Lipid load triggers migration to diapause in Arctic Calanus copepods—insights from underwater imaging

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Copepod lipids fuel the Arctic marine ecosystem, but information on the fine-scale distribution of copepods and lipids is nonexistent. This study investigated the fine-scale (1 m) vertical distribution of the copepods Calanus hyperboreus, Calanus glacialis and Metridia longa during a Lagrangian drift in the North Water Polynya using the Lightframe On-sight Keyspecies Investigation (LOKI) imaging system. A copepod species- and stage-specific automatic identification model based on machine learning, a subcategory of artificial intelligence, was used to identify images taken by LOKI. Lipids were measured from images of copepods taken over the whole water column (1 m resolution). Diel vertical migration (DVM) in all three species was detected. In C. hyperboreus and C. glacialis C4-females as well as M. longa C5-females lipid load of deep copepod individuals was significantly higher than that of shallower individuals. Vertical distribution profiles and individual lipid loads suggested that individuals with lower lipid load continued DVM, while others with high lipid load ceased migrating, remaining at depth. Calanus hyperboreus individuals seemed to migrate to diapause at lower lipid fullness (50%) than C. glacialis (60%). A bioenergetics model showed that Calanus females had enough lipids to diapause for over a year, highlighting the significant lipid overhead they use for capital breeding.

KEYWORDS: copepod lipids; DVM; diapause; fine scale vertical distribution; underwater imaging; machine learning; automatic zooplankton identification model; North Water Polynya; Arctic Ocean

INTRODUCTION

In the Arctic Ocean strong seasonality results in a pulsed availability of resources with peak phytoplankton biomass occurring during late spring and summer (Søreide et al., 2010). Zooplankton graze this overabundance of phytoplankton and can channel about a third of that biomass in the form of highly energetic lipids to higher trophic levels
such as forage fish, whales and seabirds (Welch et al., 1992; Søreide et al., 2008). Copepods constitute up to 80% of this zooplankton biomass in the Arctic and many are efficient grazers whose behavior and life-cycle strategies are finely tuned to the transient dynamics of primary producers (Auel and Hagen, 2002). However, recent and rapid environmental changes in the Arctic Ocean have already shown impacts on zooplankton distributions, survival and phenology, with the potential to disrupt this sensitive yet critical ecological balance (Falk-Petersen et al., 2007; Richardson, 2008; Søreide et al., 2010; Doney et al., 2012).

One strategy copepods display to adjust to environmental variability is extensive diel vertical migration (DVM) during which they move up and down in the water column on a daily cycle, even in the context of Arctic midnight sun during summer (Rabindranath et al., 2010; Darnis et al., 2017). During classic synchronized DVM individuals of a population move together to shallow waters at night to feed, and back to deeper waters during the day to take shelter from visual predators, mainly fish (Darnis et al., 2017), but other types of DVM exist. Such strategies allow copepods to feed efficiently and to rapidly accumulate the mass they need to develop and thrive in the extreme Arctic environment. Seasonal dynamics of lipid accumulation in Calanus hyperboreus and Calanus glacialis are well documented (Ashjian et al., 1995; Lee et al., 2006; Freese et al., 2017). Lipids accumulated by Arctic copepods contain a high proportion of energy-rich wax esters that propagate into the pelagic food web (Lee et al., 2006). This accumulation of large reserves is critical for another ubiquitous strategy displayed by the small number of large Calanus species that overwhelmingly dominate the biomass of Arctic mesozooplankton, the diapause (Thibault et al., 1999).

