Lipid content in overwintering Calanus finmarchicus across the Subpolar Eastern North Atlantic Ocean

Jónasdóttir, Sigrún H.; Wilson, Robert J.; Gislason, Astthor; Wilson, Robert J.

Published in:
Limnology and Oceanography

Link to article, DOI:
10.1002/lno.11167

Publication date:
2019

Document Version
Peer reviewed version

Citation (APA):
Jónasdóttir, S. H., Wilson, R. J., Gislason, A., & Wilson, R. J. (2019). Lipid content in overwintering Calanus finmarchicus across the Subpolar Eastern North Atlantic Ocean. *Limnology and Oceanography*, *64*(5), 2029-2043. https://doi.org/10.1002/lno.11167

Lipid content in overwintering *Calanus finmarchicus* across the Eastern Subpolar North Atlantic Ocean

Sigrún Huld Jónasdóttir[1)*], Robert J. Wilson [2)], Astthor Gislason[3)], Michael R. Heath[2)]

1) National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, Building 202, DK-2800 Kgs. Lyngby, Denmark, Email: sjo@aqua.dtu.dk

2) Department of Mathematics and Statistics, University of Strathclyde, 26 Richmond Street, Glasgow, G1 1XH, UK, Emails: robert.wilson@strath.ac.uk, m.heath@strath.ac.uk

3) Marine and Freshwater Research Institute, Skulagata 4, 101 Reykjavik, Iceland, Email: astthor.gislason@hafogvatn.is

*Corresponding author:

Sigrún Huld Jónasdóttir

National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, B-202, DK-2800 Kgs. Lyngby, Denmark

Email: sjo@aqua.dtu.dk

Tel: +45 3588 3427

Fax: + 45 3588 3333

Keywords: *Calanus finmarchicus*, wax ester reserves, diapause duration, overwintering, winter distribution, lipid pump, carbon sequestration

Running head: *Calanus finmarchicus* winter lipids

Abstract

The boreal copepod *Calanus finmarchicus* accumulates lipid reserves during summer feeding in surface ocean waters, which enable it to stay at depth and survive famine during overwintering. Respiration of lipids during prolonged overwintering at ocean depths (>1000 m in some areas) has been shown to result in a net sequestration of carbon into the deep ocean: the so called 'lipid pump'. Here we provide a comprehensive synthesis of the geographic and vertical variations in lipid content of over-wintering animals across the Subpolar Eastern North Atlantic and, on the basis of this, we revise the estimates of carbon sequestration. Wax ester (WE) content ranged from 40 to 190 µg ind^{-1} at >250 m depths, with highest concentrations in the coldest (<0°C) waters at 400-600 m depth at the slope east of Faroe Islands and east of Greenland, and lowest in the warmer (>4°C) Irminger and Rockall Basins. Our new analysis results in about 44% higher estimates of carbon sequestration at up to 11.5 gC m^{-2}.

Introduction

Diapause is a trait that many Calanoid copepod species have adapted as a strategy to ensure maximum survival during periods of food shortage. The ideal and essential place for diapause is out of reach from predators, below the deep convection layer (Krumhansl et al. 2018) and in waters with lowered temperatures; a habitat that allows the organisms to remain quiescent with reduced basal metabolism during diapause.

Wax ester (WE) accumulation before a descent to depth of the oceans for overwintering is an essential adaptation,as being lipid rich while remaining torpid requires lipids with the physical properties of wax esters (Visser and Jónasdóttir 1999; Pond et al. 2012). The depth of overwintering is likely to be a function of the trade-off between predation risk and the physiological limitation of diapause. An important part of this physiological limitation has to do with how much lipids the copepods have at the start of diapause, and how fast the wax esters are catabolised as a function of their metabolic rate (Maps et al 2014). In order to better understand lipid accumulation strategies we need to know more about copepod overwintering habitats and the physiological restraints they face in terms of different temperatures and duration of diapause.

Calanus finmarchicus is a copepod species with a wide distribution in the subpolar North Atlantic spanning from Georges Bank in the west to Norway in the East, (Conover 1988; Heath et al. 2000). It accumulates wax esters as a pre adult stage C3-C5 before overwintering usually as stage C5 in deep ocean basins for a period that lasts from 120 to >300 days (Hirche 1996; Maps et al. 2014; Melle et al. 2014; Jónasdóttir et al. 2015; Wilson et al. 2016). The winter distribution of *C. finmarchicus* is probably the best known of all copepod species (Heath et al. 2004). The winter population reaches densities of 35-

56,000 indiv. m^{-2} in the Eastern and central Norwegian Sea (Dale et al. 1999; Heath et al. 2004) and Lofoten Basin (Halvorsen et al. 2003) with pockets of high densities of 15-60,000 indiv. m^{-2} in the Nova Scotia and Newfoundland basins (Head and Pepin 2007), Gulf of Maine (Runge et al. 2015) and Iceland Sea (Gislason and Silva 2012). Overwintering populations are also found in high densities in fjords and estuaries on both sides of the Atlantic (e.g. Plourde et al. 2001; Arnkværn et al 2005; Clark et al. 2012). The overwintering parking depth varies in the different North Atlantic Basins and is most often found to be below the lower boundary of the cold intermediate layer (Heath et al. 2004; Krumhansl et al, 2018).

Lipid accumulation prior to diapause by the pre-adult copepodite stage 5 (C5) is well documented (Køgeler et al. 1987; Falk-Petersen et al. 1987; Kattner and Krause 1989; Miller et al. 1998; Jónasdóttir 1999). The lipid of late-stage C5 *C. finmarchicus* typically exceeds 50% of its dry weight (Kattner and Hagen 2009), with up to 90% of total lipids being wax esters (Lee et al. 2006). While diapause depth of *C. finmarchicus* varies, they usually are found at depth of 500 to 1500 m during winter in water temperatures of -0.5 to 5 °C (Heath et al. 2004). Overwintering at shallower depths and at higher temperatures has however, been observed in coastal fjords (e.g. Clark et al. 2012, Bagøien et al 2001). Duration of diapause is likely to be greatly influenced by storage lipid fullness, overwintering temperatures and geographical location, but increased respiration due to higher metabolism with increasing temperatures is likely the main factor affecting duration of diapause (Wilson et al. 2016).

