchicus* shows diel rhythms in vertical migration behaviour synchronized to a light-dark (LD) cycle
- The activity is reflected in daily oscillations of oxygen consumption
- Both rhythms persist under constant darkness (DD) conditions

To test the hypotheses, we conducted two laboratory experiments under varying light conditions. In the first experiment, we assessed the oxygen uptake rates of *C. finmarchicus* over three days in the laboratory, one day under a provided light/dark (LD) cycle and two days under constant darkness (DD). In the second experiment, we investigated the diel migration behaviour of *C. finmarchicus* over five days, with two days under LD and three days under DD conditions. Since the late copepodite stage (CV) performs both diel (DVM) and seasonal (Diapause) rhythms, and additionally is available almost year-round, experiments were conducted using the CV stage of *C. finmarchicus*. Animals were sampled from 'Bonawe Deep' in Loch Etive, Scotland. Most likely the special hydrographic conditions (see Chapter 2.1) restrict the numbers of the congeneric species *C. helgolandicus* in Loch Etive and leads to the isolation of a *C. finmarchicus* population at our sampling site Bonawe Deep (Hill, 2009).

2 Material and methods

2.1 Location and hydrographic conditions

Loch Etive is a sea loch situated at the west coast of Scotland (56° 27’ N, 5° 21’ W). The Loch is a glacially scoured fjord with two sills. A first sill at the Loch entrance separates the Loch from the adjacent coastal waters. The very narrow (200 m) and shallow (10 m) entrance area, named ‘Falls of Lora’, highly reduces the water exchange (Edwards & Edelsten, 1977). About 10 km east of the entrance, a second sill (‘Bonawe Sill’), lowers the water depth to only 13 m below mean high water (MHW; Nørgaard-Petersen et al., 2006) separating the Loch into a lower and an upper basin (see figure 1). The upper basin accounts for the maximum depth of Loch Etive with 140 m

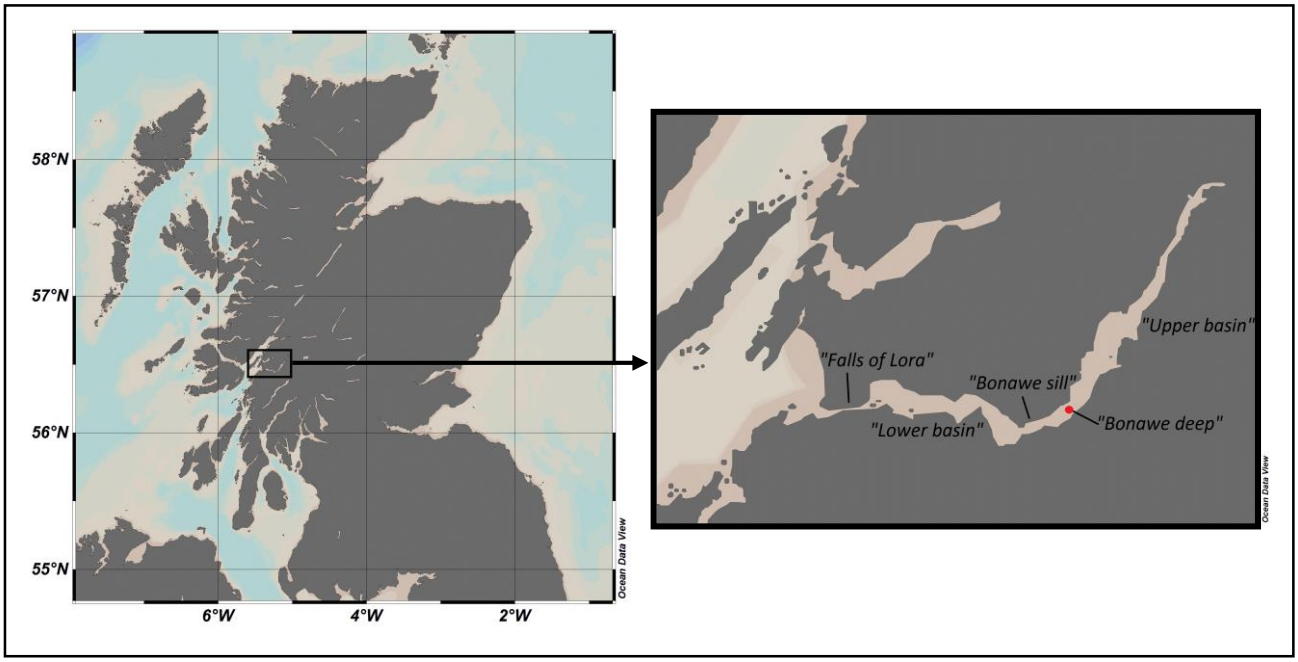


Figure 1: Location of Loch Etive on the west coast of Scotland. Right: Detailed view of Loch Etive with the location of the sampling site (red mark). Map created with ODV (Schlitzer, 2014).

at *Bonawe Deep*, a deep basin directly behind the sill. Besides the restriction of water exchange, the inflow of freshwater from river runoff mainly determines the hydrography of the Loch environment (Wood, Tett, & Edwards, 1973). The catchment area of 1400 km^2 (Wood et al., 1973) results in high runoff leading to a freshwater layer at the surface. Bonawe sill restricts the exchange of water between lower and upper basin to the brackish surface layers. Thus, the salty water in the deep layers of the upper basin may stagnate there for several months up to years. (Edwards & Edelsten, 1977). The stagnation of deep water leads to a strong oxygen depletion and only during a deep water renewal event oxygen rich waters reach the deep basins (Austin & Inall, 2002). A deep water renewal only occurs during conditions of low freshwater input and

low surface temperatures. Both increase the density of the surface layer and thus decrease the stratification, allowing the salty deep water layers to rise and flow over the sill into the upper basin (Edwards & Edelsten, 1977, Austin & Inall, 2002).

2.2 Sampling of *C. finmarchicus*

Copepods for respiration measurements were sampled on May 29th (9:00 am) with RV *Seol Mara* from the Scottish Association for Marine Science (SAMS), whereas Copepods for DVM experiments were sampled on June 19th 2015 (4:00 pm) at Bonawe Deep in Loch Etive with *RV Calanus* from SAMS.

Animals were sampled via vertical hauls, using a WP2 plankton net (Mesh size 200 µm) with a netted (50 µm) bucket cod-end. The net was equipped with an open and closing mechanism. This mechanism allows to only sample certain parts of the water column without collecting animals when lowering or hauling the net through other water layers. The net is closed when lowered to the desired depth. A weight ('Messenger'), being attached to the wire, slides down and triggers the mechanism which opens the net. The net will be hauled to a certain depth where a second messenger will be send down to close the net again. For respiration measurements, the whole water column (140 - 0 m) was sampled. During the sampling for DVM experiments a part of the *C. finmarchicus* population possibly already induced diapause (personal observation) and descended to the deep layers. Thus, the sampling was restricted to the top layer (50 - 0 m) to ensure that only active animals were obtained for migration behaviour experiments.

When back on board, the content of the cod-end was poured through a 500 µm sieve to avoid smaller early stage copepods.

For respiration measurements, the sieved sample was distributed to plastic buckets that contained filtered and chilled seawater, obtained from the aquariums sea water system at SAMS. During transport back to the laboratory (~15 km) buckets remained closed to protect animals from high light intensities (for further handling details see 2.3).

For DVM experiments, the sieved sample was distributed to a small bucket that contained seawater and directly brought to the ships laboratory for sorting (see 2.4). The seawater was obtained from 100 m water depth at the sampling site, by using 'Niskin bottles'.

In order to obtain information about the hydrographic conditions during the time of sampling, a CTD (**c**onductivity, **t**emperature, **d**epth) -instrument was used. A CTD is a cluster of sensors which measures the conductivity, temperature and pressure while it is vertically hauled through the water column. Thus, it is able to provide vertical profiles of essential parameters that provide

information about the hydrographic properties of the environment. Besides temperature, the basic equipment delivers information about the salinity, derived from conductivity, and depth, derived from pressure values. The CTD used (SBE 19 plus, SeaBird) was additionally equipped with sensors for fluorescence (WETStar, Wetlab) and dissolved oxygen (SBE 43, SeaBird). The fluorescence sensor measured the Chlorophyll *a* content of the water, which is used as a proximate measure for the phytoplankton concentration. CTD data for the sampling in May were obtained on midday of March 7th on a cruise of the *RV Calanus* from SAMS. The CTD data for the sampling in June were obtained on midday of June 19th, the day of the sampling.

2.3 Respiration measurements

Back in the laboratory bucket lids were removed and animals were allowed to acclimate to ambient conditions for 12 hours. The temperature in the 'constant temperature room' (CT-room) was set to 10 °C. Copepods were kept under a light/dark (LD) regime with 16 hours light (from 4 am to 8 pm) and 8 hours darkness, synchronized to natural conditions. The light regime was set to a constant rise and fall of light intensity throughout the day. Lights were covered with blue plastic foil and the maximum intensity did not exceed 100 Lux to simulate under water light conditions.

Directly before the start of the experiment, animals were sorted in the laboratory facilities of SAMS. The catch was examined under the microscope and sorted for species and stage. During the sorting process petri dishes were kept in cooling chambers and only red light was used to reduce temperature and photic stress to the animals. After carefully sorting under the microscope, animals were distributed to well plates containing filtered (0.7 µm) and UV-treated seawater to clean animals from bacteria and phytoplankton. This process was supposed to reduce the influence of bacteria and phytoplankton on oxygen measurements. Eight incubation bottles (DURAN Laboratory bottles) were equipped with a sensor spot on the inside and a thread at the same spot on the outside to later connect the incubation bottles to the oxygen measurement device during the experiment.

