



Photoperiodism and overwintering in boreal and sub-Arctic *Calanus finmarchicus* populations

Estelle Coguiec^{1,*}, Kim S. Last², Jonathan H. Cohen³, Laura Hobbs²,
Marvin Choquet^{4,5}, Elizaveta Ershova⁶, Jørgen Berge^{1,7}, Malin Daase¹

¹Department of Arctic and Marine Biology, UiT the Arctic University of Norway, 9019 Tromsø, Norway

²The Scottish Association for Marine Science, Oban, Argyll PA37 1QA, Scotland

³School of Marine Science and Policy, University of Delaware, Lewes, Delaware 19958, USA

⁴Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

⁵Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

⁶Institute for Marine Research, Bergen, Norway

⁷Centre for Autonomous Marine Operations and System (AMOS), Department of Biology, Norwegian University of Science and Technology, NTNU, Trondheim, Norway

ABSTRACT: The copepod *Calanus finmarchicus*, a key species in the North Atlantic, generally spends the non-productive season by descending into deep waters and entering diapause, a physiological state characterized by reduced metabolism and arrested development. In the open ocean, overwintering depths are below 600 m, where temperature and light conditions are favourable to initiate diapause. However, *C. finmarchicus* has also been reported diapausing in areas with shallow water depth such as fjords, coastal waters and shelf seas. In these environments, the temperature and light conditions are different, and it has been hypothesized that under such conditions *C. finmarchicus* may remain active throughout winter. Here, we investigated changes in the swimming activity of *C. finmarchicus* from shallow fjords in the eastern North Atlantic during overwintering in response to ambient photoperiod. We conducted monthly experiments with populations from 2 fjords from different latitudes (sub-Arctic Ramfjord, 69° N and boreal Loch Etive, 56° N), measuring the locomotor activity of individual *C. finmarchicus* stage CVs exposed to a natural light:dark cycle. At both locations, peaks in activity in response to the light cycle were observed to shift from nocturnal during the early overwintering phase to diurnal during mid and late overwintering phase, with a minimal intensity observed during the mid-overwintering phase. In Ramfjord, activity and rhythmicity were generally lower than in Loch Etive. We conclude that *C. finmarchicus* remains active throughout its overwintering period when in shallow (<200 m) locations but down-regulates its locomotor activity during the main overwintering phase, which we describe as a winter resting state as distinct from classical diapause.

KEY WORDS: Locomotor activity · Latitudinal seasonality · Lipid · Overwintering · Zooplankton

Resale or republication not permitted without written consent of the publisher

1. INTRODUCTION

The primarily herbivorous copepod *Calanus finmarchicus* is a key species in the North Atlantic marine ecosystem. It plays a major role in the transfer of

energy from phytoplankton to higher trophic levels due to its ability to accumulate large amounts of lipids (Falk-Petersen et al. 2009). *C. finmarchicus* also contributes to the biogeochemical carbon cycle by releasing organic matter at depth through its diel

and seasonal vertical migration (SVM). The lipid storage and SVM behaviour not only confer *C. finmarchicus* an essential role in the ecosystem but ensure survival through the non-productive period, i.e. when food availability is reduced, and thus ultimately improve reproductive success (Maps et al. 2011). SVM is observed in a number of pelagic organisms and describes the seasonal migration from the upper pelagic to deeper waters at the end of the productive season and an ascent to surface layers in late winter/early spring. When at depth, *C. finmarchicus* enters a physiological state, called diapause (hereafter referred to as classical diapause), characterized by reduced metabolism, as well as reduced enzymatic, feeding and swimming activity (Marshall & Orr 1955, Hirche 1983, Hirche 1996). During this time, development is arrested to a late developmental stage, which in this species is most commonly the copepodite stage V (CV) (Marshall & Orr 1955, Hirche 1983, Hirche 1996). This overwintering strategy enables *C. finmarchicus* to reduce its metabolic costs and risk of being preyed upon by visual predators during the non-productive period, due to cooler temperatures and darkness of the deep-water environment (Marshall & Orr 1955, Hirche 1996).

Diapause is a well-known behaviour in *C. finmarchicus*, but the initiation of diapause is poorly understood. Several factors are commonly recognized as involved in the initiation of diapause, including exogenous seasonal cues such as photoperiod, temperature, food availability, predation pressure, and endogenous cues such as lipid thresholds and circadian clocks (Irigoiien et al. 2004, Häfker et al. 2017). However, diapause initiation appears to be more complex than a simple response to one of these factors. It has been suggested that a combination of factors may trigger diapause, and that these factors may change geographically. Baumgartner & Tarrant (2017) suggested that cues may differ between animals inhabiting environments with shallow water depth and those inhabiting environments with deep water depth and polar environments. They contend that in locations with shallow water depth, diapause is triggered by a combination of factors such as photoperiod (Sømme 1934, Fiksen 2000), temperature (Corkett & McLaren 1979), food abundance (Rey-Rassat et al. 2002) or predation pressure (Ji 2011), while for organisms living in the deep oceanic basins and in polar environments a single seasonal cue such as photoperiod or temperature may be enough to induce diapause. For the latter, organisms inhabiting environments with shallow depth at high latitudes should respond similarly to those from deep environments.

The distribution of *C. finmarchicus* extends from 40° to 80° N (Hirche & Kosobokova 2007, Choquet et al. 2017, Grieve et al. 2017). Along this distributional range, it is exposed to a large gradient of environmental conditions including seasonal changes in day length, temperature, and prey availability. *C. Finmarchicus* has evolved its life history strategy to take advantage of a relatively predictable and extended productive period, timing its reproduction to match with the phytoplankton bloom (Miller et al. 1991). However, there is high plasticity in the timing of overwintering and reproduction within its distributional range (Johnson et al. 2008). In boreal latitudes, the overwintering period is generally shorter, lasting for a couple of months, and up to 3 generations per year can be produced (McLaren & Corkett 1986, Durbin et al. 1997, McLaren et al. 2001, Saumweber & Durbin 2006, Michaud & Taggart 2007). However, populations with 1 yr life cycles have been described as far south as the Gulf of St Lawrence and across most of the Canadian Atlantic (Plourde et al. 2001, 2009). At the northern border of the species distributional range, *C. finmarchicus* has a 1 yr life cycle (Daase et al. 2021), and the overwintering phase can last for up to 10 mo (Hirche 1996).

Overwintering depth also varies substantially across the distributional range of *C. finmarchicus*. In the deep basins of the North Atlantic, diapause occurs in the mesopelagic layer >600 m (Heath et al. 2000). However, successful overwintering of *C. finmarchicus* populations has also been observed in shallower environments such as fjords and shelf seas with bottom depth typically <300 m (Tande 1982, Hirche 1991, Durbin et al. 1995), which have a very different light climate and temperature regime. Can we, therefore, expect differences in behaviour and physiology between populations overwintering in environments of shallow and deep bottom depth? Recent observations from the Fram Strait showed that *C. finmarchicus* residing in the epipelagic at the beginning of the non-productive period were more active than those residing in the mesopelagic layers (Grigor et al. 2022). Furthermore, active feeding has been observed during the non-productive period in *C. finmarchicus* populations overwintering in shallow environments (Butler et al. 1970, Hirche 1996) as well as during periods of limited food availability such as mid-summer in the Gulf of St. Lawrence (Ohman & Runge 1994). These feeding activities during periods of low food availability are usually characterized by a dietary shift from herbivorous to omnivorous, carnivorous, and/or detritivorous. These lines of evidence suggest that populations residing in environments

that are depth-constrained remain more active during the non-productive period. A number of factors such as temperature, lipid structure, and food availability likely play a significant role causing the differences in behaviour between individuals inhabiting shallow and deep environments (Pond & Tarling 2011, Pond 2012, Pond et al. 2014). We suggest that one of the factors preventing *C. finmarchicus* from entering a classical diapause in environments with shallow water depth is the exposure to a changing photoperiod, as they cannot migrate into mesopelagic layers where the absence of a changing photoperiod would enable them to enter diapause.

Our aim was to determine locomotor activity during the overwintering period of *C. finmarchicus* in shallow environments under ambient photoperiods at 2 latitudes: the boreal Loch Etive (56°N, west Scotland) and the sub-Arctic Ramfjord (69°N, northern Norway). The main objectives of our study were to: (1) determine if *C. finmarchicus* overwintering in shallow environments enter a period of classical diapause, and (2) investigate patterns of rhythmicity in swimming activity over the diel cycle at 2 latitudes with contrasting photoperiod. Concurrently with these objectives, we characterized morphological differences between boreal and sub-Arctic *C. finmarchicus* populations to determine if differences in overwintering activities between these locations correlated with differences in body size and energy reserves.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Loch Etive, Scotland (UK), and in Ramfjord, near Tromsø (Norway). Loch Etive is located at a boreal latitude (56°45'N, 5°18'W) (Fig. 1a), where day length (i.e. from sunrise to sunset) varies from ca. 7 h in December to ca. 17 h in June (Fig. 1b). Ramfjord is located north of the Arctic circle (69°31'N, 19°02'E) (Fig. 1a). Ramfjord has an Arctic light regime with the day length increasing from 0 h between the end of November to the end of January (polar night) to a maximum of 24 h between the end of May to end of July (midnight sun) (Fig. 1b). Despite this, Ramfjord is characterized as a sub-Arctic fjord since it is not directly influenced by Arctic water masses. In both locations, the sampling station was located near the deepest point of the fjord, at 145 and 130 m in Loch Etive and Ramfjord, respectively.

2.2. Sampling

Monthly sampling was conducted from July 2017 to March 2018 in Loch Etive and from January 2019 to December 2019 in Ramfjord (Table 1). At each sampling event, a CTD profile was taken (SBE 19 in Etive, Seabird SBE 19 plus in Ramfjord, Sea-Bird electronics) (Table 1). In Loch Etive, additional CTD profiles were taken occasionally outside of the main sampling event (Table 1). *Calanus finmarchicus* was collected with a WP2 net with a closing system (Hydro-Bios) and a maximum mesh size of 200 µm. At both locations, samples were taken from a few meters above the seafloor to 50 m deep and were carefully transferred to the local laboratory, keeping the sampled organisms in a dark cooler to maintain stable temperatures, and minimize light exposure. Animals were transferred to a temperature-controlled room set to ambient seawater temperature at the collection site and kept in these conditions overnight (about 18 h) before setting up the experiments.

