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VARIABILITY IN STORAGE LIPIDS BETWEEN SPRING AND SUMMER *Calanus finmarchicus* (GUNNERUS) FROM THE IRMINGER SEA NORTH ATLANTIC OCEAN.

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ABSTRACT
Seasonal variation in lipid reserves of *Calanus finmarchicus* between seven different sampling zones namely; Central, North and Southern Irminger Sea, East Greenland Sea with characteristic current of Atlantic and Polar origin, Iceland Shelf, and Raykjanes Ridge (RR) were determined using High Performance Liquid Chromatography equipped with an Evaporative Light Scattering Detector (HPLC-ELSD). Wax ester (WE) and Triglycerides (TG) were the main lipids classes in *C. finmarchicus*. Along sampling zones in both spring and summer, the highest concentration of total storage lipids (WE+TG) were in samples from Central Irminger Sea (CIS) and the lowest in samples from Southern Irminger Sea (SIS). WE, TG as well as, the total storage lipids were significantly different between sampling zones and seasons, with WE in spring being lower than those in summer, and TG being higher in spring than in summer. Thus storage lipids varied along sampling zones between seasons in the Irminger Sea.

Key words: *C. finmarchicus*, HPLC-ELSD, Irminger Sea, North Atlantic, storage lipids

INTRODUCTION

Calanus spp. constitute a large population of the zooplankton biomass in the North Atlantic Ocean during summer (Fransz *et al.,* 1984) where they graze the phytoplankton stock efficiently and constitute a major prey for larval fish (Economou, 1991). *Calanus spp.* represents an important link in the marine food chain transferring energy from phytoplankton to zooplankton predators (Fransz *et al.,* 1991). Thus, *Calanus spp.* and their population dynamics interact with the physical oceanographic system they inhabit (Webster *et al.,* 2006; Yusuf & Webster, 2008; Yusuf *et al*., 2008).

Species of the copepod family Calanidae store lipids in a membrane-bound organ, the oil sac, which extends from the posterior end of the prosome forward into the cephalosome. The greater the quantity of oil, the further the sac extends toward the anterior end, eventually filling over half the volume of the prosome (Miller *et al*., 1998). Lee *et al*., (1970), 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 in Deep Ocean and winter temperatures and at substantial pressures (Yayanos *et al*., 1978). On the other hand, copepod storage lipids other than WE are mainly triglyceride (TG) (Lee *et al.,* 1970; Miller *et al.,* 1998; Miller *et al.,* 2000).

In *Calanus finmarchicus* (Gunnerus), accumulation of storage lipids is a characteristic feature of the life cycle, which is linked to seasonal reproductive and vertical migration patterns (Jònasdòttir, 1999; Visser & Jònasdòttir, 1999; Fiksen, 2000; Falk-Petersen *et al.,* 2000; Saito & Kotani, 2000). Lipid accumulation occurs in the surface waters during summer by copepodite development stages 4 and 5. *C. finmarchicus* survives over winter in diapause at depths of 400 – 1500 m (Jònasdòttir, 1999; Fiksen, 2000; Falk-Petersen *et al.,* 2000). The copepods are assumed to be neutrally buoyant while in diapause and their lipid content has been implicated as a determinant of the neutral buoyancy depth (Visser & Jònasdòttir, 1999). Recent studies (Campbell & Dower, 2003) provides evidence that lipid-based buoyancy of a copepod is highly unstable because slight changes in the percentage of dry mass would affect the buoyancy of a copepod. In the spring, when the overwinter survivors migrate back to the surface, remaining lipids are utilised to supplement dietary nutrition and fuel reproduction.

We studied the composition of storage lipids (WE, TG) between two seasons (spring and summer) in *C. finmarchicus* collected from seven different sampling zones in the Irminger Sea North Atlantic Ocean. We hypothesise that, variation exists in the storage lipids of *C. finmarchicus* found within the Irminger Sea in spring and summer respectively.

