Bachelorstudiengang Umweltwissenschaften

BACHELORARBEIT

The circadian clock in *Calanus finmarchicus* – Relation to diel vertical migration

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ABSTRACT

The marine copepod *Calanus finmarchicus* is an important key species in the Northern Atlantic due to his abundance and his position in the food web. It performs diel vertical migration (DVM), staying in deeper water layers during the day and ascending to the surface in the night. The exact trigger for the DVM is not known yet, but light seems to have an important influence on the position of *C. finmarchicus*. Some studies suggest an involvement of an endogenous rhythm, which controls the vertical position of *C. finmarchicus* throughout the day. In this work the DVM and respiration rate of *C. finmarchicus* were examined under natural simulated light conditions to identify possible circadian rhythms. Therefore, two laboratory experiments were performed with the CV-stage of *C. finmarchicus* under light/dark (LD) and constant darkness (DD) conditions. The position of *C. finmarchicus* in the DVM experiment showed a clear diurnal rhythm, with significant differences between day and night. The rhythm persisted in weaker form during constant darkness, indicating that an endogenous circadian clock is involved in the DVM. The results from the respiration experiment supported the assumption, revealing a rhythmicity in the oxygen uptake that also persisted under constant darkness. The light seemed to have in both experiments the role of a *Zeitgeber* that synchronises the circadian clock. For a final identification of the assumed clock a genetic analysis is necessary. However the experiments showed evidence that the DVM and the metabolic activity of *C. finmarchicus* are controlled by a circadian clock.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

1.1 The role of *Calanus finmarchicus* in the oceans

The oceans are subject to fundamental changes like the increase in temperature, the sea ice decrease and the ocean acidification due to the climate change (Pörtner et al., 2014). To predict effects on the ocean ecosystems a fundamental knowledge of the occurring organisms is necessary. The key species obtain a special role in the ecosystems because they have a large effect on their environment (Paine, 1995). This fact makes them especially important for scientific research. One of these key species is the copepod *Calanus finmarchicus* (Gunnerus 1770, Calanidae, Crustacea), who is due to his role in the food web and his abundance an important zooplankton species in the northern North Atlantic (Melle et al., 2014). *C. finmarchicus* is herbivorous and reaches sizes about 2-3 mm (Falk-Petersen et al., 2007). Many planktivorous fish and fish larvae feed on the copepod and his development stages, making it to a key species for the energy transfer from the lower to the higher trophic levels (Melle et al., 2014). The animals convert the phytoplankton bloom into high-energy lipid stores, that are a major source of energy for many fish, birds and marine mammals (Falk-Petersen et al., 2009). The life cycle of *C. finmarchicus* contains six nauplius (NI - NVI) and five copepodite stages (CI - CV) (Melle et al., 2014). The last copepodite stage (CV) is the main overwintering stage. With the beginning of the diapause in the summer and autumn the animals sink to about 500 to under 2000 m (Falk-Petersen et al., 2007). The overwintering period is characterised by a reduced metabolism and development and the use up of the existing lipid reserves (Melle et al., 2014). From mid to the end of the winter the animals arise from the diapause, develop into adults and mate during the return to the surface. The females lay their eggs depending on the food availability, so that the spawning of the eggs happens at relatively low food concentrations before the spring bloom peaks. *C. finmarchicus* shows apart from the described seasonal migration, especially in the CV-stages a diel vertical migration (DVM) (Dale and Kaartvedt, 2000). It is characterised by a migration in the daytime from the surface waters to greater depths. In the night the animals return to the food-rich surface to feed there on phytoplankton. Diel vertical migration is widespread among zooplankton and predator evasion is probably the most important ultimate reason (Hays, 2003). The control mechanism of the DVM in *C. finmarchicus* is not understood yet, but there are hints, that it is controlled by an endogenous clock (Harris, 1963, Esterly, 1919, Hardy and Paton,

1947, Enright and Hamner, 1967). Furthermore, light seems to be an important factor controlling the vertical position of the animals.

1.2 Circadian clocks as endogenous timers

Biological or endogenous clocks are inner timers, which are synchronised by periodic environmental processes but also work for limited periods under constant conditions (Aschoff, 1954). They control internal biochemical and physiological processes in organisms and are responsible for many behavioural patterns, for instance that bees fly to flowers at specific times of a day (Kuhlman et al., 2007). The first observation of a biological clock made the astronomer Jean-Jacques d'Ortous de Mairan 1729. He noticed that heliotrope plants continued their rhythmic movement when they were kept under constant darkness. Many organisms have evolved biological clocks as an adaption to the rhythmic changes in their local environment. An adaption to a 24-hour rhythm is called a circadian clock, but there are also longer (infradian) and shorter (ultradian) rhythms (Albrecht, 2010, Arechiga, 1993). The structure of a circadian rhythm is based on an input pathway, a central oscillator and an output pathway (Gardner et al., 2006). The input pathway is controlled by an environmental factor like light or temperature and is used to keep the central oscillator synchronised to its environment. The oscillator swings permanently and produces for every time point a temporary information, which is interpreted by the output pathway. At last the output regulates the metabolism and the rhythmic activity of the organism. The synchronisation happens with so called "*Zeitgebers*" (time-givers), external stimuli that reset the circadian clock (Harmer et al., 2001). If a daily rhythm persists in the absence of the *Zeitgeber* it is called a circadian rhythm and thus endogenous controlled.

Circadian rhythms with similar molecular central clock mechanisms were found in many eukaryotes such as animals, plants or fungi and some prokaryotes such as cyanobacteria (Harmer et al., 2001). Even though it is for terrestrial organisms well established that endogenous rhythms are drivers for rhythmic activity, for a long time circadian rhythms were not accepted as mechanisms of the DVM of marine zooplankton (Enright and Hamner, 1967). It was thought that the migration was only a reaction to the exogenous environmental factors, although several studies early assumed an involvement of an endogenous clock (Harris, 1963, Esterly, 1919, Hardy and Paton, 1947, Enright and Hamner, 1967). Already at the beginning of the 20th century Esterly (1919) stated, that there is evidence for an internal physiological rhythm in the swimming behaviour of *C. finmarchicus*. Also the results from the field work from Hardy and Paton

(1947) resulted in the hypotheses of an endogenous rhythm. Later, Harris (1963) assumed an internal timing in the vertical migration that determines the position during the hours of darkness, while it is dependent on the light irradiance during the day. The detection of known clock protein components in *C. finmarchicus* from Christie et al. (2013) further supports the assumption of an endogenous timing system.

To investigate the possible role of a circadian clock in C. finmarchicus DVM the recent work of Hüppe (2016) was taken up and further developed. A setup with a DVM experiment and a respiration experiment was used to clarify the question with a phenological observation if there is a circadian rhythm in the DVM and metabolic activity of *C. finmarchicus*. This is important to understand the functioning of the organism and predict possible changes of the climate change on the organism. Furthermore, due to the role of *C. finmarchicus* in the ecosystem it helps to investigate and predict the extent of the climate change on the ecosystem of the copepod.

2 MATERIAL AND METHODS

2.1 Study area

The study area was Loch Etive, a sea loch located at the west coast of Scotland near the city of Oban (56.45° N, 5.3° W). It is a 1 to 2 km wide and 30 km long fjord with two sills (Figure 1) (Nørgaard-Pedersen et al., 2006). The first one separating the loch from the sea is called “Falls of Lora”. The second sill separating the loch itself into an inner and outer basin is called “Bonawe sill”.