This diapause strategy is characterized by the building of large reserves ahead of a long period of reduced metabolism that coincides with an extensive seasonal vertical migration towards the relative security of the depths (Hirche, 1997; Darnis and Fortier, 2014). This phenomenon is not limited to the interior of the deep oceanic basins, but rather occurs all along the North Atlantic and Arctic Ocean productive shelves as long as regional bathymetry reaches ca. 150 m and below. While diapause dynamics (e.g. timing of descent to diapause, diapause depth and duration) display considerable intra- and interspecies differences (Johnson et al., 2008), it remains essentially dependent on the processes of lipid accumulation and mobilization (Johnson et al., 2008; Falk-Petersen et al., 2009; Maps et al., 2010, 2014; Tarrant et al., 2008, 2016; Baumgartner and Tarrant, 2017). The lipid accumulation window (LAW) hypothesis, for example, proposes that individual copepods initiate diapause only when they have managed to accumulate enough lipid stores to support the (reduced) metabolic demand during dormancy and the maturation process in pre-adult stages (Johnson et al., 2008; Maps et al., 2010; Baumgartner and Tarrant, 2017). Since diapause involves an ontogenetic migration towards the relative security of deeper waters, it implies that a vertical gradient in lipid content should be observed among individuals. Testing this hypothesis in situ has been challenging, owing to the coarse spatio-temporal resolution that net-based sampling methods can provide.

Until very recently, sampling for copepod lipids has been done in layers of tens of meters, essentially through multiple closing/opening nets systems (ARIES, Jonasdottir, 1999; BIONESS, Davies et al., 2015; Hydro-Bios, Laakmann et al., 2009; Pepin and Head, 2009; MOCNESS, Saumweber and Durbin, 2006). Actually, even in studies where sampling was stratified, lipid contents were provided as pooled measurements (mean ± standard deviation), sometimes split between late copepodite and adult stages. But the relative lipid content between a “surface” and a “deep” layer was reported rather anecdotally rather than as the focus of the study (e.g. Lischka and Hagen, 2007; but see Pepin and Head, 2009). As a result, there is currently no information available on the vertical distribution of total lipids (mg) or lipid fullness (LF) (%) at both the individual scale and at a fine vertical resolution of a few meters. However, such information is relevant to many biological processes such as feeding, mate finding, trophic interactions, etc. (Hays et al., 2001; Pond and Tarling, 2011; Zarubin et al., 2014). This potentially limits our detailed understanding of the ecology and life history strategies of these key copepod taxa.

Underwater imaging systems that take photographs of individual zooplankton in situ are able to overcome the low vertical resolution of nets (Hirche et al., 2014; Luo et al., 2014), and are therefore useful for studying copepod lipids. This study uses the Lightframe On-sight Keyspecies Investigation (LOKI) system (Schulz et al., 2010; Schmid et al., 2016). Images were identified using a copepod stage-specific automatic zooplankton identification model (Schmid et al., 2016) based on machine learning. The appearance of copepod lipid sacs was investigated in hundreds of LOKI images of individuals of three copepod species that dominate the biomass of mesozooplankton in the Arctic Ocean: the diapausing species C. hyperboreus and C. glacialis, and the non-diapausing Metridia longa. Individuals were automatically identified to the development stage, and their total lipid amount (mg) and LF (%) were estimated. These data of unprecedented level of resolution were used in a bioenergetics model in order to estimate the potential diapause duration of deep-dwelling individuals for the two Calanus congeners (Maps et al., 2014). The objective of this study was to use new imaging technology...
and its high resolution to explore the relationship between individual lipid content and the vertical migration behavior and diapause strategy of Arctic copepod species.

**METHOD**

**Study area**

We sampled zooplankton during the 2013 ArcticNet expedition onboard the CGGS *Amundsen*. Sampling followed a Lagrangian surface drifter on August 15 and 16. Vertical tows of LOKI were performed in the vicinity of Ellesmere Island, NU, in the North Water Polynya (NOW; Supplementary Fig. S1). The NOW, situated in northwestern Baffin Bay between Greenland and Ellesmere Island, is one of the largest recurrent polynyas in the Arctic with a size of $3-8 \times 10^3$ km$^2$ and is considered one of the most productive areas north of the Arctic Circle (Deming et al., 2002). Ice-free waters occur there as early as the beginning of April compared to mid-July in adjacent regions of the Canadian Archipelago (Fortier et al., 2002).