Lipid metabolism by copepods in diapause has recently been shown to be a substantial contributor to the North Atlantic biological carbon pump (Jónasdóttir et al. 2015; Visser et al. 2017). Respiration by the overwintering population of *Calanus finmarchicus* alone has been shown to equal the passive carbon sequestration flux in the North Atlantic and has been named “the lipid pump” (Jónasdóttir et al. 2015).

Along with accurate measures of abundance, a reliable estimate of diapause duration is crucial for assessing the lipid driven carbon sequestration. To date, to our knowledge, only two studies (Johnson et al. 2008; Melle et al. 2014) have estimated diapause duration of populations using field observations, based on percentages of C5 and adults in relation to the historical population structure in autumn and spring at given locations. Diapause duration has on the other hand been modelled by Ingvarsdóttir et. al (1999), Saumweber and Durbin (2006), Johnson et al. (2008), Maps et al. 2011 and Wilson et al. (2016). These studies and models show a broad range of durations ranging from 70 to well-over 300 days, depending on location.

Here we present results of the mapping of the lipid content of over-wintering *C. finmarchicus* in 7 basins and one shelf-sea in the Subpolar Eastern North Atlantic. The measures were conducted on a series of late autumn/winter cruises and present the most detailed geographical and vertical coverage of lipid content of this species during diapause to date. We use the observed horizontal and vertical distribution to estimate respiration and diapause duration of the different populations by applying a model presented by Visser et al. (2017), which is based on metabolic theory and isomorphism. This model is then used to re-estimate the carbon sink due to over-wintering *C. finmarchicus* respiration and mortality by applying the new measure of diapause duration, including respiration rate estimates of the C4 and C6 females observed in the overwintering populations, and adding carbon loss by mortality during the overwintering period.

Methods:

Lipid content data were derived from two sources: our own chemical analyses, and previously published studies. *Calanus finmarchicus* for our own analysis were collected for lipid analysis during 14 winter

field campaigns on RVs DANA (DTU Aqua, Denmark), Scotia (Marine Scotland Science, UK), and Bjarni Sæmundson (Marine Research Institute, Iceland). The sampling covered the periods from September to February from 1993 to 2012 in 8 different areas of the Subpolar Eastern North Atlantic. The stations sampled can be allocated to the following ocean areas: Irminger Basin (IRM), East Greenland (EGR), Iceland Sea (ICS), Iceland Basin (ICB), Rockall Basin (ROC), Western Norwegian Sea (WNS), Eastern Norwegian Sea (ENS) and the North Sea (NS) (Figure 1; Table 1). The southern EGR stations would in principle be a part of ICS while the northern stations are in the Greenland Sea but are here grouped as EGR as the copepods are sampled in the East Greenland Current at the same time of year, while the ICS copepods are sampled further east in the Iceland Sea Gyre and East Iceland Current.

During the Dana and Scotia cruises in 1993-2002, copepods were collected with the ARIES multinet system (Dunn et al. 1993) that collected samples at approximately 50 m intervals to a depth of approximately 3000 m. In some instances, ring nets (250 µm mesh, 1 m diameter mouth aperture) with a 5 L non-draining cod-end were used to sample the upper 200 m stratum. On Bjarni Sæmundsson and the 2012 Dana cruise, samples were taken with multinets in 7-10 depth layers covering the whole water column and ring nets. The ARIES was equipped with a Seabird CTD while CTD profiles were taken at all stations where multinets and ring nets were deployed. As soon as possible after retrieval of the nets, *C. finmarchicus* stages C5 and female C6s were sorted under a stereo microscope. Up to 10 intact, and preferably live, individuals were placed in cryovials, the air in the vials replaced with nitrogen gas and the samples frozen immediately in liquid nitrogen. Samples were kept on ice at all times prior to and during sorting. At the end of the cruise, the samples were transferred to a -80°C freezer for storage until analysis.

129 Prosome length (PL) of animals was measured to the nearest 0.01mm. Length measurements of
130 *Calanus finmarchicus* from a Faroe Shetland Channel transect (Dana cruises in 1993-1995) area and
131 Irminger Sea have been presented as grouped averages in Jónasdóttir (1999) and Heath et al. (2008),
132 respectively. All lipid analysis was carried out using Iatroscan TLC as described in Jónasdóttir (1999).
133 Lipid content was standardised to prosome volume (PV) in mm^3 according to Miller et al. (2000): PV=
134 $0.0292 \times PL^{3.6699}$ where PL is the average prosome length in mm for the copepodite stage at the
135 corresponding sample location and depth. Size measurements and lipid analyses were not performed on
136 the same individuals.
137
138 *Oil sac area*. Copepods from the Greenland Sea were photographed, after which the area of the lipid sac
139 was traced using the "image-J" software (Rasband , 1997– 2009). The pixel-to-mm ratio was calibrated
140 using the image of the appropriate calibration slide. The lipid sac area was converted into wax esters by
141 using the formula $\mu g\ (WE) = 0.167 \times A^{1.42}$ from Vogedes et al. (2010) where A is the area of the lipid
142 sac in mm^2.
143
144 *C. finmarchiucs* dry mass was measured from most sampling locations. Individual copepods were
145 measured for prosome length, dried in batches of 3-10 individuals at 60°C and weighed on a Cahn 26
146 Automatic Electrobalance. The dry mass (DW) therefore includes lipids. In other instances dry mass was
147 estimated from prosome length (PL) using the length/DW equation: $DW=0.0106 \times PL^{3.64}$, where DW
148 is in mg and PL in mm based on the relationship of copepods from winter in the Norwegian Sea
149 (Ingvarsdóttir et al. 1999). Carbon mass was estimated using the equation: $C=0.623 \times DW - 0.0143$
150 from Ingvarsdóttir et al. (1999).
151

Previously published data

Additional lipid data used in the analyses were compiled from our own previously published studies shown in Table 1 and locations of the samples included in Fig. 1. The original data from the Irminger Sea (Discovery 258 and 267) published as % C in Heath et al. (2008), are here re-presented on an individual and volume basis to be comparable with the present data.

