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Lipid Composition of the Copepod *Calanus finmarchicus* (Gunnerus) from the Irminger Sea in the North Atlantic Ocean Changes with Season and Life Cycle Stages

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Abstract: Accumulation of lipid reserves in the copepod *Calanus finmarchicus* (Gunnerus) is very important as it is the determinant of the time to enter into diapause for the completion of the life cycle. Storage lipids also provide energy for other metabolic activities such as gonad development and egg lying. The major lipid classes in *C. finmarchicus* Wax Ester (WE) and Triglyceride (TG) were identified using HP-TLC with the major class being WE followed by TG, while HPLC-ELSD was used to quantify the storage lipid reserves. Total storage lipids were significantly different between life cycle stages (ANOVA, $p<0.05$) with C4 stages having the highest lipid content (78.80%) of body weight. On the whole, storage lipids varied significantly between seasons in the order spring<winter<summer. Between shallow water (<100 m) and mid depth water (101-1000 m), lipid contents increased but decreased at deep water (>1000 m), thus storage lipids did not differ significantly with sampling depth (ANOVA, $p>0.05$).

Key words: *Calanus finmarchicus*, HPLC-ELSD, wax esters, triglycerides, storage lipids

INTRODUCTION

The calanoid copepod *Calanus finmarchicus* at times constitutes 70-80% of the zooplankton population biomass in the North Atlantic Ocean and is an important link in the food web between primary producers and many of the commercially exploited fish species around the North Atlantic (Heath *et al*., 2000). *C. finmarchicus* feed on phytoplankton, the primary producers and in turn are the prey of various marine animals, thus making them a key component of the food web and their life cycles and population dynamics interact with the physical oceanographic system they inhabit (Webster *et al*., 2006).

Species of the copepod family Calanidae store lipids in a membrane-bound organ, the oil sac, which extends from near the back of the prosome forward into the cephalosome. The greater the quantity of oil, the farther the sac extends toward the anterior end, eventually filling over half of the volume of the prosome (Miller *et al*., 1998). In *C. finmarchicus*, large oil sacs are most characteristic of the fifth copepodite (C5) stage, which is predominant in the resting population of summer and autumn months.

Lee *et al*. (1971) showed that calanid storage lipids include very large fractions of waxes, esters of long-chain alcohols with long-chain fatty acids (Wax Esters, WE). Most of the WE molecules have one or more cis-double bonds in both chains, producing strong curvature and making packing less efficient. This lowers the melting point such that these waxes remain liquid at Deep Ocean and winter temperatures and at substantial pressure (Yayanos *et al*., 1978). Copepod storage lipids other than WE are mainly Triglyceride (TG) (Miller *et al*., 1998, 2000).

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The accumulation of storage lipids is a characteristic feature of the life cycle of *C. finmarchicus*, linked to its seasonal reproductive and vertical migration pattern (Jònasdòttir, 1999, Visser and Jònasdòttir, 1999; Fiksen, 2000; Falk-Peterson *et al*., 2000; Saito and Kotani, 2000). Lipid accumulation occurs in the surface waters during summer by copepodite development stage 4 and 5. *C. finmarchicus* survives over wintering, without feeding, in a semi dormant state, similar to diapause, at depths of 400-1500 m (Jònasdòttir, 1999; Fiksen, 2000; Falk-Peterson *et al*., 2000). The copepods are assumed to be neutrally buoyant while in their semi dormant state (Visser and Jònasdòttir, 1999). Lipid content has been implicated as a determinant of neutral buoyancy depth. In the spring, when the over winter survivors migrate back to the surface, any remaining lipids are utilized to supplement dietary nutrition and support reproduction. The aim of this study is to determine how storage lipids in *C. finmarchicus* vary between life cycle stages and season along the North Atlantic as this important copepod plays a vital role in the marine food web hence influencing the availability or not of commercially exploited fishes.

C. finmarchicus collected from different locations in the Irminger Sea, North Atlantic during winter of 2001 and 2002 and in the spring and summer of 2002 were analysed for seasonal changes in lipid content and composition with respect to their developmental stage and sampling depth.

A number of methods have been used to quantify lipids including TG and WE. But due to their high molecular weight, HPLC rather than GC must be used to separate lipid components. However, the main challenge here is that lipids lack chromotophore which means a derivatisation step is generally required to enable their detection using spectrophotometric techniques. Lipid classes have previously being quantified after TLC separation by Flame Ionisation Detection (FID) on an Iatroscan (Jònasdòttir, 1999; Visser and Jònasdòttir, 1999; Falk-Peterson *et al*., 2000). Neutral lipids in zooplankton were also analysed spectrofluorometrically using Nile red (Alonzo and Mayzaud, 1999).