**LOKI system**

The LOKI is a mesozooplankton imaging system. A plankton concentration net, with a mouth opening of 0.28 m$^2$ and a mesh size of 200 μm is attached to the inflow of the camera (Supplementary Fig. S2). The LOKI camera-imaging channel has a length of 31.3 mm, width of 20.75 mm and height of 4 mm (i.e. a volume of 2.6 cm$^3$). A cod-end collected all zooplankton after imaging, and samples were preserved in 4% formaldehyde–seawater solution buffered with sodium borate for further analyses in the laboratory. At each time step (Table I), LOKI was deployed to 10 m above the seafloor prior to being hauled back to the surface at a constant speed of 24 m min$^{-1}$. Images of mesozooplankton were collected and stored on an internal Solid State Drive in 8-bit lossless greyscale format. Environmental conditions (temperature, oxygen, conductivity, fluorescence and pressure) were recorded during each LOKI deployment using onboard sensors. For photosynthetically active radiation ($E_{PAR}$), we used data collected by the ArcticNet main CTD ( Biospherical QCP-2300). Detailed information on LOKI design and setup are presented in Schulz et al. (2010) and Schmid et al. (2016).

**Image data preparation and automatic identification of copepod taxa using machine learning**

Every LOKI image was linked to the corresponding data from the environmental sensors based on a timestamp and calculated depth. Fifty-two image parameters including circularity, Hu moments, Fourier descriptors as well as several values describing geometry and connectivity within objects (from Morphological Spatial Pattern Analysis) were measured on all collected images using LOKI software and GUIDOS toolbox (Schulz et al., 2010; see Schmid et al., 2016 for a complete list of image parameters). Representativeness of LOKI image data was ensured by applying the ZOOMIE v 1.0 (Zooplankton Multiple Image Exclusion) software to all images (Schmid et al., 2015). ZOOMIE filters out redundant images of unique individuals. The copepod stage-specific automatic zooplankton identification model developed in Schmid et al. (2016) was applied to all images, using Salford Systems’ predictive modeler (version 7.0) implementation of the random forests (RF) algorithm (Breiman, 2001). RF is a part of machine learning, a subcategory of artificial intelligence. Schmid’s model is highly efficient at identifying images of *C. hyperboreus*, *C. glacialis* and *M. longa*, when presented with many zooplankton types. Accuracy was tested using model internal testing with withheld test data, and external testing in which automatic identification of images from deployments unknown to the model was manually verified. Externally tested accuracies (true positive samples over total manual classification) for *C. hyperboreus*, *C. glacialis* and *M. longa* were 86%, 82% and 83%, respectively. Externally tested specificities (true positive samples over total classifier prediction) for *C. hyperboreus*, *C. glacialis* and *M. longa* were 87%, 81% and 84%, respectively (Schmid et al., 2016). All images belonging to the different developmental stages of *C. hyperboreus*, *C. glacialis* and *M. longa* (Supplementary Fig. S3) were selected for further analysis. Because of morphological similarities and size overlap, the pairs of *C. glacialis* C2 and *C. hyperboreus* C1 as well as *C. glacialis* C3 and *C. hyperboreus* C2 were treated as single groups by the automatic identification model (Schmid et al., 2016). The automatic identification model does not distinguish *Calanus funmarchicus* from other congeners. Investigation of cod-end samples revealed that *C. funmarchicus* abundance was 174 ± 29 ind. m$^{-2}$ (mean ± 1 SD) during the drift, compared to 12 483 ± 213 ind. m$^{-2}$ of *C. glacialis*, as which *C. funmarchicus* would most likely be identified as by the automatic identification model.
Image observations of individuals in each taxon were binned for each 1 m of the water column. Counts of detections of each taxon in 1 m bins were corrected based on the classification probabilities (Solow et al., 2001) of all taxa from the automatic identification model (Schmid et al., 2016). The applied classification probabilities were the averages of the two confusion matrices, from external testing, developed in Schmid et al. (2016). A total of 6983 automatically identified images had been algorithmically and randomly selected over all taxa, and then manually validated, in order to generate these matrices (Schmid et al. 2016). The corrected number of observations was used to estimate taxon abundances (m$^{-3}$) per 1 m of the water column, based on the volume filtered. See Schmid et al. (2016) for additional details on each step of image processing.