Estimate of diapause duration

Respiration of copepods in diapause was calculated based on metabolic theory and the isomorphism model presented in Visser et al. (2017):

$$r\,(M,\,T) = b \times M^{3/4} \exp\,(E \times (T\text{-}T_0) \,/\, (k \times T \times T_0)) \quad \text{(Eq. 1)}$$

where M [µgC] is mass, r [µgC s^{-1}] is respiration rate, T [K] is temperature in Kelvin, b [$\mu gC^{1/4}\ s^{-1}$] is a universal scaling constant, E[eV] is the activation energy and k [eV K^{-1}] the Boltzmann constant. T_0 is base temperature, taken to be T_0= -273 °C (absolute zero). We used universal scaling constant b of 2.5 × $10^{-7}\ \mu gC^{1/4}\ s^{-1}$ as suggested by Visser et al. (2017).

The model is carbon based and includes estimates of structural mass m that scales with prosome length (PL) as: $m=\alpha PL^3$. Maximum wax ester content is expressed as $w_{max} = \beta PL^3$, and minimum wax ester content $w_{min} = \delta\beta PL^3$. The coefficients α and β are determined by a best fit regression of maximum total carbon content (α) (from dry weight) and maximum carbon based WE content (β) per prosome length observed for different sized copepods from stages C4 to C6F from the present study. WE was converted to carbon by assuming that 79% of its weight is carbon, calculated from WE fatty acid and alcohol profiles of Arctic *C. finmarchicus* summarized in Kattner and Hagen (2009). Diapause duration was estimated as the time it would take to respire the lipid to at least 20%. Therefore, minimum wax ester

content is 20% ($\delta = 0.2$) of the maximum capacity as suggested by Saumweber and Durbin (2006). We refer to the supplementary information in Visser et al. (2017) for full details of the model equations.

Estimate of carbon sequestration

The two factors that contribute to carbon sequestration during the period of overwintering are respiration and mortality. The estimated respiration rates were used to calculate the carbon sink overwintering copepods leave behind as respired CO_2 to compare with the previous estimates of the *C. finmarchicus* lipid pump (Jónasdóttir et al. 2015). The present estimate includes additional sequestration by stage C4 and C6 females, as well as a modest mortality not considered in the previous published lipid pump estimate.

Overwintering copepods face mortality due to predation, starvation and other causes. Only a few mortality rate estimates of overwintering *C. finmarchicus* are available, but they range from 0.004 d^{-1} for the Iceland Basin and the Irminger Sea (Gislason et al. 2007) and 0.007 d^{-1} for the Norwegian Sea (Bagøien et al. 2001). It is uncertain which fraction of these mortality estimates are due to predation, or non-predatory mortality or the estimate is not a mortality but advection loss. We here use a conservative estimate of 0.001 d^{-1} as non-predatory mortality that directly adds both structural and lipid bound carbon to the carbon flux on the cost of respired carbon. This non-predatory mortality is likely to differ between habitats but in lack of better knowledge we keep it constant in our calculations.

Statistics:

Differences between depths and areas were tested with one way ANOVA and if not normally distributed with Kruskal-Wallis ANOVA on ranks. When significant differences were observed, a pairwise post-

198 hoc Dunn's and Holm-Sidak tests were conducted on nonparametric and parametric data, respectively.
199 Multiple regression was run on log transformed WE data due to non-normal distribution in relation to
200 month, depth, temperature and size.
201
202 **Results:**
203 *Prosome length*
204 The size of the overwintering C5s *C. finmarchicus* differed between the geographic areas (One way
205 ANOVA on ranks, $H = 179.55$; $df = 7$; $p < 0.001$; Fig. 2a). In general, size did not differ between the
206 depth layers within an area with the exception being the individuals at the surface and at 500-1000m
207 depth in the Eastern Norwegian Sea and the two depth layers in the North Sea, surface individuals being
208 smaller in both areas. Detailed statistics of the comparison within areas is shown in Supplementary
209 Table 1. Two main size groups were observed, the larger C5s off East Greenland and the Iceland Sea
210 (2.47± 0.02 and 2.43 ± 0.03 mm ± *SE*; n=98 and 15 respectively) and smaller individuals in the Irminger
211 Sea and the North Sea (2.16 ± 0.02 and 2.22 ± 0.02 mm, $n = 39$ and 37 respectively). In the other basins
212 the C5s measured between 2.3 and 2.4 mm and did not statistically differ from the smaller and larger
213 groups. In Fig. 2a we additionally plot published sizes of *C. finmarchicus* C5 from the Labrador Sea
214 (Pepin and Head 2009) which are comparable with the larger group in off East Greenland and Iceland
215 Sea. Non-significant differences of the basin means are shown in Fig. 2a with the same lower letters
216 over the bars based on Holm-Sidak all pairwise comparisons.
217
218 *Lipid content*
219 Most *C. finmarchicus* collected were at stage C5. Individual lipid content of stage C5 differed between
220 the basins (Fig. 2b), and were highest off East Greenland where the average wax ester (WE) content per

individual measured 155 ± 7 µg ind^{-1} (± *SE*). The lowest content was observed in the Irminger Sea, Iceland Basin and the North Sea (51 ± 7, 51 ± 4 and 44 ± 6 µg WE ind^{-1} ± *SE*). As lipid storage capacity is a function of size (Fig. 3) the WE content was standardized to prosome volume (Fig. 2c). The copepods in the Greenland Sea had the highest standardized value while the copepods in the Irminger Sea had the lowest lipid content per prosome volume. The differences in standardized lipid content were not significant between most of the basins with the exception of the Greenland Sea copepods. However, the standardized lipid content in the Western Norwegian Sea and Iceland Sea were not significantly different from the East Greenland copepods (Holm-Sidak all pairwise multiple comparison).

The average triacylglycerol (TAG) content in the copepods was generally low ranging from 0.1 to 4.8 µg ind^{-1} (Table 2), with statistical difference between the basins ($H = 153.174$; $df = 6$; $p < 0.001$). Posthoc Dunn's Method on all pairwise comparison showed that the difference were due to statistically higher TAG content ($p < 0.05$) in copepods from the Norwegian Sea (East and West) and North Sea compared to the Irminger Sea, Iceland Sea, Iceland Basin and Rockall Basin. TAG was not measured in copepods off East Greenland.