Here, HPLC coupled with an evaporative light scattering detector (HPLC-ELSD) was used. The ELSD is a universal detector, which is sometimes referred to as a mass detector because it responds to a mass. An ELSD is capable of detecting any solute less volatile than the solvents and it enables lipids to be analyzed directly without derivatisation hence, preventing the risks of contamination and sample loss. However, one of the problems with the ELSD detector is its lack of linearity. This had improved in recent years and as a result these detectors are becoming widely used.

MATERIALS AND METHODS

Sample Collection

C. finmarchicus were collected from the Irminger Sea, in the North Atlantic during the winter of 2001 and 2002 and in the spring and summer of 2002. Figure 1 shows the sampling points during winter, spring and summer cruises, respectively. Zooplankton were collected using an Antarctic Reception Imagery for Enviroumental Studies (ARIES) sampling net with a mesh size of 200 μm (Dunn *et al*., 1993). Once on board *C. finmarchicus* were picked out immediately from the ARIES nets, sorted into sampling depth, stages of development i.e., copepodite stages 4, 5, 6 and sex (male and female) in sets of 10 over ice with a stereo dissecting microscope and then stored in cryo-vials flushed with nitrogen and immersed in liquid nitrogen. On return to shore the cryo-vials were stored in cryo-freezers (-170°C) until required for analysis (Webster *et al*., 2006).

Dry Weight Determination

The dry weights of individual copepods were determined gravimetrically following the method described by Ohman (1997) and modified for *C. finmarchicus* by Webster *et al*. (2006). Only intact animals were used and these were blotted against tissue to remove any excess water. Segmented Petri dishes containing pre-weighed tin discs with the individual copepods samples were transferred into

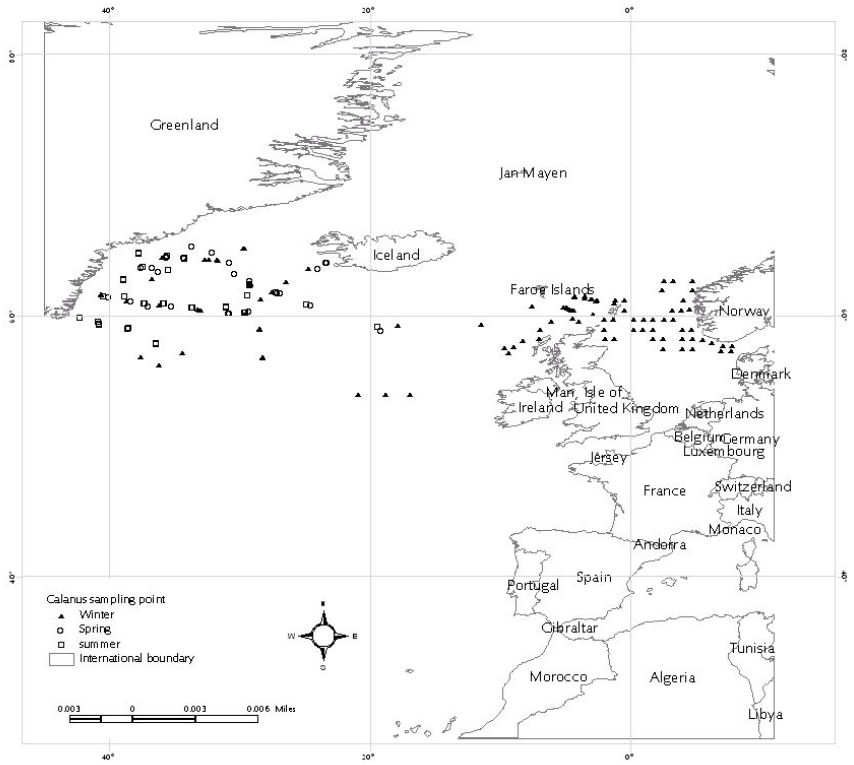

Fig. 1: Sampling points for winter, spring and summer *Calanus finmarchicus* in the Irminger Sea, North Atlantic

drying oven set at 60°C. Samples were allowed to dry for approximately 24 h, after which they are removed and allowed to cool to room temperature in a desiccator. The tin discs were reweighed and the percentage dry weight was calculated for each individual copepod. Five of the ten individual copepods in each sample were used for this procedure. The mean percentage of dry weight of the five was used when calculating the percentage of Wax Ester (WE) and Triglyceride (TG) for individual zooplankton.