MATERIALS AND METHODS

Sampling *C. finmarchicus*: *C. finmarchicus* were collected using an Antarctic Reception Imagery for Environmental Studies (ARIES) sampling net with a mesh size of 200 µm (Dunn *et al.,* 1993) during the spring and summer of 2002 (Fig. 1). Copepods were collected based on encounters at a particular sampling station. Samples were collected from Central Irmiger Sea (CIS), Northern Irminger Sea (NIS), Southern Irminger Sea (SIS), East Greenland of Artic origin (EGC-A), East Greenland of Polar origin (ECG-P), Iceland Shelf (IcS) and Raykjanes Ridge (RR). Once on board *C. finmarchicus* were picked out immediately from the nets, sorted into sampling depth, stages of development (copepodite stages 4, 5, 6 and sexed into male and female) in sets of 10 over ice with a stereo dissecting microscope. These are then stored in cryo-vials flushed with nitrogen and immersed in liquid nitrogen. On return to shore, cryo-vials were stored in cryo-freezers at a temperature of -170 ^{o}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). Five intact copepods out of the ten from each sample vial were used for this procedure. These were blotted with tissue paper to remove any excess water and placed on a solvent rinsed pre-weighed 30 mm tin discs (Elemental Microanalysis Limited UK catalogue no. D1066) in segmented Petri dishes. These were then transferred into drying oven at 60 ^{o}C for 24 hours; Tin discs were then desiccated and reweighed to obtain the percentage dry weight which was used to calculate the concentration of lipids in each 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) dissolved in a chloroform/methanol mixture (2:1 v/v; 180 ml)). The remaining five zooplankton from those used for dry weight measurement, were extracted in chloroform/methanol/BHT mixture (6 ml) for at least 24 hours in a 15 ml screw-top test tube. After which, 1.5 ml aqueous potassium

