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

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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.

Prosome length (PL) of animals was measured to the nearest 0.01mm. Length measurements of *Calanus finmarchicus* from a Faroe Shetland Channel transect (Dana cruises in 1993-1995) area and Irminger Sea have been presented as grouped averages in Jónasdóttir (1999) and Heath et al. (2008), respectively. All lipid analysis was carried out using Iatroscan TLC as described in Jónasdóttir (1999). Lipid content was standardised to prosome volume (PV) in mm^3 according to Miller et al. (2000): $PV = 0.0292 \times PL^{3.6699}$ where PL is the average prosome length in mm for the copepodite stage at the corresponding sample location and depth. Size measurements and lipid analyses were not performed on the same individuals.

Oil sac area. Copepods from the Greenland Sea were photographed, after which the area of the lipid sac was traced using the “image-J” software (Rasband , 1997– 2009). The pixel-to-mm ratio was calibrated using the image of the appropriate calibration slide. The lipid sac area was converted into wax esters by 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 sac in mm^2.

C. finmarchiucs dry mass was measured from most sampling locations. Individual copepods were measured for prosome length, dried in batches of 3-10 individuals at 60°C and weighed on a Cahn 26 Automatic Electrobalance. The dry mass (DW) therefore includes lipids. In other instances dry mass was estimated from prosome length (PL) using the length/DW equation: $DW=0.0106 \times PL^{3.64}$, where DW is in mg and PL in mm based on the relationship of copepods from winter in the Norwegian Sea (Ingvarsdóttir et al. 1999). Carbon mass was estimated using the equation: $C=0.623 \times DW - 0.0143$ from Ingvarsdóttir et al. (1999).

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 posthoc Dunn's and Holm-Sidak tests were conducted on nonparametric and parametric data, respectively. Multiple regression was run on log transformed WE data due to non-normal distribution in relation to month, depth, temperature and size.

Results:

Prosome length

The size of the overwintering C5s *C. finmarchicus* differed between the geographic areas (One way ANOVA on ranks, $H = 179.55$; $df = 7$; $p < 0.001$; Fig. 2a). In general, size did not differ between the depth layers within an area with the exception being the individuals at the surface and at 500-1000m depth in the Eastern Norwegian Sea and the two depth layers in the North Sea, surface individuals being smaller in both areas. Detailed statistics of the comparison within areas is shown in Supplementary Table 1. Two main size groups were observed, the larger C5s off East Greenland and the Iceland Sea (2.47± 0.02 and 2.43 ± 0.03 mm ± *SE*; n=98 and 15 respectively) and smaller individuals in the Irminger Sea and the North Sea (2.16 ± 0.02 and 2.22 ± 0.02 mm, $n = 39$ and 37 respectively). In the other basins the C5s measured between 2.3 and 2.4 mm and did not statistically differ from the smaller and larger groups. In Fig. 2a we additionally plot published sizes of *C. finmarchicus* C5 from the Labrador Sea (Pepin and Head 2009) which are comparable with the larger group in off East Greenland and Iceland Sea. Non-significant differences of the basin means are shown in Fig. 2a with the same lower letters over the bars based on Holm-Sidak all pairwise comparisons.

Lipid content

Most *C. finmarchicus* collected were at stage C5. Individual lipid content of stage C5 differed between 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

The carbon vital mass (C_{max}) and maximum wax ester content (w_{max} as carbon) as a function of prosome length are shown in Figure 7, where $\alpha = 19$ and $\beta = 14$. Therefore, the maximum WE carbon content that best fitted the data, $w_{max}= 14\ PL^3$ and the structural mass, $C_{max} =19\ PL^3$. The minimum WE carbon content w_{min} is presented as 20% of the w_{max}.

Stage based individual respiration rates varied 3 fold from 0.65 (C4 in ENS) to 2.30 µgC d^{-1} (Female in LS) dependent on size and temperature (Table 3). This is similar to the reported values for dormant *C. finmarchicus* at 0°C from the Faroe Shetland Channel in December and January of 0.1 to 0.7 µgC d^{-1} (Ingvarsdóttir et al. 1999) and 0.4-0.9 µgC d^{-1} at 6°C (Hirche 1983). Maximum estimated overwintering period for C5 *C. finmarchicus,* assuming minimum WE reserve level of 20%, was shortest in the Rockall Basin and North Sea (89 and 81 days respectively), Iceland Basin (119 days), and longest in the Iceland Sea (204 days).

Integrating the respiration rate over diapause duration and stage abundance gives the respired carbon ranging from 0.4 to 8.8 gC m^{-2} (Fig.8). This value was highest in the Eastern Norwegian Sea due to high copepod abundance and long diapause duration (187 days for C5). Estimated mortality added about 12-25% to the respired carbon flux as combined structural and lipid bound carbon resulting values as high as 11.5 gC m^{-2}.

Discussion:

The present study comprises the most extensive geographic, horizontal and vertical coverage of *Calanus finmarchicus* winter-lipid content to date. It represents a collage of a 6 month overwintering period (September to February) spanning over 8 different years. Our data synthesis has revealed a clear and unique picture of the different thermal habitats of this species in the Subpolar North Atlantic basins and offers a valuable resolution of overwintering copepods for further modelling of diapause duration (*cf.* Pierson et al 2013). Admittedly, the study does not cover all overwintering areas of this species, with notable exception being the populations in the deeper waters off Newfoundland and the Scotian Shelf. As open-ocean winter sampling is treacherous and haphazard due to bad weather conditions, only a few earlier winter data were available that we could use for our analysis in the present study.