Oxygen uptake rates of CV stage *C. finmarchicus* were assessed for three days under varying light conditions. Therefore, incubation bottles (DURAN Laboratory bottles) with a total volume of 320 ml were cleaned with Ethanol (95 %), rinsed and filled with filtered (0.7 µm) and UV treated seawater. Bottles were open for about 30 min. to aerate and level to oxygen content of the atmosphere. Eight sorted CV-stage copepods each were transferred to six incubation bottles, using a small spoon made of mull to not to harm the animals during the transfer. To avoid air bubbles inside, the bottles were closed in a basin filled with the same seawater used

in the incubation bottles. While closing the bottles, a piece of mull was used to cover the bottle neck and thus preventing the copepods from escaping. Two incubation bottles without animals were prepared as controls, which allow to correct the measured oxygen values for bacterial O_2 consumption afterwards. The prepared incubation bottles were connected to the oxygen measurement device by attaching optical fibre cable to the threads. All bottles were kept in an upright position and inside a cooling basin (volume 36.5 L) to buffer against external temperature fluctuations. During the experiment, two *OXY-4 mini 4-Channel fibre optic oxygen transmitter* (PreSens) were used to log the oxygen saturation in eight incubation bottles parallel. The principle of measurement is based on the effect of *dynamic luminescence quenching* by molecular oxygen. Thereby, the device measures a light signal which changes depending on the oxygen content of the water.

Respiration measurements were conducted for three days (midnight 30th of May to midnight 1st of June, 72 hours). The light regime varied from an LD cycle (16 h:8 h) during the first 24 hours to constant darkness conditions (DD) during the following two days. For temperature observation a temperature logger (Star Oddi) measured the temperature in a five-minute interval inside the cooling basin. Additionally, the temperature in the cooling basin was measured manually using a digital thermometer (Hannah Instruments).

After the end of the experiment, the animals were inspected under the microscope. The number, stage and condition of the copepods of each bottle were noted and the individuals were distributed to pre-weighed tin capsules. Tin capsules were frozen at -80 °C and transferred to the Alfred-Wegener-Institute in Bremerhaven for further processing.

2.4 Diel vertical migration experiment

For DVM experiments animals were sorted directly after the haul in the ships laboratory. 200 CV stage *C. finmarchicus* were sorted under the microscope as described above and transferred to a bucket with seawater from the sampling site. Animals with signs of activity, such as a filled gut and a small lipid sack, were selected to avoid the use of diapausing animals. In a closed bucket animals were transported to the laboratory facility of SAMS.

To investigate the diel swimming behaviour of *C. finmarchicus* a transparent tube was set up in a CT-room at SAMS. The column with a total length of 90 cm and a diameter of 10 cm is closed at the bottom and stands in an upright position. The column was coated in black foil to ensure natural conditions with light penetration just from above. Marks on the outside separate the column into eight different depth layers with a height of 10 cm each (0 - 80 cm), whereas 0 - 10 cm represents the surface layer and 70 – 80 cm the deepest layer (see fig. 2). The tube is filled

with filtered and UV treated seawater. The water surface is equal to the top mark of the surface layer resulting in a water column with a depth of 80 cm (volume: 6.3 L). The light conditions were the same as in the respiration experiment (LD, 16:8). The temperature in the laboratory was set to 11 °C.

After arriving at the laboratory facilities copepods were transferred to the water column and were allowed to acclimate to laboratory conditions for four hours before the start of the experiment.

The experiment to the diel migration behaviour of 200 CV stage *C. finmarchicus* were conducted for five days (midnight 20th – midnight 24th of June, 120 hours) under varying light conditions. Over the time of the experiment, the number of animals in each water layer was counted and noted hourly. Therefore, a part of the black coat was removed to get access to the column. To count the animals, a red-light head torch was used, a white bucket lit behind the column ensured a better contrast. The light regime varied from an LD cycle (16 h:8 h) during the first two days to constant darkness conditions during the remaining 3 days. After each counting, the temperature in the surface layer was measured manually using a digital thermometer (Hannah Instruments), additionally during the LD cycles, the Light intensity at the top of the column was measured hourly using a Photometer (ILM-1337, ISO-TECH).

In order to assess the oxygen depletion of the water column after the experiment, the oxygen content in each water layer was measured using an 'Oxygen Dipping Probe' (DP-PSt3, PreSens), connected to the oxygen measurement device used in the respiration experiment.

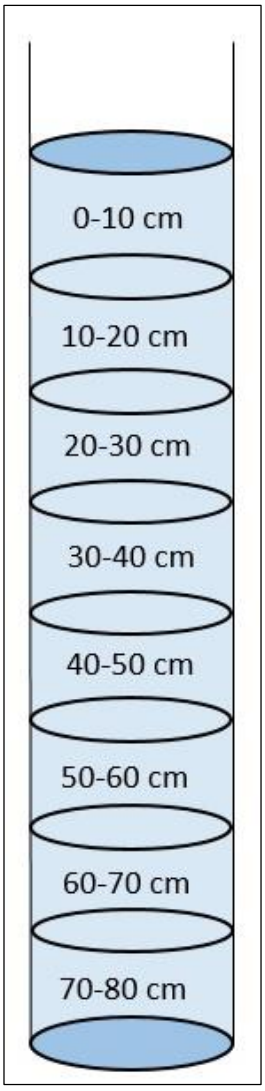


Figure 2: Sketch of the DVM column showing the eight depth layers.

2.5 Statistics

CTD data were uploaded from the instruments internal memory and pooled to 1 m intervals for further analysis. Figures of the hydrographic data were produced using the statistical program R.

The data from respiration measurements first needed to be corrected for temperature and bacterial O_2 consumption. Due to temperature fluctuations of the CT-room during respiration measurements, the temperature inside the water basin varied of about 1 °C. The sensitivity of the solubility of oxygen in water to changes in temperature makes it necessary to correct the measured O_2 values for temperature. The manufacture of the measurement device (PreSens) provides information about the change in the measured oxygen value at a

constant oxygen content and a change in temperature of ±1 °C. These values show a linear trend in changes of the measured oxygen for different oxygen contents and a change in temperature of ±1 °C. The linear equation (1) of the regression line over these given values was used to calculate a correction factor *k* for the measured O_2 content.

(1) $y = 0{,}0154\ x + 0{,}0569$

(2) $k = y * (t_x - 10°C)$

By setting in the measured oxygen values (in % air sat.) for *x* gives a correction factor for a change of ±1 °C (*y*). By multiplying the difference between the set measurement temperature (10 °C) and the actual measured temperature (t_x) by the correction factor from equation (1); delivers a temperature correction factor *k* for each O_2 value (2). This correction factor *k* is > 0 for measured temperatures under 10 °C, correcting the O_2 value up, and < 0 for temperatures over 10°C, correcting the O_2 value down. To finally correct the data, *k* was calculated for each O_2 value and being added to the relevant value. Furthermore, the values were converted from % air saturation into mg/L for further processing.

After temperature correction, the data were also corrected for bacterial O_2 consumption. Therefore, the mean O_2 values of the control bottles were subtracted from O_2 values of the bottles containing copepods. To obtain the uptake rates of the copepods, the difference in measured oxygen content from one time-point to another was calculated. Thereafter, a moving average for a period of 12 h was calculated to demask possible rhythms in oxygen uptake rates. To cover the whole range of time-points, the moving average was calculated three times: one by averaging 12 h after a given time point, a second averaging the previous 12 h and a third, averaging the time span from 6 h before to after a given time point.

To analyse the DVM data, the percentage of animals in each depth layer was calculated as well as the mean depth of the animals over the time of the experiment. Additionally, a moving average (period 5) was used to demask possible diel rhythms under short term fluctuations. For each time point the previous five values (including the time point value) were averaged to produce a period-5 moving average. The package RAIN (R/Bioconducter) for the statistical program R was used to detect diel rhythms in migration behaviour. The RAIN analysis was done using the moving average of the mean depth values. Analysis with p-values <0.05 were accepted as significant.

3 Results

3.1 Hydrographic properties

Profiles of the hydrographic properties at the sampling site were obtained using a profiling CTD (Seabird), additionally equipped with sensors for dissolved oxygen and chlorophyll *a* fluorescence measurements.