2.3. Locomotor activity

2.3.1. Experimental settings

For each location and time point, *Calanus* spp. CV copepodites were carefully picked using a stereomicroscope and soft forceps (see Section 2.5 for species identification) (Table 1). Individual copepods (copepodite stage CV) were placed in clear uncapped acrylic tubes (volume 5 ml; diameter 10 mm; length 64 mm) filled with filtered seawater. The sorting was performed in a temperature-controlled room in darkness, but the organisms were exposed to light from the stereomicroscope while being sorted. To monitor the locomotor activity of individual CVs, the test tubes were placed in modified LAM10 activity monitors (TriKinetics). These are infrared light (IR) beam arrays which detect the movement of an individual copepod in each test tube. IR beams were located at 30 mm above the bottom of each tube, i.e. the middle of the filled tubes. Beam breaks were registered every 30 s on a laptop computer using proprietary TriKinetics software (filescan.exe). The monitors were placed in a temperature-controlled room with a controlled light environment where the local ambient photoperiod was reproduced (Table 1). The light environment was controlled with LED red green blue (RGB) ribbons set on white light. The

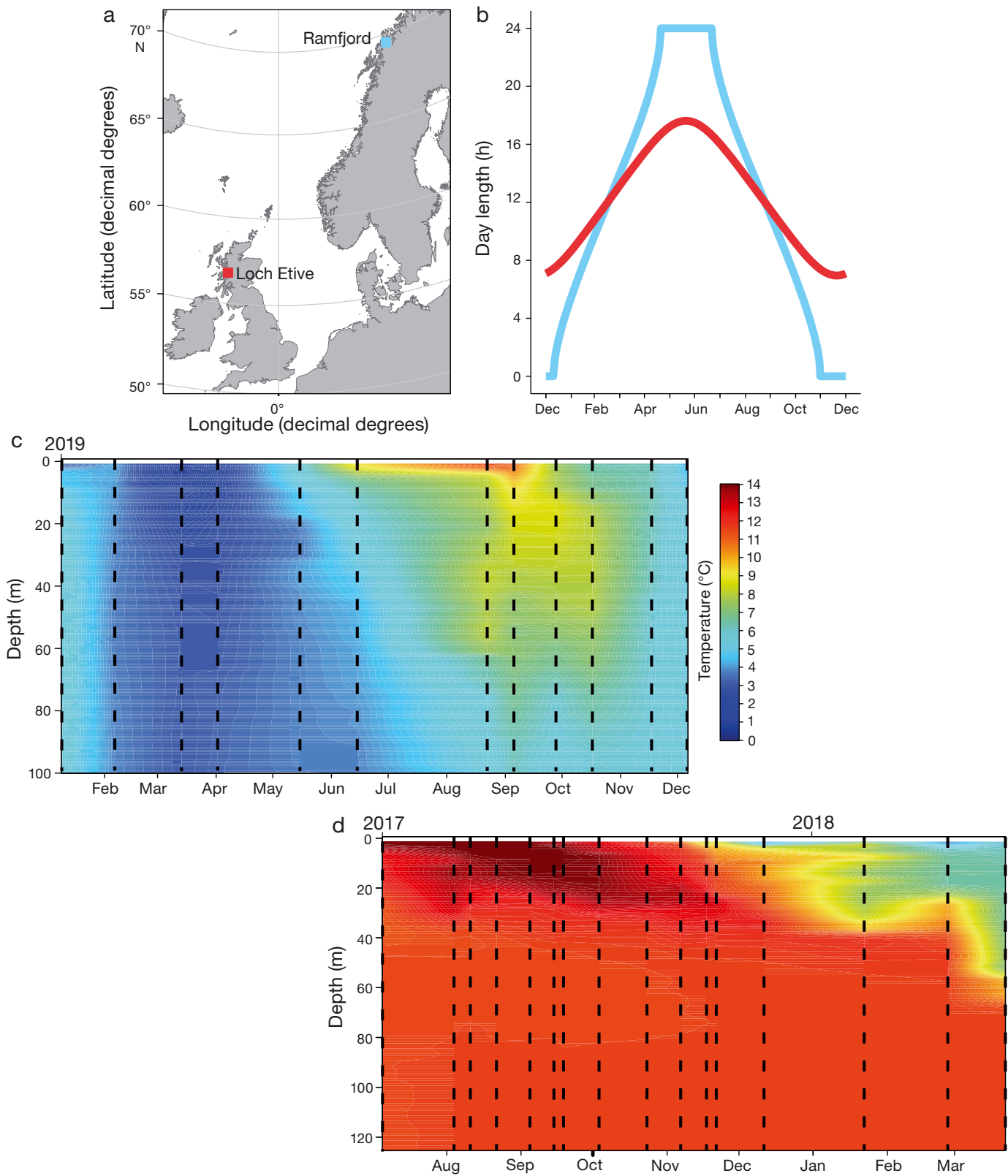


Fig. 1. (a) Sampling station locations, (b) day length curve. Temperature contour plot in (c) Ramfjord from January to December 2019 and (d) Loch Etive from July 2017 to March 2018. Vertical black dashed lines in the contour plots show time of the CTD profile collection

Table 1. Sampling events, experiment setting and composition of each experiment. CV: *Calanus* stage CV. From the CV copepodites the number dead, *C. finmarchicus* (*C. fin*), *C. glacialis* (*C. gla*), and unknown are indicated. Empty cells: no data

Location	Date (d/mo/yr)	CTD	Nets	Expt duration (d)	Light cycle (h light: h dark d ⁻¹)	No. <i>Calanus</i> sampled	No. <i>Calanus</i> CV	No. <i>Calanus</i> dead	<i>C. fin</i>	<i>C. gla</i>	Unknown
Ramfjord	09/01/2019	x	x	4	4.5:19.5	64	54	5	43	5	1
	06/02/2019	x	x	6	8.5:15.5	64	36	7	27	0	2
	13/03/2019	x	x	6	13.5:10.5	32	1	0	1	0	0
	01/04/2019	x	x	6	17:7	30	4	0	2	2	0
	14/04/2019	x	x	5	19:5	50	2	0	0	2	0
	14/05/2019	x	x	4	24:0	32	10	0	0	10	0
	13/06/2019	x	x	6	24:0	64	52	0	26	25	1
	20/08/2019	x	x	7	22:2	64	50	10	11	28	1
	03/09/2019	x	x	7	15:9	122	92	3	30	24	35
	25/09/2019	x	x	7	13:11	96	78	1	50	23	4
	14/10/2019	x	x	6	11:13	128	117	1	60	51	5
	14/11/2019	x	x	5	7:17	128	108	2	50	56	0
	03/12/2019	x	x	6	4:20	116	100	0	72	23	5
Loch Etive	04/07/2017		x	5	16:8	94	89	23	65		
	11/07/2017	x									
	03/08/2017	x									
	10/08/2017	x									
	21/08/2017	x									
	23/08/2017		x	5	14.5:9.5	93	87	3	83		
	04/09/2017	x									
	14/09/2017	x									
	18/09/2017	x	x	5	13:11	96	80	0	80		
	03/10/2017	x									
	23/10/2017	x	x	6	10: 14	96	67	1	67		
	06/11/2017	x									
	17/11/2017	x									
	21/11/2017	x	x	8	8:16	96	79	0	79		
	27/11/2017	x									
	11/12/2017	x	x	6	7.5:16.5	96	88	1	87		
	22/01/2018	x	x	6	8.5:15.5	96	95	32	63		
26/02/2018	x	x	6	10.5:13.5	96	19	0	19			
23/03/2018	x	x	5	12.5:11.5	96	7	0	7			

light was installed above the monitors (about 20–30 cm above). In Ramfjord, the individuals were exposed to a day length equivalent to the time when the sun is above the horizon (real day length) plus the civil twilight (the period when the sun is between the horizon and -6° below the horizon) to simulate a photoperiod as close as possible to the natural light climate. Thus, experiments conducted for Ramfjord during the polar night (December and January) were not maintained under continuous darkness but rather under a short photoperiod (Table 1). Meanwhile, individuals from Loch Etive were exposed to a day length equivalent to the time between sunrise and sunset (Table 1).

2.3.2. Data processing

At the end of each experiment, the number of beam breaks for each individual was summed over 30 min time bins with the DAMFileScan111 software (TriKinetics) and defined as activity intensity (Act_{int}). The average Act_{int} over the entire experiment, called activity level (Act_{level}) was calculated for each individual. Individuals which were dead at the end of the experiment (usually less than 10%; Table 1) were removed from the data set. Individuals with an $Act_{level} > 60$ breaks per 30 min were assumed to occupy a chamber with a faulty sensor (based on an analysis of outliers) and likewise were removed from the data set.

2.4. Morphology

After the experiment, each individual was photographed using a stereomicroscope. The resulting image was used to measure the prosome length (PL), the prosome area (PA), and the lipid sac area (LA) of each individual using Image J software (Schneider et al. 2012). The lipid sac area to PA ratio (LAPA) was calculated for each individual as an index of the amount of lipids stored with each individual independent of body size (i.e. indicating lipid fullness). Values are presented as means with standard deviation (mean \pm SD). Additionally, LA was used to calculate the total lipid content (TL) in mg following Vogedes et al. (2010).

$$TL = 0.197 LA^{1.38} \quad (1)$$

The lipid consumption over the overwintering period (%) was calculated by deducting the average LAPA during late overwintering phase from the average LAPA during early diapause.