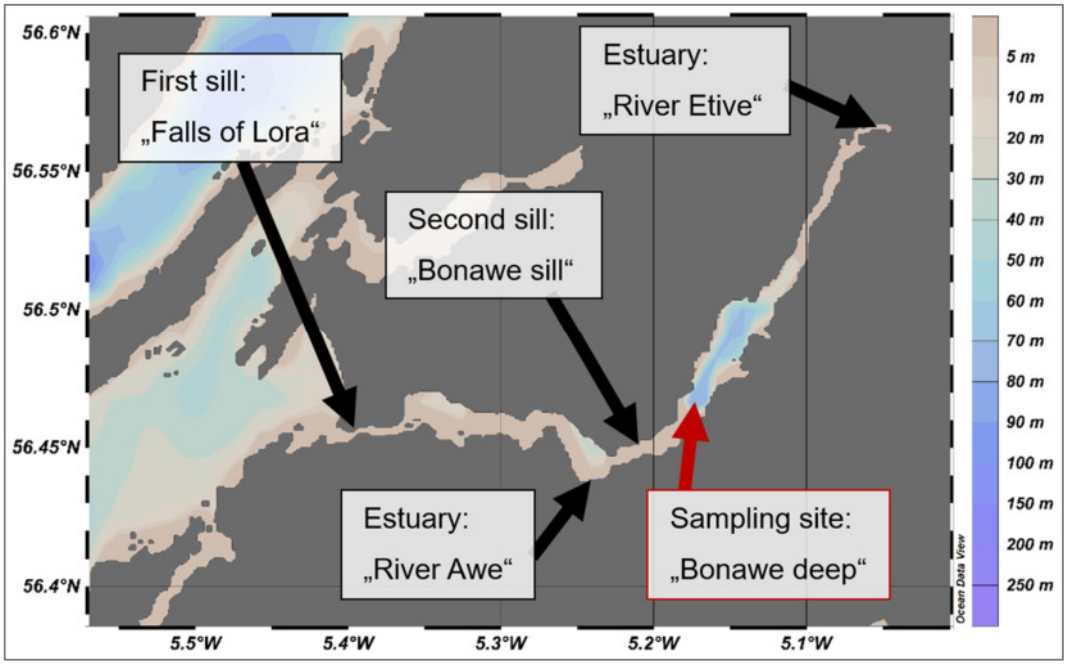

Figure 1: View of Loch Etive with water depth profile and the sampling site, the two sills and river estuaries marked (Ocean Data View).

The “Falls of Lora” sill is 300 m wide and 10 m deep, which results in a reduced water exchange and a to 2 m lowered tidal range in Loch Etive (Edwards and Edelsten, 1977). The two rivers “River Etive” (at the end) and “River Awe” (in the middle) flow into the sea loch, providing a relatively large freshwater input resulting in a reduced salinity especially in the surface water. Next to the Estuary from the “River Awe” the second sill called “Bonawe sill” is located. It is around 13 m deep below medium high water (MHW) and restricts the deep water exchange between the inner and the outer basin under normal conditions (Nørgaard-Pedersen et al., 2006). Near the “Bonawe sill” the sampling site “Bonawe deep” is situated, where Loch Etive reaches the maximum depth of 150 m. Part of the inner basin has an isolated layer of salty water in the deep, where the oxygen saturation is very low (Edwards and Edelsten, 1977, Austin and Inall, 2002). Only when

the bottom water density gets lower than the surface water density a renewal event happens leading to a water exchange (Nørgaard-Pedersen et al., 2006). This can occur when the surface water temperature is relatively low and the freshwater input is reduced. The mean repetition time of these aperiodic renewal events is 18 months (Edwards and Edelsten, 1977).

The Conductivity-Temperature-Depth (CTD) data from Hüppe (2016), taken at the 7th of May 2015 show the hydrographic conditions at Bonawe deep during a similar time of the year as the samplings of *Calanus finmarchicus* were done (Figure 2).

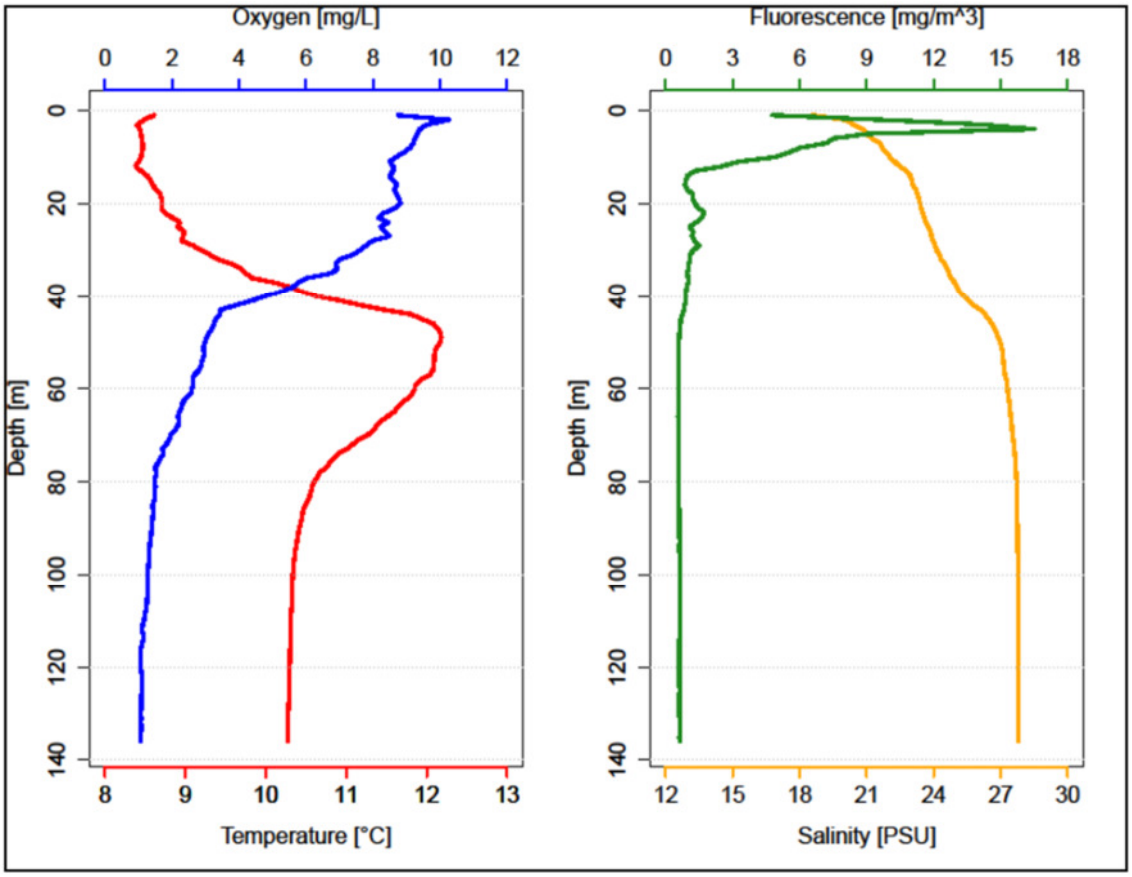

Figure 2: CTD-data from Hüppe (2016) taken on the 7th of May 2015 at Bonawe deep. Blue: dissolved oxygen. Red: temperature. Green: chlorophyll a fluorescence. Yellow: salinity.

The water has a thermocline at about 40 m which reveals the stratification of the water column. The oxygen saturation shows the low saturation in the deep water below 40 m. The high fluorescence values in the top 10 to 15 m indicate the developed spring bloom at the surface water (Hüppe, 2016). The salinity graph shows the clear difference between the salty water in the deep and the freshwater input on the surface. The halocline at 45 m displays again the stratified water at the study site “Loch Etive”. The

specific hydrographic conditions are supposed to be the reason for an isolated population of *C. finmarchicus* in the inner basin of Loch Etive (Hill, 2009).

2.2 Sampling of *Calanus finmarchicus*

The study organisms *C. finmarchicus* were obtained with the ships "*RV Seol Mara"* and *"RV Calanus"* from the Scottish Association for Marine Science. The copepods for the respiration experiment were taken on the 23th of May 2016 (1:30 p.m.) with the "*RV Calanus*" and for the diel vertical migration experiment on the 3rd of June 2016 (10:50 a.m.) with the "*RV Seol Mara*". At first, water for the animals was collected with two Niskin bottles (10 L) from 60 m depth and the water was filled into buckets. Afterwards the copepods were sampled with a WP2-plankton net (diameter: 57 cm) with 200 µm mesh size. The net was lowered in closed position to 60 m depth and then opened by the trigger. In the open position the net was pulled up to 10 m depth and then closed by a second trigger, before taking it back to the ship. The animals from the net were poured through a 500 µm sieve to filter out the early stage *C. finmarchicus* before filling them in the prepared water buckets. The range between 10 and 60 m depth for the net was chosen because the animals in the top 10 m seemed to be inactive, which might be due to the low salinity in the surface water. In greater depths the animals seem to be inactive, because they may have already induced the diapause, so they were not supposed to be valuable in contributing to the research question. The copepods in the closed buckets were then brought to the laboratory of the Scottish Association for Marine Science and were kept dark during the transport. They were put in a constant temperature (CT) room set to 9°C where the lid was removed, so they could acclimate to the CT-room conditions.

