Wax-ester mobilization by female *Calanus finmarchicus* (Gunnerus) during spring ascendance and advection to the Faroe Shelf

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Time of ascendance and initiation of reproduction in *Calanus finmarchicus* is closely correlated with the phytoplankton spring bloom. However, significant egg production can take place before the bloom, fuelled by mobilizing stored wax-ester lipids. *Calanus finmarchicus* from stations on the Faroe Shelf were compared with specimens collected off the shelf. Biological parameters such as gut contents, egg production, developmental stage, and lipid content were determined and correlated with phytoplankton concentration and spatial distribution along two transects in late April 2003 across the shelf northeast and southwest of the Faroe Islands. Grazing by *C. finmarchicus* on phytoplankton was significantly lower northeast of the Faroe plateau. However, the egg production was generally high for a pre-bloom situation, with significantly higher rates of egg production on the shelf than off it, along both transects. Wax-ester content of female *C. finmarchicus* was significantly higher and more variable at off-shelf stations than on the shelf (<2 μg female^{-1}). From this, we suggest that off-shelf *C. finmarchicus* had recently emerged from overwintering depths, in contrast to individuals from stations on the shelf, which had been in the upper water masses for some time. Females (from off-shelf stations) most likely supported the initial egg production from their wax-ester reserves.

Keywords: ascendance, *Calanus finmarchicus*, copepod, egg production, HPLC–ELSD, lipid mobilization, wax esters.

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Introduction

Ascent from hibernation habitats and reproduction by the oceanic copepod *Calanus finmarchicus* (Gunnerus) are correlated with the onset of the diatom spring bloom. Although around the Faroe Islands the bloom generally takes place in May (Gaard, 2003), significant egg rates (4–25 eggs female^{-1} d^{-1}) before the spring bloom have been reported (Richardson *et al.*, 1999; Debes *et al.*, 2005; Debes and Eliasen, 2006). It is likely that *C. finmarchicus* is not dependent on phytoplankton intake to initiate reproduction (Richardson *et al.*, 1999), but the fecundity of females is often positively correlated with food availability (e.g. Diel and Tande, 1992; Plourde and Runge, 1993; Nielsen and Hansen, 1995; Gaard, 1999; Niehoff *et al.*, 1999). As wax esters (WE) are the main storage lipid class in *C. finmarchicus*, a better knowledge of the dynamics of this lipid class is essential for understanding the life-history strategy and reproduction of this key calanoid species in the Faroe Shelf area.

The Faroe Islands are situated on a ridge extending from Greenland, over Iceland, to Scotland (Figure 1, upper panel), separating the Atlantic Ocean southwest of the ridge from the Norwegian Sea to the northeast. The hydrography of the Faroe Shelf is well described (Hansen and Østerhus, 2000), and the important features for *C. finmarchicus* are a clear separation between, and a variable exchange of, on- and off-shelf water masses (Gaard *et al.*, 1998; Gaard and Hansen, 2000). Variable exchange of the shelf water affects the composition and abundance of copepods on the shelf during spring and summer. When the inflow is low, the copepod community is dominated by neritic copepods, but during periods of high inflow, the oceanic copepod *C. finmarchicus* is abundant (Gaard, 1999, 2003; Debes and Eliasen, 2006).

Calanus finmarchicus that are advected onto the northern and southern Faroe Shelf may originate from two different water masses with different physical properties. The area to the north and northeast of the shelf is influenced by cold water which likely originates from the Norwegian Sea and the East Icelandic Current Waters (EICW). From the Faroe Bank Channel, warm Atlantic Water (AW) with high salinity is advected onto the western shelf. The temperature of the water masses is higher in the Faroe Bank Channel than north of the shelf, likely affecting the suggested delay in reproduction in the northern offshore areas compared with that in the south (Gaard and Nattestad, 2002). In most years, the spring bloom in Faroese waters commences earlier on the shelf in well-mixed water than off the shelf, where stratification of the water column is necessary for the bloom to begin (Gaard, 2000).