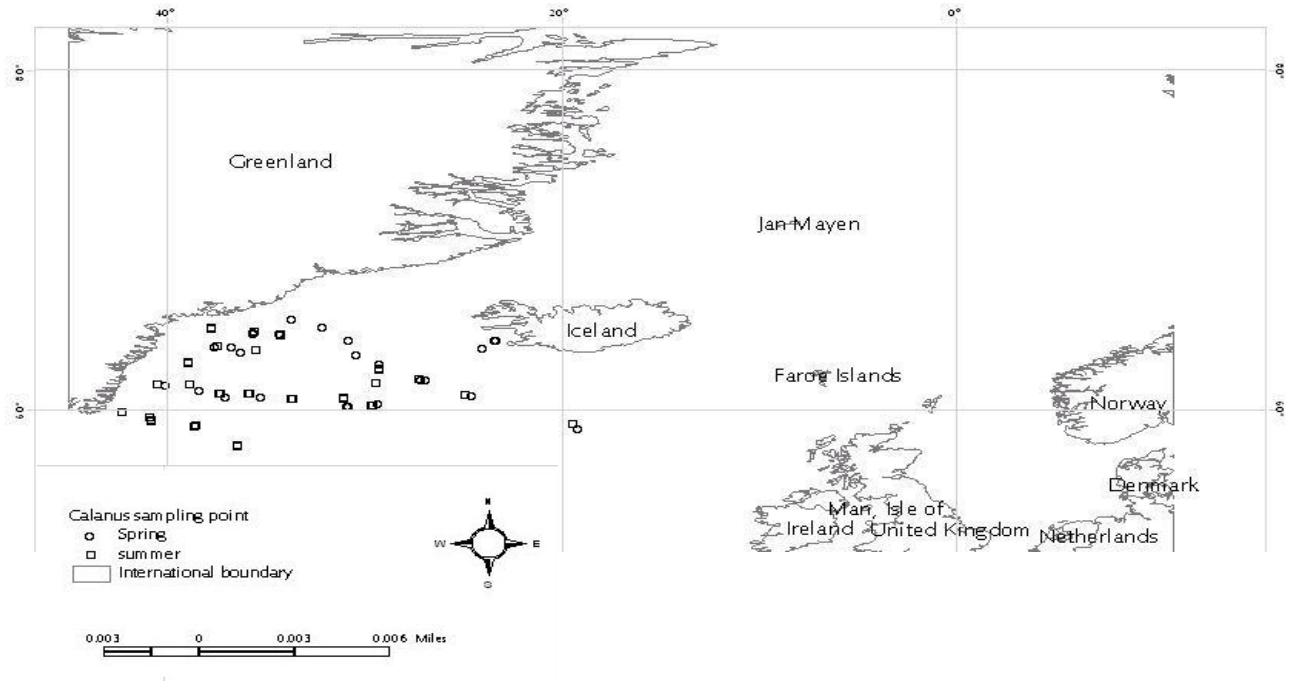

FIG. 1 SAMPLING POINTS FOR *Calanus finmarchicus* IN THE NORTH ATLANTIC DURING SPRING AND SUMMER CRUISES IN 2002. (Circles represent spring, while squares represents summer sampling points respectively).

chloride (0.88 % w/v) was added to form an emulsified mixture of 8:4:3 v/v/v chloroform, methanol and water. The mixture was then centrifuged to separate the organic aqueous layers. Chloroform extracts were transferred into 2 ml vials and evaporated under a gentle stream of charcoal scrubbed nitrogen, and desiccated for 12 hours. The resulting lipid extract was re-suspended in 2 ml *iso*-hexane and stored at –25 ºC until required for analysis.

Quantitative lipid class analysis using HPLC coupled with ELSD (HPLC-ELSD): This was carried out following the method described in Webster *et al.* (2006). About one eight of the total lipid extract (250 µl) was transferred into a HPLC vial using a calibrated syringe. 10 µg/ml of the internal standard Fatty acid methyl ester (FAME) 22:5 (n - 3) was added. A spherisob 3 µm Silica (100 mm ×4.6 mm) column was used for the separation, using a column heater set at 30 °C on a Hewlett Packard HPLC 1050 series quaternary HPLC pump equipped with an Evaporative Light Scattering Detector (ELSD 1000 version 3, Polymer Laboratories). Lipids were separated by gradient elution using Tetrahydrofuran (THF)/*iso*-hexane (0.5 % v/v), and THF/*iso*-propanol/*iso*-hexane (1:1:3 v/v) as a mobile phase. TG (Tripalmitin) and a WE (Linoleyl behenate), in the range 1 - 50 µg/ml were analysed (0.02 - 1 µg on column) as external standards. The concentration of WE and TG in the samples were calculated relative to the external standards and expressed as µg per individual copepod.

All data were subjected to Analysis of Variance (ANOVA) using Minitab statistical software (version 14).

RESULTS

Dry weights: Dry weights ranged from 45.0 to 516.0 µg with a mean of 239.0 ± 88.0 µg (n = 92), and this varied with developmental stages. The mean dry weight for the copepods collected in spring and summer were 217.1 ± 76.0 µg (n = 34) and 254.1 ± 106.5 µg (n = 64). Dry weights were significantly different (ANOVA, P < 0.05) between the two seasons.

Lipid classes between season and sampling zones: Mean concentrations of WE and TG in µg per individual and total storage lipids between season and sampling zones are shown in Table 1 and Fig. 2 for spring and summer, respectively.

TABLE 1. CONCENTRATION OF WAX ESTER (WE) AND TRIGLYCERIDE (TG) IN *C. finmarchicus* WITHIN SAMPLING SITES IN SPRING AND SUMMER*.

Lipid class	*Zone*	Season			
		Spring		Summer	
		N	*Mean (µg) ± SE*	*N*	*Mean (µg) ± SE*
WE	CIS	9	7.26 ± 1.41	21	23.91 ± 4.25
TG		9	0.69 ± 0.06	21	0.27 ± 0.04
WE	NIS	10	3.33 ± 0.81	5	13.56 ± 3.41
TG		10	0.58 ± 0.10	5	0.42 ± 0.08
WE	SIS	3	4.25 ± 1.67	4	11.36 ± 5.36
TG		3	0.70 ± 0.08	4	0.53 ± 0.07
WE	ECG-A	16	2.94 ± 1.08	36	14.34 ± 1.44
TG		16	0.54 ± 0.14	36	0.22 ± 0.03
WE	ECG-P	14	2.31 ± 0.93	9	19.07 ± 3.13
TG		14	0.37 ± 0.04	9	0.34 ± 0.08
WE	IcS	7	3.56 ± 0.27	1	68.65*
TG		7	1..29 ± 0.21	1	0.86
WE	RR	6	4.31 ± 1.22	16	16.39 ± 3.79
TG		6	0.96 ± 0.22	16	0.44 ± 0.05