2.3 Diel vertical migration experiment

To identify circadian rhythms and a possible involvement of a biological clock in the diel vertical migration of *C. finmarchicus* a laboratory setup with water columns and an artificial light setup was used. It should simulate the natural conditions in Loch Etive on a smaller scale in an aquarium. Therefor four plastic columns (dimensions: 10*8*100 cm, fill level: 90 cm) with black plastic film on the back side were placed in the CT-room at the aquarium of the Scottish Association for Marine Science. In each case two columns were placed next to each other and measuring tape was attached on the front side

between them to act as a scale. On each side three infrared lamps were placed above each other to light the whole columns from the side (Figure 3).

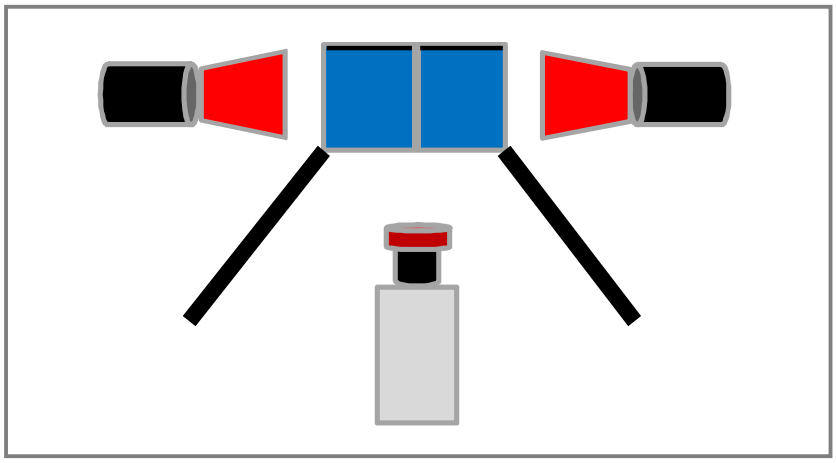

Figure 3: Sketch in top-down perspective of the DVM experiment setup. In the middle are the two water columns (black plastic film on the back side) with the infrared lights at the sides, in front of the columns are the cameras with IR-filters. Two covers were placed between the IR-lights and the cameras to avoid light disturbances during recording.

At the front side six cameras were assembled above each other, each one covering a water layer of 15 cm in the column (0-15, 15-30, 30-45, 45-60, 60-75, 75-90 cm). A lens with an IR-filter which only lets infrared light pass was attached to the cameras. Two covers between the lamps and the cameras should prevent the camera images to bloom. Sea water from the aquarium supply was taken and mixed with Milli-Q water to a salinity of 28 PSU. Shortly before the sampling the columns were filled with the mixed water to a fill level of 90 cm (ca. 10 a.m. of the 3rd of June 2016). After the sampling the animals were sorted under a binocular microscope in petri dishes cooled by a water chiller. Only red light was used during the sorting process to avoid unnecessary stress and disturbance. The CV-stage *C. finmarchicus* were counted and separated to use them for the experiments. At 5:20 p.m. 50 *C. finmarchicus* (CV-stage) were transferred in each water column. The experiment had a runtime of five days (120 hours), from 1:00 a.m. on 5th of June to 1:00 a.m. on 10th of June 2016 local time. Because of the Western European Summer Time there is a difference of 1 hour between the local and the experiment time (e.g. 1:00 local time → 0:00 experiment time). The CT-room was equipped with LEDs, which had a day-night cycle with fluent changes in the light intensity during the day (Mitras Lightbar oceanic blue, GHL Advanced Technology GmbH & Co. Kg, Germany). The light cycle should simulate the natural light conditions in the loch at about 50 m depth and had a maximum light intensity of 5.43 lux at midday, measured with a luxmeter (ILM-1337, ISO-TECH). The animals were exposed to a diurnal light cycle (16 h light, 8 h darkness) for the first two days (LD), followed by two days of

complete darkness (DD). The fifth and last day was again a simulated day-night cycle (LD). The cameras were connected to a computer, recording the videos using the program webCCTV (Quadrox, Belgium). Caused by a mistake the experiment started 24 hours later than planned. The animals stayed in the water columns during that time and had a normal LD-light cycle. A temperature logger (SBE56, Sea-Bird Electronics) measured the temperature of a fifth water filled column, to look for temperature fluctuations in the CT-room during the experiment.

After the end of the experiments an oxygen profile of one water column was measured using an oxygen dipping probe (PreSens) to look for an oxygen gradient inside the columns, which may have influenced the results. Then the number of *C. finmarchicus* in every layer of the videos was counted every full hour. The counting of the video data was done by three persons and the counts were averaged afterwards to minimize personal miscounts. The lowest layer (0-15 cm) was not counted because it had the most copepods and thus was prone to miscounts. It was calculated using the total number of *C. finmarchicus* (50) minus the sum of animals counted in the other layers. Because a part of the animals stayed in the lowest layer and showed no movement during the experiments the lowest counted number of animals in this layer was set as zero to exclude the passive animals from the DVM analysis. After that the mean depth (±SD) of the animals in all water columns was computed for each hourly time point (Figure 4). Finally, the relative abundance of *C. finmarchicus* in the different layers of the water columns was plotted (Figure 5). With the statistic software program R and the package RAIN (Rhythmicity Analysis Incorporating Non-parametric Methods) a rhythm analysis of the data was done. The mean depth of all water columns together was used to detect diel rhythms in the vertical migration of *Calanus finmarchicus* in the experiment. The rhythms with p-values lower than 0.05 were accepted as significant rhythms. It was searched for 20-28 hour rhythms for all experiment days separately as well as LD and DD days together. In addition, an analysis with a set period length of 24 hours for the DD days was done.

2.4 Respiration experiment

With the second experiment a possible involvement of a biological clock in the respiration activity of *C. finmarchicus* was investigated. To look for circadian rhythms the artificial light setup from the DVM experiment was used again and the oxygen consumption of the copepods in closed water bottles was measured. Therefore, a water filled box was

placed in the CT-room. It was connected with a water chiller providing a water flow through the box to constantly keep the temperature between 9.5 and 10°C. Then eight Schott flasks were filled up to the top with 305 mL filtrated seawater (0.22 µm filter) taken from 60 m depth at Bonawe deep (see 2.2). As in the DVM experiment (see 2.3) the copepods were sorted and the CV-stages separated and counted. Just before midnight on the 23th of May 2016 ten *C. finmarchicus* CV-stages each were put in six of the eight bottles. Two bottles were kept without copepods to act as a control. After removing all air bubbles inside, the bottles were closed using parafilm strained over the opening and the cap. The bottles were equipped with a light sensor spot for oxygen measurements. Each spot was connected via a fibreglass cable to one of the two OXY-4 Multi-channel fiber optic oxygen transmitters (PreSens) measuring the oxygen saturation in the bottles. They were connected to two computers recording the oxygen concentration during the experiment. The LED setup was the same as in 2.3, but the runtime was only three days (72 hours), from 1:00 a.m. on 24th of May to 1:00 a.m. on 27th of May 2016 local time (local time - 1 h = experiment time). The first day was a simulated LD cycle, followed by two days in DD. The water temperature in the box was measured manually with a digital thermometer (Hannah instruments) during the first hours. Upon 9:30 a.m. of the 25th of May a temperature logger (SBE56, Sea-Bird Electronics) measured the temperature automatically every minute. At the end of the experiment the animals were counted and checked using a binocular microscope.