Jónasdóttir (1999) and Richardson *et al.* (1999) reported that WE in *C. finmarchicus* from this area primarily are used for moulting of

CVs into adult females, for gonadogenesis, and for oogenesis (see Lee *et al.*, 2006). Oocytes develop to gonadogenesis during winter and early spring, before the onset of the spring bloom (Plourde and Runge, 1993; Hirche, 1996). Although it is widely accepted that gonad formation may be fuelled by internal lipid reserves, several researchers have pointed out that ingestion is needed for final gonad maturation in several copepod species, including *C. finmarchicus* (Plourde and Runge, 1993; Rey *et al.*, 1999).

The effects of different water masses on the ascent, reproduction, and lipid patterns of *C. finmarchicus* populations during early spring were studied from transects across the Faroe Shelf system. We explored whether *C. finmarchicus* originating in the Norwegian Sea are delayed in ascent and reproduction compared with *C. finmarchicus* originating in the Faroe Bank Channel. We expected this delay, if present, to be reflected in (i) the developmental stage composition of *C. finmarchicus* on the shelf, (ii) the stage of female utilization of the stored WE, and (iii) the egg production at the time of sampling.

Material and methods

Biological and physical measurements were collected from 14 stations along two transects crossing the Faroe Shelf front (Figure 1) in a pre-bloom situation (25–29 April) in 2003 on a cruise aboard the RV "Magnus Heinason". The transects (Figure 1, lower panel) were selected to sample *C. finmarchicus* from different water masses north (T_1) and south (T_2) of the Faroe Shelf. Water masses along T_1 change from the Faroe Shelf Water (FSW) on the shelf to AW, probably influenced by EICW outside the shelf front. Deeper water strata consisted of cold Norwegian Sea Deep Water, which constitutes an overwintering habitat for *C. finmarchicus*. T_2 began on the southwestern part of the shelf (FSW) and extended southeast into the Faroe Bank Channel (AW).

A distinction between on- and off-shelf was determined from the descriptions of the shelf system (Gaard, 2000) and was set to follow approximately the 150-m bottom contour. This distinction was supported by hydrographic data (Figure 2). Stations 14, 14a, and 14b along T_1 and stations 54 and 55 along T_2 were considered to be off-shelf stations.

Salinity and temperature were determined from CTD profiles (Seabird Electronics SBE 911plus). Measured salinities were calibrated with an Autosal 8400A salinometer, using seawater samples collected from the deepest sampling depth at each station using 1.7 l Niskin bottles mounted on the CTD. *In situ*