Total lipids in C5s ranged from being 27-56% of dry weight, except for Irminger Sea (6.2-7.8%), and wax esters from 54-92% of total lipids (Figure 4, Table 2). The general trend in lipid accumulation was over 50% of the total dry mass off East Greenland, the Iceland Sea and Norwegian Sea (East and West) but between 30 and 40% in Rockall and the Iceland Basins and the North Sea. In most areas 80% of the total lipids were composed of WE with the exception of the North Sea where the WE composition was 54%.

There were 2 distinctive temperature systems in the 7 basins; the areas where the deep water was at about 0°C (EGR, ICS, WNS and ENS) and the areas where the water was >4°C (IRM, ICB, ROC) (Fig. 5a). Standardized wax ester content was generally higher in copepods residing in colder deep water mass (white bars in Fig. 5b). This difference was statistically significant at most depths, with the exception at the average 875 m depth (statistics shown in the figure and in supplementary Table 2A). The wax ester content was also higher in the cold water group when binned at their ambient temperature, with significant differences at temperatures from 2-8 °C (see statistics in the figure and in supplementary Table 2A), but the difference between the groups was not significant in ambient waters of >8 °C reflecting the surface waters in the cold water group (Fig. 5c).

The most frequent sampling of *C. finmarchicus* lipid content was from the Faroe Shetland Channel. The winter WE content in stage C5 *C. finmarchicus* at >600 m depth differed statistically between years from 1997-2003 (One Way ANOVA: $F_7 = 4.8$, $p < 0.001$) where the WE content during 99-00 was higher than in 93-94, 94-95 and 95-96 (Holm-Sidak Pairwise Multiple comparison t = 3.9, 3.8 and 3.8 and P= 0.004, 0.005 and 0.005 respectively) (Fig. 6A). When standardized to size the difference in WE content (WE/Vol Fig 6B) in 99-00 was higher than 94-95 (Kruskal-Wallis One Way ANOVA on ranks H_6=16.14, P=0.013, Pairwise Multiple comparison Q=3.43, P=0.13). Combining monthly measures from all sampling years a difference was observed for WE content (Kruskal-Wallis One Way ANOVA on ranks H_5=16.84, P=0.005) (Fig. 6C) while Dunn´s post-hoc test could not isolate the groups that caused that difference (all pairs with P> 0.08). No measurable statistical difference could be observed in standardized WE content (WE/Vol) between the months.

Diapause duration and carbon sequestration

267 The carbon vital mass (C_{max}) and maximum wax ester content (w_{max} as carbon) as a function of prosome
268 length are shown in Figure 7, where $\alpha = 19$ and $\beta = 14$. Therefore, the maximum WE carbon content that
269 best fitted the data, w_{max}= 14 PL^3 and the structural mass, C_{max} =19 PL^3. The minimum WE carbon
270 content w_{min} is presented as 20% of the w_{max}.
271
272 Stage based individual respiration rates varied 3 fold from 0.65 (C4 in ENS) to 2.30 µgC d^{-1} (Female in
273 LS) dependent on size and temperature (Table 3). This is similar to the reported values for dormant *C.*
274 *finmarchicus* at 0°C from the Faroe Shetland Channel in December and January of 0.1 to 0.7 µgC d^{-1}
275 (Ingvarsdóttir et al. 1999) and 0.4-0.9 µgC d^{-1} at 6°C (Hirche 1983). Maximum estimated overwintering
276 period for C5 *C. finmarchicus,* assuming minimum WE reserve level of 20%, was shortest in the
277 Rockall Basin and North Sea (89 and 81 days respectively), Iceland Basin (119 days), and longest in the
278 Iceland Sea (204 days).
279
280 Integrating the respiration rate over diapause duration and stage abundance gives the respired carbon
281 ranging from 0.4 to 8.8 gC m^{-2} (Fig.8). This value was highest in the Eastern Norwegian Sea due to high
282 copepod abundance and long diapause duration (187 days for C5). Estimated mortality added about 12-
283 25% to the respired carbon flux as combined structural and lipid bound carbon resulting values as high
284 as 11.5 gC m^{-2}.
285
286 **Discussion:**
287 The present study comprises the most extensive geographic, horizontal and vertical coverage of *Calanus*
288 *finmarchicus* winter-lipid content to date. It represents a collage of a 6 month overwintering period
289 (September to February) spanning over 8 different years. Our data synthesis has revealed a clear and

290 unique picture of the different thermal habitats of this species in the Subpolar North Atlantic basins and
291 offers a valuable resolution of overwintering copepods for further modelling of diapause duration (*cf.*
292 Pierson et al 2013). Admittedly, the study does not cover all overwintering areas of this species, with
293 notable exception being the populations in the deeper waters off Newfoundland and the Scotian Shelf.
294 As open-ocean winter sampling is treacherous and haphazard due to bad weather conditions, only a few
295 earlier winter data were available that we could use for our analysis in the present study.
296
297 The results have implication for our understanding of the role of advection in the population dynamics
298 of *C. finmarchicus* in the central Atlantic. The prosome lengths of the Irminger Sea C5 *C. finmarchicus*
299 in diapause were smaller (<2.2mm) than expected considering the observed C5 prosome length of 2.4-
300 3.0 mm in the upper 100 m in the Irminger Sea during late spring and summer (Yusuf and Webster
301 2008; Jónasdóttir personal observations). The overwintering sizes of the C5s correspond well to the ones
302 in the Iceland Basin, but not in the Iceland Sea and off the Greenland coast. Therefore, it is possible that
303 the Irminger Sea overwintering population may originate from the Iceland Basin, while the summer and
304 spring surface populations are advected along with the Greenland Coastal current from the Greenland
305 Sea. The low lipid content in copepods sampled deeper than 500 m in November and December in the
306 Irminger Sea and the Iceland Basin comprise less than 21% (ca 43 µgC indiv^{-1}) of the estimated
307 maximum WE content for that size (w_{max} = 192 µgC indiv^{-1}). Therefore, based on the assumption of the
308 model of 20% remaining for ascent, the copepods would not survive the overwintering period and will
309 probably not be able to make it to the surface in spring by lipid driven buoyancy assisted force. The lipid
310 reserve would last about 40 days with the estimated respiration rate of 1.26 µgC day^{-1} during diapause at
311 5 °C. Therefore, these individuals are probably lost from the spring recruitment except if they have
312 other assisted forces (such as upward mixing by the aid of the deep convection layer) to bring them to