2.5. Species identification

In Loch Etive, the *Calanus* population consists exclusively of *C. finmarchicus* (Choquet et al. 2017), but in Ramfjord 4 species of *Calanus* occur sympatrically: *C. finmarchicus*, *C. glacialis*, *C. helgolandicus*, and *C. hyperboreus* (Coguiec et al. 2021). *C. hyperboreus* was identified visually based on the presence of a spine on the last prosome segment, but molecular tools were needed to discriminate between the 3 other species, as they are morphologically similar and overlap in size. A modified version of the insertion-deletion markers (InDels) protocol described by Choquet et al. (2017) was therefore used to identify the individuals used in the experiment in Ramfjord to species level. After the completion of the experiment, DNA was extracted from individual *Calanus* using the HotShot method (Montero-Pau et al. 2008). The InDel molecular marker G-150 from Smolina et al. (2014) was amplified by polymerase chain reaction (PCR). The resulting amplicons were loaded on a 2% agarose gel, and species identification was carried out based on fragment lengths. The InDel marker G-150 is diagnostic to discriminate between *C. finmarchicus* and *C. glacialis* but not *C. helgolandicus*. The abundance of *C. helgolandicus* in the fjord is very low compared to other *Calanus* spp. (Coguiec et al. 2021), and in most cases, the marker G-150 does not amplify for that species (see dataset from Choquet et al. 2017); hence, we would remove individuals with no amplifi-

cation from our dataset. As G-150 is not diagnostic between *C. finmarchicus* and *C. helgolandicus*, there is a slight chance for a genotype identical to *C. finmarchicus* to appear in *C. helgolandicus* for that marker. However, due to the above, we consider that the likelihood of having mistakenly included *C. helgolandicus* in our dataset is very low, and henceforth this is not problematic for our findings.

2.6. Data analysis

2.6.1. Processing

For the experiment carried out in Ramfjord, data recorded in March, April, and May were excluded from further analysis since not enough *C. finmarchicus* CV were present in net samples (<2 ind. per sample), which we considered not statistically representative of the population.

A Lomb-Scargle periodogram analysis (R package lomb version 2.0) (Ruf 1999) was conducted for each individual to determine whether it exhibited a rhythmic pattern in locomotor activity, i.e. activity response to the imposed laboratory light/dark cycle. Lomb-Scargle is a particularly robust statistical analysis of rhythmic time-series data, especially for analysis of marine chronobiological data (Chabot et al. 2007, Lambert et al. 2019). Its particular strength lies in the analysis of unequally spaced or missing data (Ruf 1999), but it is also considered equally powerful in the analysis of continuous data. Lomb-Scargle analysis is derived from the principles of Bayesian probability theory and combines the strength of Fournier analysis with least-square methods (VanderPlas 2018). The analysis was run for periods <30 h. All individuals that had a significant period ($p < 0.05$) were classified as rhythmic individuals, while the others were categorized as arrhythmic. From this categorization the percentage of rhythmicity (RH) per experiment was defined as:

$$RH = \frac{\text{No. rhythmic indiv}}{\text{No. total indiv}} \times 100 \quad (2)$$

The rhythm strength of each rhythmic individual was defined using the peak normalized power in the periodogram (PNmax) from the Lomb-Scargle analysis (Lambert et al. 2019). A higher value of PNmax indicates a stronger rhythm.

The experiments were grouped according to overwintering phases which the animals had theoretically entered at the time of the sampling, based on population dynamics and timing of *C. finmarchicus*

overwintering in Loch Etive (Häfker et al. 2018) and Ramfjord (Coguiec et al. 2021). Following Hirche (1996), we defined 3 overwintering phases: (1) the early overwintering ('early' on figures), which defines the period when part of the *C. finmarchicus* CV population had descended to depth (between June and August in both locations) and is equivalent to Hirche's 'induction phase'; (2) the mid-overwintering ('mid') defined as the period when the majority of the *C. finmarchicus* population was found at overwintering depth (from September to November in Loch Etive, September to December in Ramfjord), equivalent to Hirche's 'refractory phase'; (3) the late overwintering ('late') defined as the time when a part of the population had ascended higher up in the water column to feed and moult prior to reproduction (from December to March in Loch Etive, January and February in Ramfjord), and is equivalent to Hirche's 'termination phase'.

To identify differences in the timing of increased population-level activity over the diel cycle, the population's daily activity was calculated as the average Act_{int} of all individuals mo^{-1} for each 30 min interval using data from the first 3 d of each experiment, thereby allowing for robust chronobiological analysis whilst minimizing the impacts of prolonged laboratory conditions. The data were visualized using a double-plotted actogram. For each experiment, the uniformity of the daily activity over a 24 h period was tested with a Rayleigh test on this daily activity data. All months in both locations showed non-uniform distributions (Rayleigh test, $p < 0.05$). Accordingly, the mean angle (μ), i.e. the time around which the peak of activity was centered, and the mean resultant length (ρ), i.e. the peak Act_{int} , were calculated for each experiment. All circular statistics were done in R with the circular package (Agostinelli & Lund 2022).

2.6.2. Statistics

The normality of PL, LAPA, Act_{level} , PNmax and TL were tested with a Shapiro test, but none met the normality conditions ($p < 0.05$). The variance homogeneity for each location between the different diapause phases as well as for each diapause phase between the locations was tested with a Fligner test. The variances were not homogenous ($p < 0.05$), except for PL and LAPA between each diapause phase in Loch Etive ($p > 0.05$). Due to the absence of normality and homoscedasticity, only non-parametric tests were applied.

For each location, the variation in PL, LAPA, Act_{level} and PNmax over the diapause phases was tested with a Kruskal-Wallis test. If significant ($p < 0.05$), then differences among diapause phases were determined by pairwise Wilcoxon tests with a Holm's correction. In addition, for LAPA and TL in Loch Etive, the effect size was calculated using Cohen d method (R package *effsize*; Torchiano 2016).

For each diapause phase, PL, LAPA, Act_{level} , and PNmax were compared between Loch Etive and Ramfjord with unilateral Wilcoxon tests.

All statistical tests were performed with a 5% level of significance. All the graphs and statistical tests were performed in R (version 4.01) (R Core Team 2020).

3. RESULTS

3.1. Hydrography

In Ramfjord, during the mid-overwintering phase (September–December), surface waters cooled from ~ 12 to 6°C , decreasing gradually with depth to 5°C at the bottom (Fig. 1c). Between January and May (i.e. late overwintering phase and the active period), the water temperature decreased slightly from $4/5$ to $1/2^\circ\text{C}$. During this period the water column was particularly homogenous in terms of temperature. From May the water column start to stratify in terms of temperature with the surface water warmer than that of the bottom. The water temperature increased between May and September (early overwintering phase). In Loch Etive, during the mid-overwintering phase (September–November) the water column was relatively warm (12°C) and homogenous (Fig. 1d). Loch Etive had a stable water column temperature below 50 m deep from July to November.

3.2. Morphology

The average PL of *Calanus finmarchicus* CV was higher in Ramfjord (early: 2.6 ± 0.1 mm; mid: 2.4 ± 0.2 mm; late: 2.3 ± 0.2 mm) than in Loch Etive (early: 2.4 ± 0.1 mm; mid: 2.3 ± 0.1 mm; late: 2.3 ± 0.1 mm) during the early and mid-overwintering phase (bilateral Wilcoxon $p < 0.05$), while during the late overwintering phase there was no significant difference on average PL between Ramfjord and Loch Etive (bilateral Wilcoxon test $p = 0.18$) (Fig. 2a). In Ramfjord, the average PL decreased with each subsequent overwintering phase (pairwise Wilcoxon $p <$

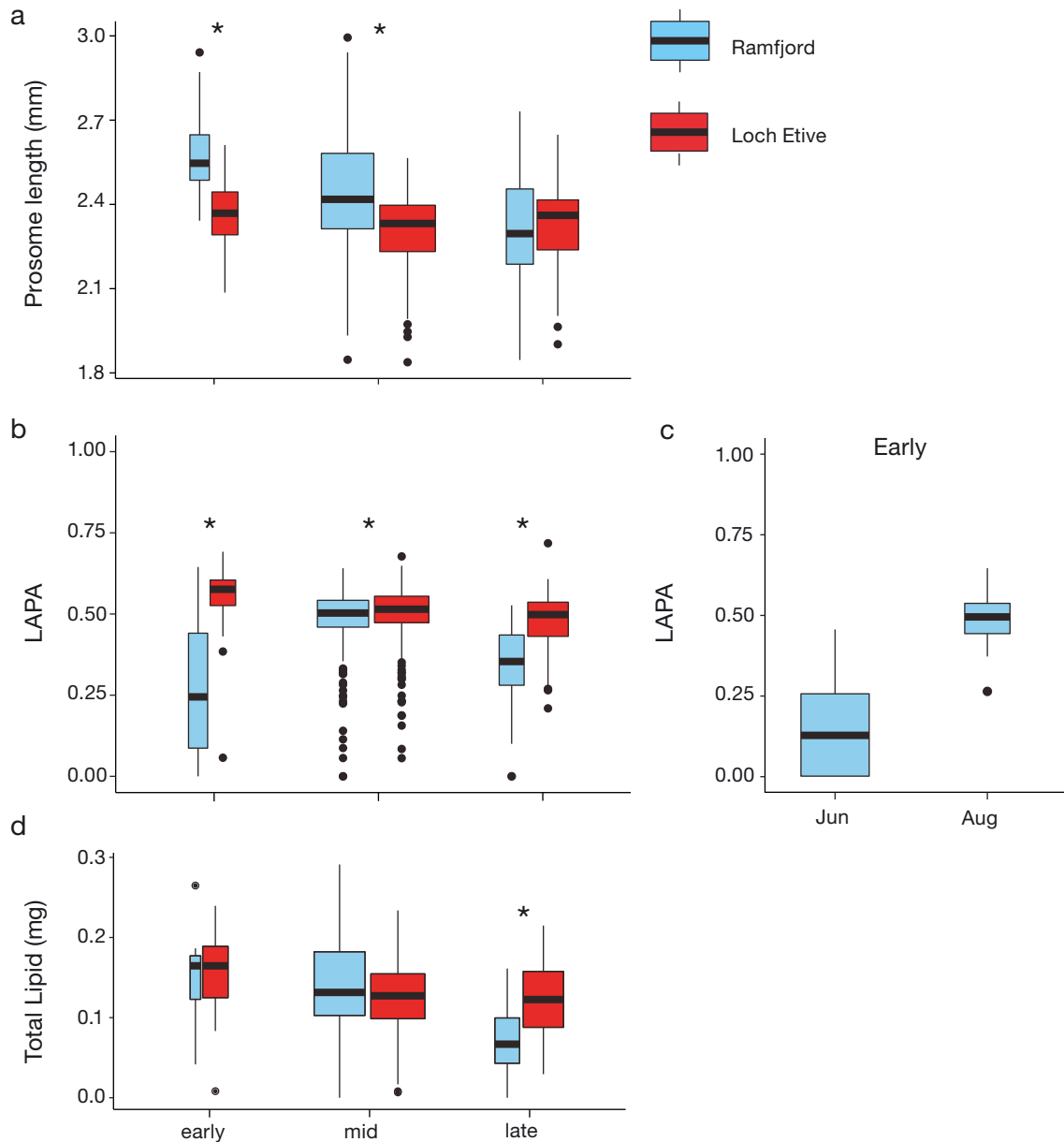


Fig. 2. (a) Variation in prosome length, (b) lipid sac area to prosome area ratio (LAPA), and (d) total amount of lipid of *Calanus finmarchicus* CV during the different overwintering phases in Ramfjord (blue) and Loch Etive (red) and (c) variation of the LAPA during the early overwintering in Ramfjord. Early total lipid values do not include measurements from June, but June is included for the prosome length and LAPA. *Significantly different between Ramfjord and Loch Etive for a given phase. Width of each box is proportional to the number of individuals used during each overwintering phase; bold line: median; top and bottom of box: 75th percentile and 25th percentile, respectively; whiskers extend 1.5 times the interquartile range of the sample; values outside this range are represented by circles. The p-values of the pairwise Wilcoxon test are presented in Table 2