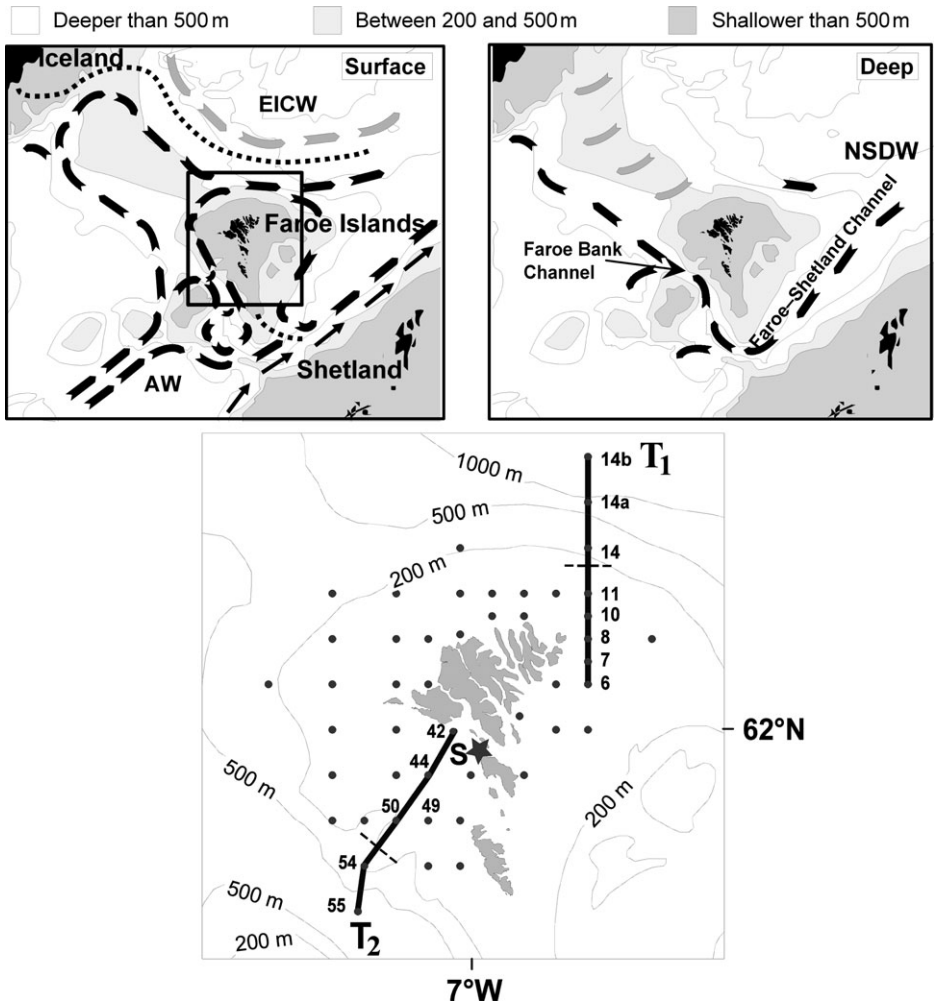

Figure 1. Location of the two transects (T_1 and T_2) on the Faroe Shelf with dominant ocean currents. EICW, East Atlantic Current Water; NSDW, Norwegian Sea Deep Water. Thick black dashed lines indicate separation of on- and off-shelf stations. S indicates Station S.