Extraction of Lipids

Lipids were extracted using a modification of the Folch *et al.* (1957) method. 2,6-Di-tert-butyl-p-cresol (1.8 mg, Butylated Hydroxtoluene (BHT) was dissolved in chloroform/methanol (2:1 v/v; 180 mL). Samples, consisting of five zooplankton, were allowed to stand in the chloroform/methanol/BHT mixture (6 mL) for at least 24 h in a 15 mL screw top test tube for pooled samples, or a 2 mL screw top glass vial for single copepods, in a refrigerator (2-8°C). After this, aqueous potassium chloride (0.88% w/v, 1.5 mL) was added to form an emulsified mixture of 8:4:3 v/v/v chloroform, methanol and water. Centrifugation was used to separate the organic aqueous layers. The chloroform extracts were transferred into vials and evaporated under a gentle stream of charcoal scrubbed nitrogen. The extracts were then desiccated, for 12 h, to remove any additional water present. The lipid was re-suspended in iso-hexane (2 mL for pooled samples, 0.2 mL for single copepods) and the extracts stored at -25°C in 1.75 mL glass vials until required for analysis.

Qualitative Analysis of Lipid Classes Using High-Performance TLC (HP-TLC)

Excess gel was scrapped from the LHP-K linear HP-TLC plate (20×10 cm, Whatman Labsales, Maidstone, Kent and UK). Samples and standards were streaked on the preabsorbent layer of the plates at concentrations <5 mg mL^{-1}. The first solvent mixture, comprising iso-hexane/diethyl ether (70:30 v/v), was allowed to travel 4 cm beyond the preabsorbent layer before the plate was removed and the solvent allowed to evaporate. Once dried, the plate was redeveloped in iso-hexane/diethyl ether (97:3 v/v) until the solvent front was within 0.3-1.0 cm of the top of the plate. The solvent was again allowed to evaporate before final development to the top of the plate with *iso*-hexane. The lipids were visualised by spraying with copper (II) sulphate (10% w/v) in aqueous orthophosphoric acid (8% v/v) followed by heating at 180°C for 10 min.

Quantitative Lipid Class Analysis Using HPLC Coupled with ELSD (HPLC-ELSD)

An aliquot of the lipid extract (250 µL) was transferred into glass HPLC vial using a calibrated 250 µL syringe. This left a further 250 µL in case samples were too dilute or too concentrated and the analysis had to be repeated. This volume represents one eight of the total lipid extract obtained from the sample. To this was added the internal standard, Fatty Acid Methyl Ester (FAME) 22:5 (n-3), (10 µg mL^{-1}). The HPLC method was based on the method of Nordbäck and Lundberg (1999). A spherisob 3 µm Silica (100×4.6 mm) column was used for the separation, using a column heater set at 30°C. A Hewlett Packard 1050 series quaternary HPLC pump was used together with an ELSD (ELSD 1000 version 3, Polymer Laboratories). The system was also equipped with a Hewlett Packard 1050 Autosampler. Lipid classes were separated by gradient elution (Table 1) consisting of Tetrahydrofuran (THF)/iso-hexane (0.5% v/v) and THF/iso-propanol/iso-hexane (1:1:3 v/v). The flow rate was set at 0.6 mL min^{-1} and the injection volume was 20 µL. The oven temperature of the ELSD was set at 30°C whilst the nebuliser and evaporator temperatures were seen at 40 and 60°C, respectively. With each batch of samples eight calibration standards, containing triglyceride (tripalmitin) and a wax ester (Linoleyl behenate), in the range 1-50 µg mL^{-1} were analysed (0.02-1 µg on column). Each calibration standard contained the internal standard; FAME 22:5 (n-3), at a concentration of 10 µg mL^{-1}. The data were quantified using the Hewlett Packard Chemstation (Revision A. 02.02) data system. WE and TG were expressed as a percentage of the dry weight (% dw) per individual copepod.

Quality Control

Gloves were worn throughout the analytical procedure to prevent contamination from lipids on the skin. All analytical glassware were washed in a dishwasher using Camacid and Camclean (Camlab, Cambridge, UK) and then oven-dried at 85°C. After cooling and just prior to use, the glassware was rinsed with dichloromethane and then with iso-hexane, the latter being allowed to evaporate before proceeding. With each batch of samples, both cod liver oil Laboratory Reference Material (LRM) and an orange roughy oil LRM (1.0±1.0 mg) were analysed. Procedural blanks were also analysed with each batch of samples.

Table 1: HPLC solvent gradient used to elute wax esters and triglycerides from *C. finmarchicus*

Time (min)	Solvent (%) A	Solvent (%) B	Flow (mL min^{-1})
0	100	0	0.6
4	100	0	0.6
10	15	85	0.6
12	10	90	0.6
13	0	100	0.6
25	100	0	0.6

The cod liver oil (triglyceride) LRM and orange roughy oil (wax ester) LRM were analysed by HPLC-ELSD along with each batch following addition of the FAME 22:5 (n-3) internal standard. The percentage of triglyceride in the cod liver oil and the percentage of WE in the orange roughly oil were calculated. The data obtained from the LRMs were transferred onto NWA Quality Analyst and Shewhart charts were produced with warning and action limits being drawn at ±2 and ±3 SD of the mean.