The oxygen concentration results needed to be corrected for the temperature fluctuations, because the oxygen solubility is strongly correlated to the water temperature. The provided information of the manufacturer of the used oxygen measurement device (PreSens) was used for the changes in the measured oxygen saturation for temperature fluctuations of ±1°C (1). Thereby, a correlation factor *k* was calculated for every time point separately using the equation (2). The used standardised temperature was 10°C. At last the calculated factor *k* was added to every oxygen concentration value to get the corrected oxygen concentrations.

$$(\mathbf{1})\ y = 0.0154 * x + 0.0569 \qquad x = measured\ oxygen\ saturation\ [\%\ air\ sat.]$$

$$(\mathbf{2})\ k = y * (10\ °C - \mathrm{t_x}) \qquad \mathrm{t_x} = measured\ temperature$$

After that the results also needed to be corrected for possible bacterial oxygen consumption. To correct the results, the mean oxygen saturation from the two control bottles without copepods was subtracted from each bottle with copepods. Then three different moving averages were calculated for each of the six bottles with copepods to

obtain the changes in the oxygen consumption over the whole experiment (Figure 8). The moving averages had a 12-hour period with different start points at 0 h, 6 h and 12 h. The one starting at 0 h subtracted the averaged values of the 12 hours after the time point. The moving average that started at 6 h subtracted the averaged values of the 6 hours before and after the time point, and the one at 12 h the averaged values of the 12 hours before the time point. Finally, the complete data were inverted, to ensure that high values represent high oxygen consumption. This was necessary as the measured factor was oxygen content and a relatively low oxygen content corresponds to a relatively high oxygen consumption. Similar as in 2.3 a RAIN-analysis of the data was done. For the analysis the three inverted moving averages were calculated separately for every bottle with copepods. Then the pooled values of every hour were used by taking the mean value of the five values before and after a time point (e.g. 0:55 – 1:05 for 1:00). After that the mean values of the moving averages were calculated to get the three different mean moving averages with the start points 0 h, 6 h and 12 h. The data were included in the RAIN-analysis to detect possible rhythms in the respiration activity of *C. finmarchicus*. The rhythms with p-values lower than 0.05 were accepted as significant rhythms. It was searched for 20-28 hour rhythms and for a set 24-hour rhythm for all three moving averages. The moving average that started at 0 h was analysed for 20-28 hour rhythms and for a set 24-hour rhythm during the LD day separately. The same was done with the moving average that started at 12 h for the DD days.

3 RESULTS

3.1 Diel vertical migration experiment

The position of *C. finmarchicus* in the DVM experiment showed a clear synchronisation to the given day/night cycle (Figure 4). The rhythm persisted in a weaker form during DD even though the pattern got blurred the longer the darkness was. The mean depth during the two first days (LD) was lower in the daytime than in the nights. When it was dark the animals stayed at about 65 - 70 cm and sank to about 80 cm depth when the day began. In the night between the second day LD and the first day DD the mean depth was similar to the ones before (ca. 65 cm). During the first day DD the mean depth was between 65 and 70 cm. The following night the mean depth rose to about 55 cm, before dropping to about 60 cm in the first hours of the second day DD. During the second day constant darkness the mean depth increased continuously to about 50 cm. The trend went on during the following night until a mean depth of 45 cm, before the light of the last day (LD) began. This is where the mean depth dropped instantly to 70 cm and reaches about 75 cm in the second half of the fifth day. In the last night of the experiment the mean depth then rose at about 50 cm.

The distribution of *C. finmarchicus* showed, that most copepods were during LD daytime located at the bottom of the water columns while in constant darkness they were more spread of the different layers (Figure 5). During the first night about 70 % of the animals were located in the lowest layer (75 – 90 cm) of the water columns. The remaining ones were spread over the other five layers. With the first light of the first day (LD) at 5:00 a.m. more than 90 % of the *C. finmarchicus* were located in the lowest layer and stayed there during the day. In the following night again about 70 % of the animals were found in the lowest layer. The rest was mostly located in the top layer (0-15 cm). The second day LD was very similar to the first day LD. During the third night the percentage of copepods in the lowest layer dropped to about 55 %, while in the beginning of the first day DD it increased to about 65 %. In the course of the day the amount dropped to about 50 % while already about 15 % of the animals were located in the top layer. In the next night only about 40 % of the animals were located in the lowest layer. The other copepods were mostly in the second lowest and in the top layer. The second day DD had in the beginning about 45 % of the animals in the lowest layer, but the number dropped during the day to about 30 %. In the following night the lowest numbers of *C. finmarchicus* (ca. 25 %) were in the lowest layer located, but the amounts were unsteady. With about 30 %

most copepods were then found in the top layer. During the last day (LD) initially about 55 % of the animals were in the lowest layer. The percentage increased during the day to about 70 %. During the last night the percentage of *C. finmarchicus* was about 30 % in the top layer as well as in the lowest layer.

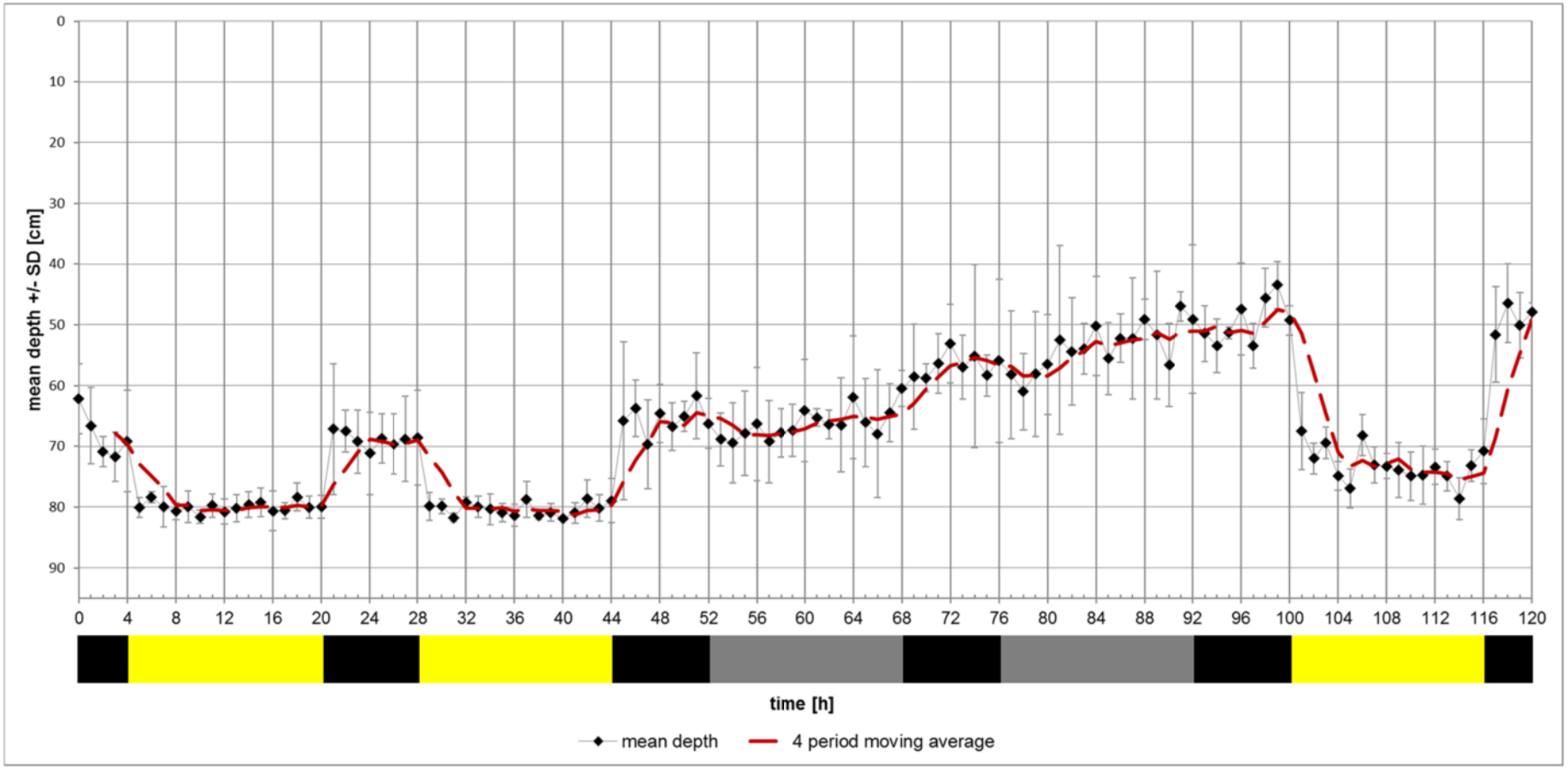

Figure 4: Mean depth [cm] (black points) +/- standard deviation of the *C. finmarchicus* (CV-stage) in the water columns during the experiment with the different light settings (LD LD DD DD LD). The red line shows a moving average of the mean depth over the previous 3 h. The yellow bars represent the daylight, the black bars the night and the grey ones the subjective day when no light was given.