Lipid analysis

We used two complementary measures of individual lipid content: total lipids (TL, in mg) and LF (in % of body volume). Since inter- and intraspecific variability in individual size can be very large (Forster and Hirst, 2012), total lipid measures were not directly comparable among stages and species, whereas LF could provide a more general picture of the individuals’ condition and lipid metabolism among copepods. Total lipids and LF were estimated for a total of 822 LOKI images of the development stages known to accumulate lipids in a visible lipid sac in their prosome. Images of individual C. hyperboreus stages C3 to adult female (F), C. glacialis C4 to F and M. longa stages C5 to F were sourced from two of the five deployments: one during daytime (18:45 h DST, UTC – 4; 380 μE m$^{-2}$ s$^{-1}$ incident EPAR), and one at night (2:40 h, 18 μE m$^{-2}$ s$^{-1}$ incident EPAR). For C. hyperboreus C3–C5, C. glacialis C4/C5 and M. longa C5/F, 50 individuals were analyzed from each of the two deployments. For C. glacialis and C. hyperboreus F, a total of 42 and 80 individuals respectively were analyzed, due to their different relative availabilities. Copepod images for lipid analysis were sampled randomly from the whole water column, but images where the copepod was not in a lateral position had to be discarded due to the methodology used (Vogedes et al. 2010), and a new image was drawn randomly. For all individuals, the area of the lipid sac (A, in mm$^2$) was measured (Fig. 1), and used to estimate total lipids according to Vogedes et al. (2010):

$$TL = 0.197A^{1.38},$$  \hspace{1cm} (1)

LF was simply defined as the area of the lipid sac divided by the area of the prosome times 100.

Potential diapause duration estimation

Potential diapause duration was computed for the stages and species known to perform extensive diapause in the area, i.e. C. hyperboreus C3 to F and C. glacialis C4 to F, following Maps et al. (2014):

$$D = \frac{4}{-M}\left(\frac{1}{C_{\text{out}}} - \frac{1}{C_{\text{in}}}\right)$$  \hspace{1cm} (2)

where $M$ (μg C$^{1/4}$ s$^{-1}$) is the metabolic constant for dormant individuals at 0°C. We did not include any effect of temperature since the observed temperature at the expected depth of diapause (>150 m) was for the largest part constant at 0°C (Supplementary Fig. S4). $C_{\text{in}}$ and $C_{\text{out}}$ are the individual body mass (μg carbon) at the onset and at the end of dormancy, respectively (see Equations (3) and (4) below). To estimate these critical values, we made the assumption that the maximum potential diapause duration would be reached upon lipid stores depletion. Total lipid values estimated from the images were converted into carbon using a carbon to lipid conversion factor λ = 0.81, based on the average lipid composition of calanoid copepods (Kattner and Graeve, 1991). Total body carbon of each individual was calculated from the lipid carbon and LF:

$$C_{\text{in}} = \frac{\lambda TL}{LF}.$$  \hspace{1cm} (3)

Then the difference between $C_{\text{out}}$ and the lipid carbon gave the minimum (structural) carbon content $C_{\text{out}}$:

$$C_{\text{out}} = C_{\text{in}} - \lambda TL.$$  \hspace{1cm} (4)

We likely overestimated the minimal carbon content at the end of diapause since the structural component of the calanoid copepods’ body contains much less carbon per unit of dry weight than its lipid stores, as revealed by comparing the elemental C to N ratios of lipid full vs. lipid poor individuals (e.g. Pepin and Head, 2009). As a result, we consider our estimate of potential maximum diapause duration to be conservative. Data and R-code (R Core Team, 2017) to reproduce these results can be found at https://zenodo.org/record/1203741.