RESULTS AND DISCUSSION

Due to the small number of animals (maximum of ten per sample station) available for this study, an accurate gravimetric determination of the total lipid was not practicable. Therefore, a chromatographic technique had to be developed and applied to quantify zooplankton storage lipids, following an appropriate extraction methodology. To enable the percentage of storage lipids to be determined for the *C. finmarchicus* the dry weights were measured. Dry weights were determined on individual intact copepods since free water can remain trapped between animals if pooled samples are used. Handling the copepods had to be minimised as damage to the copepods could affect the weight obtained. Therefore, dry weights were determined for five of the individual copepods from each sample of ten and the mean dry weight calculated. Dry weights ranged from 45.0-516.0 μg with a mean of 239.0 μg (SD = 88.0 μg, n = 136) and were dependent on the developmental stage. The mean dry weight for the copepods collected in spring, summer and winter were 217.1 μg (SD = 76.0 μg, n = 34), 254.1 μg (SD = 106.5 μg, n = 58) and 231.4 μg (SD = 79.1 μg, n = 34), respectively. Dry weights within each life cycle stage were broadly similar as samples were initially sorted according to their developmental stage and sex.

Most lipid extraction techniques, such as those of Folch *et al*. (1957) and Bligh and Dyer (1959), require at least 1 g of tissue. However, these techniques cannot be applied directly to the extraction of zooplankton due to their small size (i.e., <500 μg). The remaining five copepods (~ mg) from each sample of ten animals were used for the lipid extraction. A variation of the Folch *et al*. (1957) method was used, the samples being allowed to stand in chloroform/methanol mixture for atleast 24 h. Samples were not homogenised or mixed to avoid any losses of the copepods or associated lipids. Due to the small sample size, solvent volumes were reduced to <2 mL. Centrifugation was used to separate the organic and aqueous layers. Transferring the sample between glassware was kept to a minimum, to prevent loss of lipids. The number of glass vessels used during this procedure was also reduced as far as practicable to minimise loses.

Qualitative Analysis to Determine the Major Lipid Classes in *C. finmarchicus*

HP-TLC was used to establish the major lipid classes in *C. finmarchicus*. The HP-TLC confirmed that the major lipid classes in *C. finmarchicus* were WE followed by TG (Jònasdòttir, 1999; Visser and Jònasdòttir, 1999; Falk-Peterson *et al*., 2000). In all *C. finmarchicus* samples the WE band was split into two components on the HP-TLC plate. The R_f values of both components were close to the R_f of the wax ester standard and, therefore, were attributed to two WE groups. Two WE groups were also identified in copepods by Saito and Kotani (2000) with the first WE group being a less polar ester and the second component a more polar ester.

Quantitative Analysis of the Major Lipid Classes in *C. finmarchicus* by HPLC-ELSD

The total proportion of storage lipid by dry weight in *C. finmarchicus* was calculated using a sub-sample of the sample analysed for lipid classes. Results for total lipids are expressed as percentage dry weight (% dw) per individual. The HPLC method used underestimates the amount of

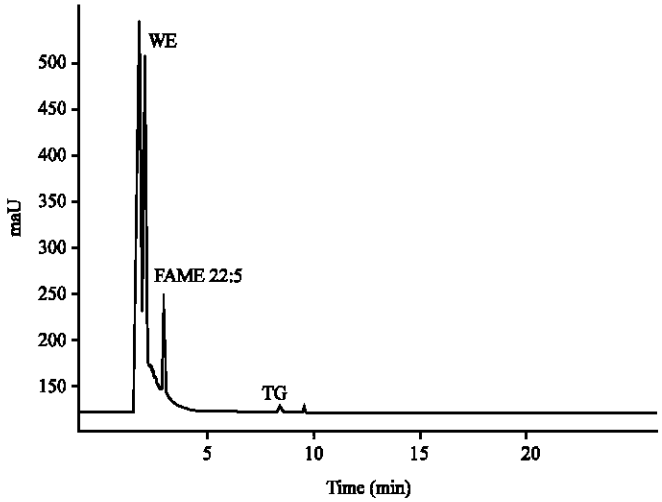

Fig. 2: HPLC-ELSD chromatogram showing the lipid class profile of a pooled (five individuals) copepod sample from the Irminger Sea. Two closely eluting peaks at 2.6 min are the Wax Esters (WE), the FAME internal standard (22:5) is at 3.6 min and the Triglyceride (TG) was eluted at 8.6 min.