0.05, Table 2). For Loch Etive, the average PL during early overwintering was significantly higher compared to the mid-overwintering phase (pairwise Wilcoxon $p < 0.05$, Table 2). However, the average PL was not significantly different between mid-overwintering and late overwintering and between late and early overwintering (pairwise Wilcoxon $p = 0.1071$ and $p = 0.1175$, respectively, Table 2).

The average LAPA of CVs was lower in Ramfjord (early: 0.25 ± 0.2 ; mid: 0.49 ± 0.1 ; late: 0.34 ± 0.12) than in Loch Etive (early: 0.55 ± 0.09 ; mid: 0.50 ± 0.09 ; late: 0.48 ± 0.08) during each of the overwintering phases (bilateral Wilcoxon $p < 0.05$) (Fig. 2b). In both locations, the average LAPA decreased in each subsequent overwintering phase (pairwise Wilcoxon $p < 0.05$, Table 2; in Loch Etive, Cohen's $d > 0.6$),

Table 2. Table summarizing the p-value of the pairwise Wilcoxon test comparing the variable (prosome length, lipid sac area to prosome area ratio [LAPA], total lipid, activity level, and peak normalized power [PNmax]) over the overwintering phase for each location. **Bold:** significant

		Ramfjord		Loch Etive	
		Early	Mid	Early	Mid
Prosome length	Mid	0.0001	–	0.0059	–
	Late	1.10E-09	4.40E-06	0.1175	0.1071
LAPA	Mid	1.30E-10	–	6.10E-09	–
	Late	0.043	<2e-16	1.90E-11	0.0022
Total lipid	Mid	0.38613	–	3.60E-07	–
	Late	0.00019	5.40E-15	8.60E-07	0.65
Activity level	Mid	0.92	–	0.42	–
	Late	0.92	0.0003	0.42	0.99
PNmax	Mid	0.049	–	0.11	–
	Late	0.065	0.499	0.11	0.67

except for Ramfjord where the average LAPA was lowest in the early overwintering phase. However, during that phase the variation in the LAPA was twice as high as during any other overwintering phase in Ramfjord or Loch Etive. This large variability is caused by a low LAPA in June (the lowest LAPA measured over the study period, 0.16 ± 0.15), while in August (0.47 ± 0.10) the LAPA was higher and relatively close to the values measured during the other overwintering phase (Fig. 2c).

The TL was similar in Ramfjord (early: 0.15 mg, without June; mid: 0.14 mg) and Loch Etive (early: 0.16 mg; mid: 0.12 mg) during the early and mid-overwintering phases (bilateral Wilcoxon $p > 0.05$), but TL was lower in Ramfjord (0.07 mg) than in Loch Etive (0.12 mg) (unilateral Wilcoxon $p < 0.05$) during the late phase. In Ramfjord, the TL during the late overwintering phase was significantly lower than during the early and mid-overwintering phase (pairwise Wilcoxon $p < 0.05$, Table 2) while in Loch Etive the TL during the early overwintering phase was significantly higher than during the mid and late overwintering phase (pairwise Wilcoxon $p < 0.05$, Table 2 and Cohen's $d > 0.8$).

3.3. Locomotor activity

The Act_{level} was consistently lower in individuals from Ramfjord (early: 8.8 ± 9.7 ; mid: 7.0 ± 6.0 ; late: 5.1 ± 6.0) compared to individuals from Loch Etive (early: 14.7 ± 13.0 ; mid: 11.1 ± 8.4 ; late: 11.3 ± 9.5) (unilateral Wilcoxon tests $p < 0.05$). The average Act_{level} in Ramfjord was reduced slightly during late overwintering when compared to earlier phases (Fig. 3a), with significant differences observed only when compared

to mid-overwintering (Kruskal-Wallis $p = 0.0009$, pairwise Wilcoxon $p < 0.05$, Table 2). For Loch Etive, the Act_{level} was consistent across overwintering phases (Kruskal-Wallis $p = 0.31$).

Rhythmic activity with a period in the circadian range (20 to 28 h) was detected in both locations and for each experiment (Table 3). In Ramfjord, there was high seasonal variability in the percentage of individuals expressing rhythmic swimming activity under ambient temperature and photoperiod conditions, varying from 7% in January to 82% in August (Table 3). On average, $39 \pm 25\%$ of the Ramfjord population displayed rhythmic activity. In Loch

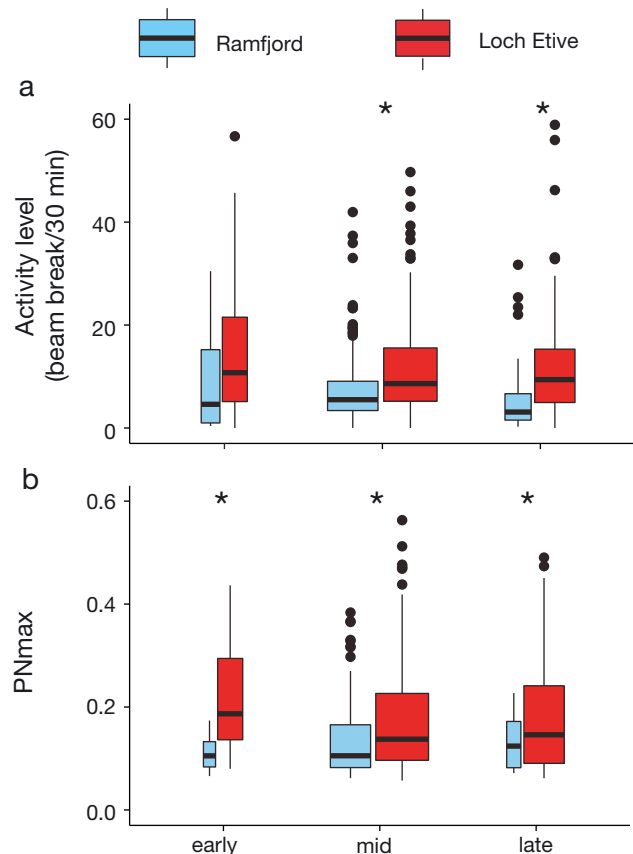


Fig. 3. Variation in (a) activity level, (b) peak normalized power (PNmax) of *Calanus finmarchicus* CV during different overwintering phases in Ramfjord (blue) and Loch Etive (red). *Significantly different between Ramfjord and Loch Etive for a given phase. Width of each box is proportional to the number of individuals used during each overwintering phase; bold line: median; top and bottom of box: 75th percentile and 25th percentile, respectively; whiskers extend 1.5 times the interquartile range of the sample; values outside this range are represented by circles. The p-values of the pairwise Wilcoxon test are presented in Table 2

Table 3. Percentage of rhythmicity for each experiment. Empty cells: data not available

	Jan	Feb	Mar	Jun	Jul	Aug	Early Sep	Late Sep	Oct	Nov	Dec
Ramfjord	6.98	44.4		8		81.8	63.8	68	44.1	32	36.1
Loch Etive	69	78.6	57.1		75	65.1		83.5	81.8	59.4	77

Etive, the percentage of rhythmic individuals was higher and relatively stable throughout the different experiments, varying from 57% in January to 83% in September (Table 3) and averaging to $71 \pm 9\%$ over the entire study.

Rhythm strength (PNmax) did not show a clear seasonal variation in Ramfjord (Fig. 3b). The average PNmax differed significantly between the mid and late overwintering phase (pairwise Wilcoxon $p = 0.049$), while the average PNmax was not significantly different between early and mid-overwintering and between late and early overwintering phase. In Loch Etive, PNmax did not vary among overwintering phases (Kruskal-Wallis $p = 0.08$) (Fig. 3b).

A significant peak in activity over the diel cycle was detected for each experiment for populations from both locations (Rayleigh tests $p < 0.05$). In early overwintering, a nocturnal peak of activity was detected, while during mid-overwintering and late overwintering this peak was diurnal, in both locations. In Ramfjord, daytime peaks in activity were detected between October and February. These peaks were centred over a wide range of time, 12:00 h in October, 14:30 h in November, 10:00 h in December, 14:30 h in January and 13:00 h in February (Fig. 4c). From October to December and in January the Act_{int} during the peak in activity was relatively low (shortest mean resultant length, ρ) (Fig. 4c). In August and September, the peaks in activity happened during times when individuals were exposed to darkness, with peaks centred around 01:00 h in August and 00:00 h in both September experiments (Fig. 4a,c). During these months, the Act_{int} during the peak was high (longest ρ) (Fig. 4c). During the experiment in June, the individuals were exposed to constant illumination, and the peak in activity was centred around 20:00 h with a relatively high Act_{int} over the entire 24 h period and a short mean resultant length (Fig. 4a,c). In Loch Etive, the peaks in activity were centred around mid-day (Fig. 4d), except for July and November. In July and November, the peaks in activity were observed during nighttime around 03:30 and 06:00 h, respectively (Fig. 4b,d). In July, the Act_{int} was relatively high throughout the 24 h period. The peaks in activity were more marked during the late dia-

pause in February and March (Fig. 4b,d). The shortest mean resultant length was observed in October and November.