RESULTS

Environmental setting

A dicothermal layer prevailed during the drift study with relatively warm (4.3°C) surface waters sitting on a glacial (−1.5°C) layer centered around 100 m over
warmer deep waters (Supplementary Fig. S4). A strong halocline with salinity 31.4 at the surface and 34.1 at depth provided vertical stability. Fluorescence in surface waters reached 1.4 mg m$^{-3}$ by the end of the drift (Supplementary Fig. S4). Daylight prevailed from 7:00 to 23:00 with maximum EPAR in the water column reaching 239 µE m$^{-2}$ s$^{-1}$ (Supplementary Fig. S4). A constant T-S signature among proplanktonic taxa was maintained during the 24 h drift over a 15 km distance.

Vertical distributions of copepods

The mean abundances ($\pm$1 SD) of *Calanus hyperboreus*, *C. glacialis* and *M. longa* during the drift, as estimated from automatically identified underwater imagery, were $11,463 \pm 320$, $12,563 \pm 265$ and $15,668 \pm 378$ ind. m$^{-2}$, respectively. The abundance of each species varied little among profiles. While the combined young *Calanus* copepodite stages C2/C1 distributed vertically according to the fluorescence profile, especially at night (2:40 h profile, Fig. 2), the older C3/C2 showed tendencies of avoiding the immediate surface layer (<10 m) during daytime, before congregating in or slightly under the subsurface chlorophyll maximum (SCM) during night. Older *C. hyperboreus* development stages presented more distinct vertical distribution patterns. C3 congregated in the SCM at night (2:40 h profile), moved between 100 and 200 m well below the SCM during the day and then moved back towards it at dusk (Fig. 3). Similarly, the bulk of the abundant C4 stage was distributed around 200 m in daytime, moving towards the SCM at dusk and converging into it at night. C5 were distributed all over the water column with greater abundance at depth near the bottom. Females were distributed in the deeper half of the water column (Fig. 3). *Calanus glacialis* C1 distribution matched chlorophyll $a$ fluorescence at all times within the 0–120 m layer and displayed little vertical movement throughout the diel cycle (Fig. 4). The abundant C4 and C5 were distributed over the entire water column with a noticeable increase in density immediately above and below the SCM at night. The rare female *C. glacialis* were distributed close to the seafloor at all times [Fig. 4]. Vertical distributions of *M. longa* C1–C4 remained similar throughout the drift (Fig. 5; Supplementary Fig. S5). C4, C5 and females were found throughout the water column but avoided the immediate surface layer (<10 m) in daytime and concentrated in the SCM at night. Males were rare, and always situated deep in the water column (Supplementary Fig. S5).

Copepod lipids

In *C. hyperboreus*, we observed maximum mean total lipids of 2.45 mg and maximum mean lipid fullness of 79% in females, while minimum mean total lipids of 0.09 mg and minimum mean lipid fullness of 39% were found in C3 (Fig. 6; see Supplementary Table S1 for all means and standard errors). *Calanus glacialis* female individuals contained maximum mean total lipids of 0.52 mg and maximum mean lipid fullness of 80%, while minimum mean total lipids of 0.17 mg and minimum mean lipid fullness of 43% were found in C4 (Fig. 6; Supplementary Table S1). Finally, *M. longa* females contained maximum mean total lipids of 0.1 mg and maximum mean lipid fullness of 36%, while minimum mean total lipids of 0.01 mg and minimum mean lipid fullness of 12% were found in C5 (Fig. 6; Supplementary Table S1). While in most taxa total lipids and lipid fullness followed normal distributions, *C. hyperboreus* C3 and female lipid fullness showed a tendency towards a bimodal distribution (Supplementary Fig. S6).