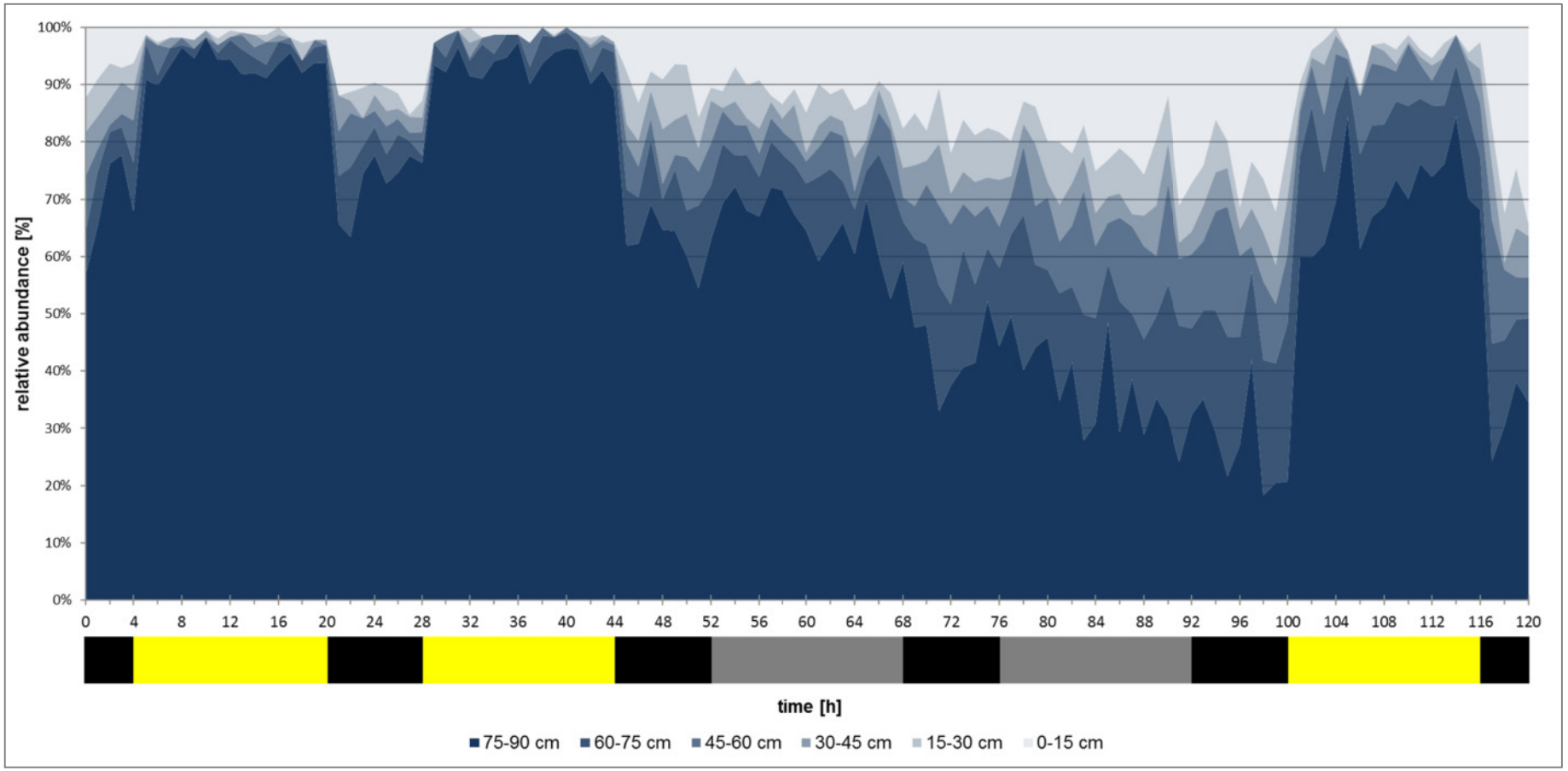

Figure 5: Relative abundance [%] of *C. finmarchicus* (CV-stage) in the different layers of the water columns during the experiment with the different light settings (LD LD DD DD LD). The yellow bars represent the daylight, the black bars the night and the grey ones the subjective day when no light was given.

The rhythm analysis with the package RAIN for R showed that there are significant diurnal rhythms in the DVM of C. finmarchicus for the LD days as well as for the DD days (Table 1). The analysis with all days separately showed shorter rhythms for the LD days with period lengths from 20 to 23 hours. The DD days have longer rhythms from 25 to 26 hours. The 20 to 28 hours analysis of the LD and DD days respectively together exposed 25-hour rhythms, but only the rhythm for the LD days is significant. For the following two days with constant darkness the p-value is not significant. The analysis with a set 24-hour rhythm demonstrated for both light settings significant rhythms.

Table 1: RAIN-analysis of the DVM experiment for all days separately and the LD and DD days respectively together. It was searched for 20 to 28 hour rhythms and additionally a set 24-hour rhythm for the LD and DD days together.

All days separately	**p value**	**period**
day1_LD_20-28 h	2.208870E-14	22
day2_LD_20-28 h	1.170763E-15	23
day3_DD_20-28 h	7.005230E-06	26
day4_DD_20-28 h	2.468765E-03	25
day5_LD_20-28 h	1.642805E-18	20
LD and DD respectively together		
day1&2_LD_20-28 h	1.774040E-09	25
day1&2_LD_24 h	7.645055E-10	24
day3&4_DD_20-28 h	1.464739E-01	25
day3&4_DD_24 h	3.855342E-02	24

The mean incubation temperature over the five-day experiment was 10.13°C ± 0.36 SD. The temperature stayed during the first two days LD of the experiment at around 9.8°C with low fluctuations about 0.1°C (Figure 6). After 44 hours the temperature dropped slightly (9.6 to 9.7°C). During the first day DD, at about 60 hours after the start of the experiment, the temperature started to rise with bigger fluctuations of about 0.3°C. From 80 hours until the end of the experiment the temperature stayed then at about 10.6°C with fluctuations of about 0.3°C. No connection could be found between temperature change and DVM behaviour.

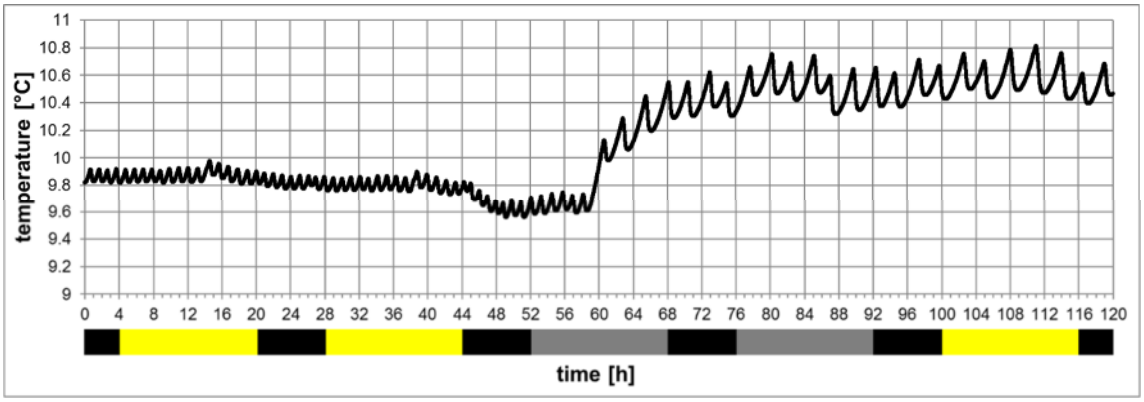

Figure 6: Water temperature [°C] in the additional column during the DVM experiment with the different light settings (LD LD DD DD LD). The yellow bars represent the daylight, the black bars the night and the grey ones the subjective day when no light was given.