4. DISCUSSION

Swimming activity of *Calanus finmarchicus* under seasonal ambient photoperiods was used to characterize behaviour during the overwintering period in shallow fjords at sub-Arctic and boreal latitude. We found that regardless of latitude, *C. finmarchicus* maintains diel patterns of swimming activity consistent with a resting state rather than a torpid state of 'classical' diapause as encountered in the mesopelagic (Hirche 1996, Kaartvedt 1996, Grigor et al. 2022). However, at the sub-Arctic latitude the individuals were larger, less fat, and less active compared to the boreal latitude. The differences between the 2 locations and their implications for *C. finmarchicus* metabolism are discussed below.

4.1. *C. finmarchicus*' winter resting

In both Loch Etive and Ramfjord, seasonal changes in swimming activity suggest that *C. finmarchicus* displays a different behaviour whilst overwintering in shallow fjordic environments compared to a 'classical' diapause as observed in deep Atlantic/Arctic off-shelf waters. Grigor et al. (2022) used the locomotor activity monitor methodology to compare the swimming activity of *C. finmarchicus* in the Fram strait from the epipelagic layer which were assumed not to be overwintering to individuals from the mesopelagic which were considered to be diapausing. They found that individuals residing in mesopelagic layers in late August maintain very low swimming activity (between 1 and 2 beam breaks 30 min^{-1}) compared to the epipelagic layer (12 to 15 breaks 30 min^{-1}). While we detected peaks in activity in each experiment at both locations in the early and late overwintering phases, the peak in activity throughout the mid-overwintering phase was low. Since swimming accounts for 30 to 40% of the metabolic costs in copepods (Alcaraz &

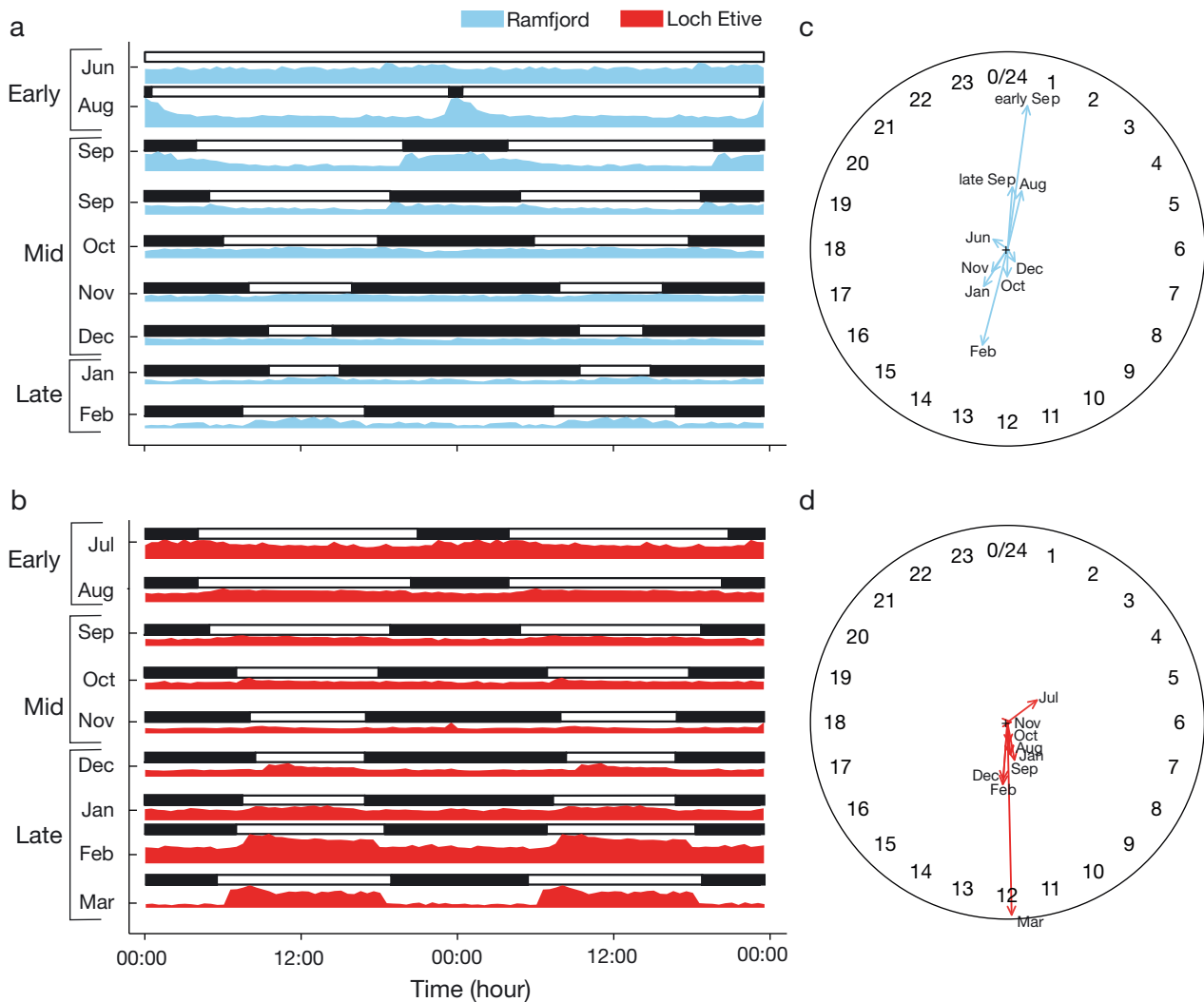


Fig. 4. Actogram of the daily activity (left panels) and the mean angle (μ) and mean resultant length (ρ) (right panel) of the daily activity over the 3 first days of the experiment obtained by combining all the individuals sampled during each month in (a,c) Ramfjord and (b,d) Loch Etive. For the actogram, the 24 h were doubled and joined to have better visualisation during the nighttime. Black segments on top of each actogram indicate period when the light was off. Overwintering phases corresponding to the month indicated on the left side of the y-axis. All months had non-uniform circular distributions (Rayleigh test $p < 0.05$). Mean angles correspond to the time of day for peak activity in a given month, with the mean resultant length (plotted here as $\rho \times 2.5$, for clarity) corresponding to the strength of that activity. Months are labelled for each mean vector

Strickler 1988), we interpreted the low intensity of the peak in activity during mid-overwintering as a reduction in metabolism. *C. finmarchicus* diapause is defined as a physiological state characterized by a reduced metabolism and torpidity, a definition based on observations of animals from deep environments (Hirche 1996). Conversely, studies from shallow environments on the eastern side of the Atlantic suggest that *C. finmarchicus* may remain active during the overwintering period (Tande 1982, Hirche 1991), which is supported by our observations. The Act_{level} measured during our study (5 to 8 breaks 30 min^{-1} in

Ramfjord and 11 to 14 breaks 30 min^{-1} in Loch Etive) are close to those reported for *C. finmarchicus* residing in epipelagic layers (12 to 15 breaks 30 min^{-1}) in the Fram Strait in late August (Grigor et al. 2022). In that study, mesopelagic *C. finmarchicus* displayed considerably lower Act_{level} (and respiration rates) than epipelagic individuals, suggesting that the mesopelagic copepods were in deep diapause (Grigor et al. 2022). Grigor et al. also observed higher variance in the swimming activity in the population residing in epipelagic layers (10.8 to 17.9) compared to those at mesopelagic layer (2.11 to 3.63), indicating higher

heterogeneity in the activity within the epipelagic population (some were more active than others). We observed a variance close to what Grigor et al. (2022) observed in the epipelagic for all overwintering phases (Ramfjord: 6 to 9, Loch Etive: 8.4 to 13). Furthermore, Grigor et al. (2022) found that ~5 beam breaks 30 min^{-1} marked the upper limit of mesopelagic activity and the lower limit of epipelagic activity, which corresponds to the lowest limited found in Ramfjord. These observations further strengthen our interpretation that *C. finmarchicus* in our study never reached a state of diapause comparable to that observed in deep-dwelling copepods by Grigor et al. (2022). It is still not fully understood what regulates the activity of overwintering copepods. Freese et al. (2016) showed that the closely related *C. glacialis* overwintering in a shallow Arctic fjord substantially reduce their digestive enzymatic activities in winter, but this reduction seemed to be regulated by food availability rather than being triggered by internal or behavioural factors related to overwintering. The term 'active diapause' has previously been used for other copepod species such as *Acartia longiremis* and *Pseudocalanus acuspes* to describe a state where metabolism is reduced but the organisms remain partially active (Corner et al. 1974, Elgmork 1980, Williams & Conway 1982, 1984, Næss & Nilssen 1991, Norrbin 1996, Svetlichny et al. 1998, Grigor et al. 2022). Since diapause is generally described as a phase of dormancy with delayed development and reduced metabolic activity, the term 'active' diapause is somewhat contradictory, and instead, we suggest describing the state of overwintering *C. finmarchicus* in shallow environments as entering a 'winter resting' state.

During the winter resting phase, rhythmicity in the population in response to ambient photoperiods was overall lower, the rhythms in activity were weaker, and the individuals were less active at the sub-Arctic latitude than at the boreal location. Furthermore, the Act_{level} remained constant during winter resting in Loch Etive, while in Ramfjord the animals became less active during the late overwintering phase. In flies, temperature and photoperiod have been described as factors with an important influence on flying, with high temperatures resulting in more flight and increased rhythmicity while both long and short photoperiods induce lower rhythmicity (Kauranen et al. 2012, 2016). It has been suggested that these factors synergistically impact the flying activity of flies, with a combination of temperature and photoperiod supporting a more robust rhythm and higher Act_{level} (Kauranen et al. 2012, 2016). We suggest that for *C.*

finmarchicus the photoperiod and temperature conditions encountered in Loch Etive facilitate increased and rhythmic swimming behaviour.