313 the surface in spring or the copepods have even lower metabolic rates than we use in our model
314 predictions.
315
316 It is clear from the present observations that size matters. Comparing *C. finmarchicus* in the basins with
317 ~4°C overwintering habitats, the C5s in the Labrador Sea were larger than the C5s in the Irminger and
318 Iceland Basins. Being large is beneficial in warmer overwintering waters, having more lipid storage
319 capacity and relatively lower metabolic rates. Our calculations show that the Labrador Sea copepods can
320 survive a whole month longer overwintering periods (142 d) than those in the Irminger (121 d) and
321 Iceland Basin (119 d) copepods.
322
323 A month can make a huge difference for survival in spring. Large differences are observed in the
324 remaining lipid reserves of *C. finmarchicus* in spring between Eastern and Western Subpolar North
325 Atlantic basins. In the Norwegian Sea Basins and Iceland Sea to the East, the lipid reserves after ascent
326 are about 150 µg WE ind^{-1} for a C5 and 80-100 for a C6 female (Jónasdóttir 1999; Jónasdóttir et al.
327 2008) allowing gonad development to take place before the spring bloom (Niehoff et al. 1999) and
328 initiate some lipid based spawning (Richardson et al. 1999; Jónasdóttir et al. 2008). In the Labrador Sea
329 and Disko Bay, West Greenland final gonad maturation and hence spawning do not take place until after
330 the initiation of the spring bloom. In Disko Bay the females loose about 47% of their WE (from 75µg to
331 40 µg) before the spring bloom starts, but do not seem to direct their energy into development of gonads
332 or to produce eggs, indicating lipid catabolism is used for basic metabolism only (Niehoff et al. 2002;
333 Swalethorp et al. 2011). The bet-hedging strategy of pre-bloom spawning used by the populations on
334 the eastern side of the Atlantic appear not to be beneficial for the western populations. The timing of the

spring bloom is therefore critical for the Western Atlantic populations and may play an important role in the success of populations with shorter overwintering periods.

Jónasdóttir et al. (2015) and Visser et al. (2017) showed the importance of copepod respiration during overwintering for the overall carbon sequestrations of the oceans and named it the lipid pump. The lipid pump estimates for *C. finmarchicus* in Jónasdóttir et al. (2015) were based on the model approach of Saumweber and Durbin (2006) using their respiration rates but otherwise the same *C. finmarchicus* data presented here (compared in Fig. 8). However, Jónasdóttir et al. (2015) did not include any mortality in the estimates of carbon sequestration and included only stage C5. Here, we use the most recent method presented in Visser et al. (2017) based on metabolic theory as presented in Maps et al. (2014) which results in higher estimates of the lipid pump than in Jónasdóttir et al. (2105). The Saumweber's approach used in Jónasdóttir is nonlinear while Visser's approach is linear.

Diapause duration is a crucial factor in life history of overwintering copepods (Dahms 1995, Pierson et al. 2013). While it is challenging to estimate from field observations the modelled durations used in our lipid pump estimates are consistent with those few observations that do exist. Our model resulted in diapause durations between 90 and 205 days which is slightly shorter but comparable to the field based estimates of Melle et al. (2014) and in good agreement with the model estimates of Maps et al. (2014) while falling mid-way between the estimates of Saumweber and Durbin (2006) and Johnson et al. (2008). Our carbon sequestration estimates are on average 44% higher than presented Jónasdóttir et al. (2015) due to longer diapause duration, mortality (structural and lipid mass carbon) and higher population abundance due to inclucion of C4s and females. The two models show calculations within the same order of magnitude in the respired based carbon, with the largest difference between the

358 models in the coldest water masses (ICS, WNS and ENS). Respiration is the largest contributor to
359 carbon sequestration by diapausing *C. finmarchicus*, but even low mortality rate add 10-30% to the
360 estimates of respired carbon.
361
362 The calculations of the lipid pump and the potential diapause duration face two significant uncertainties
363 that can potentially be resolved by future research: overwintering respiration rates and the relationship
364 between prosome length and lipid levels. Indeed, *C. finmarchicus* appears to have particularly low
365 metabolic rates (Maps et al 2014) and fast development rates (Banas and Campbell 2016) compared to
366 other copepod species. Lowering of the metabolic rates to even lower as shown for *C. finmarchicus*
367 sized copepods in Maps et al (2014) would result in lower respiration rates, but in turn also slower lipid
368 catabolism allowing longer duration of diapause. Relationships between body size, temperature and
369 overwintering respiration rates have now been established for a number of copepod species (Maps et al.,
370 2014) but a large amount of uncertainty remains about the precise relationships and whether they are
371 geographically consistent. Furthermore, it is unclear and possibly impossible to establish if the
372 respiration rates, which are estimated using diapausers brought to the surface, are truly reflective of
373 those in deep waters of up to 1000 m.
374
375 Similarly, the relationship between prosome length and maximum wax ester levels in ours and other
376 studies are variable and potentially imply that there is significant geographic range in this relationship.
377 For example, the relationships in Miller et al. (2000) and Saumweber and Durbin (2006) give much
378 higher storage lipid estimates than observed in other studies while that of by Pepin and Head (2009) fits
379 our data very well. The model of diapause duration reported here therefore differs from those of Wilson
380 et al. (2016) and Saumweber and Durbin (2006) due to being derived from different prosome length-wax

ester data sets. Future work should therefore establish if this geographic variation in the relationship between prosome length and lipids is due to regional adaptations to differing overwintering habitats or to variation in growth conditions before diapause.