Vertical distribution of lipids

In *C. hyperboreus* C4, C5 and F, total lipids and lipid fullness significantly increased with depth during both day and night (Fig. 7; see Supplementary Table S2 for a linear regression summary). Vertical profiles of total lipids and lipid fullness differed significantly between day and night sampling only in *C. hyperboreus* C4 (Fig. 7; see Supplementary Table S3 for an analysis of covariance (ANCOVA) summary). Total lipids and lipid fullness of *C. glacialis* from C4 to female stages were significantly higher in deeper water compared to shallower water during both day and night samplings (Fig. 8; see Supplementary Table S2). Vertical profiles of lipid fullness in individuals differed significantly between day and night samplings in *C. glacialis* C4 and C5 (Fig. 8; Supplementary Table S3), while for total lipids this was only the case in *C. glacialis* C4. Total lipids and lipid fullness of *M. longa* C5 and females were significantly higher in deeper water compared to shallower water during night.
sampling (Fig. 9; Supplementary Table S2), but not during the daytime.

**Potential diapause duration**

Potential maximum diapause duration (D) estimated for all the developmental stages of the *Calanus* congeners increased almost linearly with their size (Fig. 10). The youngest developmental stages thought to be able to undergo diapause, i.e. *C. hyperboreus* C3 and *C. glacialis* C4, could on average be diapausing during 78 and 112 days, respectively. Subsequent stages could all exceed an arbitrary duration threshold of six months. It also appeared that *C. glacialis* females could remain dormant for almost a year, whereas *C. hyperboreus* females potential maximum diapause duration could well exceed this duration.

**DISCUSSION**

**Diel vertical migration**

In this study, underwater images of zooplankton species and stages taken by the LOKI system were automatically, and with high accuracy, identified using a previously developed machine learning model (Schmid et al., 2016). The resulting fine-scale vertical distribution data (1 m resolution) showed clear differences in the DVM of the different developmental stages. The observed DVM in *C. hyperboreus*, *C. glacialis* and *M. longa* corresponded to the classic patterns reported in previous Arctic studies (Kosobokova, 1978; Fortier et al., 2001; Falk-Petersen et al., 2008; Daase et al., 2008, 2015). Our observations are consistent with studies showing the clearest signs of classic DVM in copepods during late Arctic summer and autumn (Kosobokova, 1978; Cottier et al., 2006; Wallace et al., 2010). The ultimate cause for DVM is thought to be predator avoidance, by reducing detection from visual predators such as *Themisto* sp. and polar cod in surface layers (Hays, 2003), while the proximate cause for DVM is thought to be changes in light intensity (Cohen and Forward, 2009). Local hydrography has also been shown to affect DVM patterns, for instance through thermal optima at depth (Berge et al., 2014), water mass differences (Lawson et al., 2004) and thermoclines (Williams, 1985). However, the observations of individual copepods by means of underwater imaging and at multiple time steps in this study showed that also the small/young copepodite stages (see for instance the *C. glacialis* C3 / *C. hyperboreus* C2 complex, Fig. 2) readily migrated from immediate surface waters to deeper waters, and back again, crossing thermo- and halocline. This suggests that hydrography, and especially clines, were not the most important drivers, and not inhibiting, in this study. Since smaller *C. glacialis* C3 and *C. hyperboreus* C2 stages swim at lower Reynolds numbers than larger stages, an effect of clines would be visible first in these weaker swimmers. The youngest and smallest copepodite stages (i.e. *C. glacialis* C2 / *C. hyperboreus* C1 complex and *M. longa* C1) showed no signs of DVM,
which can be explained by a reduced visual predation pressure in their size range (Brooks and Dodson, 1965; Fortier et al., 2001; Daase et al., 2015). Instead, these young stages were vertically distributed in proportion to the abundance of their algal food throughout the drift. Especially during the night-time deployment at 2:40, individuals of all copepodite stages except Calanus females accumulated within the SCM (Rabindranath et al., 2010), most likely in order to feed (Falk-Petersen et al., 2008; Daase et al., 2008, 2015). Our results suggest that inter- and intraspecific differences observed in the DVM patterns are linked to the condition of individual copepods (Ohman, 1990; Hays et al., 2001), specifically their lipid fullness. Furthermore, the distribution profiles shown in this study demonstrate that the majority of a given copepod population (e.g. C. glacialis C5, Fig 4.) was constantly vertically migrating, rather than being at a certain “resting depth”, a term which is often used in DVM studies.