triglyceride. Cod liver oil LRM was analysed with each batch of samples. Using an external standard method an average of 54.03% (SD = 0.06, n = 6) triglyceride was found in the LRM. The amount of triglyceride in cod liver oil should be close to 100%. Therefore this method underestimates the amount of triglyceride by approximately 45.97%. A possible explanation for this is that the exterual standard (tripalmitin) used for the quantification may give a different response on the ELSD compared to the natural triglycerides found in the samples. Orange roughy oil was used as an LRM for the wax ester component of the lipid. Orange roughy oil contains predominately wax esters. Using the same HPLC method and Linolely behenate as the external standard for the wax ester, a mean of 113% (SD = 0.08, n = 6) wax ester was found in the orange roughy oil. A typical chromatogram of the lipid class profile of a pooled (five individuals) *C. finmarchicus* is shown in Fig. 2. The two closely eluting peaks at 2.6 min are the Wax Esters (WE), the FAME (22:5) is at 3.6 min and the Triglyceride (TG) was eluted at 8.6 min.

Variation in Percentage Wax Ester (WE), Triglyceride (TG) and WE+TG Between Sample Stages

Only one C3 stage sample was analysed and gave a %WE of 0.27% (n = 1). In C4 animals, the % WE ranged from 0.83 to 74.84 (mean = 12.63, SD = 20.50, n = 12). In C5 animals the range was from 0.57% dw to a maximum of 77.40% dw with mean = 14.56% dw (SD = 13.02, n = 166). WE in C6 adults ranged from 0.50 to 60.71 % dw with a mean of 6.18% dw (SD = 9.55, n = 66). There was a significant difference between %WE in relation to sample stage (ANOVA, $p<0.05$). As expected the mean proportion of WE was highest in stage C4 and C5 as at these stages *C. finmarchicus* had accumulated lipids and are preparing to enter diapause. Whereas in adult C6, lipid reserves have been used for metabolic activities so as to cater for shortages after exit from diapause.

The %dw of TG in the ouly stage C3 zooplankton analysed was 6.27% dw (n = 1), in C4 TG ranged from 0.07% dw to 3.96% dw with a mean of 1.09% dw (SD = 0.99, n = 12). The range of TG in C5 animals was 0.0 to 1.55% dw, mean 0.25% dw (SD = 0.27, n = 166) while in C6 the range was 0.0 to 1.74% dw (mean = 0.29% dw, SD = 0.27, n = 66). Again there was a significant difference in the amount of TG with sample stage (ANOVA, $p<0.05$) (Fig. 3a-c).

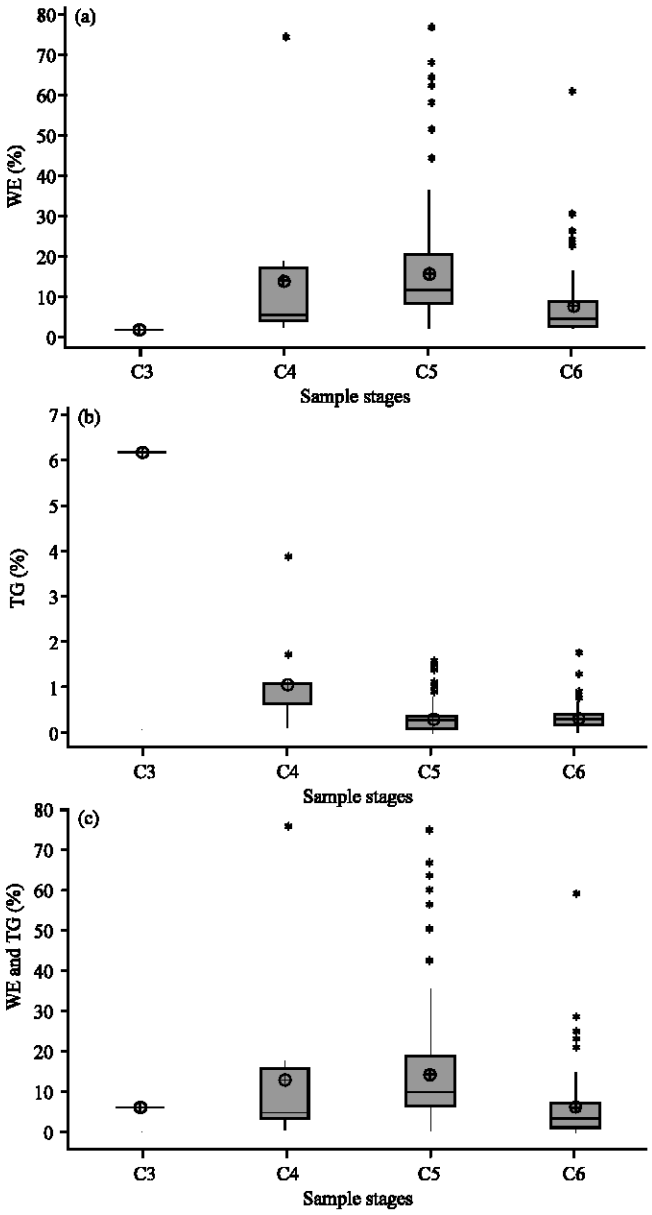

Fig. 3: (a) and (b) and (c) Box and whisker plots showing the % WE (a), (b) % TG and WE+TG (c) % between sample stages in *C. finmarchicus* from all seasons and depths. Circles represent the mean, horizontal lines cutting through the boxes represent the median. Asterisks represent outliers (values which are 1.5 times away from the range), C3, C4, C5 and C6 represents copepodite stages in *C. finmarchicus*. Note the high amount of TG in the only C3 sample analysed