C. finmarchicus being a key species in the North Atlantic food web makes prediction of the fate of the species with warming of the North Atlantic crucial. While warming of the deep overwintering habitats are not seen as an imminent threat in the deep ocean basins, the warming of the surface waters is predicted to increase more rapidly in the Arctic and Subarctic regions compared to other oceanic regions (IPCC 2014). *C. finmarchicus* populations appear to be moving further north with the warmer currents (e.g. Chust et al. 2014) with implications for export of carbon flux across the whole North Atlantic Basin (Brun et al. 2019). However, while overwintering habitats may stay stable and within the tolerable range for successful overwintering, the warmer surface waters will inevitably result in faster growth and smaller sized copepods (Campbell et al. 2001, Forster and Hirst, 2012). Maps et al. (2014) cautioned against using surface conditions during growing seasons only for estimating climate impacts on diapausing copepods. However, warming of the surface waters will affect overwintering indirectly by reduced lipid storage capacity that may in turn affect the maximum duration of diapause (Pierson et al. 2013), similar to what the present study shows to be the case in the Irminger Sea and Iceland Basin. The importance of warmer surface water for overwintering is therefore more related to the reduced lipid storage capacity that may in turn affect the maximum duration of diapause has currently an unforeseen consequence for this key species across the Northern North Atlantic ecosystem.

Acknowledgements:

This work was funded by the EU MAS3 program TASC (MAS3_CT95-0039) (SHJ, MRH, AG), The Scottish Executive Environment and Rural Affairs Department project MF0754 (MRH), the Danish Council for Strategic Research under the NAACOS (SHJ) and RW was supported by the UK Natural Environment Research Council [grant number NE/P005985/1] as part of the Changing Arctic Ocean Programme DIAPOD project. We thank the captains and the crew on the research vessels Dana, Scotia and Bjarni Sæmundsson, and Anna Ingvarsdóttir, Steve Hay, John Dunn, Mikkel Sand, Hildur Pétursdóttir and Anna Rósa Böðvarsdóttir and many others for their assistance on the cruises.

References:

Arnkværn, G., M. Daase, and K. Eiane. 2005. Dynamics of coexisting *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* populations in a high-Arctic fjord. Polar Biology **28**: 528-538.

Bagøien, E., S. Kaartveidt, D. L. Aksnes, and K. Eiane. 2001. Vertical distribution and mortality of overwintering Calanus. Limnol. Oceanogr. **46**: 1494–1510.

Banas, N. S., and R. G. Campbell. 2016. Traits controlling body size in copepods: separating general constraints from species-specific strategies. Mar. Ecol Progr. Ser. **558:** 21-33.

Bergvik, M., O. Leiknes, D. Altin, K. R. Dahl, and Y. Olsen. 2012. Dynamics of the lipid content and biomass of *Calanus finmarchicus* (copepodite V) in a Norwegian Fjord. Lipids **47**: 881–95.

Brun, P., K. Stamieszkin, A. W. Visser, P. Licandro, M. R. Payne, T. Kiørboe. 2019. Climate change has altered zooplankton-fuelled carbon export in the North Atlantic. Nature Ecology & Evolution.

Campbell, R. G., M. Wagner, G. Teegarden, C. Boudreau, and E. Durbin. 2001. Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. Mar. Ecol. Prog. Ser. **221**: 161–183.

Chust, G., C. Castellani, P. Licandro, L. Ibaibarriaga, Y. Sagarminaga, X. Irigoien. 2014. Are *Calanus* spp. shifting poleward in the North Atlantic? A habitat modelling approach. ICES J. Mar. Sci. **71**:241–253,

Clark, K. A. J., A. S. Brierley, and D. W. Pond. 2012. Composition of wax esters is linked to diapause behavior of *Calanus finmarchicus* in a sea loch environment. Limnol. Oceanogr. **57**: 65–75.

Conover, R. J. 1988. Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. Hydrobiologia **167–168**: 127–142.

Dale, T., E. Bagøien, W. Melle, and S. Kaartvedt. 1999. Can predator avoidance explain varying overwintering depth of *Calanus* in different oceanic water masses? Mar. Ecol. Prog. Ser. **179**: 113–121.

Dahms, H.U., 1995. Dormancy in the Copepoda—an overview. Hydrobiologia, 306:199-211.

Dunn, J., C. Hall, M. R. Heath, R. Mitchell, and B. Ritchie. 1993. ARIES - a system for concurrent physical, biological and chemical sampling at sea. Deep. Res. I **40**:867–878.

Falk-Petersen, S., J. R. Sargent, and K. S. Tande. 1987. Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol. **8**: 115–120.

Forster, J., and A. G. Hirst. 2012. The temperature-size rule emerges from ontogenetic differences between growth and development rates. Functional Ecology **26**:483-492.

Gislason, A., K. Eiane, and P. Reynisson. 2007. Vertical distribution and mortality of *Calanus finmarchicus* during overwintering in oceanic waters southwest of Iceland. Mar. Biol. **150**: 1253–1263.

Gislason, A., and T. Silva. 2012. Abundance, composition, and development of zooplankton in the Subarctic Iceland Sea in 2006, 2007, and 2008. ICES J. Mar. Sci. **69**: 1263–1276.

Halvorsen, E., K. S. Tande, A. Edvardsen, D. Slagstad, and O. P. Pedersen. 2003. Habitat selection of overwintering *Calanus finmarchicus* in the NE Norwegian Sea and shelf waters off Northern Norway in 2000-02. Fish. Oceanogr. **12**: 339–351.

Head, E., and P. Pepin. 2007. Variations in overwintering depth distributions of *Calanus finmarchicus* in the slope waters of the NW Atlantic continental shelf and the Labrador Sea. J. Northwest Atl. Fish. Sci. **39**: 49–69.

Heath, M. R., P. Boyle, A. Gislason, W. Gurney, S. Hay, E. J. H. Head, S. Holmes, A. Ingvarsdóttir, S. H. Jónasdóttir, and P. Lindeque. 2004. Comparative ecology of over-wintering *Calanus finmarchicus* in the northern North Atlantic, and implications for life-cycle patterns. ICES J. Mar. Sci. **61**: 698–708.

Heath, M. R., J. G. Fraser, A. Gislason, S. J. Hay, S. H. Jónasdóttir, and K. Richardson. 2000. Winter distribution of *Calanus finmarchicus* in the Northeast Atlantic. ICES J. Mar. Sci. **57**: 1628–1635.