The role of lipids in vertical migration and diapause

In addition to the diapause requirements, C. hyperboreus depends on its lipid reserves for egg spawning in winter (Conover, 1967; Hirche, 1991), while C. glacialis utilizes stored lipids but also grazes on ice algae to spawn during early spring (Hirche and Kattner, 1993; Søreide et al., 2010). As an interesting counterpoint, M. longa is a smaller but abundant omnivorous species that differs...
from *Calanus* spp. in that it feeds and reproduces year-round. Since diapause is not a feature of its life cycle (Darnis and Fortier, 2014), it does not accumulate lipid reserves to the level found in *Calanus* spp. (Ashjian et al., 1995). As a result, we expected very different vertical gradients in lipid content between these species and among their developmental stages. Our individual-based observations obtained on finely resolved vertical and temporal scales showed that lipid-rich *C. hyperboreus C5-F*, as well as *C. glacialis F*, were found almost exclusively below 150 m. The lack of any significant increase in numbers within the surface layer at night strongly suggests that these individuals were already diapausing. On the contrary, the changes in lipid fullness observed in younger *C. glacialis C4* and *C. hyperboreus C4* suggest that some individuals with lower lipid fullness were still migrating from their deep refuge during the day towards the surface layer during the night, most likely for feeding (Figs 7–9; Supplementary Table S3), while individuals from the same stages with higher lipid fullness remained deep and did not migrate back up to surface waters. These results support the LAW hypothesis and may even allow estimation of the threshold beyond which active DVM would stop for a given developmental stage. Lipid fullness observations of *C. hyperboreus C4* and *C. glacialis C5* suggested that *C. hyperboreus C4* individuals were able to start diapausing at lower lipid fullness (∼50%) than *C. glacialis C5* (∼60%).

Observations of lipid-filled *Calanus* sp. at depth are consistent with the understanding that *Calanus* sp. are strong
seasonal migrators (Darnis and Fortier, 2014) that can diapause as soon as stage C3 for *C. hyperboreus* (Hirche, 1997) or C4 for *C. glacialis* (Melle and Skjoldal, 1998; Falk-Petersen *et al.*, 2009). While total lipids reported in this study for *C. hyperboreus* C4–C5, *C. glacialis* C4–C5 and *M. longa* females are within the ranges reported in the literature (Hirche and Kattner, 1993; Ashjian *et al.*, 1995; Scott *et al.*, 2000; Auel *et al.*, 2003), no comparable study

Fig. 5. Vertical distribution of *Metridia longa* C4 to female (F) copepodite stages (black bars) and phytoplankton fluorescence (green line) for five different profiles over the 24-h duration of a drift study in the North Water. Sampling time and incident $E_{PAR}$ ($\mu$E m$^{-2}$ s$^{-1}$) at the beginning of a profile are given above each panel. Note the differences in maximum depth among profiles. The vertical distributions of the remaining *M. longa* stages are given in Supplementary Fig. S5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6. Total lipids (a) and lipid fullness (b) for different copepodite stages and female (F) *Calanus hyperboreus*, *C. glacialis* and *Metridia longa*.
Fig. 7. Total lipid amounts (left panels) and lipid fullness (right panels) in *Calanus hyperboreus* individuals located at different depths. C3–C5, copepodite stages 3, 4 and 5; F, females. In each panel, the light blue crosses show daytime (18:45 h) results. The dark blue crosses show nighttime (2:40 h) results. Regression lines for the two datasets (day and night) are shown as light blue and dark blue lines with the 95% confidence intervals shown as gray areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
has ever shown total lipids and lipid fullness for *C. hyperboreus* C3 and *M. longa* C5. The average lipid fullness in *C. hyperboreus* and *C. glacialis* females was around 80%, which is higher than what was reported by previous studies elsewhere around the Arctic (Hirche and Kattner, 1993; Ashjian et al., 1995; Scott et al., 2000; Auel et al., 2003). Total lipids and lipid fullness differences between studies can be explained by the individual feeding success (Miller et al., 1998; Baumgartner et al., 2011) as well as seasonal and geographical variability in environmental conditions. High amounts of total lipids and replete oil sacs of *Calanus* sp. found in our study suggest that these copepods found plenty of suitable phytoplankton food during the productive spring/summer of 2013.