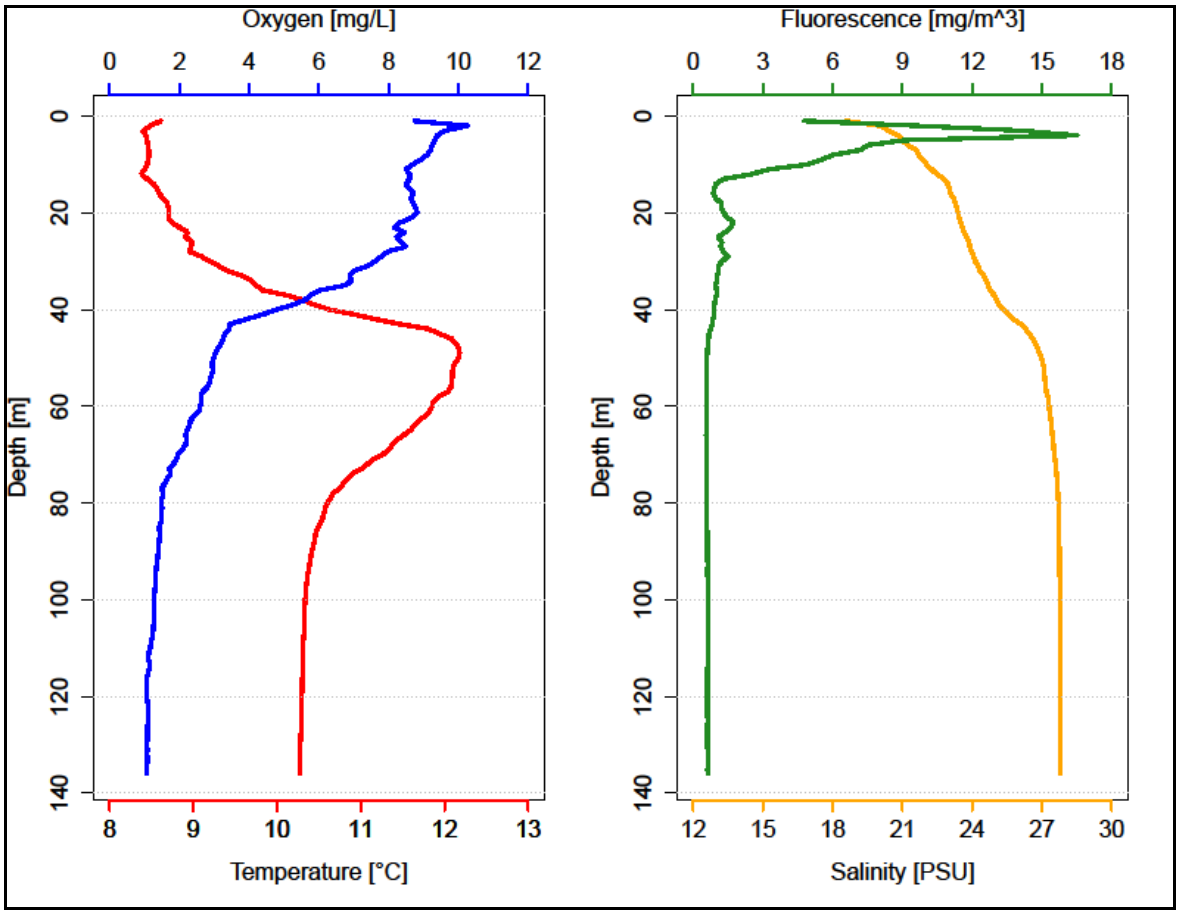


Figure 3: Hydrographic properties at Bonawe Deep on midday of May 7th. Left: vertical profile of dissolved oxygen (blue) and temperature (red). Right: vertical profile of Chlorophyll a fluorescence (green) and salinity (orange).

CTD data for the May sampling showed temperatures of about 8°C for the upper 30 meters. Between 30 m and 45 m temperatures rose to about 12.5°C, indicating a thermocline (Fig. 3, left). Thereafter, water temperatures declined again reaching 10.3°C at 80 m. The water temperatures remained constant throughout the bottom layer (>80 meters). Values for dissolved oxygen ranged between 8 to 10 mg/L in the upper 30 meters. Thereafter, oxygen concentrations declined to 1 to 2 mg/L to a depth of 80 m and remained constant in deeper layers, indicating anoxic conditions below the thermocline. The salinity at Bonawe Deep in May increased with increasing water depth from 19 to 27 PSU over the upper 50 m. Thereafter, salinity was constant with 27 PSU (Fig. 3, right). Chlorophyll *a* in the water column, peaked

between 3 and 4 m with values between 14 and 16.5 mg/m^3 (Fig. 3, right). Thereafter, chlorophyll *a* decreased rapidly and showed values of <1 mg/m^3 throughout the rest of the water column.

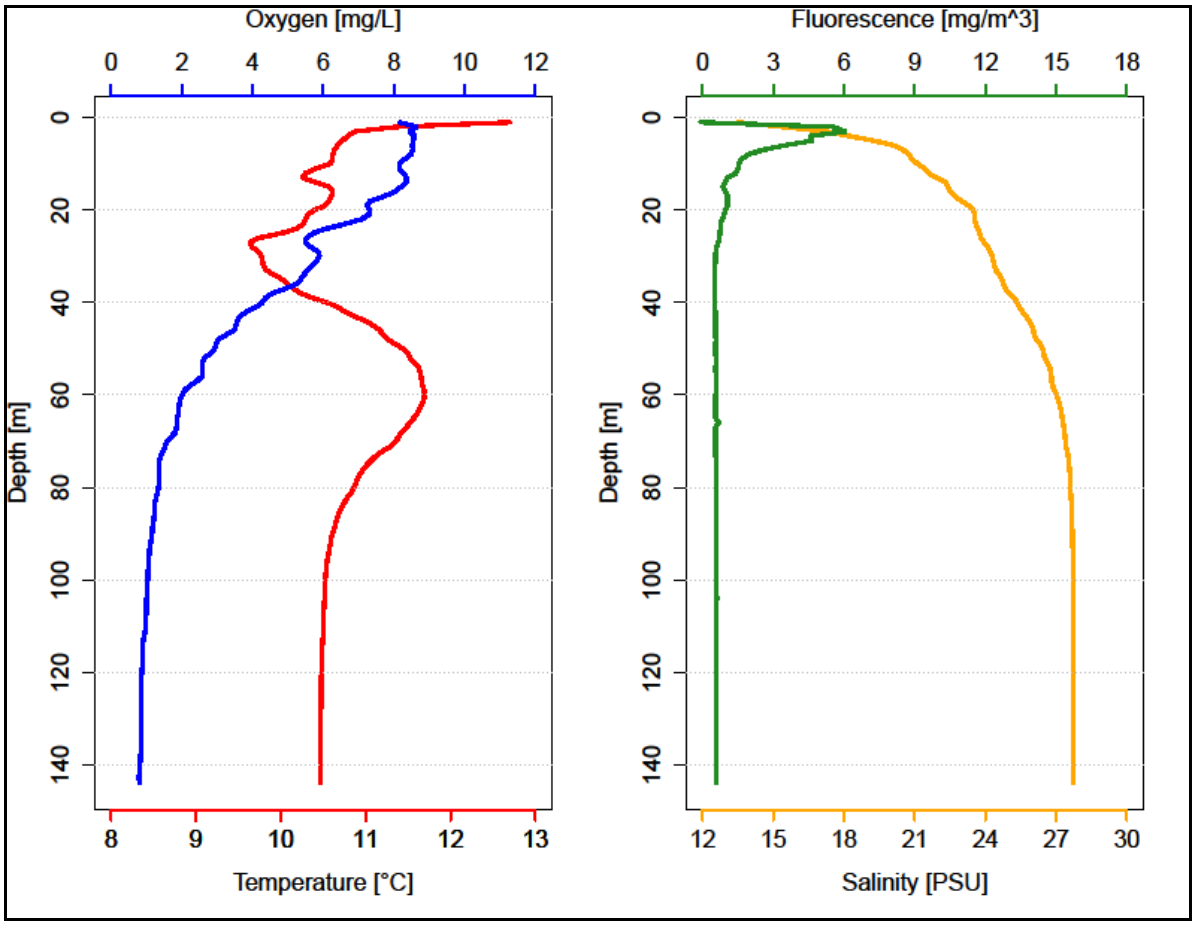


Figure 4: Hydrographic properties at Bonawe Deep on midday of June 19th. Left: vertical profile of dissolved oxygen (blue) and temperature (red). Right: vertical profile of Chlorophyll a fluorescence (green) and salinity (orange).

CTD data for June showed generally similar conditions (Fig. 4). They differed mainly in higher surface water temperatures and lower chlorophyll *a* values towards May conditions. Water temperatures of the upper 2 m showed values of 10 to 12°C but declined to <10°C until ~30 m. A thermocline is notable at 60 m, where temperatures rose again and peaked with 11.7°C. Water temperatures remained constant throughout the bottom layer at about 10.5°C. The dissolved oxygen showed values of 8 mg/L in the upper 20 m and thereafter decreased constantly, reaching very low values of <2 mg/L at 80 m water depth (Fig. 4, left). Salinity and chlorophyll *a* data were similar to May data, but with a smaller chlorophyll *a* peak (5.5 – 6.5 mg/m^3) compared to May conditions between 2 to 3 m and values <1 mg/m^3 for lower water layers >20 m. Salinity was low in the upper 5 m with values between 13 and 19 PSU and increased to >27 PSU for water depths >60 m (Fig. 4, right).

3.2 Respiration measurements

The oxygen uptake rates of the CV stage of *Calanus finmarchicus* were assessed by incubation experiments over three days, with one day under LD (16:8) and two days under DD conditions. Oxygen values were corrected for temperature fluctuations and bacterial O_2 consumption. To detect possible rhythmic changes in oxygen uptake of the copepods, three moving averages were calculated by averaging the data of 12 h after a time point (*next 12 h*), 12 h *previous* to a time point (*previous 12 h*) and 6 h *before and after* a time point (*previous and next 6 h*).

The oxygen uptake rates of *C. finmarchicus* showed almost no changes during the first 20 hours of the experiment under LD conditions (Fig. 5). A first change is visible just before the first midnight showing a short increase in oxygen uptake of about 0.005 mg O_2 L^{-1} $individual^{-1}$ (hereafter noted without units). The small peak during the first night is visible in all three moving averages. Moving average for *next 12 h* showed a constant decline in oxygen consumption from morning of the first subjective day until late afternoon of about 0.01. It was followed by an increase until the early subjective morning of about 0.04. Moving average values for the *previous and next 6 h* remain constant during the first subjective day. During subjective night they showed an increase in oxygen uptake of about 0.015 peaking at late night. The moving average calculated for the *previous 12 h* increased from midday of the first subjective day until late night with a change in oxygen uptake rates of 0.02. From late subjective night values of all moving averages decrease again until midday of the second subjective day. A beginning rise in oxygen uptake is visible in the moving average of *previous 12 h* until the end of the experiment (midnight of second subjective night).