Heath, M. R., and others 2008. Spatial demography of *Calanus finmarchicus* in the Irminger Sea. Prog. Oceanogr. **76**: 39–88.

Hirche, H.-J. 1983. Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. Mar. Ecol. Prog. Ser. **11**: 281–290.

Hirche, H.-J. 1996. Diapause in the marine copepod, *Calanus finmarchicus* - A review. Ophelia **44**: 129–143

Ingvarsdóttir, A., D. F. Houlihan, M. R. Heath, and S. Hay. 1999. Seasonal changes in respiration rates of copepodite stage V *Calanus finmarchicus* (Gunnerus). Fish. Oceanogr. **8** (Suppl 1): 73–83.

IPCC (2014). Climate change 2014: Synthesis report. In R. K. Pachauri, L. A. Meyer & Core Writing Team (Eds.), Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change (p. 151). Geneva, Switzerland: IPCC.

Johnson, C. L., A. W. Leising, J. A. Runge, E. J. H. Head, P. Pepin, S. Plourde, and E. G. Durbin. 2008. Characteristics of *Calanus finmarchicus* dormancy patterns in the Northwest Atlantic. ICES J. Mar. Sci. **63**:339–350.

Jónasdóttir, S. H. 1999. Lipid content of *Calanus finmarchicus* during overwintering in the Faroe–

Shetland Channel. Fish. Oceanogr. **8**: 61–72.

Jónasdóttir, S. H., K. Richardson, M. R. Heath, A. Ingvarsdóttir, and A. Christoffersen. 2008. Spring production of *Calanus finmarchicus* at the Iceland–Scotland Ridge. Deep Sea Res. Part I Oceanogr. Res. Pap. **55**: 471–489.

Jónasdóttir, S. H., A. W. Visser, K. Richardson, and M. R. Heath. 2015. Seasonal copepod lipid pump promotes carbon sequestration in the deep North Atlantic. Proc. Natl. Acad. Sci. **112**: 12122–12126.

Kattner, G., and W. Hagen. 2009. Lipids in marine copepods: Latitudinal characteristics and perspective to global warming, p. 257–280. *In* M. Kainz, M.T. Brett, and M.T. Arts [eds.], Lipids in Aquatic Ecosystems. Springer New York.

Kattner, G., and M. Krause. 1989. Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. Mar. Chem. **26**: 261–275.

Køgeler, J., S. Falk-Petersen, Å. Kristensen, F. Pettersen, and J. Dalen. 1987. Density-and sound speed contrasts in sub-Arctic zooplankton. Polar Biol. **7**: 231–235.

Krumhansl, K. A., E. J. H. Head, P. Pepin, S. Plourde, N. R. Record, J. A. Runge, and C. L. Johnson. 2018. Environmental drivers of vertical distribution in diapausing *Calanus* copepods in the Northwest Atlantic. Progr. Oceanogr. **162**: 202-222.

Lee, R. F., W. Hagen, and G. Kattner. 2006. Lipid storage in marine zooplankton. Mar. Ecol. Prog. Ser. **307**: 273–306.

Madsen, S., T. Nielsen, and B. Hansen. 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., N. R. Record, and A. J. Pershing. 2014. A metabolic approach to dormancy in pelagic copepods helps explaining inter-and intra-specific variability in life-history strategies. J. Plankton Res. **36:**18-30.

Maps, F., J. A. Runge, A. Leising, A. J. Pershing, N. R. Record, S. Plourde, and J. J. Pierson. 2011. Modelling the timing and duration of dormancy in populations of *Calanus finmarchicus* from the Northwest Atlantic shelf. J. Plankton Res. **34**: 36-54.

Melle, W., and others. 2014. The North Atlantic Ocean as habitat for *Calanus finmarchicus*: Environmental factors and life history traits. Prog. Oceanogr. **129**: 244–284.

Miller, C., J. Crain, and C. Morgan. 2000. Oil storage variability in *Calanus finmarchicus*. ICES J. Mar. Sci. **57**: 1786–1799.

Miller, C., C. Morgan, F. Prahl, and M. Sparrow. 1998. Storage lipids of the copepod *Calanus finmarchicus* from Georges Bank and the Gulf of Maine. Limnol. Oceanogr. **43**: 488–497.

Niehoff, B., U. Klenke, H.-J. Hirche, X. Irigoien, R. Head, and R. Harris. 1999. A high frequency time series at Weathership M, Norwegian Sea , during the 1997 spring bloom : the reproductive biology of *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. **176**: 81–92.

Niehoff, B., S. D. Madsen, B. W. Hansen, and T. G. Nielsen. 2002. Reproductive cycles of three dominant *Calanus* species in Disko Bay, West Greenland. Mar. Biol. **140**: 567–576.

Pepin, P., and E. J. H. Head. 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. Part I Oceanogr. Res. Pap. **56**: 989–1002.

Pierson, J. J., H. Batchelder, W. Saumweber, A. Leising, and J. Runge. 2013. The impact of increasing temperatures on dormancy duration in *Calanus finmarchicus*. J. Plankton Res. **35:**504-512.

Plourde, S., P. Joly, J. A. Runge, B. Zakardjian, and J. J. Dodson. 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.

Pond, D. W., G. Tarling, P. Ward, and D. J. Mayor. 2012. Wax ester composition influences the diapause patterns in the copepod *Calanoides acutus*. Deep Sea Res. Part II **59–60**: 93–104.

Rasband, W. ImageJ 1997-2008. http://rsb.info.nih.gov/ij/.

Richardson, K., S. H. Jónasdóttir, S. Hay, and A. Christoffersen. 1999. *Calanus finmarchicus* egg production and food availability in the Faroe–Shetland Channel and northern North Sea: October–March. Fish. Oceanogr. **8**: 153–162.

Runge, J. A., R. Ji, C. R.S. Thompson, N. R. Record, C. Chen, D. C. Vandemark, J. E. Salisbury, F. Maps. 2015. Persistence of *Calanus finmarchicus* in the western Gulf of Maine during recent extreme warming, J. Plankton Res.**37:**221–232

Saumweber, W. J., and E. G. Durbin. 2006. Estimating potential diapause duration in *Calanus finmarchicus.* Deep Sea Res. Part II Top. Stud. Oceanogr. **53**: 2597–2617.