Differences in % TG between sample stages could be attributed to the fact that in stages C4 and C5 lipid reserves are accumulated in preparation for diapause. In the adult stage (C6) TG is either converted into WE or used for metabolic activities e.g., gonad and egg formation (Miller *et al*., 1998).

Total storage lipids (WE+TG) in C3 was 6.47% dw (n = 1), in C4 WE+TG were in the range of 0.86 to 78.80% dw mean 13.64% dw (SD = 21.34, n = 12), in C5 it was between 0.61% dw to 77.52% dw (mean = 14.81%, SD = 13. 08, n = 166). In C6 total storage lipids ranged from 0.65% dw to 60.91% (mean = 6.47% dw, SD = 9.56, n =66) (Fig. 3a). Similar to the TG and WE, there was significant difference in total storage lipids with sample stage (ANOVA, $p<0.05$) with the highest maximum storage lipids in C4 (78.80% dw) and C5 animals (77.52%).

Total lipids in C3 were lower than those reported by Mayzaud *et al*. (1998) i.e., 6.54% dw as against the reported 12% dw. Although only one representative sample was measured which is not enough to make a population set for comparison. Storage lipids in C4 (mean = 13.63% dw, SD = 21.34, n = 12) were also lower than 17% dw reported by Mayzaud *et al*. (1998) for *C. propinquus* collected in the Indian Ocean between 1981 and 1995. In stage C5 animals, collected during this study, the total lipid (WE and TG) contents ranged from 10.35 to 77.52% dw (mean = 14.81, SD = 13.08, n = 166). Jónasdóttir (1999) reported total storage lipid (WE and TG), using Thin layer chromatography/flame ionisation detector ranging from 30-55% dw) when studying over wintering C5 and females stages of *C. finmarchicus* from the upper 1000 m along the Faroe- Shetland.

Variation in Percentage Wax Ester (WE), Triglyceride (TG) and WE+TG Between Seasons

The mean, media and range of WE, TG and WE +TG in *C. finmarchicus* from all areas and depth in winter summer and spring is shown in Fig. 4a-c.

In winter, over wintering *C. finmarchicus* are made mainly of the C5 stages with few adults from the previous year's stock. In spring, the population is made mainly of the adults (C6) who have emerged from diapause full of metabolic activities. The popnlation of *C. finmarchicus* in summer is made up of the C3, C4, C5 and the C6 adult stages (Heath *et al*., 2000).

The proportion of WE in winter (mainly C5 stages) ranged from 0.57 to 85.53% dw (mean = 13.50% dw, SD = 14.42, n = 91). TG were in the range of 0.00% dw to 1.55% dw (mean = 0.26% dw, SD = 0.28, n = 91). Total storage lipids had minimum of 0.61% to maximum of 86.05% dw (mean = 13.75% dw, SD = 14.54, n = 91).

The proportion of WE in spring (mainly C6 stages) ranged from 0.50 to 34.35% dw (mean = 4.35% dw, SD = 5.56, n = 69). Percentage of TG was in the range of 0.08 to 1.74% dw (mean = 0.45% dw, SD = 0.36, n = 69). Percentage of totals storage lipids (WE and TG) ranged from 0.65 to 35.04% dw (mean = 4.79% dw, SD = 5.64, n = 69) (Fig. 4a-c).

In summer, there was an increase in the amount of storage lipids with WE ranging from 0.27 to 77.40% dw (mean = 17.73% dw, SD = 15.12, n = 102), TG ranged from 0.00to 6.27% dw (mean = 0.31% dw, SD = 0.73, n = 102). The amount of total storage lipids nearly doubled those obtained in spring with a range of 0.86 to 78.80% dw (mean = 18.04% dw, SD = 15.24, n = 102) compared to the range of 0.65 to 35.04% (mean = 4.79%, SD = 5.64, n = 69) in spring. In spring *C. finmarchicus* (C5) emerge from diapause with their storage lipids depleted due to overwintering. In summer, *C. finmarchicus* contain a higher proportion of storage lipids as they accumnlate storage lipids then in preparation for decent in winter to enter diapause (Jónasdóttir, 1999).