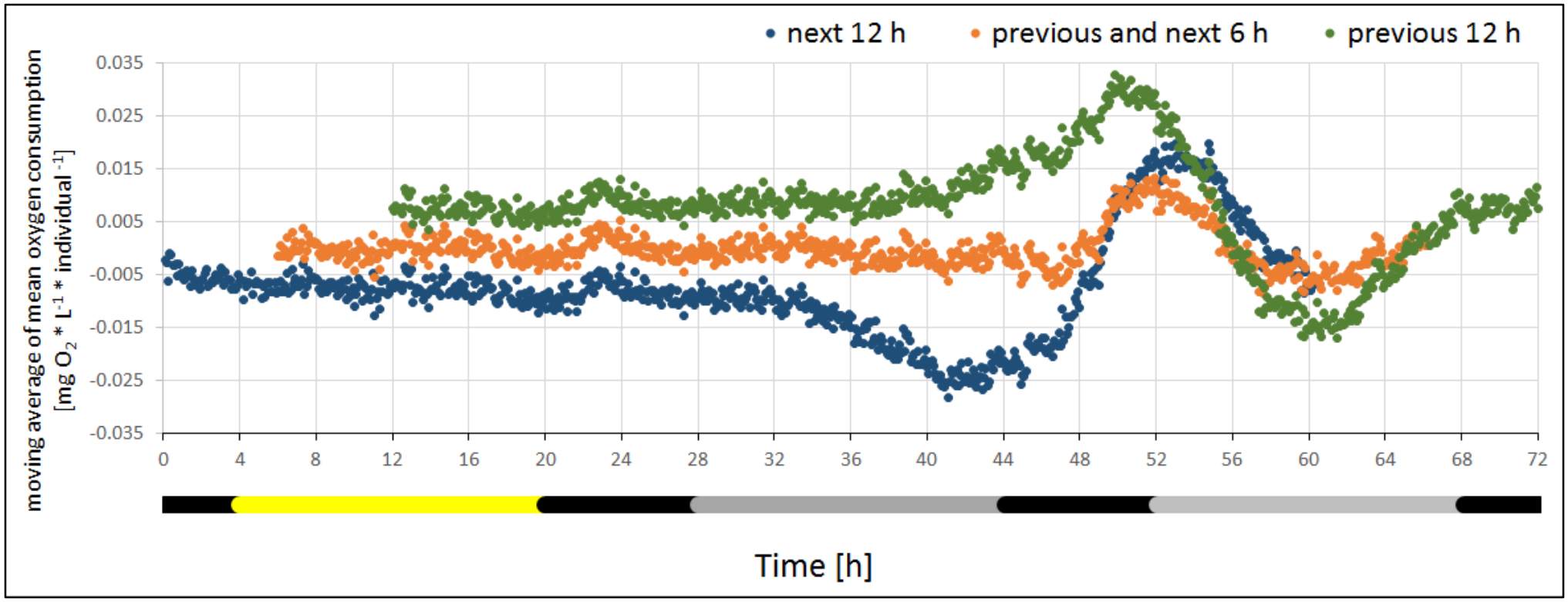


Figure 5: Moving average of the change in mean oxygen consumption (mg O_2 L^{-1} $individual^{-1}$) over the duration of the experiment (3 days, 72 hours). Green: Moving average using the data of 12 h previous to a time point. Orange: Moving average using the data of 6 h before and 6 h after a time point. Blue: Moving average using the data of the next 12 h after time point. The bar under the graph indicates the light regime with night (black), day (yellow) and subjective day (grey).

3.3 Diel vertical migration experiment

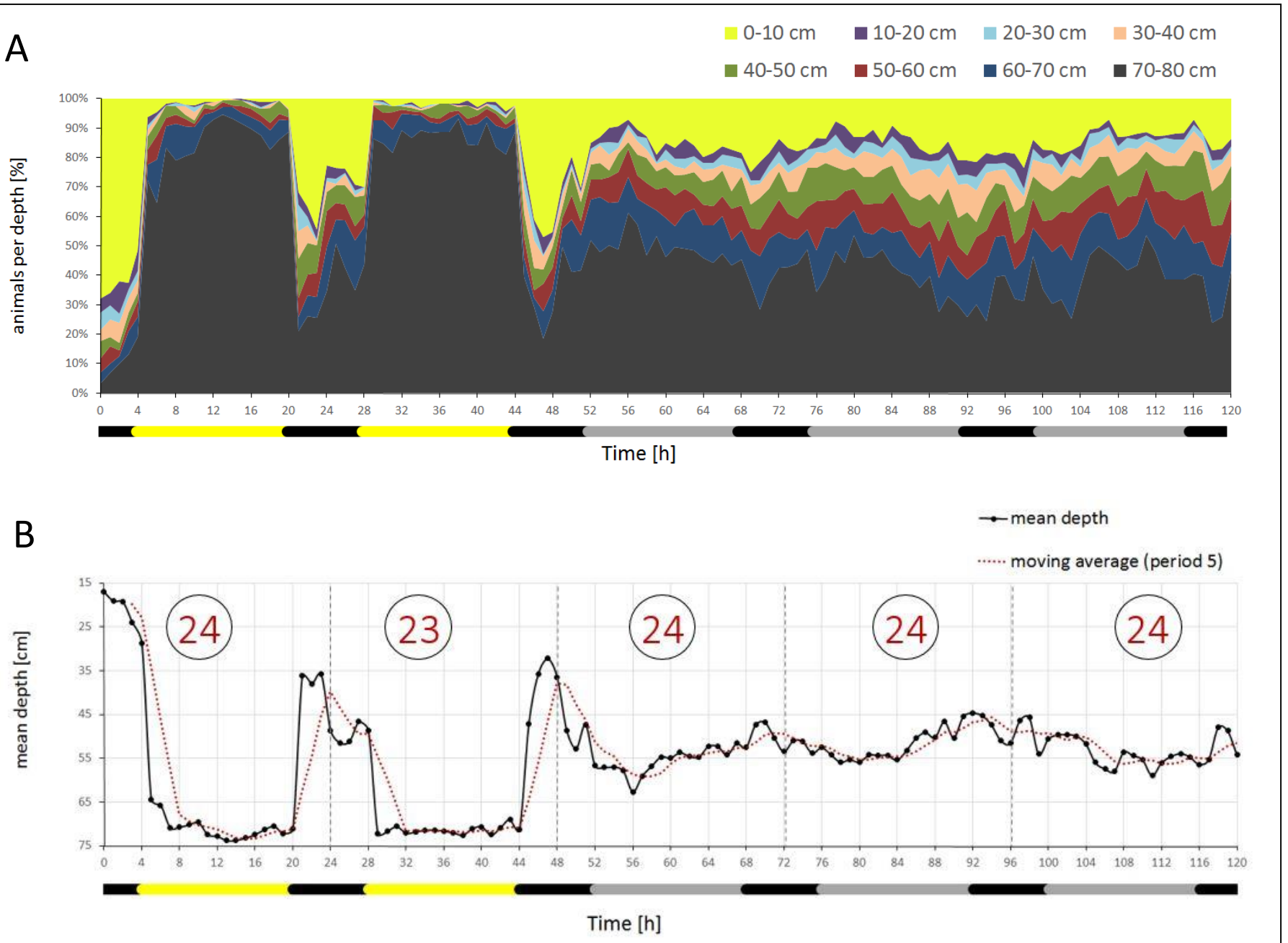
The experiment to the diel swimming behaviour of *C. finmarchicus* was performed over five days, with two days under LD (16:8) and three days under DD conditions.

The results revealed strong fluctuations between the day- and nighttime distribution of the copepods (Fig. 6). Day-night differences were strongest under LD conditions. Percentages in the upper 10 cm varied strongly from up to 70 % during the night to 0-6 % during the day (Fig. 6, A). The occurrence of animals in the bottom layer (lower 10 cm) showed a reverse pattern with high numbers of animals (65 – 94%) during the first two days and relatively low numbers (<50%) during the nights. Copepods in the middle layers (10 -70 cm) averaged to 19% (SD = ±11.1 %) during the LD cycle.

The following 3 days under constant darkness showed lower variations in copepod distribution between subjective day and subjective night (Fig. 6, A). Percentages of *C. finmachicus* in the bottom layer during subjective nights varied between 23% and 51% (mean = 37.8%, SD = ±7.9%). However, during subjective day values were slightly higher in the bottom layer with a mean of 43.37% (SD = ± 7.9%). In the top layer, during subjective night values were in the mean 5% higher than at subjective daytime (mean = 13.84%, SD = ± 3.8%). The proportion of animals in the middle layers increased during constant darkness conditions of about 24% towards LD conditions, wich resulted in a mean of 43.03% (SD = ±7.5%).

Likewise the distribution of copepods per depth layer, the mean depth values of *C. finmarchicus* copepods displayed clear differences between day and night under LD conditions (Fig. 6, B). During daytime, mean depth ranged between 70 to 75 cm, whereas mean depth range during the night was generally lower (35 – 50 cm). Additionally, a fluctuation in the mean depth during the night was visible under LD conditions. Lowest values occured before midnight, with a mean depth around 35 cm. Over midnight mean depth of copepods dropped to a depth of >50 cm, before a smaller second peak, at 45 to 50 cm, occured during late night (three hours after midnight). RAIN analysis of the moving average of the mean depth values (period 5) indicated a highly significant 24 h oscillation (RAIN:$p<0.06*10^{-16}$) during the first day under LD conditions (Fig. 6, B).

Figure 6 (next page): A: Percentage of animals per depth layer over the duration of the experiment. B: Mean depth of animals over the duration of the experiment (black line) and moving average of the mean depth (period 5) (red dotted line). Numbers in circles indicate the period of the oscillation by the moving average, calculated from RAIN analysis. Values are significant for all days (p<0.01*10-9). Bars at the bottom of each graph indicate the light regime, with day (yellow), night (black) and subjective day (grey). For a higher resolution of days 3 to 5 see Fig. 7.


A
0-10 cm
10-20 cm
20-30 cm
30-40 cm
40-50 cm
50-60 cm
60-70 cm
70-80 cm
animals per depth [%]
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104 108 112 116 120
Time [h]
B
mean depth
moving average (period 5)
mean depth [cm]
15
25
35
45
55
65
75
24
23
24
24
24
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104 108 112 116 120
Time [h]

During constant darkness conditions mean depth values showed lower day/night differences in vertical copepod distribution (Fig. 7). However, mean depth values were still higher during subjective day, indicating most copepods in the lower part of the water column at this time. During subjective night, lowest mean depth values occurred around midnight, with peaks ranging between 45 and 48 cm. Furthermore, moving average data still indicated clear differences in mean depth between subjective night and subjective day ranging between 5 and 10 cm (Fig 7).

Despite damped day/night differences in *C. finmarchicus* mean depth, analysis of rhythmic oscillations indicated significant 24 h oscillations of the moving average (RAIN: $p<0.01*10^{-9}$) under DD conditions for all three days (Fig. 6, B).