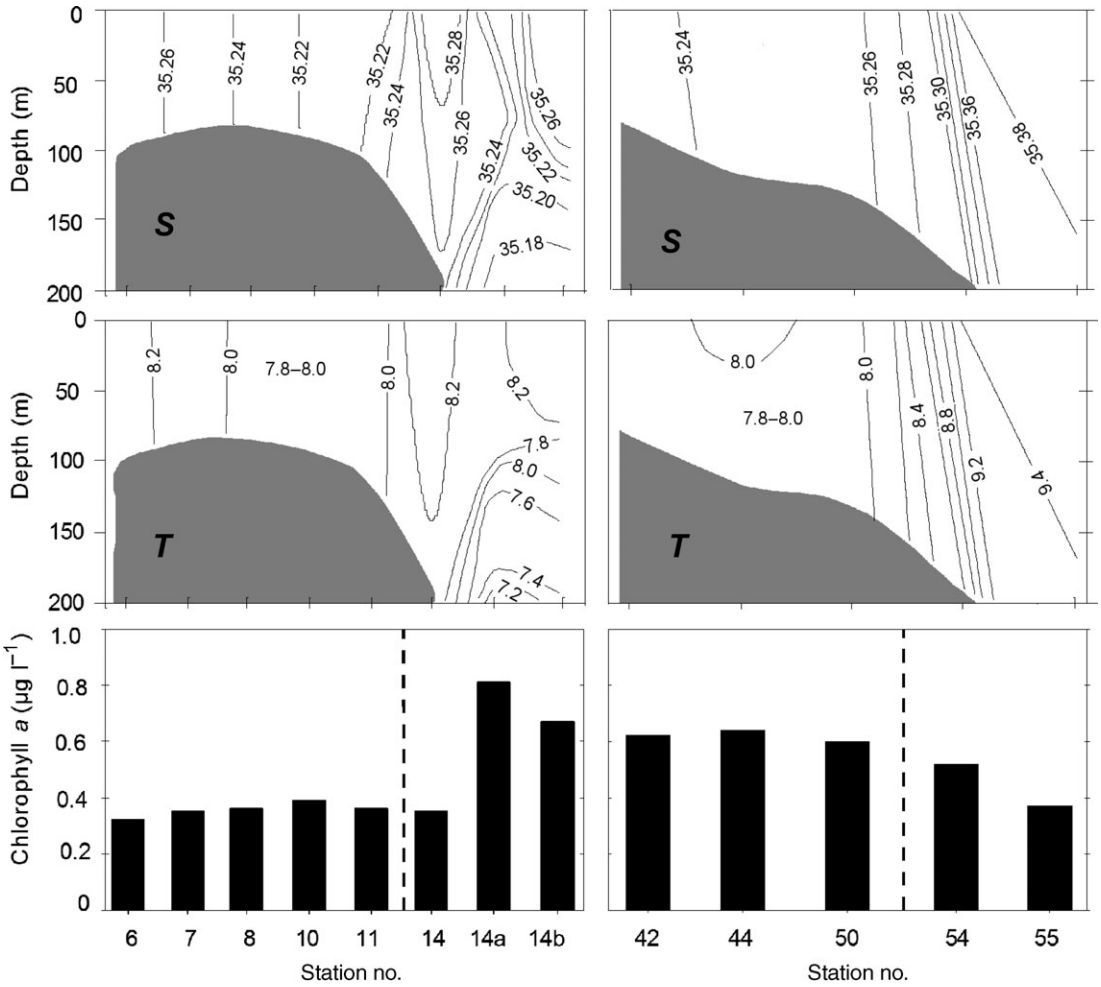

Figure 2. Salinity (*S*), temperature (*T*), and integrated chlorophyll *a* concentration of the water column along the two transects on the Faroe Shelf. Dashed lines indicate separation between on- and off-shelf water masses.

fluorescence (Figure 2) was determined with a Sea Tech fluorometer mounted on the CTD, calibrated against chlorophyll *a* (Chl *a*) measurements, as described by Parsons *et al.* (1984). Calculations were made according to Jeffrey and Humphrey (1975).

Calanus finmarchicus for enumeration and stage identification from the upper 50 m, were sampled with a bongo net (diameter 60 cm, mesh size 200 μm) equipped with a Hydro-Bios flowmeter, towed at ~1.3 m s^{-1}. The samples were preserved in 4% buffered formaldehyde. For gut content, egg production, and lipid analysis, females were sampled by vertical hauls from the uppermost 50 m with a WP-2 (200 μm) plankton net, mounted with a non-filtering codend of 1 l. Deeper samples were collected with vertical WP-2 nets equipped with a closing device and at a towing speed of 0.3–0.5 m s^{-1} for enumeration, stage identification, gut content, and lipid analysis of females. Younger stages are most likely undersampled using such a coarse mesh (Nichols and Thompson, 1991; Munk *et al.*, 2003).

Copepod gut fluorescence measurements were performed to obtain an instantaneous index of grazing activity. Individual *C. finmarchicus* females were quick-frozen with cryo-spray immediately after retrieval and kept at −18°C until analysis. At each station, ~30 females were divided into subsamples, each containing five copepods, then extracted in the dark for 24 h in 5 ml of 90% acetone. Gut fluorescence was measured on a Turner Design fluorometer (Båmstedt *et al.*, 2000). The fluorescence was calibrated against Chl *a* standards (from the Danish Hydraulic Institute). Calculation of chlorophyll equivalents was performed as described by Parsons *et al.* (1984).

Secondary production was determined during the cruise as the daily numerical egg production per female. One or two females were kept at *in situ* temperature in 500 ml filtered (60 μm) seawater in spawning containers with a false bottom (1000 μm) to avoid egg cannibalism. This was replicated 9–18 times per station. After 24 h, the eggs were filtered onto a net (30 μm) and counted. The rate of egg production (G_f) was calculated according to Runge and Roff (2000).

Prosome length of live, adult female *C. finmarchicus* collected for lipid analyses was measured immediately after retrieval, and the females were placed in glass GC–MS vials with 1 ml of chloroform:methanol (2:1). Oxygen was removed with nitrogen gas, and the vial was closed and frozen in liquid nitrogen. After the cruise, the samples were transported on solid CO_2 and subsequently stored at −80°C until analysis.