4.2. *C. finmarchicus*' seasonal response to photoperiod

Photoperiodic responses were characterized by the appearance of peaks in activity coincident with either the light or dark phase of the laboratory diel cycle, i.e. day or night. We hypothesize that the response to light is a negative phototactic reaction (Miljeteig et al. 2014) with the individual increasing the number of beam breaks as it moves throughout the tube trying to escape the light (Miljeteig et al. 2014). However, this hypothesis does not explain the behaviour observed in individuals from Ramfjord during the June experiment, when the individuals were exposed to constant illumination and nevertheless showed a distinct peak in activity in the population. The maintenance of an activity rhythm under constant light conditions in the absence of any other environmental cue is a sign that the internal clock can entrain a rhythm, which has been demonstrated in *C. finmarchicus* (Häfker et al. 2017). Such endogenous rhythms are commonly observed in locomotor activity studies (Bregazzi & Naylor 1972, Sánchez-Vázquez & Tabata 1998), particularly in flies, which can maintain rhythms for weeks under constant environmental conditions (Dubruille & Emery 2008). However, in terrestrial environments constant illumination is also associated with a decrease or an absence of rhythmicity (Van Oort et al. 2005, Kauranen et al. 2012, Menegazzi et al. 2017, Beauchamp et al. 2018, Bertolini et al. 2019). In Ramfjord during mid-summer, under constant illumination, only 8% of the population was rhythmic, one of the lowest rhythmicity percentages recorded over the entire study. This low percentage of rhythmic individuals indicates that a peak in activity is only present in a low proportion of the studied individuals while the majority of the population did not show a peak in activity. It is likely that *C. finmarchicus*, like many marine organisms (Naylor 2010), cannot maintain rhythmic activity for a long time in absence of environmental cues (Häfker et al. 2017). In its natural environment in Ramfjord, *C. finmarchicus* is exposed to daily variations in light intensity and spectral quality, despite the presence of midnight sun, due to diel shift in the sun's altitude above the horizon and the light attenuation in the water column (Miller et al. 1991, Cohen et al. 2020). Thus, we hypothesize that *C. finmarchicus* did possess a functional biological clock

prior to the experiment (Häfker et al. 2017), but the overt absence of environmental cues during the experiment led to the rapid loss of the rhythm. The peak of activity during night in August and September in Ramfjord and July and November in Loch Etive remains unexplained. Under natural conditions, such a behaviour could indicate active feeding during nighttime, which *a priori* is plausible in summer, as more food is available than during the overwintering period. However, as our experiments were conducted without food supplied, we are not able to conclude if this behaviour reflects feeding activity. Furthermore, physiological differences (such as change in metabolic rate) may also be involved in this behaviour. Based on our experiments we are not able to explain this peak in activity during nighttime, and additional studies are needed to test multiple factors (such as food concentration, temperature changes, predator presence) that could evoke this behaviour.

4.3. *C. finmarchicus*' morphology

While we found differences in body size and lipid content between the 2 study sites, these were most likely related to differences in temperature and food availability, rather than photoperiod. However, seasonal changes in the morphological parameters provide some insights into the seasonal changes in activity. We show that body size (i.e. PL) was within ranges previously reported for *C. finmarchicus* CV in similar environments as our sampling locations, e.g. Disko Bay (Madsen et al. 2001) and Georges Bank (Miller et al. 2000). Overall, the individuals from Ramfjord were bigger but had proportionally smaller lipid reserves than the individuals from Loch Etive. *C. finmarchicus* growth is temperature dependant. Low temperatures favour long development times and an investment in reserve accumulation rather than fast turnover, while at higher temperatures, short development times and fast turnover are stimulated. This leads to *C. finmarchicus* individuals being bigger with higher lipid reserves at high temperatures than at low temperatures (Huntley & Lopez 1992, Campbell et al. 2001, Møller et al. 2012). We suggest that this temperature dependency of *C. finmarchicus* may explain these size differences.

Since low temperatures lead to a longer development time and a higher investment in reserve building, it was expected for *C. finmarchicus* in Ramfjord would have more lipid reserve than those in Loch Etive (Pepin & Head 2009). However, we observed a lower LAPA in Ramfjord with an amount of lipid sim-

ilar to Loch Etive, indicating that *C. finmarchicus* in Ramfjord did not accumulate more lipid than in Loch Etive as expected. Furthermore, a higher loss of lipid was observed in Ramfjord with a loss of 27% of the lipid reserves in Ramfjord and 12% in Loch Etive. In Ramfjord, the LAPA and total amount of lipid was lower during the early overwintering phase compared to the other overwintering phase because of the low LAPA and TL content in June. We concluded that most CV individuals did not accumulate enough lipid in June, and were probably not yet ready to overwinter despite residing at depth. Consequently, we excluded June from the early overwintering phase when calculating the lipid consumption over the overwintering period. Overall, we see a clear decrease in lipid content over the overwintering period (August–February) at both locations. This loss of lipid throughout the overwintering period is in agreement with previous studies showing high lipid consumption during overwintering in shallow environments (e.g. 50% of the lipid reserve), as compared to the limited lipid consumption during diapause in deeper environments (5% of the lipid reserve) (Jónasdóttir 1999, Campbell et al. 2004, Clark et al. 2012). An important loss of lipid during the overwintering has also been reported in deep environment in the Labrador Sea (Pepin & Head 2009). We suggest that the more marked lipid decrease in Ramfjord compared to Loch Etive is the result of more active feeding activity during overwintering in Loch Etive compared to Ramfjord and/or a higher consumption of lipid in Ramfjord. The light cycle at high latitudes constrains primary production. At boreal latitudes, light may reduce the primary productivity during autumn and winter, but under favourable nutrient conditions, elevated primary production is possible year-round (Wood et al. 1973, Hicks & Brand 2018). Furthermore, Loch Etive has considerable suspended organic matter (Ansell 1974, Hicks & Brand 2018), which could enhance *C. finmarchicus* feeding activity during overwintering, as this species can switch its dietary preferences e.g. to detritivory when preferred food items become scarce (Marshall & Orr 1958, Butler et al. 1970, Corner et al. 1974, Ohman & Runge 1994, Hirche 1996). The larger body sizes observed in Ramfjord are energetically more costly in terms of maintenance (Saumweber & Durbin 2006) and could explain a higher consumption of lipid than in Loch Etive. However, in Loch Etive the individuals are also subject to high lipid consumption due to the high temperature of their environment. Consequently, it is impossible to establish if lipid consumption is higher in Ramfjord than in Loch Etive with the

data collected in this study. A more detailed energetic budget would be necessary to do so. The combination of lipid consumption rate and food availability explain both the counter-intuitive higher lipid fullness at boreal latitudes and the higher Act_{level} . In summary, at boreal latitudes, more energy can be allocated to swimming activity and larger lipid reserve can be accumulated, due to better availability of food resources and possibly reduced costs of metabolic maintenance.

C. finmarchicus CV body size decreased progressively through the overwintering period in sub-Arctic latitudes but not at boreal latitudes. The production of a second generation has previously been suggested for the population in Ramfjord, with a first generation being produced around the spring bloom (i.e. April/May) and the second one in early autumn (Coguiec et al. 2021). Since *C. finmarchicus* growth is temperature dependent (Huntley & Lopez 1992, Campbell et al. 2001, Møller et al. 2012), and the surface water is warming from late spring, the ontogenetic development of the second generation is shorter than that of the first generation, which could result in animals with a smaller body size entering the overwintering population later in the season. This could explain the decrease in average PL in Ramfjord, especially between the early and mid-diapause phases. A difference in PL between 2 generations of the same population due to differences in surface water temperature has previously been described for the Newfoundland continental shelf and the Labrador Sea (Pepin & Head 2009). The advection of the second generation in Ramfjord from Atlantic water could similarly explain the decrease in PL. Advection in Ramfjord has previously been reported at the time of the mid-overwintering period (Coguiec et al. 2021). Changes in average PL during overwintering can also be explained by early moulting of the bigger individuals to adults. Kosobokova (1998) found that during autumn/winter in the White Sea, *C. glacialis* males derive from larger CV individuals and appear earlier than females in the population; such mechanisms would lead to a decrease in PL of the CV population. Finally, we cannot exclude that a seasonal change in predation pressure on larger individuals may have pushed the population towards smaller body sizes. In Loch Etive, it has been shown that *C. finmarchicus* produces a single generation (Hill 2009, Clark et al. 2012), but a more recent study found 2 generations in Loch Etive, and suggests that the nutrient enrichment due to fish aquaculture might explain the transition to population dynamics involving 2 generations a year (Häfer et

al. 2018). Our PL data do not indicate a change in the population size structure that may indicate the appearance of a second generation. However, given the rather stable water surface temperature during spring and winter in Loch Etive, a second generation may not necessarily be characterized by a difference in PL and would therefore not be detectable as a change in average PL.

4.4. Methodological consideration

A disturbance of the diapause state due to rapid changes in pressure, temperature and light during sampling as well as due to the light exposure during sorting is a concern with the method we used. To keep light exposure to a minimum, the sampled organisms were transferred to a container which was sealed immediately after collection, and the organisms were kept in darkness until the start of the sorting. During the sorting, the room was kept dark, but organisms were exposed to the light emitted by the stereo microscope. However, Miller et al. (1991) demonstrated that following an exposure that triggers an exit from diapause, a minimum of 10 d is needed to reverse the diapause state. Thus, we find it unlikely that sampling and setting up the experiments has severely disturbed the diapause state. We are convinced that our method is suitable to detect diapause/overwintering, especially since the focus is on a comparison with non-overwintering individuals, either looking at seasonal variability (this study) or spatial variability (Grigor et al. 2022). But swimming activity as an indicator of overwintering and/or diapause is mainly applicable at population level, while characterizing the mechanism of diapause at the individual level remains a challenge. This limitation complicates the investigation of the associated physiological processes, particularly for populations inhabiting shallow environments, as metabolism reduction is not as marked as in deeper environments. The development of molecular markers to identify diapause and winter resting is essential to increasing understanding of the physiological processes behind overwintering (Tarrant et al. 2016, Skottene et al. 2019, Lenz et al. 2021).