Attributed to the small sizes of copepods and counting with the naked eye, it was not possible to count all 200 individuals during the experiment. The total number of counted animals varied therefor between 130 and 192 individuals, with a mean count of 164 animals (SD = ±15 animals). Oxygen measurements at the end of the experiment indicated a nearly linear decrease in dissolved oxygen with depth from 11.1 mg/L in the surface layer to 9.1 mg/L in the bottom layer.

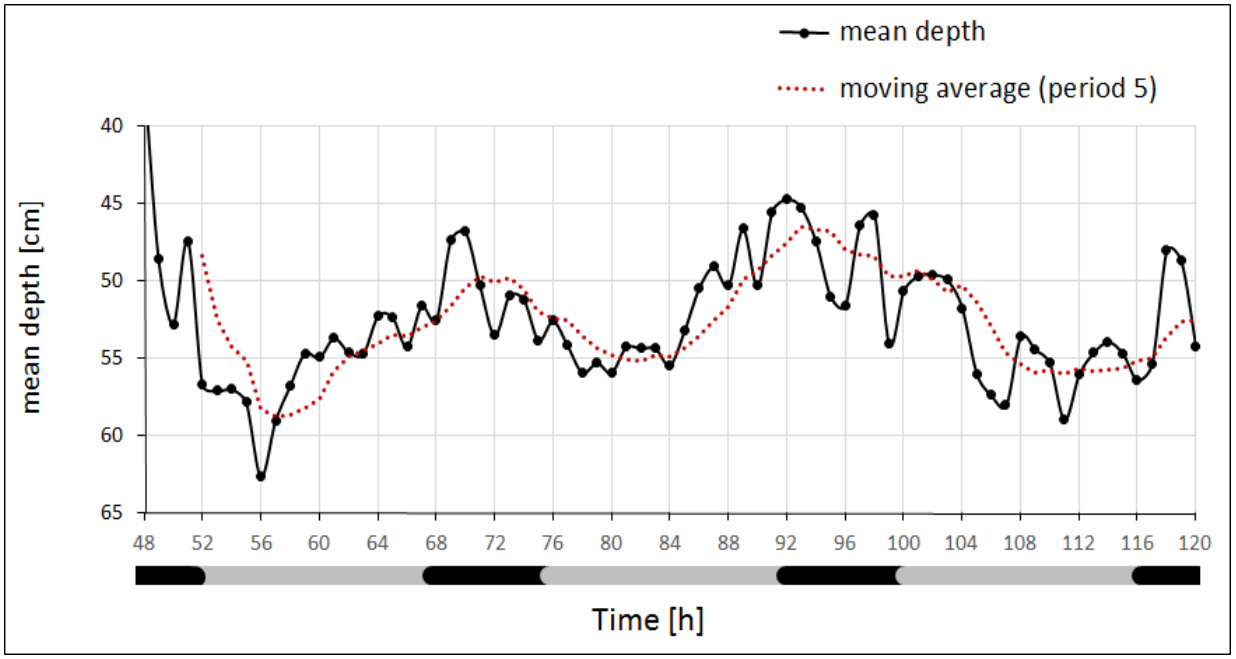


Figure 7: Mean depth of animals during constant darkness conditions. Dotted red line indicates the moving average (period 5) of the mean depth. Bar under the graph indicates subjective day (grey) and subjective night (black).

4 Discussion

The aim of this work was to assess the role of light (photoperiod) on DVM and diel metabolic processes in *C. finmarchicus* and to detect the possible involvement of endogenous rhythmicity in these processes. To test this, diel swimming behaviour and oxygen uptake rates were invetigated under LD (16 h:8 h) and DD conditions in laboratory experiments, using the CV stage of *Calanus finmarchicus*. Active copepods were sampled in May (for respiration measurements) and June (migration experiments) from Bonawe Deep in Loch Etive, Scotland. In the laboratory experiments copepods showed a migration behaviour that is highly synchronized to the LD cycle, whereas a damped migration continued under DD conditions. Significant 24-hour oscillations in the vertical distribution were found in the migration experiment during the first day under LD conditions and during all three days under constant darkness. Also an oscillation in oxygen uptake rates was found under DD conditions. Despite, that these findings underlie some restrictions (see 4.8) they overall stress the importance of light as stimulus for DVM in *C. finmarchicus* and suggest the involvement of an endogenous rhythm in diel patterns of vertical migration and metabolic processes. Therefore, this work may help to improve further DVM research, that implicates the involvement of biological clocks.

4.1 Hydrographic conditions

The hydrography at the sampling site Bonawe deep is highly determined by the special topography of two sills separating the Loch from the coastal area (see fig. 1) as well as a high freshwater inflow by two rivers draining into Loch Etive (Edwards & Edelsten, 1977; Nørgaard-Pedersen et al., 2006). The restricted exchange of water between the basins of the Loch and the coast may lead to long periods of stagnating water bodies, especially in the deep basin of Bonawe Deep (Edwards & Edelsten, 1977). The periods of stagnation are characterized by a strong stratification of the waterbody as well as low dissolved oxygen values in the deep layer. The hydrographic data obtained in May (Fig. 3) and June (Fig. 4) show a clear stratification, with a thermocline at ~45 m in May. Temperatures were at 9°C above and 12°C below the thermocline. June data showed relatively high surface layer temperatures of >12°C declining rapidly in the upper 5 m. Increasing temperatures at ~ 60 m indicate the thermocline, with about 10°C above and 11.5°C below it. Lower surface water salinity in June indicate an increased fresh water inflow between the first and the second sampling. The low surface water salinities intensify the stratification of the water body. Just before a deep water renewal event in 2000 dissolved oxygen values reached <1 mg/L in the deep layers of Bonawe deep (Austin & Inall, 2002). In agreement with these findings, our data indicated very low dissolved oxygen values

(<2 mg/L) below the thermocline. Both the strong stratification as well as the very low oxygen values in the deep layers suggest a long stagnation period of at least several months prior to our samplings (Edwards & Edelsten, 1977; Austin & Inall, 2002). As a deep water renewal event is indicated by a marked increase in bottom layer oxygen values and a decrease in stratification of the overlying water masses (Edwards & Edelsten, 1977) we assume that no renewal of deep water occurred during our sampling period.

The onset of the annual phytoplankton spring bloom in Loch Etive usually occurs mid to end of March (Wood et al., 1973; Solórzano & Gratham, 1975), whereas peak values are reached in May and June (Okumus & Stirling, 1998). Chlorophyll *a* fluorescence measured in May showed peak values of 16 mg/m^3 in the upper 5 m and declined to 6 mg/m^3 until the end of June. Although chlorophyll *a* fluorescence is just a rough estimate of the true chlorophyll *a* concentration, our measured values indicate that the spring phytoplankton bloom has started and May sampling was done during full bloom. Lower values at the end of June indicate a first decrease of the bloom. The concentration of the phytoplankton in the surface layer (<5 m) shows the influence of the inflowing, light freshwater keeping the algae at the surface (McKee et al., 2002). Based on the findings of Hill (2009) *C. finmarchicus* population in Loch Etive start to emerge from diapause during January, where they moult to adults and ascend to the surface to reproduce. In April already older copepodite stages occur and throughout May about half of the new generation reached the latest juvenile stage (CV). In June more than 80% of the population consist of the CV stage (Hill, 2009). The findings suggest that during our sampling period in May and June most of the population consisted of CV stage of *C. finmarchicus*, which actively grazed on phytoplankton in the surface layer to accumulate lipid reserves in preparation of diapause and performed DVM (Hirche, 1996). As sampling in May was done in the morning (9 am), it is likely that copepods were in the deeper layers at the time of sampling and in a satiated state due to feeding at night in surface layers. In June copepods were sampled in the afternoon (4 pm). Regarding the DVM behaviour, we assume that copepods were still at their daytime depth in deeper layers at the time of sampling but in a hungry state, since they are not supposed to feed in deeper layers during the day.

4.2 Migration under a light/dark cycle

The diel swimming behaviour of *C. finmarchicus* showed a clear synchronization to the provided LD cycle (16:8) in the laboratory (Fig. 6 A and B). Animals descended in the early morning to the deepest layer of the column, residing there for the whole period of illumination. As soon as the lights were switched off, a bigger fraction of animals started to migrate upwards to spend the

night hours in the surface layers. During both nights of the LD cycle the mean depth indicates a descend over midnight, followed by another ascend just before the early morning. RAIN analysis detected a significant 24-hour oscillation in the vertical distribution for the first day under LD. In the following, the factors that possibly underlie the observed migration behaviour under the LD cycle are discussed.

The **morning descent** of animals showed a strong response to the onset of illumination (Fig. 6). A study of the sensitivity of calanoid copepods in the arctic revealed that copepods may react highly sensitive to smallest amounts of light (Båtnes et al., 2013). Especially white, blue, and green wavebands evoked negative phototactic responses of copepods to lowest intensities (10^{-8} - 10^{-6} µmol photons m^{-2} s^{-1}). Despite the fact, that organisms of high latitudes might be better adapted to low levels of irradiance than those inhabiting temperate regions, the study shows that copepods may respond to very small amounts of light with negative phototaxis. Cohen and Forward (2005a) suggest the morning descend of the copepod *C. americana* to be a response to either high absolute intensities of light or a high relative rate of change in light intensity during the morning hours. Due to the fact that copepods are highly sensitive to small light intensities and light in the laboratory increased to up to 60 Lux within one hour, it is probable that the morning descend of *C. finmarchicus* in the LD phase of our experiment is the result of a light avoidance response rather than an endogenously controlled rhythm. This suggestion is supported by the strong accumulation of animals (up to 90%) in the deepest layer during the whole period of illumination. Thorisson (2006) also suggested that the morning decent in *C. finmarchicus* is a light avoidance response but hypothesized that only satiated animals show this behaviour. This stands in contrast to our findings, where animals were kept without any food during the duration of the experiment.