Total lipid from individual females was extracted using the Folch extraction method (Folch *et al.*, 1957). Non-polar and polar lipid classes were separated on an NH_2–SPE column using

a Gilson 233 sampling injector. The extracted lipids were dissolved in 1 ml chloroform and added to the SPE column, which was conditioned using *n*-hexane. Non-polar hydrocarbons, WE, triacylglycerols (TAG), and cholesterol were eluted with 2 ml chloroform:2-propanol (2:1), and the polar lipids, including phospholipids, were eluted with 2 ml of acetic acid (6%) in methanol. The solvent was evaporated with nitrogen gas, and the non-polar lipids redissolved in 200 μl 0.5% tetrahydrofurane (THF) in *n*-hexane for analysis on the HPLC–ELSD.

The polar lipids, represented by the phospholipids, were treated as described by Zhou and Arthur (1992). After 20–30 min (Vaskovsky, 1975), the absorbance at 660 nm was measured spectrophotometrically (Milton Ray, Spectronic Genesys 5). Dark brown or black samples were discarded. Calibration curves of diheptadecanoyl phosphatidylcholine (PC-17) were used for quantification.

The neutral lipid classes, WE and TAG, were quantified on a Dionex HPLC system (Dionex P80A Low Pressure pump and Dionex Gina 50 autosampler) with an Alltech MKIII evaporative light scattering detector (ELSD). Peaks were integrated using Chromeleon Client v. 6.40 (SP1 Build 711) software from Dionex. Detector response was quantified from peak areas. We used the method described by Nordbäck and Lundberg (1999) modified to our chromatographic system and optimized for WE and TAG analyses on individual adult female *C. finmarchicus*. Mobile phase B (20% 2-propanol and 20% THF in *n*-hexane) was added to mobile phase A (0.5% THF in *n*-hexane) from 4 min (0%) to 20 min (43.1%), and reduced to 0% (24 min). Total run time was 60 min at 0.6 ml min^{-1}. Retention times of the lipid classes WE and TAG were $t = 2$ and $t = 14$ min after the void (t_0).

The response of the detector is not equal for all compounds, and quantification should be done using response factors from external standard curves of each compound of interest (Hopia and Ollilainen, 1993; Webster *et al.*, 2006). The response of the ELSD has been reported to be linear (Murphy *et al.*, 1996), sigmoid (Hopia and Ollilainen, 1993), or to follow a second-degree polynomial (Webster *et al.*, 2006). The calibration curves of the chosen lipid classes on our chromatographic system could best be described as second-degree polynomial. The underestimation of TAG, reported by Webster *et al.* (2006), was not regarded as a problem in our study, because all quantification was done from external standard curves of a complex mixture of TAGs in fishoil.

Differences in Chl *a* between the two transects were determined using a non-parametric Mann–Whitney *U*-test. Differences in gut fluorescence of the females were tested using two-way (on-/off-shelf and north–south) ANOVA on untransformed data. Differences in egg production data were tested using Kolmogorov–Smirnov tests (two-sided probabilities), to take different distributional patterns into account. Differences in WE content between on- and off-shelf stations and between the two transects were determined using a non-parametric Mann–Whitney *U*-test. TAG differences were determined from two-way ANOVA tests on $\log_{10}$-transformed data. All comparisons were considered significant at a 95% confidence level.

Results

There was a clear separation of the FSW from the water masses around the Faroe Shelf (Figure 2). The less-saline water (<35.25) of the northern shelf was distinctly separated from the more-saline water masses of the Norwegian Sea along the ~150-m depth contour. The inflow of water to the shelf was greatest at the southwestern part of the shelf, from the Faroe Bank Channel. There was no stratification of the water column on the shelf, but there was a clear difference in vertical profiles of salinity and temperature in the water column north of the Faroe Shelf. The high-salinity, warm AW dominated the surface layers (0–100 m) outside the shelf (off-shelf stations 14, 14a, 14b, 54, and 55), and the less-saline, colder water from the EICW was clearly identifiable below the surface waters (>100 m). Warm AW was also found in the Faroe–Shetland Channel, but there was no stratification of the water column in the top 200 m there.