5. CONCLUSIONS

This study helps improve the definition of the overwintering period in *Calanus finmarchicus* as a gradient in behaviour and physiology. It provides addi-

tional evidence towards the adaptability of *C. finmarchicus* to local environments (Tarling et al. 2022a) and may have implications for *C. finmarchicus* in a future warmer world, specifically winter survival, since increased swimming activity may deplete lipid reserves faster (Tarling et al. 2022b). This adaptability may be constrained where and when winter food supplies are restricted. Indeed, high winter mortality in shallow environments in Svalbard in both *C. finmarchicus* and *C. glacialis* have been attributed to insufficient energy stores to sustain activities throughout winter (Daase & Søreide 2021). However, our observations from Loch Etive suggest that elevated food availability throughout winter allows for sustained activity and less dependence on lipid reserves, supporting a recent model study which showed that exiting diapause early does not lead to poorer fitness as long as food is available (Hobbs et al. 2020). It will now be important to determine how elevated swimming activity during overwintering may affect the energy budget of *C. finmarchicus* in predicting how climate warming may affect their fitness during overwintering. Finally, a gradient in swimming activity of overwintering copepods provides an opportunity for molecular studies to elucidate fundamental processes in diapause by characterizing different overwintering phenotypes in relation to genotype.

Acknowledgements. We appreciate the support of the captains and crew of RV 'Hyas' and 'Seøl Mara' during the fieldwork in Ramfjord and Loch Etive. Funding was provided by the following: CHASE project (NE/R012733/1), part of the Changing Arctic Ocean (CAO) program, jointly funded by the UKRI Natural Environment Research Council (NERC) and the German Federal Ministry of Education and Research (BMBF); The Marine Alliance for Science and Technology for Scotland (MASTS) pooling initiative, which is funded by the Scottish Funding Council (grant reference HR09011); 'CalAct' funded by Sentinel Nord through the Université Laval / University of Tromsø research partnership; and by the Norwegian Research Council through the projects Arctic ABC (project no 244319) and Deep Impact (project no 300333). This study is a contribution to the ARCTOS research network (arctos.uit.no). E.E. was partially supported by Research Council of Norway (norges forskningsråd) through the CoastRisk initiative (NFR 299554/F40).

LITERATURE CITED

- Agostinelli C, Lund U (2022) R package 'circular': circular statistics (version 0.4-94). <https://rdocumentation.org/packages/circular/versions/0.4-95>
- Alcaraz M, Strickler J (1988) Locomotion in copepods: pattern of movements and energetics of *Cyclops*. *Hydrobiologia* 167:409–414
- Ansell AD (1974) Sedimentation of organic detritus in Lochs Etive and Creran, Argyll, Scotland. *Mar Biol* 27:263–273
- Baumgartner MF, Tarrant AM (2017) The physiology and ecology of diapause in marine copepods. *Annu Rev Mar Sci* 9:387–411
- Beauchamp M, Bertolini E, Deppisch P, Steubing J, Menegazzi P, Helfrich-Förster C (2018) Closely related fruit fly species living at different latitudes diverge in their circadian clock anatomy and rhythmic behavior. *J Biol Rhythms* 33:602–613
- Bertolini E, Schubert FK, Zanini D, Sehadová H, Helfrich-Förster C, Menegazzi P (2019) Life at high latitudes does not require circadian behavioral rhythmicity under constant darkness. *Curr Biol* 29:3936.e3
- Bregazzi P, Naylor E (1972) The locomotor activity rhythm of *Talitrus saltator* (Montagu)(Crustacea, Amphipoda). *J Exp Biol* 57:375–391
- Butler E, Corner E, Marshall S (1970) On the nutrition and metabolism of zooplankton. VII. Seasonal survey of nitrogen and phosphorus excretion by *Calanus* in the Clyde Sea-area. *J Mar Biol Assoc UK* 50:525–560
- Campbell RG, Wagner MM, Teegarden GJ, Boudreau CA, Durbin EG (2001) Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Mar Ecol Prog Ser* 221:161–183
- Campbell RW, Boutillier P, Dower JF (2004) Ecophysiology of overwintering in the copepod *Neocalanus plumchrus*: changes in lipid and protein contents over a seasonal cycle. *Mar Ecol Prog Ser* 280:211–226
- Chabot CC, Betournay SH, Braley NR, Watson WH III (2007) Endogenous rhythms of locomotion in the American horseshoe crab, *Limulus polyphemus*. *J Exp Mar Biol Ecol* 345:79–89
- Choquet M, Hatlebakk M, Dhanasiri AKS, Kosobokova K and others (2017) Genetics redraws pelagic biogeography of *Calanus*. *Biol Lett* 13:20170588
- Clark K, Brierley A, Pond D (2012) Composition of wax esters is linked to diapause behavior of *Calanus finmarchicus* in a sea loch environment. *Limnol Oceanogr* 57:65–75
- Coguiec E, Daase M, Ershova EA, Vonnahme TR and others (2021) Seasonal variability in the zooplankton community structure in a sub-Arctic fjord as revealed by morphological and molecular approaches. *Front Mar Sci* 8:705042
- Cohen JH, Berge J, Moline MA, Johnsen G, Zolich AP (2020) Light in the polar night. In: Berge J, Johnsen G, Cohen JH (eds) *Polar night marine ecology*. Springer, Bremen, p 37–66
- Corkett CJ, McLaren IA (1979) The biology of *Pseudocalanus*. *Adv Mar Biol* 15:1–231
- Corner E, Head R, Kilvington C, Marshall S (1974) On the nutrition and metabolism of zooplankton. IX. Studies relating to the nutrition of over-wintering *Calanus*. *J Mar Biol Assoc UK* 54:319–331
- Daase M, Søreide JE (2021) Seasonal variability in non-consumptive mortality of Arctic zooplankton. *J Plankton Res* 43:565–585
- Daase M, Berge J, Søreide JE, Falk-Petersen S (2021) Ecology of Arctic pelagic communities. In: Thomas DN (ed) *Arctic ecology*. John Wiley & Sons, Helsinki, p 219–259
- Dubruille R, Emery P (2008) A plastic clock: how circadian rhythms respond to environmental cues in *Drosophila*. *Mol Neurobiol* 38:129–145
- Durbin EG, Gilman SL, Campbell RG (1995) Abundance, biomass, vertical migration and estimated development rate of the copepod *Calanus finmarchicus* in the southern Gulf of Maine during late spring. *Cont Shelf Res* 15:571–591

- Durbin EG, Runge JA, Campbell RG, Garrahan PR, Casas MC, Plourde S (1997) Late fall–early winter recruitment of *Calanus finmarchicus* on Georges Bank. *Mar Ecol Prog Ser* 151:103–114
- Elgmork K (1980) Evolutionary aspects of diapause in freshwater copepods. In Kerfoot WC (ed) *Evolution and ecology of zooplankton communities*. University Press New England, Hanover, NH, p 411–417
- Falk-Petersen S, Mayzaud P, Kattner G, Sargent J (2009) Lipids and life strategy of Arctic *Calanus*. *Mar Biol Res* 5: 18–39
- Fiksen Ø (2000) The adaptive timing of diapause — a search for evolutionarily robust strategies in *Calanus finmarchicus*. *ICES J Mar Sci* 57:1825–1833
- Freese D, Søreide JE, Niehoff B (2016) A year-round study on digestive enzymes in the Arctic copepod *Calanus glacialis*: implications for its capability to adjust to changing environmental conditions. *Polar Biol* 39:2241–2252
- Grieve BD, Hare JA, Saba VS (2017) Projecting the effects of climate change on *Calanus finmarchicus* distribution within the US Northeast Continental Shelf. *Sci Rep* 7:6264
- Grigor JJ, Freer JJ, Tarling GA, Cohen JH, Last KS (2022) Swimming activity as an indicator of seasonal diapause in the copepod *Calanus finmarchicus*. *Front Mar Sci* 9:909528
- Häfker NS, Meyer B, Last KS, Pond DW, Huppe L, Teschke M (2017) Circadian clock involvement in zooplankton diel vertical migration. *Curr Biol* 27:2194
- Häfker NS, Teschke M, Last KS, Pond DW, Hüppe L, Meyer B (2018) *Calanus finmarchicus* seasonal cycle and diapause in relation to gene expression, physiology, and endogenous clocks. *Limnol Oceanogr* 63:2815–2838
- Heath MR, Fraser JG, Gislason A, Hay SJ, Jonasdottir SH, Richardson K (2000) Winter distribution of *Calanus finmarchicus* in the Northeast Atlantic. *ICES J Mar Sci* 57: 1628–1635
- Hicks N, Brand T (2018) Loch Etive case study: MASTS case study workshop report. https://www.masts.ac.uk/media/36494/loch-etive-workshop-report_final-report.pdf
- Hill KA (2009) Changes in gene expression, lipid class and fatty acid composition associated with diapause in the marine copepod *Calanus finmarchicus* from Loch Etive, Scotland. University of St Andrews
- Hirche HJ (1983) Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. *Mar Ecol Prog Ser* 11: 281–290
- Hirche HJ (1991) Distribution of dominant calanoid copepod species in the Greenland Sea during late fall. *Polar Biol* 11:351–362
- Hirche HJ (1996) Diapause in the marine copepod, *Calanus finmarchicus* — a review. *Ophelia* 44:129–143
- Hirche HJ, Kosobokova K (2007) Distribution of *Calanus finmarchicus* in the northern North Atlantic and Arctic Ocean — expatriation and potential colonization. *Deep Sea Res II* 54:2729–2747
- Hobbs L, Banas NS, Cottier FR, Berge J, Daase M (2020) Eat or sleep: availability of winter prey explains mid-winter and spring activity in an Arctic *Calanus* population. *Front Mar Sci* 7:541564
- Huntley ME, Lopez MDG (1992) Temperature-dependent production of marine copepods — a global synthesis. *Am Nat* 140:201–242
- Irigoien X, Conway DVP, Harris RP (2004) Flexible diel vertical migration behaviour of zooplankton in the Irish Sea. *Mar Ecol Prog Ser* 267:85–97
- Ji R (2011) *Calanus finmarchicus* diapause initiation: new view from traditional life history-based model. *Mar Ecol Prog Ser* 440:105–114
- Johnson CL, Leising AW, Runge JA, Head EJH, Pepin P, Plourde S, Durbin EG (2008) Characteristics of *Calanus finmarchicus* dormancy patterns in the Northwest Atlantic. *ICES J Mar Sci* 65:339–350
- Jónasdóttir SH (1999) Lipid content of *Calanus finmarchicus* during overwintering in the Faroe–Shetland Channel. *Fish Oceanogr* 8:61–72
- Kaartvedt S (1996) Habitat preference during overwintering and timing of seasonal vertical migration of *Calanus finmarchicus*. *Ophelia* 44:145–156
- Kauranen H, Menegazzi P, Costa R, Helfrich-Förster C, Kankainen A, Hoikkala A (2012) Flies in the north: locomotor behavior and clock neuron organization of *Drosophila montana*. *J Biol Rhythms* 27:377–387
- Kauranen H, Ala-Honkola O, Kankare M, Hoikkala A (2016) Circadian clock of *Drosophila montana* is adapted to high variation in summer day lengths and temperatures prevailing at high latitudes. *J Insect Physiol* 89:9–18
- Kosobokova KN (1998) New data on the life cycle of *Calanus glacialis* in the White Sea (based on the seasonal observations of the development of its genital system development). *Oceanology* 38:347–355
- Lambert S, Tragin M, Lozano JC, Ghiglione JF, Vaulot D, Bouget FY, Galand PE (2019) Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations. *ISME J* 13:388–401
- Lenz PH, Roncalli V, Cieslak MC, Tarrant AM, Castelfranco AM, Hartline DK (2021) Diapause vs. reproductive programs: transcriptional phenotypes in a keystone copepod. *Commun Biol* 4:426
- Madsen SD, Nielsen TG, Hansen BW (2001) Annual population development and production by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. *Mar Biol* 139:75–93
- Maps F, Zakardjian BA, Plourde S, Saucier FJ (2011) Modeling the interactions between the seasonal and diel migration behaviors of *Calanus finmarchicus* and the circulation in the Gulf of St. Lawrence (Canada). *J Mar Syst* 88:183–202
- Marshall SM, Orr AP (1955) The biology of a marine copepod (*Calanus finmarchicus* Gunnerus). Oliver & Boyd; reprint Springer-Verlag, Berlin
- Marshall S, Orr A (1958) On the biology of *Calanus finmarchicus* X. Seasonal changes in oxygen consumption. *J Mar Biol Assoc UK* 37:459–472
- McLaren I, Corkett CJ (1986) Life cycles and production of two copepods on the Scotian shelf, eastern Canada. In: Schriever G, Schminke HK, Shih CT (eds) *Syllogeus* 58. Proc 2nd Int Conf on Copepoda 13–17 August. The National Museum of Canada, Ottawa, p 362–368
- McLaren IA, Head E, Sameoto DD (2001) Life cycles and seasonal distributions of *Calanus finmarchicus* on the central Scotian Shelf. *Can J Fish Aquat Sci* 58:659–670
- Menegazzi P, Dalla Benetta E, Beauchamp M, Schlichting M, Steffan-Dewenter I, Helfrich-Förster C (2017) Adaptation of circadian neuronal network to photoperiod in high-latitude European drosophilids. *Curr Biol* 27:833–839
- Michaud J, Taggart CT (2007) Lipid and gross energy content of North Atlantic right whale food, *Calanus finmarchicus*, in the Bay of Fundy. *Endang Species Res* 3:77–94
- Miljeteig C, Olsen AJ, Båtnes AS, Altin D, and others (2014) Sex and life stage dependent phototactic response of the marine copepod *Calanus finmarchicus* (Copepoda: Calanoida). *J Exp Mar Biol Ecol* 451:16–24
- Miller CB, Cowles TJ, Wiebe PH, Copley NJ, Grigg H (1991) Phenology in *Calanus finmarchicus*; hypotheses about control mechanisms. *Mar Ecol Prog Ser* 72:79–91