* Note: Only one sample collected and analysed.
Values are means (µg) per individual copepod ± SE, N= number of samples, CIS = Central Irminger Sea, NIS = North Irminger Sea, SIS = Southern Irminger Sea, EGC-A and EGC-P = East Greenland Current of Atlantic and Polar origins, respectively, IcS= Iceland Shelf and RR = Raykjanes Ridge.

Concentration of storage lipids in spring and summer along sampling zones: *C. finmarchicus* collected in spring during the study contained WE in the range of 7.26 to 2.31 µg/individual copepod (Table 1). Concentration of WE in these samples were in the order CIS > RR > SIS > IcS > NIS > EGC-A > ECG-P respectively. In summer WE ranged from 11.36 – 23.90 µg/individual copepod (Table 1).

Amounts of WE between sampling zones in summer were in the order CIS > ECC-P > RR > ECG-A > NIS > SIS (Table 1). It is worth noting that; only one sample was collected from the IcS in summer which recorded 68.65 µg/individual copepod (Table 1). This was however not considered in the comparison due to small sample size. Overall, WE was significantly more in summer samples (ANOVA, $P < 0.05$, $N = 64$).

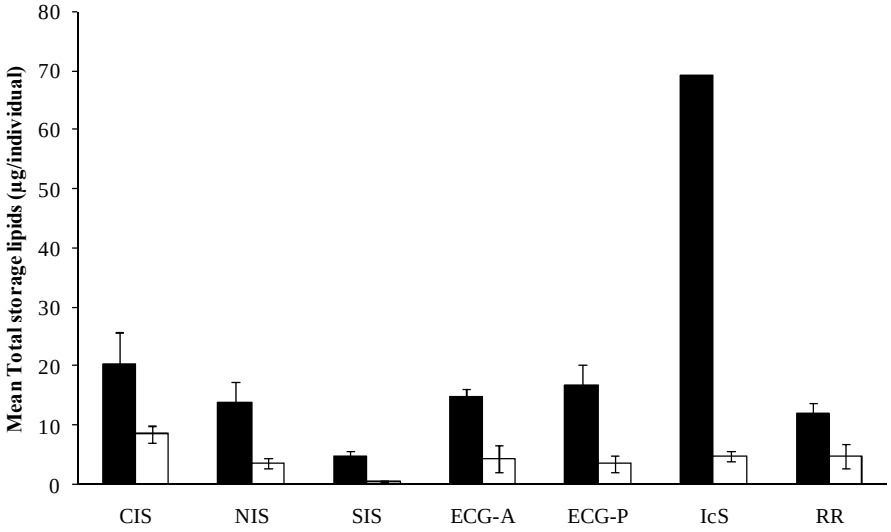

FIG. 2. CONCENTRATIONS OF TOTAL STORAGE LIPIDS IN SUMMER AND SPRING *C. finmarchicus* FROM THE SAMPLING ZONES IN THE NORTH ATLANTICOCEAN.

CIS = Central Irminger Sea, NIS = Northern Irminger Sea, SIS = Southern Irminger Sea, ECG-A = East Greenland of Atlantic origin, ECG-P = East Greenland of Polar origin, IcS = Iceland Shelf and RR = Raykjanes ridge. (Black filled bars represent total storage lipids in summer, while white bars represent total storage lipids in spring). Note only one sample was collected from IcS in summer.

TG in spring samples were between 0.37 to 1.29 µg/individual copepod; with samples from IcS, RR, SIS, CIS, NIS, ECG-A and ECG-P having concentration of TG in descending order respectively (Table 1). Concentration of TG in summer copepods were lower than those of spring ranging from 0.27 to 0.53 µg/individual copepod (Table 1). IcS was excluded because of problems with sample size.