A gradual decrease in the oxygen concentration was measured after the experiment, but even the strongest decrease was not exceeding 16 % compared to the oxygen concentration before the experiment (Figure 7). After the experiment the top layer (0-15 cm) had an oxygen concentration of about 9.25 mg/L which dropped to about 9.1 mg/L in the second layer (15-30 cm). In the third layer (30-45 cm) the oxygen concentration was about 9 mg/L, followed by about 8.9 mg/L in the fourth layer (45-60 cm). The oxygen concentration in the fifth layer (60-75 cm) was about 8.8 mg/L and about 8.75 mg/L in the lowest layer (75-90 cm).

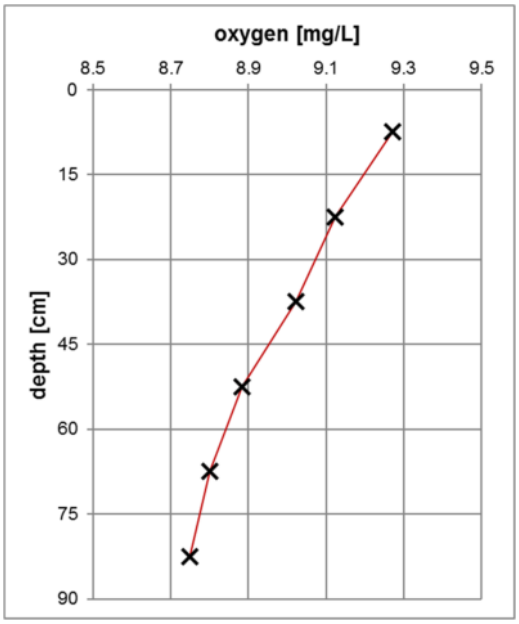

Figure 7: Profile of the oxygen concentration [mg/L] in the 6 different layers (0-90 cm) of one water column after the end of the DVM experiment.

3.2 Respiration experiment

The relative change in the oxygen consumption of *C. finmarchicus*, which was corrected by the bacterial oxygen consumption and the fluctuations in the temperature, showed a rhythmicity with the given day/night cycle (Figure 8). The relative change in the oxygen consumption was lower during the days and peaked in the nights under LD as well as DD light conditions. The under LD conditions noticed drop in the oxygen consumption at the beginning of the night shifted under DD conditions to the end of the night. The relative change in the oxygen consumption showed an increase from about -0.15 at 0 hours in the first night to about 0.1 in the beginning of the second night after the first day (LD). From 20 hours on it dropped constantly until it reached -0.025 at 28 hours after the beginning of the experiment. Then the three different moving averages differed from each other. The moving averages that started at 6 h and 12h stayed on the level during the second day (DD) while the moving average that started at 0 h dropped to about -0.75. At about 44 hours (beginning third night) all three moving averages showed an increase in the relative change in the oxygen consumption to about 0.05 at 52 hours. With the beginning of the third day (DD) the relative change in the oxygen consumption dropped again to about -0.025. The moving average that started at 12 hours showed, that in the last night the oxygen consumption rose again to about 0.05, before the experiment ended.

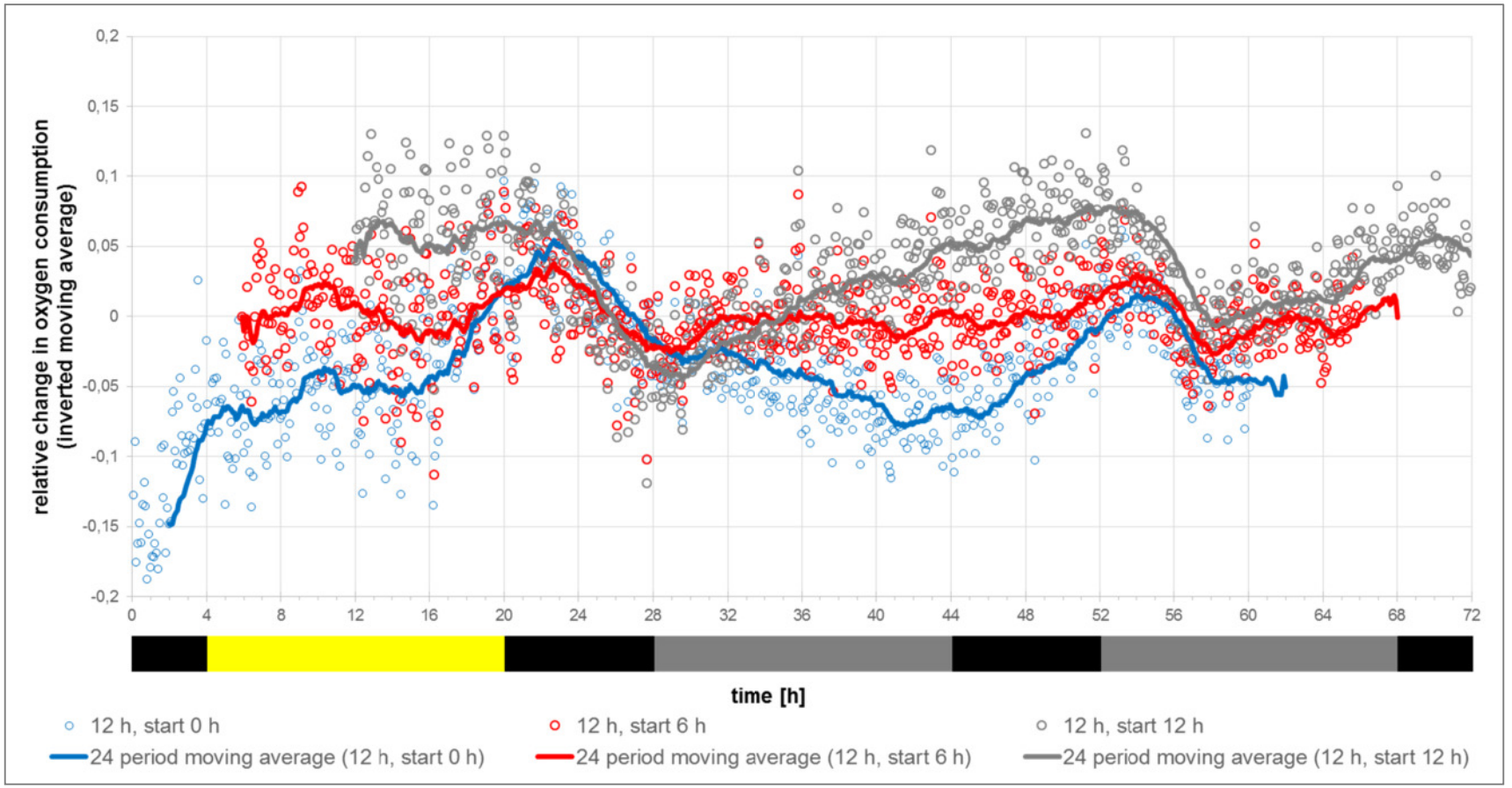

Figure 8: Three 12 h moving averages of the relative change in the oxygen consumption of *C. finmarchicus* (CV-stage) with the different light settings (LD LD DD DD LD). The first moving average started at 0h (blue dots) and used the values 12 h after the time point. The second one started at 6 h (red dots) and used the values 6 hours before and after the time point and the third moving average started at 12 h (grey dots) and used the values 12 hours after the time point. The red, blue and grey lines show a 24 h moving average of the respective 12 h moving average. The yellow bar represents the daylight, the black bars the night and the grey ones the subjective day when no light was given.

The rhythm analysis of the three different moving averages showed diurnal rhythms for LD and DD days (Table 2). The respiration rhythm of *C. finmarchicus* seems to be longer than a daily 24-hour rhythm because all analysis from 20 to 28 hours showed significant 28-hour rhythms. For the analysis of the moving averages that started at 6 and 12 h with all days together also significant 24-hour rhythms were found. For the moving average that started at 0 h no significant 24-hour rhythm is found. The analysis of set 24-hour rhythms for the LD and DD days respectively together exposed significant rhythms.

Table 2: RAIN-analysis of the respiration experiment for all days together and the LD and DD days respectively together. It was searched for 20 to 28 hour rhythms and a set 24-hour rhythm.