Swalethorp, R., S. Kjellerup, M. Dünweber, T. Nielsen, E. Møller, S. Rysgaard, and B. Hansen. 2011. Grazing, egg production, and biochemical evidence of differences in the life strategies of *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* in Disko Bay, western Greenland. Mar. Ecol. Prog. Ser. **429**: 125–144.

Visser, A. W., J. Grønning, and S. H. Jónasdóttir. 2017. *Calanus hyperboreus* and the lipid pump.

Limnol. Ocean. **62**:1155-1165.

Visser, A. W., and S. H. Jónasdóttir. 1999. Lipids , buoyancy and the seasonal vertical migration of *Calanus finmarchicus*. Fish. Oceanogr. **8**: 100–106.

Vogedes, D., Ø. Varpe, J. E. Søreide, M. Graeve, J. Berge, and S. Falk-Petersen. 2010. Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods. J. Plankton Res. **32**: 1471–1477.

Wilson, R. J., N. S. Banas, M. R. Heath, and D. C. Speirs. 2016. Projected impacts of 21st century climate change on diapause in *Calanus finmarchicus*. Glob. Chang. Biol. 1–9.

Yusuf, A. A., and L. Webster. 2008. Seasonal variation in the physical characteristics of the copepod *Calanus finmarchicus* (Gunnerus) along the North Atlantic. J. Biol. Sci. **8**: 95–100.

549 **Figure Legends:**

550 **Figure 1.** Sampling locations for lipid content of *C. finmarchicus* during winter. The dotted lined boxes
551 surround the different ocean basins IRM: Irminger Sea, EGR: East Greenland, ICS: Iceland Sea, ICB:
552 Iceland Basin, ROC: Rockall Basin, WNS: Western Norwegian Sea, ENS: Eastern Norwegian Sea, NS:
553 North Sea. LS: Labrador Sea from which literature data were obtained.

554

555 **Figure 2.** *Calanus finmarchicus*, average ± *SE* of a) prosome length (mm) b) wax ester content (µg
556 individual^{-1}) and c) Wax ester per unit prosome volume (µg mm^{-3}) in 4 different depth layers across the
557 Subpolar North Atlantic basin (from light grey to black, 0-250, 250-500, 500-1000m and >1000m depth
558 layers). The same lower case letters above the clusters of bars indicate no significant difference between
559 the basins as tested by Dunnet's post hoc pairwise comparison. LS: size from Pepin and Head (2009).
560 Ocean Basin abbreviations as in Figure 1.

561

562 **Figure 3.** Wax ester content (µg individual^{-1}) of *C. finmarchicus* as a function of prosome length (mm).
563 Current study: black dots. Comparison to other studies open diamonds: Miller et al. (2000) their
564 equation: oil sac volume OSV = 0.5822 PL – 1.0208 adjusted to µg WE; open circles: Pepin and Head
565 (2009) only showing their maximum measures of the lateral oil sac area adjusted to WE using the
566 equation of Vogedes et al. (2010); open downwards triangles: Bergvik et al. (2012) transposing oil sac
567 volume to WE by using 900µg/mm^3 as suggested by Miller et al. (1998); and open upwards triangles:
568 Vogedes et al. (2010 in supplementary information).

569

570 **Figure 4**. *C. finmarchicus* total lipid (TL) and wax ester (WE) content as % fraction of dry weight (DW)
571 ± *SE*, in the different North Atlantic Basins. Abbreviations same as in Table 1. The WE fraction of total

lipids is listed at the end of the bars. For EGR the WE proportion is assumed to be the same as in the ICS.

Figure 5. A) Temperature and depth for the samples collected and analysed for lipids from all cruises. B) Mean (dotted line), median (solid line) and 5th and 95th percentile of WE/Vol (µg mm^{-3}) in C5 *C. finmarchicus* at different B) depth layers and C) temperature ranges. Open symbols and white boxes: sampling in EGR, ICS, WNS and ENS; grey symbols and boxes: sampling in IRM, ICB, ROC and NS. Results from One-Way ANOVA on Ranks: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Figure 6. Average (dotted line), median (solid line) and 5th and 95th percentile of WE (µg ind^{-1}) content and WE/Vol (µg mm^{-3}) in stage C5 *C. finmarchicus* from >600 m depth in the Faro Shetland Channel during A) and B) 8 winters (September to March); C and D) 6 winter months all years averaged. *n*: number of samples behind in the analyses, the same lower case letters above the bars in A and B indicate a statistical difference.

Figure 7. Wax ester content (ω [µgC]) as function of prosome length (PL [mm]) across stages C5 for *C. finmarchicus*. Solid dots are the maximum WE content as carbon per unit length from the present study. Power law regressions to the observations (black dotted line, excluding the largest individuals with relatively low lipids) suggest ω[µgC] = 2.18 PL$^{4.79}$ ($R^2 = 0.82$). Superimposed (black solid lines) are estimates of the maximum and minimum carbon based wax ester content $\omega_{max} = \beta PL^3$ and $\omega_{min} = \delta\beta PL^3$ respectively, and carbon based vital (structural) mass $C_{max} = \alpha PL^3$ based on isometric scaling (se methods for details). Carbon mass regressions in light grey dotted lines are results from Ingvarsdóttir et al. (1999), Madsen et al. (2001) and Swalethorp et al. (2011).

Figure 8. Regional estimates of the carbon flux associated with overwintering populations of *C. finmarchicus*. Estimates are broken down as to respired flux (grey fill), mortality structural mass all stages (green fill) and mortality lipid mass all stages (dark grey fill). Regional estimates Labrador Sea (LS), Irminger Sea (IRM), East Greenland (EGR), Iceland Sea (ICS), Iceland Basin (ICB), Rockall Basin (ROC), E Norwegian Sea (ENS), W Norwegian Sea (WNS) and North Sea (NS). White line indicates contribution of stage C5 to the flux. Parallel shaded column is the results from Jónasdóttir et al. (2015) based on C5 flux only without mortality.