The results have implication for our understanding of the role of advection in the population dynamics of *C. finmarchicus* in the central Atlantic. The prosome lengths of the Irminger Sea C5 *C. finmarchicus* in diapause were smaller (<2.2mm) than expected considering the observed C5 prosome length of 2.4-3.0 mm in the upper 100 m in the Irminger Sea during late spring and summer (Yusuf and Webster 2008; Jónasdóttir personal observations). The overwintering sizes of the C5s correspond well to the ones in the Iceland Basin, but not in the Iceland Sea and off the Greenland coast. Therefore, it is possible that the Irminger Sea overwintering population may originate from the Iceland Basin, while the summer and spring surface populations are advected along with the Greenland Coastal current from the Greenland Sea. The low lipid content in copepods sampled deeper than 500 m in November and December in the Irminger Sea and the Iceland Basin comprise less than 21% (ca 43 µgC indiv^{-1}) of the estimated maximum WE content for that size ($w_{\max}$ = 192 µgC indiv^{-1}). Therefore, based on the assumption of the model of 20% remaining for ascent, the copepods would not survive the overwintering period and will probably not be able to make it to the surface in spring by lipid driven buoyancy assisted force. The lipid reserve would last about 40 days with the estimated respiration rate of 1.26 µgC day^{-1} during diapause at 5 °C. Therefore, these individuals are probably lost from the spring recruitment except if they have other assisted forces (such as upward mixing by the aid of the deep convection layer) to bring them to the surface in spring or the copepods have even lower metabolic rates than we use in our model predictions.

It is clear from the present observations that size matters. Comparing *C. finmarchicus* in the basins with ~4°C overwintering habitats, the C5s in the Labrador Sea were larger than the C5s in the Irminger and Iceland Basins. Being large is beneficial in warmer overwintering waters, having more lipid storage capacity and relatively lower metabolic rates. Our calculations show that the Labrador Sea copepods can survive a whole month longer overwintering periods (142 d) than those in the Irminger (121 d) and Iceland Basin (119 d) copepods.

A month can make a huge difference for survival in spring. Large differences are observed in the remaining lipid reserves of *C. finmarchicus* in spring between Eastern and Western Subpolar North Atlantic basins. In the Norwegian Sea Basins and Iceland Sea to the East, the lipid reserves after ascent are about 150 µg WE ind^{-1} for a C5 and 80-100 for a C6 female (Jónasdóttir 1999; Jónasdóttir et al. 2008) allowing gonad development to take place before the spring bloom (Niehoff et al. 1999) and initiate some lipid based spawning (Richardson et al. 1999; Jónasdóttir et al. 2008). In the Labrador Sea and Disko Bay, West Greenland final gonad maturation and hence spawning do not take place until after the initiation of the spring bloom. In Disko Bay the females loose about 47% of their WE (from 75µg to 40 µg) before the spring bloom starts, but do not seem to direct their energy into development of gonads or to produce eggs, indicating lipid catabolism is used for basic metabolism only (Niehoff et al. 2002; Swalethorp et al. 2011). The bet-hedging strategy of pre-bloom spawning used by the populations on 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 models in the coldest water masses (ICS, WNS and ENS). Respiration is the largest contributor to carbon sequestration by diapausing *C. finmarchicus*, but even low mortality rate add 10-30% to the estimates of respired carbon.

The calculations of the lipid pump and the potential diapause duration face two significant uncertainties that can potentially be resolved by future research: overwintering respiration rates and the relationship between prosome length and lipid levels. Indeed, *C. finmarchicus* appears to have particularly low metabolic rates (Maps et al 2014) and fast development rates (Banas and Campbell 2016) compared to other copepod species. Lowering of the metabolic rates to even lower as shown for *C. finmarchicus* sized copepods in Maps et al (2014) would result in lower respiration rates, but in turn also slower lipid catabolism allowing longer duration of diapause. Relationships between body size, temperature and overwintering respiration rates have now been established for a number of copepod species (Maps et al., 2014) but a large amount of uncertainty remains about the precise relationships and whether they are geographically consistent. Furthermore, it is unclear and possibly impossible to establish if the respiration rates, which are estimated using diapausers brought to the surface, are truly reflective of those in deep waters of up to 1000 m.

Similarly, the relationship between prosome length and maximum wax ester levels in ours and other studies are variable and potentially imply that there is significant geographic range in this relationship. For example, the relationships in Miller et al. (2000) and Saumweber and Durbin (2006) give much higher storage lipid estimates than observed in other studies while that of by Pepin and Head (2009) fits our data very well. The model of diapause duration reported here therefore differs from those of Wilson 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.

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Figure Legends:

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

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

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

Figure 4. *C. finmarchicus* total lipid (TL) and wax ester (WE) content as % fraction of dry weight (DW) ± *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 $\omega_{\mathrm{max}} = \beta PL^3$ and $\omega_{\mathrm{min}} = \delta\beta PL^3$ 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.