All days together	**p value**	**period**
start 0 h_20-28 h	2.282576E-06	28
start 0 h_24 h	4.949016E-01	24
start 6 h_20-28 h	1.700545E-02	28
start 6 h_24 h	2.282391E-02	24
start 12 h_20-28 h	6.909741E-14	28
start 12 h_24 h	2.985380E-05	24
LD and DD respectively together		
LD_start 0 h_20-28 h	2.663468E-05	28
LD_start 0 h_24 h	4.231868E-04	24
DD_start 12 h_20-28 h	1.108463E-11	28
DD_start 12 h_24 h	8.472465E-06	24

The mean water temperature in the box over the three-day experiment was 9.87°C ± 0.17 SD. The temperature of the first 9.5 hours of the experiment was measured manually and stayed at about 9.55°C. After that a temperature logger was used and the temperature sank slowly from about 9.55°C to about 9.5°C at 16 hours after the start with fluctuations of about 0.1°C (Figure 9). Then the temperature rose first slowly and with the beginning night after 20 hours quicker to about 10°C at 32 hours. At about 38 hours the temperature dropped a little bit to about 9.5°C where it stayed until the end of the experiment. The fluctuations during that time where at about 0.1°C.

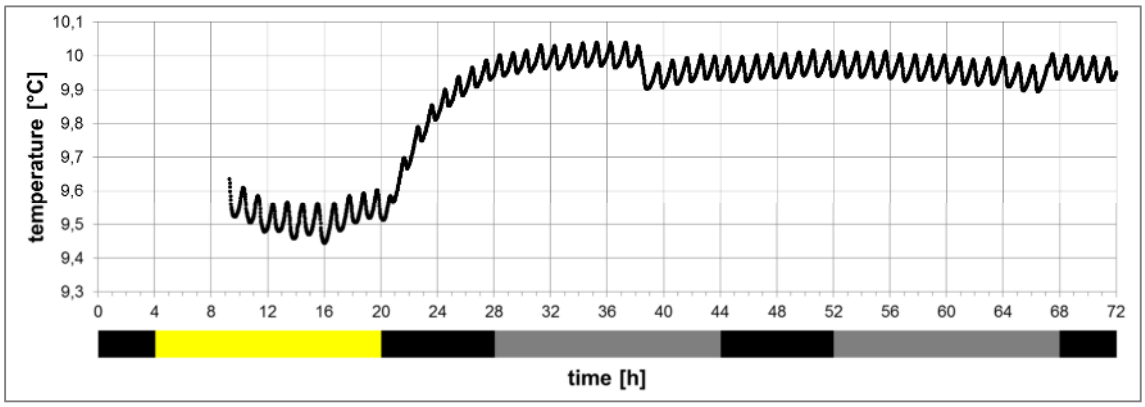

Figure 9: Water temperature [°C] in the box with the bottles during the respiration experiment with the different light settings (LD DD LD). The yellow bar represents the daylight, the black bars the night and the grey ones the subjective day when no light was given.

The inspection after the experiment showed alive and active animals. Some copepods had slightly damaged tails, but they were overall in a good condition.

4 DISCUSSION

4.1 Circadian rhythms in the diel vertical migration of *Calanus finmarchicus*

The results from the diel vertical migration experiments showed, that the position of *Calanus finmarchicus* in the water column is strongly depended to the light irradiation. During the LD light conditions in the daytime clearly more copepods were located in the depth than in the night time. With the beginning of the night, a part of the animals started swimming closer to the surface of the water columns, where they stayed during the night until the next light incidence occurred. The diel vertical migration in the water columns was synchronized to the given LD-light cycle. Therefore, a strong effect of the light on the diel vertical migration of *C. finmarchicus* is shown as a negative phototaxis. Ringelberg (1999) showed the effect of light on the diel vertical migration of *Daphnia spp*. as a model organism for zooplankton and considered phototaxis as the most important mechanism basic to the diel vertical migration. The results from the rhythm analysis with RAIN for the days with LD light-cycles revealed significant rhythms with period lengths between 20 and 23 hours (Table 1). The RAIN analysis of single days cuts off half of the nights, which results in a shorter period, because the end of one night and the beginning of another are rated as one peak. Because of that, an analysis with the first two LD days merged were done. They showed significant 25 and 24-hour rhythms, which could be a circadian rhythm in the diel vertical migration of *C. finmarchicus* under simulated light conditions with a day/night-cycle. Nevertheless, under LD conditions light seemed to be the controlling factor for the position of the copepods.

Under DD conditions it was observable that still more animals were located in the depth during the subjective day, even though no light was given. The number of animals which performed DVM was clearly lower than under LD conditions, but with the beginning of the subjective day some copepods still started to sink to the lower layers and rose to the surface when the subjective night began. During the long constant darkness time in the experiment the initially clear DVM was more and more blurred. This was caused because more copepods swam to the surface the longer the darkness lasted. A reason for that may be that no food was given in the experiment and the copepods searched at the surface where they normally feed instead of performing DVM. The observation indicated, that light cannot be the only factor to control the DVM of *C. finmarchicus*, because otherwise there would not be a difference identifiable between the position during night

and subjective day under DD conditions. The results from the RAIN analysis showed significant 25 and 26-hour rhythms for the DD days separately and a 25-hour rhythm for the DD days merged (Table 1). An analysis of a set 24-hour rhythm for the merged DD days revealed, that also this rhythm is significant. Therefore, a circadian rhythm could also be assumed under DD conditions. The temperature fluctuations during the experiment do not seem to be as a factor to falsify the results because they were overall rather small (±0.6°C) and did not show any rhythmicity which may have explained a diurnal rhythm in the DVM. The oxygen measurement after the experiment showed a decline in the oxygen saturation with an increasing depth. No influence in the behaviour and the vitality due to a lack of oxygen is estimated because the gradient was not very strong (0.4 mg/L) and the overall oxygen decrease (<16%) was rather small. Nevertheless, it may be another reason for the animals to swim more and more to the surface during the two days of constant darkness.

The results of the DVM under DD conditions cannot be explained with the light irradiation as the results under LD conditions, because there was no light given, which could have induced a rhythm. Thus light cannot be the only factor controlling the DVM. Instead they are a hint for an internal controlled mechanism that directs the DVM in *C. finmarchicus*. In early works Esterly (1919) stated, that there is evidence for a physiological rhythm in the swimming behaviour of *C. finmarchicus*. The field work from Hardy and Paton (1947) showed no significant differences in the DVM of *C. finmarchicus* between normal light and during constant darkness, which resulted in the hypotheses of an endogenous rhythm. In their work Harris (1963) observed the same distribution of animals during the night as in the DVM experiment with a group of active animals at the surface and a passive group of animals at the bottom. They assumed an internal timing in the vertical migration that determines the position during the hours of darkness, while during the day it is dependent on the light irradiance. The experimental studies of Enright and Hamner (1967) on different marine zooplankton species showed, that the DVM of some species seem to be controlled by an internal rhythm while in other species it is a response to the light irradiance. The results from the DVM experiment indicates, that the DVM of *C. finmarchicus* may also be controlled by a circadian rhythm. The role of the light could be the one of the *Zeitgeber* that synchronises the rhythm.

4.2 Circadian rhythms in the respiration of *Calanus finmarchicus*

The changes in the oxygen consumption showed a rhythmicity with a peak during the night followed by a sharp decrease in the oxygen uptake. The visible pattern persisted under DD conditions even though the drop in the oxygen uptake shifted from the beginning to the end of the night. The oxygen consumption was seen as an indicator of the biological activity of an organism. The measured respiration activity was higher during the nights, which matches with a higher biological activity and therefore a higher energy demand due to DVM and feeding activity of *C. finmarchicus* in the night (Simard et al., 1985). The results from the RAIN-analysis showed for all three moving averages significant 28-hour rhythms (Table 2). The rhythms with a 24-hour period were only significant for the moving averages starting at 6 h and 12 h. An analysis with the different light settings (LD & DD) analysed respectively together showed for LD and DD significant 28-hour rhythms. The same analysis for a 24-hour rhythm resulted in significant rhythms for both LD and DD days. This could mean, that there is a circadian rhythm in the respiration activity of *C. finmarchicus*. The period length seems to be longer than the normal 24 hours, but circadian rhythms are known to be just roughly matched the earth's rotation (Harmer et al., 2001). The longer period could explain the noticed shift in the oxygen uptake drop during the DD nights. The temperature fluctuations during the experiment did not seem to influence the rhythmicity, because they were corrected in the oxygen uptake and did not have a rhythm that could have induced a rhythmic respiration activity. The good condition of the animals after the experiment is an indicator for a rather low stress level caused by the experiment setup.