Variability of total lipid contents in spring and summer samples between sampling zones: Fig. 2 compares the total lipid contents of *C. finmarchicus* in spring and summer between the sampling zones. In general, the variations in lipid contents exhibited within the sampling zones by season were significantly different (ANOVA, $P < 0.05$) with the total storage lipids in spring being lower than those for summer *C. finmarchicus* collected from all the sampling zones (Fig. 2). Among all the sampling zones CIS had the highest amount of storage lipids in both seasons, followed by ECG-P > ECG-A > NIS > RR > SIS.

DISCUSSION

Dry weights obtained in this study in the range of 45.0 – 516.0 µg/individual exhibits similar trends of increase in dry weights from one life cycle stage to the next as reported by Mayzaud *et al.,* (1998) when studying over wintering *C. sinmulimus* along the Atlantic Ocean.

Variation in the lipids contents of *C. finmarchicus* from the central, northern, and southern Irminger Sea could be explained by the findings of Pickard and Lavender *et al.,* (2000) on the deep convection which occurs periodically in the Irminger Sea. Another reason could be due to the variable strength of convection and the circulation on decadal timescales, which affects the thickness and properties of the Labrador Sea water (LSW) which rapidly enters the Irminger Sea on a timescale of six months (Sy *et al.,* 1997). This perhaps explain the high storage lipid contents in *C. finmarchicus* found in the Central Irminger in spring and summer because it is less affected by these decadal flows of the LSW than the north and south. The collection of mainly C5 stages copepods from the CIS and both C4 and C5 stages in summer (Yusuf *et al*., 2008) could also be a reason for the high amount of lipids within the CIS. Higher amount of storage lipids in *C. finmarchicus* from the EGC-A than in those from the EGC-P in spring could be explained by the collection of both C4, C5 and C6 copepod stages from the EGC-A where as in the EGC-P only C5 and C6 stages (Yusuf *et al*., 2008) were collected during the same sampling period. However, this was not the case in summer as the EGC-P had copepods with higher lipid contents. This was expected because during that sampling period, samples collected from the EGC-P were about four times those collected from the EGC-A. The RR serves as a route for an observed tight gyre which indicates a re-circulation cell with series of sub-basin scale (Lavender *et al*., 2000) which returns as a tight sub-polar gyre round the southern tip. This path which continues north up the western flank has a physical objective as it is believed to be the route for *C. finmarchicus* into the Iceland basin (Sy *et al*., 1997).

The observed amount of storage lipid in summer in the region suggest the presence of well fed *C. finmarchicus* ready to pass into the Iceland basin to overwinter as the ridge is not deep enough for such descent. Samples collected from IcS in spring had higher amounts of lipids than NIS, but due to the fact that only one sample was collected during summer, comparison was not possible with other sampling points. Reason for not collecting many samples from the IcS in summer could be due to the harsh weather and atmospheric conditions in the IcS during sampling (Astthorsoon & Vilhjalmsson, 2002) which affects the Marine

ecosystem seasonally especially in summer with fluctuations in temperature and salinity.

Results from the present study had shown that storage lipids in *C. finmarchicus* vary in spring and summer within the seven sampling points in the North Atlantic Ocean. With summer copepods containing more total storage lipids than spring an indication of well feed and matured adults in summer ready to go into diapause. This has further confirmed findings from our earlier work that; storage lipids in *C. finmarchicus* varied between copepod development stages and season as well as, the correlation between total copepod length and prosome length (see Yusuf & Webster, 2008; & Yusuf *et al*., 2008). However, there is the need for more studies aimed at sampling in all seasons with repeated sampling over years. Results from such studies will rule out the possibility of sampling bias and enable models to be generated which can be used to predict variability in storage lipids in *C. finmarchicus.* Modeling storage lipids in this important zooplankton can help boast commercial fisheries in the North Atlantic where fishes have being on the decline since the *el-nino* in 1990's.

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