A significant difference was found in the % storage lipid (WE) and total (WE+TG) per dry weight with season (ANOVA, $p<0.05$). However, the % dw of TG was not significantly different with season (ANOVA, $p>0.05$). As expected, the increase in WE between season was in the order spring <winter <summer. This trend shows copepods coming out of diapause in spring utilising their lipid reserves and well fed copepods in summer with accumnlated reserves ready to enter diapause. In winter, during diapause, the metabolic activities of copepods cease, hence using little of their lipid reserves.

In this study, total storage lipids in summer were higher than those in spring and winter. Thus deviating from the findings of Jónasdóttir (1999) who reported total storage lipid (WE and TG) contents of greater than 76% for over wintering C5 and female *C. finmarchicus*. Percentage storage lipids in spring were within the range of 30-35% dw as reported by Jónasdóttir (1999).

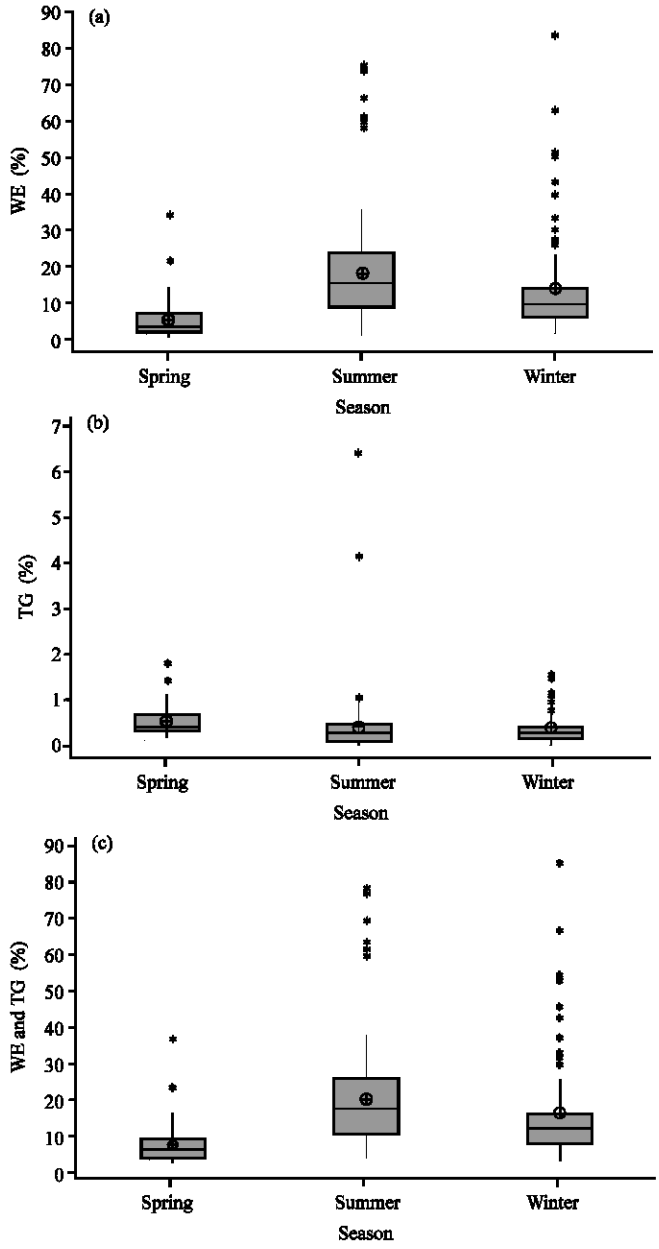

Fig. 4: (a), (b) and (c) Box and whisker plots of %WE (a), %TG (b) and %WE+TG (c) in *C. finmarchicus* from all areas and depths in spring summer and winter. Circles represents mean, horizontal lines represents the median, asterisk denotes outliers values lying 1.5 times away from the range. Upper and lower lines represent the first and third quartiles

The presence of high amounts of TG in summer (6.27% dw) in one C3 sample suggests an indication of feeding activity as TG is used in *Calanus* sp. as a dietary lipid (Hakanson, 1984). The significant difference between total storage lipid (WE+TG) and WE with season confirms the findings

of (Mayzaud *et al*., 1998; Jònasdòttir, 1999) and those by Miller *et al*. (2000) that storage lipids varies with season, with the highest proportion of storage lipids being found in summer and the lowest in spring.

Variation in Storage Lipids with Sampling Depth

In shallow water, WE ranges from 0.50 to 68.65% dw (mean = 10.61% dw, SD = 12.40, n = 90). TG had a minimum of 0.00% dw reaching a maximum of 1.74% dw (mean = 0.37% dw, SD = 0.36, n = 90). Total storage lipids (WE+TG) ranged between 0.70-69.24% dw (mean = 10.98% dw, SD = 12.44, n = 90).