The rhythmicity of the respiration activity cannot be explained through the light cycle, because it persisted under constant darkness. Because the factors that could induce a rhythm were tried to be eliminated in the experiment, an internally controlled circadian rhythm could be the controlling factor for the respiration activity. In another experiment the respiration measurements of different migratory copepods showed daily variations with significant differences between day and night (Pavlova, 1994). They were seen as an effect of the natural migratory rhythm and the search for food. So the increase in the oxygen uptake of *C. finmarchicus* seems to be directly connected with the DVM swimming behaviour. In freshwater zooplankton endogenous diel rhythms in the respiration rate and feeding activity were found (Duval and Geen, 1976). This encourages the assumption that this also applies for marine zooplankton as *C. finmarchicus*.

Hüppe (2016) also assumed an involvement of a circadian clock in the metabolic activity of *C. finmarchicus* even though the respiration experiments had some strong limitations regarding the methods in the experiment. As this work is a direct advancement based on the work of Hüppe the methodological differences are listed. For the respiration experiment Hüppe used copepods from the whole water column in Loch Etive (0-140 m), but observation showed, that the animals in the top 10 m seem to be inactive due to the freshwater layer and the animals below 60 m may already have induced diapause. Therefore, only the animals from 10 m to 60 m depth were used for this experiment to enhance the number of active animals. In the experiment of Hüppe the bottles were closed underwater, which involves the risk of escaping animals and remaining air bubbles in the bottles. He recommended to fill the bottles up to the top with water and the usage of plastic foil, to cover the bottles and exclude air bubbles. Both suggestions were realized in this experiment. The respiration experiment of Hüppe had issues with strong fluctuations in the water temperature. With a water chiller providing a constant water flow the fluctuations were reduced in this experiment. The result was a lowered temperature fluctuation from ±1°C to ±0.3°C. As a result of the further developed methodology it was now possible to detect a clear rhythmicity in the respiratory activity during LD and DD light settings.

4.3 The circadian clock in *Calanus finmarchicus*

In the DVM and the respiration activity of *C. finmarchicus* diurnal rhythms were found. The controlling factor did not seem to be the light irradiance and both experiments provide some evidence, that there is a circadian clock in *C. finmarchicus* controlling these processes. The light that seemed to determine the migration under LD light conditions could have the role of the *Zeitgeber* as an important part of the circadian rhythm. It may synchronise the circadian clock and thereby ensures the functioning of the rhythm. However, it needs to be considered, that the experimental setups were artificial, only striving to simulate the natural conditions. The animals were taken from there natural ecosystem in the loch and put into small bottles and 1 m high plastic columns. The sampling is always related with stress in the animals that can change the behaviour of the animals and distort the results. The copepods in the respiration experiment had no place for a vertical migration and stayed in the same water for three days. In the water columns the vertical migration was limited to 1 m, while the water at the sampling site of Loch Etive is about 150 m deep. Furthermore, the animals in the lowest layer tended to be crowded, which might have affected their activity. When considering these limitations,

it is even more impressing that the diurnal rhythms were still visible in the laboratory experiments. This enhances the significance of the experimental setups and allows assumptions about the controlling factor of the respiration and the DVM. The work of Pavlova (1994) showed, that only the respiration rate of copepods that perform DVM have a diurnal cycle, which leads to the assumption, that the respiration rate and the DVM are directly connected and both are controlled by a circadian clock. The results from the DVM and respiration experiments support the assumption that this also applies for *C. finmarchicus*. However, for a final identification of a circadian clock a genetic analysis is necessary. The detection of known clock protein components in *C. finmarchicus* from Christie et al. (2013) marks an important step. With a further genetic analysis possible clock genes may be identified, which would help to understand the functioning of the assumed clock.

4.4 *Calanus finmarchicus* in times of climate change

As an effect of climate change the marine ecosystems are exposed to important changes like rising temperature, ocean acidification or expansion of hypoxic zones (Pörtner et al., 2014). Shifts in marine productivity, biodiversity and ecosystem structure are expected as well as regional extinctions of species. A general shift of the range of many species towards higher latitudes is assumed. This involves greater risks of extinction for polar species that have no place to shift. Beaugrand et al. (2002) demonstrated that the biogeographical northwards shift of many calanoid copepods including *C. finmarchicus* is associated with a decline of the number of cold-water species. Zooplankton is crucially important for the functioning of the ocean food webs because they are an important link in the energy transfer between the primary producers and the higher trophic levels due to their abundance (Richardson, 2008). It is also involved in the lock up of CO_2 in the sediment as a part of the biological pump and thus in the extent and pace of the climate change. *C. finmarchicus* is a key species in the northern North Atlantic and therefore an important factor to predict the effects of the climate change on the ecosystem (Melle et al., 2014). Due to the northwards shift, *C. finmarchicus* has been replaced in the North Sea with *Calanus helgolandicus* which has a different seasonal cycle. (Beaugrand et al., 2003, Richardson, 2008). The peak abundance of *C. finmarchicus* is reached in spring while *C. helgolandicus* peaks in autumn (Richardson, 2008). This is critical, because the Atlantic cod larvae spawning at spring are dependent on a large copepod biomass in the North Sea. The shift of *C. finmarchicus* is believed to have caused a decrease of Atlantic cod recruitment due to changes in mean prey size, seasonal timing, and abundance of

plankton (Beaugrand et al., 2003). The northwards shift *C. finmarchicus* could also alter the arctic ecosystem because it may replace the polar copepods *Calanus glacialis* and *Calanus hyperboreus* which have higher amounts of the for the important lipids (Falk-Petersen et al., 2009). The timing of seasonal events of zooplankton is known to be highly sensitive to global warming (Richardson, 2008). This can result in mismatch situations when the timing of the species of a food web do not react identical to ocean warming. A less efficient energy transfer between the trophic levels can be the consequence. It is known that the reduction of the sea ice thickness and coverage will alter the timing of the arctic phytoplankton bloom and could result in a mismatch with the reproductive cycle of *Calanus glacialis* (Søreide et al., 2010). An important timing event of *C. finmarchicus* is the diapause, that is expected to be shortened by global warming due to increased metabolic rates and reduced body size and lipid stores (Wilson et al., 2016). It is assumed that a reduction in diapause will have ecological consequences, but they are difficult to predict. Possible mismatch situations like in *C. glacialis* could also affect *C. finmarchicus* with negative consequences for the ecosystem. Aside from the seasonal processes also diurnal process like DVM may be vulnerable to climate change induced processes. A possible circadian clock in *C. finmarchicus* may be distorted by altered environmental conditions due to a northwards shift to habitats with more extreme photoperiods. A not properly working circadian clock could result in a restricted DVM and therefore in a reduced viability due to predator pressure. This could lead to major changes on the ecosystem in the North Atlantic because of the role of *C. finmarchicus*. However, to predict possible changes, also research on the general functioning of diurnal processes like DVM is necessary.

4.5 Conclusion & Outlook

To conclude, the position of *C. finmarchicus* in the water columns showed a clear diurnal rhythm, with significant differences between day and night. During the LD light setting the position was strongly depending on the light irradiance. This was seen as a negative phototaxis. Since the rhythm persisted during constant darkness, light cannot be the only factor controlling the vertical migration. Instead an involvement of an endogenous circadian clock is assumed. The results from the respiration experiment supported the assumption, because the rhythmicity in the oxygen uptake also persisted under constant darkness. The light seemed to have the role of a *Zeitgeber* that synchronises the circadian rhythm. Even though the results are seen as a strong sign towards an endogenous rhythm, they are only a phenological view on the topic. For a final

identification of a circadian clock a genetic analysis is necessary. The identification and functioning of a circadian clock is an important part of understanding the organism *C. finmarchicus* and the marine ecosystems. Possible effects of the climate change on circadian clocks may be detected, that could influence the functioning of whole ecosystems. The achieved knowledge could also help to identify possible endogenous rhythms on a seasonal scale, which may control important parts of the life cycle of *C. finmarchicus* and allow a further understanding of marine ecosystems.

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SELBSTSTÄNDIGKEITSERKLÄRUNG

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Jorin Hamer