The **daytime residence** during LD conditions was characterized by a large fraction (<90%) of copepods that remained in the deepest layer (lower 10 cm) during the full period of illumination. The daytime residence in deep layers in *C. americana* is suggested to be caused by high absolute irradiance as well as inactivity due to endogenous rhythmicity (Cohen & Forward, 2005a). The authors suggest that the inactivity of an endogenous rhythm during the day may lower the response to changing light intensities and thus prevent an ascend respond to temporal decreases in light probably due to cloud cover. This suggestion is supported by findings of Hardy & Bainbridge (1954), who found no ascend response of *C. finmarchicus* to a sudden black out of light during daytime. As mentioned above, our suggestion to the strong accumulation of animals in the bottom layer is that high light intensities supress an ascend of the animals, which confirms the assumption of Thorisson (2006). To what extend a possible endogenous rhythm is involved in the determination of the daytime distribution is not assessable from our findings in LD.

An **evening ascend** response of the copepods was found as soon as it was dark, marked by increasing numbers in the upper layers of the column. A high rate of change in light intensity as well as a decrease in absolute intensity is thought to be involved in triggering the upward migration (Stearns & Forward, 1984; Cohen & Forward, 2005). Since the evening upward migration during the LD cycle started not before the first hour of complete darkness, it is likely that in our experiment the relief from overhead light penetration is a necessity to allow the onset of a migration. However, the simple relief from light penetration itself is not thought to trigger an ascend behaviour. On the one hand, the previous high rate of change in light intensity during the last hours of daytime may play a role in inducing a migration reaction. On the other hand, earlier studies that took the rate of change in light intensity into account noticed an immediate ascend response to rapid changing light conditions (Stearns & Forward, 1984b; Ringelberg, 1999; Cohen & Forward, 2005;). However, in our experiment illumination from above could have delayed the ascent response to the hours of complete darkness. Thorisson (2006) suggests the evening ascend to be triggered by 'food smell'. Since the copepods in our experiment were not exposed to any food, we assume that it is unlikely that food plays a role in triggering DVM. This in turn confirms multiple studies, which state that diel rhythms in feeding activity occur in addition to vertical migrations rather than being connected to it (Mackas & Bohrer, 1976; Baars & Oosterhuis, 1984). As the high light intensities during daytime caused an accumulation of animals in the bottom layer the effect of crowding (Wong et al., 1986) as well as random swimming needs to be considered. We assume that the ascent migration in the early night is a delayed response to high rates of change in light intensity, which in addition might be underpinned by biological rhythmicity, causing an increase in photosensitivity.

The following section discusses the causes of an observed '**night time residence and midnight sink'** behaviour of a large fraction of copepods. During the night, we observed a pattern with two peaks in vertical distribution. The first maximum occurred right after the onset of the evening ascend. However, during midnight the mean depth was lowered before a second smaller peak was visible before the morning descent. Although the pattern did not show large amplitudes, the possibility of a midnight sink will be discussed in the following as *C. finmarchicus* is known to exhibit this behaviour in the natural environment (Tarling et al., 2002).

The hypothesis of 'predator avoidance' is probably the most accepted for the ultimate reason of upward migration and night time residence in the surface waters for many organisms performing DVM (Ringelberg, 1999; Hays, 2003; Marcus & Scheef, 2010; Brierley, 2014). During the bright day hours, predation pressure of visual hunting predators forces copepods to deeper and darker water layers. Hence, the organisms use the screen of the dark night hours were visual

hunting is limited to feed in the surface layer. Thus, the most important cause for the night residence in the surface layer is the need to feed.

The occurrence of a so called midnight sink is a known behaviour of some zooplankton organisms (Cushing 1951; Simard et al., 1985), including *C. finmarchicus* (Tarling et al., 2002). The behaviour may be seen as a variation of the normal DVM pattern and is characterized by a sinking of organisms from the surface to intermediate depths around midnight. Several studies consider food to be an important factor and suggest the midnight sink to be caused by increased inactivity of satiated copepods (Gauld, 1953; Mackas & Bohrer, 1976). Contrary, a work by Tarling et al. (2002) on *C. finmarchicus* in the Clyde Sea suggested that the attendance of tactile hunting predators around midnight, such as krill, cause *C. finmarchicus* to decent in order to avoid predation. The absence of food in our experiment excludes the possibility of a satiation driven midnight sink to be responsible for the pattern. Also the absence of predators in our experiment stays in contrast to the hypothesis of a direct response to predation. From personal observation we can say that potential predators of *C. finmarchicus*, such as *Euphausiids* (Bamstedt & Karlson, 1998) and Chaetognats (Irigoien et al., 2004), are attendant at Bonawe Deep. Work on the copepod *C. americana* by Cohen and Forward (2005a) suggests an endogenous driven decrease in activity to cause a sinking respond during midnight. Although, the pattern was not observed during DD conditions in our experiment.

4.3 Migration under constant darkness conditions

After exposing the copepods to a LD cycle (16:8) for two days, the animals were kept in constant darkness (DD) for the following three days of the vertical migration experiment. Mean depth values indicated an ongoing vertical migration, with a lower mean depth during subjective night than during subjective day (see Fig. 7). Although damped in the amplitude, the oscillation of the moving average showed a significant rhythm with a period of 24 h (Fig. 6 B). Bearing in mind that circadian rhythms persist under constant conditions with a period of ~24 hours (Kuhlman et al., 2007) our findings clearly point towards the involvement of circadian rhythmicity in *C. finmarchicus* DVM.

As light may be excluded as a factor influencing the migration under DD conditions, temperature changes in the laboratory remain as a possible exogenous factor. There is evidence from freshwater zooplankter that migrate in response to diel changes in temperature (Haney, 1988; Ringelberg & van Gool, 2003). Since the dynamic system of the marine environment does usually not show large diel temperature fluctuations, it is not thought that marine zooplankter use temperature fluctuations on a diel scale to time their migration.

Moreover, in our experiment the water temperature in the surface layer of the column varied of <1°C without any rhythmicity. It is therefore unlikely, that temperature fluctuations are responsible for the direct initiation of the migration. The gradual depletion of dissolved oxygen, especially in the bottom layers of the column over the duration of the experiment might have affected the behaviour of the animals. However, up to 50% of the animals resided in the bottom layer even after five days, which suggests, that the decrease in oxygen concentration has no significant effect on the cause of the migration.

Light is the most reliable and thus most widely used cue to time events that occur on a diel basis (Aschoff, 1954; Marcus & Scheef, 2010). The strong migration response to the LD cycle during the first two days of our migration experiment, as well as the lower amplitude during DD conditions stresses the importance of light in DVM of *C. finmarchicus*. However, the ongoing migration under DD indicates that at least another factor is involved. During constant conditions (DD) we widely excluded possible *Zeitgeber.* The ongoing migration observed under constant conditions therefore provides evidence that DVM in *C. finmarchicus* is underpinned by an endogenous rhythm.

4.4 Respiration measurements

During the LD cycle of our respiration measurements, we found only weak changes in oxygen uptake by *C. finmarchicus*. A small peak in oxygen consumption during the first night (LD) and a stronger peak during the second night (DD) point to a general pattern of a higher oxygen demand during the night than during day time. However, the data of the respiration experiments have to be interpreted carefully as they underlie some major restrictions (see chapter 4.8).

The respiration rate may be seen as a measure of overall biological activity (Mayzaud et al. 2005). A normal DVM pattern is supposed to cause an increase in oxygen demand during the night due to the active migration and feeding activity of the copepods (Pavlova, 1994). The low change in oxygen consumption under LD conditions stay in contrast to our findings of a strong migration response under LD in the migration experiment. The handling of animals just before the start of the measurements may have induced stress and thus might have in some way supressed a more distinct diurnal pattern in oxygen consumption during the first day of the experiment. However, stress is usually accompanied by higher rates in oxygen consumption (Marshall & Orr, 1935). The stronger increase in oxygen uptake during subjective night might reflect the recovery of a diel migration pattern after a full LD cycle under experimental conditions. The shift of the peak in oxygen consumption from midnight into the early morning

might be caused by a lack of synchronization due to the absence of a Zeitgeber under constant conditions.

Besides changes in locomotor activity, a higher metabolic rate may have caused an increase in oxygen uptake. Several studies found increasing activities of digestive enzymes like amilase or trypsin in *Calanus finmarchicus* (Tande & Slagstad, 1982) and other copepod species (Mayzaud et al., 1984) during night time. Although often related to feeding activity, Mayzaud et al. (1984) and Head et el. (1984) found that digestive enzyme activity and feeding are not directly linked. Since no food was provided in our experiment, a potentially increased activity of digestive enzymes may have caused the higher oxygen demand during the night. Moreover, Mayzaud et al. (1984) suggested an internal clock to be responsible for diurnal rhythms in digestive enzyme activity. This suggestion agrees with our findings of a higher oxygen demand during night time under constant darkness conditions. In this regard, an enduring activity of digestive enzymes into the early morning hours could also be responsible for the shift of the peak in oxygen consumption. However, analysis of the activity level of digestive enzymes need to be done on a diel scale to support our suggestions.

4.5 Laboratory experiment synthesis

C. finmarchicus exhibits rhythms on a diel (Falkenhaug et al., 1997; Dale & Kaartvedt, 2000a; Marcus & Scheef, 2010) and a seasonal (Diapause; Hirche, 1996) scale that show synchronization to either the diel light dark cycle or the seasonal change photoperiod (Marcus, 1986; Marcus & Scheef, 2010). There is evidence that at least diel rhythms in *C. finmarchicus* underlie an endogenous rhythm (Harris, 1963; Enright & Hamner, 1967). Circadian clocks are widespread among a broad range of organisms, synchronizing several physiological and behavioural functions to the 24 hour fluctuation of day and night (Dunlap, 1999; Tessmar-Raible et al., 2011). These 24-hour rhythms are generated on a molecular basis by cycling inhibition and activation of so called clock genes (Dunlap, 1999; Kuhlman et al., 2007). The timing information may be used to synchronize behavioural and physiological processes to diel fluctuations in environmental parameters. The genetically produced ~24-hour rhythm is entrained by external cues to keep time with the environment. The most widely used and thus most important cue for such a synchronisation is light (Aschoff, 1954; Marcus, 1986).