Such individual conditions led to high potential diapause duration estimates for *Calanus* spp. copepodite stages. Substantial lipid reserves of *C. hyperboreus* C4-F and *C. glacialis* C5-F were sufficient for a potential diapause from late August until next spring, when the first production peak of ice algae starts (late March–early April). *Calanus hyperboreus* C5 and both congener’s females even had a sizable lipid surplus, enabling potential diapause of over a year in females. Obviously, females of either species do not remain in diapause for over a year. However, both are...
known to produce eggs from their lipid reserves, and while *C. glacialis* reaches its maximum egg production during the spring-summer phytoplankton bloom, *C. hyperboreus* is a strict capital breeder (Hirche, 1991). This lipid overhead shows that all female individuals of the two *Calanus* congeners would be able to safely undergo diapause and

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**Fig. 9.** Total lipid amounts (left panels) and lipid fullness (right panels) in *Metridia longa* individuals located at different depths. C5, copepodite stage C5; F, females. In each panel, the light blue crosses show daytime (18:45 h) results. The dark blue crosses show night-time (2:40 h) results. Regression lines for the two datasets (day and night) are shown as light blue and dark blue lines with the 95% confidence intervals shown as gray areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 10.** Estimated maximum potential diapause duration for observed development stages of *Calanus hyperboreus* and *Calanus glacialis*. Dotted line: 6-month threshold; dashed line: 1-year threshold.
survive through the long Arctic winter as well as successfully reproduce. While *M. longa* does not go into diapause, the substantial lipid fullness of its females reinforces the hypothesis that *M. longa* relies on a mixed strategy of active feeding and accumulating lipids to overcome the harsh winter conditions of the Arctic marine ecosystem, and even produce some eggs (Conover and Huntley, 1991). Higher temperatures of ambient water can substantially increase the metabolic demand of diapausing copepods, leading to a shortening of potential diapause length (Maps et al., 2014). However, temperature of deeper Arctic waters (>200 m) is not expected to increase to a level that would negatively affect diapause length in the near future (Seidov et al., 2015). Climate change-induced mismatch scenarios earlier in the season (Falk-Petersen et al., 2007), however, have the potential to disrupt lipid accumulation and could lead to starvation of copepods during diapause (Auel et al., 2003). This would also negatively affect the capital breeding strategy of Arctic-adapted *Calanus* species. In such a scenario, *M. longa* would likely fare better than *Calanus sp.* owing to its omnivorous, year-round feeding strategy, buffered by some lipid-accumulating capabilities.

CONCLUSION

This study used a machine learning—artificial intelligence approach to automatically identify underwater imagery, leading to fine-scale (1 m) resolution data on the distribution of individual copepods and species life stages as well as environmental parameters. Data derived from LOKI can be used in studies that aim at integrating the physiology and ecology of copepods, as was demonstrated here with the *in situ* lipid data of copepods. The present study shows that for *Calanus* individuals within a given developmental stage, individuals that accumulated high lipid fullness had entered diapause and ceased DVM, while individuals with lower lipid fullness still performed DVM and had not yet entered diapause. The lipid fullness threshold for migration to diapause varied between *Calanus* species and stages. Potential diapause durations of over a year were estimated in *Calanus* females, highlighting the large amounts of lipids going into egg production. It is hoped that the fine scale insights on copepod abundance, total lipids and lipid fullness provided in this study will be useful for a range of studies, especially for modeling experiments (e.g. Dufour et al., 2016).

SUPPLEMENTARY DATA

Supplementary data can be found online at *Journal of Plankton Research* online.

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