- ✦ Miller CB, Crain JA, Morgan CA (2000) Oil storage variability in *Calanus finmarchicus*. *ICES J Mar Sci* 57: 1786–1799
- ✦ Møller EF, Maar M, Jónasdóttir SH, Nielsen TG, Tønnesson K (2012) The effect of changes in temperature and food on the development of *Calanus finmarchicus* and *Calanus helgolandicus* populations. *Limnol Oceanogr* 57:211–220
- ✦ Montero-Pau J, Gómez A, Muñoz J (2008) Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnol Oceanogr Methods* 6: 218–222
- ✦ Næss T, Nilssen JP (1991) Diapausing fertilized adults. *Oecologia* 86:368–371
- Naylor E (2010) *Chronobiology of marine organisms*. Cambridge University Press, Cambridge
- ✦ Norrbin MF (1996) Timing of diapause in relation to the onset of winter in the high-latitude copepods *Pseudocalanus acuspes* and *Acartia longiremis*. *Mar Ecol Prog Ser* 142:99–109
- ✦ Ohman MD, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply — omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. *Limnol Oceanogr* 39:21–36
- ✦ Pepin P, Head EJH (2009) Seasonal and depth-dependent variations in the size and lipid contents of stage 5 copepodites of *Calanus finmarchicus* in the waters of the Newfoundland Shelf and the Labrador Sea. *Deep Sea Res I* 56:989–1002
- ✦ Plourde S, Joly P, Runge JA, Zakardjian B, Dodson JJ (2001) Life cycle of *Calanus finmarchicus* in the lower St. Lawrence Estuary: the imprint of circulation and late timing of the spring phytoplankton bloom. *Can J Fish Aquat Sci* 58:647–658
- ✦ Plourde S, Maps F, Joly P (2009) Mortality and survival in early stages control recruitment in *Calanus finmarchicus*. *J Plankton Res* 31:371–388
- ✦ Pond DW (2012) The physical properties of lipids and their role in controlling the distribution of zooplankton in the oceans. *J Plankton Res* 34:443–453
- ✦ Pond DW, Tarling GA (2011) Phase transitions of wax esters adjust buoyancy in diapausing *Calanoides acutus*. *Limnol Oceanogr* 56:1310–1318
- ✦ Pond DW, Tarling GA, Mayor DJ (2014) Hydrostatic pressure and temperature effects on the membranes of a seasonally migrating marine copepod. *PLOS ONE* 9: e111043
- R Core Team (2020) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org>
- ✦ Rey-Rassat C, Irigoien X, Harris R, Carlotti F (2002) Energetic cost of gonad development in *Calanus finmarchicus* and *C. helgolandicus*. *Mar Ecol Prog Ser* 238:301–306
- ✦ Ruf T (1999) The Lomb-Scargle periodogram in biological rhythm research: analysis of incomplete and unequally spaced time-series. *Biol Rhythm Res* 30:178–201
- ✦ Sánchez-Vázquez F, Tabata M (1998) Circadian rhythms of demand-feeding and locomotor activity in rainbow trout. *J Fish Biol* 52:255–267
- ✦ Saumweber WJ, Durbin EG (2006) Estimating potential diapause duration in *Calanus finmarchicus*. *Deep Sea Res II* 53:2597–2617
- ✦ Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9: 671–675
- ✦ Skottene E, Tarrant AM, Olsen AJ, Altin D and others (2019) The β -oxidation pathway is downregulated during diapause termination in *Calanus* copepods. *Sci Rep* 9:16686
- ✦ Smolina I, Kollias S, Poortvliet M, Nielsen TG and others (2014) Genome- and transcriptome-assisted development of nuclear insertion/deletion markers for *Calanus* species (Copepoda: Calanoida) identification. *Mol Ecol Resour* 14:1072–1079
- Sømme JD (1934) *Animal plankton of the Norwegian coast waters and the open sea. I. Production of Calanus finmarchicus* (Gunnerus) and *Calanus hyperboreus* (Krøyer) in the Lofoten area. *Fiskeridir Skr (Havunders) IV:1–163* (Reports on Norwegian Fishery and Marine Investigations)
- Svetlichny L, Hubareva E, Arashkevich E (1998) Physiological and behavioural response to hypoxia in active and diapausing stage V copepodites of *Calanus euxinus*. *Arch Hydrobiol Spec Issues Adv Limnol* 52:507–519
- ✦ Tande KS (1982) Ecological investigations on the zooplankton community of Balsfjorden, Northern Norwa — generation cycles, and variations in body-weight and body content of carbon and nitrogen related to overwintering and reproduction in the copepod *Calanus finmarchicus* (Gunnerus). *J Exp Mar Biol Ecol* 62:129–142
- ✦ Tarling GA, Belcher A, Blackwell M, Castellani C and others (2022a) Carbon and lipid contents of the copepod *Calanus finmarchicus* entering diapause in the Fram Strait and their contribution to the boreal and Arctic lipid pump. *Front Mar Sci* 9:926462
- ✦ Tarling GA, Freer JJ, Banas NS, Belcher A and others (2022b) Can a key boreal *Calanus* copepod species now complete its life-cycle in the Arctic? Evidence and implications for Arctic food-webs. *Ambio* 51:333–344
- ✦ Tarrant AM, Baumgartner MF, Lysiak NSJ, Altin D, Størseth TR, Hansen BH (2016) Transcriptional profiling of metabolic transitions during development and diapause preparation in the copepod *Calanus finmarchicus*. *Integr Comp Biol* 56:1157–1169
- Torchiano M (2016) Effsize—a package for efficient effect-size computation. <https://cran.r-project.org/web/packages/effsize/index.html>
- ✦ van Oort BE, Tyler NJ, Gerkema MP, Folkow L, Blix AS, Stokkan KA (2005) Circadian organization in reindeer. *Nature* 438:1095–1096
- ✦ VanderPlas JT (2018) Understanding the Lomb–Scargle periodogram. *Astrophys J Suppl Ser* 236:16
- ✦ Vogedes D, Varpe O, Soreide JE, Graeve M, Berge J, Falk-Petersen S (2010) Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods. *J Plankton Res* 32:1471–1477
- ✦ Williams R, Conway D (1982) Population growth and vertical distribution of *Calanus helgolandicus* in the Celtic Sea. *Neth J Sea Res* 16:185–194
- ✦ Williams R, Conway D (1984) Vertical distribution, and seasonal and diurnal migration of *Calanus helgolandicus* in the Celtic Sea. *Mar Biol* 79:63–73
- ✦ Wood B, Tett P, Edwards A (1973) An introduction to the phytoplankton, primary production and relevant hydrography of Loch Etive. *J Ecol* 61:569–585

Editorial responsibility: Myron Peck,
Den Burg, The Netherlands

Reviewed by: F. Maps, A. M. Tarrant and 1 anonymous
referee

Submitted: November 11, 2022

Accepted: April 6, 2023

Proofs received from author(s): June 5, 2023