Our findings of ongoing rhythmic vertical migration under constant conditions clearly point towards the existence of a circadian clock in *C. finmarchicus*. Despite the limitations in the respiration measurements, the higher oxygen uptake rates during the night under both LD and DD conditions tend to support the suggestion. It is obvious from several studies that light is the

most important cue being involved in DVM of Zooplankton organisms (Ringelberg, 1999; Cohen & Forward, 2002; Marcus & Scheef, 2010) and our findings agree with the importance of light for DVM and potentially for the activity of digestive enzymes in *C. finmarchicus*. A putative endogenous timing system in *C. finmarchicus* is therefore likely to be entrained by light. Moreover, the timing information might be used to entrain both diel and seasonal processes (Oster et al., 2002; Kuhlman et al., 2007). On the diel scale a circadian clock might be involved in increased locomotor activity during night time causing a migration response. Furthermore, metabolic processes like the activity of digestive enzymes might be underpinned by a clock mechanism to synchronize the excretion of digestive enzymes to the time of feeding (Mayzaud et al., 1984). On the seasonal scale a circadian clock might be involved in the initiation and termination of diapause (Oster et al., 2002). There is evidence, that *C. finmarchicus* relies to a great extent on photoperiod (day length) as a cue to time its annual initiation and termination of diapause (Miller et al., 1991b). Furthermore, different studies suggest a circadian timing system to be involved in measuring photoperiod and thus receive information on the progression of the season (Pittendrigh & Minis, 1964; Miller et al., 1991b; Davis, 2002; Oster et al., 2002). There are different approaches and theories to describe how circadian clocks measure photoperiod whereof two basic models are considered in the following. The first so called 'external coincidence model' splits the circadian 24-hour system into two 12 h cycles. One of cycle is photosensitive and the other one photoinsensitive. When days are short, the photosensitive cycle receives little stimulus telling the organism that it is late in the season and induces e.g. the initiation of diapause. When days become longer more light stimulates the photosensitive cycle allowing organisms to react to the start of the season (e.g. termination of diapause; Bünning, 1960; Oster et al., 2002). The second model termed 'internal coincident model' assumes the existence of at least two independent circadian systems, whereof one is entrained at dusk and one at dawn. A changing photoperiod over the course of the season alters the phase relation of the two circadian systems and thus provides information on the progression of the season (Pittendrigh & Minis, 1964; Davis, 2002). In this respect, the detection of a circadian timing system in *C. finmarchicus* could greatly contribute to the understanding of mechanisms that are involved in the timing of seasonal events such as diapause. However, knowledge about such mechanisms in Crustacea is scarce (Strauss & Dircksen, 2010) and further research is needed to assess the involvement of circadian clocks in the timing of seasonal events in *C. finmarchicus*.

4.6 *Calanus finmarchicus* DVM in Loch Etive

The hydrographic properties of Bonawe Deep in Loch Etive assessed in this study indicate that the local *C. finmarchicus* population inhabits a highly stratified environment. Furthermore, the animals are exposed to strong variations in surface layer temperature and salinity, as well as hypoxic conditions in deep water layers below the thermocline.

Studies of different copepod species showed that DVM pattern are highly variable on both geographical and temporal scales (Marshall & Orr, 1960; Frost, 1988; Hays, 1996; Dale & Kaartvedt, 2000a). The migration pattern may be shaped by several environmental parameters including food availability, temperature, and oxygen concentration (Harris, 1988; Fragopoulu & Lykakis, 1990; Cohen & Forward, 2002; Irigoien et al., 2004). Hydrographic properties may therefore have a fundamental influence on the pattern of DVM (Sameoto, 1984; Fragopoulu & Lykakis, 1990). We assume that the high stratification in Loch Etive throughout the year may have a major influence on the migration pattern in the field. On the one hand, the strong thermocline itself, as well as the low oxygen concentrations below the thermocline may restrict the daytime depth of active migrating *C. finmarchicus*. On the other hand, high freshwater run off, especially during the winter months, form a fresh surface layer influencing the vertical distribution of phytoplankton (McKee et al., 2002). The reduced density of the fresh surface layer restricts the mass development of phytoplankton during the phytoplankton bloom to the upper few meters. The low salinity of the surface layer may prevent *C. finmarchicus* from feeding on the high concentrations of phytoplankton we found in the upper 5 m. The night time distribution of migrating copepods might therefore be restricted to the upper 10 to 15 m, were salinity increases and phytoplankton concentrations are still higher than throughout the rest of the water column (see fig. 3 and 4).

The properties of the water column in Loch Etive may vary throughout the year (Wood et al., 1973; Edwards & Edelsten, 1977; Hill, 2009). The migration range may therefore vary with the position of the thermocline as well as the expansion of the freshwater layer at the surface. While the hydrography of the water column may provide boundaries for the migration of zooplankton organisms, it is also thought that the organisms in turn affect the hydrography (Bianchi et al., 2013). Bianchi et al. found that vertical migrating organisms may affect the expansion of oxygen minimum zones. They suggested that organisms migrate down into the upper margin of oxygen minimum zones to seek refuge from predators. The consumption of oxygen in these layer by a large number of organisms is thought to promote the expansion of low oxygen layers, especially in poorly ventilated areas. The long stagnation periods of bottom water in Loch Etive (Edwards & Edelsten, 1977) may therefore facilitate such processes.

From our findings during the laboratory experiments we suggest that an endogenous timing system is involved in *C. finmarchicus* DVM. As discussed above, an endogenous rhythm is likely to be entrained by light. Therefore, it is crucial to the organisms to perceive light also in greater depths to time the migration. Hill (2009) calculated the downward irradiance for Bonawe Deep throughout the year. Results showed that light is absorbed quickly above 50 m, reaching a low irradiance level of $<5*10^{5}$ $W*m^{-2}$ between 50 and 60 m (Hill, 2009). Recent studies revealed that copepods are sensitive to smallest intensities of irradiance (Berge et al., 2009; Båtnes et al., 2013). The study showed that Calanus spp. shows phototactic responses to light intensities as low as $0.05 * 10^{-6}$ µmol photons m^{-2} s^{-1}. Thus low light intensities at 50 to 60 m might be still sufficient to entrain an endogenous rhythm in *C. finmarchicus*. However, irradiance levels provided by Hill were not directly measured but calculated from surface irradiance. Further in situ measurements of the underwater light climate are necessary to assess the light intensity and range of wave lengths available to animals at greater depth.

In conclusion, we assume that due to a strong stratification as well as low surface layer salinities and low bottom layer oxygen concentrations at Bonawe Deep, *C. finmarchicus* performes DVM within a limited range. The amplitude of the migration may vary due to the position of the thermocline and the expansion of the oxygen minimum zone, as well as to the expansion of the freshwater layer at the surface. The recent recovery of an underwater mooring equipped with a number of oceanographic sensors and Acoustic profiling devices provided first insight into DVM patterns at Bonawe Deep in Loch Etive. A first analysis of the ADCP data widely agree with the assumptions on the range of DVM described above.

4.7 *Calanus finmarchicus* in a changing environment

Over the last decades a rise in atmospheric CO_2 caused a rise in sea surface temperatures (IPCC, 2007; Jackson, 2008). Higher water temperatures may affect a broad range of physical processes that are directly coupled to changes in temperature such as oxygen concentration and stratification (Helaouet & Beaugrand, 2007). The decrease in surface water density through rising surface temperatures increases the stratification of the ocean (Schmittner, 2005). A higher stratification may reduce vertical mixing processes that in turn decreases the upwelling of nutrients from deeper layers and prevents a downward transport of 'fresh' oxygen-rich water (Beaugrand et al., 2001; Schmittner, 2005). A lack of nutrients may decrease the annual productivity of phytoplankton (Behrenfeld et al., 2006) meaning a loss of energy at the very basis of marine food webs. Furthermore, an increase in stratification is believed to increase the number and size of hypoxic zones (Diaz & Rosenberger, 2008).

Calanus finmarchicus is well adapted to an oceanic environment. High mixing rates during the winter month as well as high surface oxygen concentrations and nutrient levels are typical characteristics of environments inhabited by *C. finmarchicus* (Helaouet & Beaugrand, 2007).
An increase in seas surface temperatures, along with increased stratification, acidification and a decreased primary production may impact processes of zooplankton organisms on both a diel and seasonal scale.
As stated above, increasing temperatures and stratification may cause a reduction in total primary production (Behrenfeld et al., 2006). A decrease in nutrient availability is thought to affect zooplankton behaviour on a diel scale (Hirst & Batten, 1998). Different calanoid copepods showed responses in DVM strength to a varying food supply (Huntley & Brooks, 1982; Dagg et al., 1997). It has been observed that copepods intensified their vertical migration pattern when food supply increased (Huntley & Brooks, 1982; Dagg et al., 1997). In turn, low surface phytoplankton concentration may lead to a prolonged stay of species in the surface layer (Huntley & Brooks, 1982). Hypoxic zones are known to expand (Diaz & Rosenberger, 2008) and may limit the amplitude of diel migration patterns, which is particularly important for poorly ventilated environments (Bianchi et al., 2013). The increasing stratification through rising temperatures and decreasing ventilation (Schmittner, 2005) may therefore facilitate this process. The expansion of oxygen minimum zones and an increasing stratification of the oceans may restrict the extent of diel migrations of *C. finmarchicus.* Late stage *C. finmarchicus* are known to be a major prey to different ontogenetic stages of commercially important fish stocks like Atlantic cod (*Gadus morhua*, Sundby, 2000; Drinkwater, 2005) and herring (Dalpadado et al., 2000). Furthermore, there is evidence that fish species that mainly feed on copepods track the diel vertical migrations of their prey (Cardinale et al., 2003; Hays, 2003). Thus, a change in diel migration patterns of important prey species like *C. finmarchicus* may have a large impact on higher predators and is likely to cascade through higher trophic levels. Vertical migrating zooplankter are thought to be major contributors to the transfer of carbon from the surface to deeper layers and thus are involved in the storage of atmospheric CO_2 into the interior of the ocean (Longhurst & Harrison, 1988). As the oceans take up large amounts of atmospheric CO_2, a part of it is fixed by the primary production of phytoplankton organisms. As copepods usually feed on the phytoplankton at nights they take up fixed carbon and either repack it to fast sinking fecal pellets (Ducklow et al., 2001) or actively transport it downwards by descending at dusk and store it in deep layers below the thermocline by respiring (Steinberg et al., 2000). A possible restriction of DVM to zones above the thermocline might reduce the amount of carbon stored at the bottom of the oceans and affect the benthic-pelagic coupling. However, most studies focus on the impact of climate change on seasonal processes (discussed below) and knowledge

about the impact on diel processes like DVM is limited (Brierley, 2014). It is therefore important to investigate the impact of global warming on diel processes like DVM, to make predictions about changes in benthic-pelagic exchange processes.