In the mid depth layer (n = 106), there was an increase in the amounts of lipids, with WE ranging from 0.51 to 77.40% dw (mean = 13.90% dw, SD = 14.17). TG ranged from 0.00% dw to as high as 3.96% dw (mean = 0.28% dw, SD = 0.44). Percentage total lipids (WE and TG) were between 0.65 to 78.80% dw (mean = 14.17, SD = 14.34). With further increase in depth (n = 28) to the deeper water layer (>1000 m), amount of storage lipids decreased to a range of 0.57 to 29.30% dw for WE (mean = 8.64, SD = 6.63). TG was between 0.02 to 0.86% dw (mean = 0.19% dw, SD = 0.20), total storage lipids ranged from 0.61% dw to maximum of 29.59% dw (mean = 8.80% dw, SD = 6.63). However, the lipids contents did not differ significantly with depth (ANOVA, $p>0.05$). The results in shallow and mid depth, which revealed an increase in WE, TG and total storage lipids agree with previous studies like those by (Mayzaud *et al*., 1998; Jònasdòttir, 1999; Miller *et al*., 2000). Decrease in lipid contents with further increase in depth was not shown in previous studies because they did not collect samples beyond 1000 m. High amounts of WE in C4 (74.84% dw), C5 (77.40% dw) and in C6 (60.71% dw) (Table 2) which accounts for approximately 80% of total lipids per dw in *C. finmarchicus* agrees with findings in previous literature that WE was the major lipid component with maximum values of 70-80% according to (Lee *et al*., 1971; Miller *et al*., 1998; Falk-Peterson *et al*., 2000).

Despite the underestimation of TG by the HPLC system, the maximum range for total lipid content (TG and WE) presented here for summer (78.80%) were higher than those previously reported. However, the underestimated TG component is a minor component of the total lipid content. Lipid content is said to vary, with sampling depth and season. This was reported by Lee *et al*. (1971), while studying the distribution of lipids in marine copepods. Kaltner and Krause (1987) reported variation in storage lipids while studying change in lipids during development in *C. finmarchicus* from copepod to adult. Kaltner *et al*. (1989) also reported variation in storage lipid while studying the seasonal variation in lipids in calaniod copepods found in the North Sea. Similarly, Smith (1990) reported variations in lipid content while studying egg production and feeding by copepods prior to

Table 2: Minimum, maximum, mean ± 1 standard deviation from mean percentage of WE, TG and WE+TG per dry weight of individual *C. finmarchicus* based on sampling depth*

Lipid class	Depth	N	Minimum % dw	Median % dw	Maximum % dw	Mean±SD % dw
WE	Shallow	90	0.50	7.13	68.65	10.61±12.40
TG		90	0.00	0.27	1.74	0.37±0.36
WE+TG		90	0.70	7.35	69.24	10.98±12.44
WE	Mid depth	106	0.51	10.30	77.40	13.90±14.17
TG		106	0.00	0.19	3.96	0.28±0.44
WE+TG		106	0.65	10.39	78.80	14.17±14.34
WE	Deep	28	0.57	8.19	29.20	8.64±6.63
TG		28	0.02	0.07	0.86	0.19±0.20
WE+TG		28	0.61	8.24	29.59	8.80±6.63

*: N represents the number of samples, Deep samples were those collected >1000 m, mid depth (samples collected between 101-1000 m) while shallow samples were those collected from 0-100 m). Note results for TG were underestimated by the HPLC-ELSD

spring bloom in the Greenland Sea. Another reason conld be because a more quantitative method of detecting the storage lipids (WE, TG) using the HPLC-ELSD with external calibration standards was used here rather than the traditional Thin Layer Chromatography (TLC) used in previous studies which is more of a qualitative than quantitative method of analysis.

CONCLUSION

From this study, we were able to extract lipids from the zooplankton *C. finmarchicus* using a modification of (Folch *et al*., 1957; Bligh and Dyer, 1959) methods. With this modification sample lost and contamination was reduced to the barest minimum following the qnality control protocols earlier highlighted.

This study reveals that, total storage lipids vary significantly between sample stages and season (spring<winter<summer) but not with sampling depth. Shallow water (<100 m depth) and mid water (101-100 m depth) revealed an increase in WE, TG and total lipids (WE+TG). Although the HPLC-ELSD method used here underestimates the TG component of the *C. finmarchicus* it gave a good estimate of the WE which is the main storage lipid in this important copepod. And could serve as a very good quantitative way of measuring and assessing zooplankton lipids as it prove promising to giving as idea of how lipids in *C. finmarchicus* changes in the Irminger Sea with sample stages, season and depth.

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