The impact of climate change on the seasonal scale may have a severe effect on the development of zooplankton population, as well as on the spatial distribution and timing of seasonal events (Beaugrand et al., 2001; Beaugrand, 2002; Søreide et al., 2010; Reygondeau & Beaugrand, 2011). Rising temperatures and the ongoing ocean acidification may both impact the hatching success of *C. finmarchicus* (Hanssen, 2014; Mayor et al., 2007). Additionally, a decrease in primary productivity during the summer months may affect the successful development of younger stages and may therefore reduce the abundance of the population. Moreover a reduced food supply may restrict the generations *C. finmarchicus* can produce per season, as it is the situation in Loch Etive, and may delay the onset of diapause (Hill, 2009).

Rising temperatures are already found to be directly involved in regime shifts of zooplankton communities (Beaugrand, 2002; Hays et al., 2005; Helaouet & Beaugrand, 2007). Over the last decades, a northward shift of calanoid copepods like *C. finmarchicus* has been observed (Beaugrand, 2002). The northward shift may implicate a loss of energy for the local food webs and may therefore have severe consequences for higher predators (Hirche & Kosobokova, 2007). Moreover, recent studies also found northward shifts in the distribution of several marine fish species, including direct predators of *C. finmarchicus* like cod (Perry et al., 2005; Rose, 2005; Beaugrand & Kirby, 2010). Beaugrand and Kirmy (2010) suggest that the rapid declines in cod stocks over the last decades are coupled to temperature induced changes in the spatial distribution of *C. finmarchicus.* Also a northward shift in herring stocks is thought to be related to rising temperatures and the shift in spatial distribution of *C. finmarchicus* (Beaugrand, 2002; Corten, 2001). These examples illustrate the strong effects a temperature induced latitudinal shift in important zooplankton species may have on the whole *Calanus* based ecosystem. Regarding the commercial importance of species like cod or herring for the fishing industry, it is likely that changes in *C. finmarchicus* also have strong impacts on an economical level.

A further increase in global warming is predicted for the following decades (IPCC, 2007). Modelling the impacts of ongoing global warming on the spatial distribution of *C. finmarchicus* revealed, that an ongoing northward shift of the population and a further expatriation into arctic regions is probable (Reygondeau & Beaugrand, 2011). With ongoing climate-mediated changes the inflow of Atlantic water into arctic regions may produce more favourable conditions for *C. finmarchicus* and could possibly lead to a shift from *C. glacialis* to *C. finmarchicus* (Kosobokova, 1999; Hirche & Kosobokova, 2007). The lower lipid content of *C. finmarchicus* may in turn imply a lack of energy for the local food web (Hirche & Kosobokova, 2007; Falk-Petersen et al., 2009).

The initiation and termination of diapause is possibly timed by a circadian clock that measures the photoperiod. Using photoperiod as a cue implies the risk of a timing mismatch between the onset of phytoplankton blooms and the emergence from diapause. Regarding the predicted shifts in phytoplankton blooms, it is of great importance to understand the mechanisms that time the initiation and termination of diapause.

The relatively harsh environmental conditions in Loch Etive, with a strong stratification almost throughout the year, low oxygen concentrations below the thermocline as well as a high variability in surface temperatures and salinity, might reflect conditions that are believed to increase in oceanic environments in the course of climate change. The successful persistence of *C. finmarchicus* in Loch Etive shows its great tolerance to a range of environmental conditions. This may lead to the suggestion, that *C. finmarchicus* is able to cope with future changes that come a long with a further increase in atmospheric CO_2 and its effects on the marine environment. However, competition with congeneric species like *C. helgolandicus* is low in Loch Etive which facilitates the successful persistence of *C. finmarchicus* (Hill, 2009). In this course, further research on both diel seasonal scales is needed to assess the impact of climate change on the distribution and development of *C. finmarchicus* populations.

4.8 Limitations and Outlook

Analysing the results of the respiration measurements revealed some deficiencies in experiment conduction, which will be analysed in the following.

Capture and handling stress are known to cause an increase in the respiration rate of *C. finmarchicus* (Marshall et al., 1935). To reduce the handling stress to the animals right before the start of the experiment, copepods should be sorted right after the sampling and distributed to prepared incubation bottles to allow for acclimatization to laboratory conditions and to avoid direct handling right before the start of the experiment. From results of the respiration measurements we assume that no migration behaviour took place during the first day of the experiment. However, the changes in oxygen uptake rates during the second day may reflect a recovery of the migration behaviour. Therefore, it should be considered to prolong the entrainment period, e.g. to a full LD cycle.

Closing the incubation bottles underwater involves the risk that copepods escape from the bottles or that air bubbles cannot be excluded totally. Thereby, a stable measurement of the oxygen concentration is restricted. To prevent this, incubation bottles should be filled up with seawater slightly above the rim (using surface tension) right before the start of experiment. To

close the bottles, the opening should carefully be covered with a plastic foil to exclude any air bubbles and thereafter closed with lids. This method reduces both stress to the animals and allows more precise work.

During our respiration measurements the temperature of the cooling basin varied of about ±1°C. To obtain high quality respiration data it is important to keep the measurement temperature on a constant level throughout the whole experiment. Lower temperatures lead to higher solubility of oxygen in water and alter the measured oxygen concentration. To buffer against external fluctuations of temperature the cooling tanks for the measurement bottles should provide a high volume. Moreover, a flow through system in the cooling tanks may help to keep the temperature on a constant level.

The oxygen measurement device used (see chapter 2.2) measures the oxygen saturation directly at a sensor spot. To obtain representative information about the oxygen content inside the measurement bottle, it is important to ensure an even distribution of dissolved oxygen throughout the incubation bottle. Equipping the bottles with a magnetic stirring bar would ensure a constant oxygen level in the whole bottle throughout the whole experiment. In this case, copepods should be protected against strong turbulences or direct contact with the stirring bar as this may induce stress and increase the oxygen demand.

The results of the migration experiment strongly suggest the involvement of endogenous rhythmicity in *C. finmarchicus* DVM. Due to increased oxygen demand during the night time, respiration measurements tend to support the suggestion. However, the limitations discussed above need to be considered when interpreting the respiration results. To support our findings both migration and respiration experiments should be repeated in parallel, considering the improvements that are described above. Furthermore, light intensities during the LD cycle should be lowered considerably and diffuse lighting from the sides should be provided to exclude the factor of light avoidance. This may help to identify what feature of the diel light cycle is involved in triggering DVM in *C. finmarchicus*.

To finally prove the involvement of a circadian clock in *C. finmarchicus* DVM the genetic analysis of clock gene expression patterns on a diel scale should be performed. Additionally, a clock knock out experiment associated with migration experiments could reinforce the importance of a circadian clock for DVM in *C. finmarchicus*. Furthermore, the investigation of different proteins may help to understand the influence of a circadian clock on other processes, such as digestive enzyme activity or diapause, and will allow further insight into the function of the clock. A fundamental understanding of the underlying processes of diel and seasonal events in *C. finmarchicus* will help to make predictions of the future development of this species and of the effects on the *Calanus* based ecosystem.

4.9 Conclusion

The variation in the vertical position of the calanoid Copepod *Calanus finmarchicus* in the water column shows strong synchronicity to the daily fluctuation of light (day and night). Although known for decades and subject of multiple studies (Hardy & Bainbridge, 1954; Harris, 1963; Fortier et al., 2001; Cohen & Forward, 2005b; Berge et al., 2009), researchers were not able to isolate a single or a combination of factors yet that cause the diel migration behaviour of zooplankton (Cohen & Forward , 2005a; Marcus & Scheef, 2010).

The use of endogenous timing systems as a tool to time the migration has been suggested in a few earlier studies (Harris, 1963; Enright & Hamner, 1967), which were able to show ongoing migration under constant darkness conditions in the laboratory. The recent identification of components of an endogenous clock in *C. finmarchicus* by Christie et al. (2013) strongly supports the suggestion of an endogenous timing system in this species that may be involved in the timing of rhythmic behaviour.

During this study we performed laboratory experiments to the diel swimming behaviour of the copepod *C. finmarchicus* under light/dark and constant darkness conditions. Additionally, respiration measurements were performed to assess diurnal fluctuations of metabolic processes. Our findings showed a strong correlation between the provided LD cycle and the vertical position of copepods during migration experiments. We suggest a strong light avoidance response to be responsible for the distinct migration pattern under LD conditions. However, ongoing migration was found under constant darkness conditions with a significant 24-hour oscillation, which clearly point towards the involvement of endogenous rhythmicity in *C. finmarchicus* DVM. The respiration measurements showed higher oxygen demands during the nights under LD and DD conditions. Although these results underlie restrictions due to errors in experiment conduction, they seem to support our findings and allow the suggestion that also diurnal fluctuations in metabolic processes of *C. finmarchicus* may by governed by a circadian timing mechanism.

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Hiermit versichere ich, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Außerdem versichere ich, dass ich die allgemeinen Prinzipien wissenschaftlicher Arbeit und Veröffentlichung, wie sie in den Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg festgelegt sind, befolgt habe.

Lukas Hüppe