Phytoplankton

In general, the mean depth-integrated phytoplankton biomass at the two transects reflects a pre-bloom situation with a low Chl *a* biomass (0.3–0.8 μg l^{-1}; Figure 2). The concentration of Chl *a* was higher at T_2 than at T_1, but this difference was not significant. At T_1, the highest concentrations of Chl *a* were at the northernmost stations outside the shelf (0.81 μg l^{-1}), whereas the highest Chl *a* levels along T_2 were found on the shelf (~0.6 μg l^{-1}).

Copepods

At T_1, gut fluorescence (Table 1) was generally low (0.96–3.85 ng Chl equivalents $female^{-1}$), with a significantly lower gut fluorescence off the shelf (two-way ANOVA $p < 0.018$, $n = 43$). At T_2, there was no significant difference in gut fluorescence between *C. finmarchicus* females caught on- and off-shelf (two-way ANOVA $p < 0.476$, $n = 33$).

Females from on-shelf stations at T_2 had significantly greater gut fluorescence (two-way ANOVA $p < 0.0001$, $n = 50$) than females from on-shelf stations at T_1. A similar highly significant difference (two-way ANOVA $p < 0.0001$, $n = 26$) was found between females collected off-shelf on the two transects. The general pattern showed higher gut fluorescence levels along T_2 than along T_1.

Calanus finmarchicus reproduction was initiated, but a considerable fraction of the females was not producing eggs (Table 1). Fewer females were unproductive on T_2 than on T_1, except for the off-shelf station 54, where 81.8% of females were unproductive. The mean egg production was high for a pre-bloom situation. The egg production was significantly higher on the shelf than off it on both T_1 [Kolmogorov–Smirnov (two-sided probabilities) $p < 0.026$, $n = 102$] and T_2 [Kolmogorov–Smirnov (two-sided probabilities) $p < 0.049$, $n = 87$]. There was no correlation between Chl *a* concentration and egg production.

The abundance of *C. finmarchicus* on and around the Faroe Shelf varied considerably. At T_1, abundance was greatest at the northernmost stations 14a and 14b, at 145 and 198 m^{-3}, respectively (Figure 3, upper panel). Transect 1 was dominated by overwintered stages (CIV, CV, and females), which constituted >80% of the total abundance outside the shelf (stations 14, 14a, and 14b), and >75% on the shelf, except at the innermost station (station 7), where the concentration of 77 nauplii–CIII m^{-3} made up more than half the population (Figure 3, lower panel).

Along T_2, the scenario was different. The overwintering stages dominated the off-shelf stations, but the relative presence of younger development stages was profoundly higher than at T_1. At station 55, overwintering stages accounted for >90% of the total abundance of *C. finmarchicus*. The dominance of overwintering stages was lower for stations on the shelf. At station 49, situated in the transition water between on- and off-shelf, numbers of

Table 1. Mean ± s.d. numerical egg production of *Calanus finmarchicus*, the fraction of females not reproducing, and mean ± s.d. female gut content values, based on fluorescence measurements.

Station	Sampling depth (m)	Egg production (G_f)			Gut content	
		n	Eggs female^{-1} d^{-1} ± s.d.	Non-reproducing females (%)	Replicates[a]	Chlorophyll equivalents (ng female^{-1}) ± s.d.
Transect 1 on-shelf						
6	0–50	12	21.2 ± 20	25.0	6	1.0 ± 0.3
7	0–50	10	22.5 ± 11	10.0	6	1.5 ± 0.5
8	0–50	15	26.5 ± 13	6.7	6	2.5 ± 1.3
10	0–50	10	10.8 ± 11	20.0	7	3.9 ± 1.2
11	0–50	15	21.8 ± 21	28.6	1	1.6
Transect 1 off-shelf						
14	0–50	15	11.3 ± 15	28.6	6	1.6 ± 0.6
14a	0–50	16	25.3 ± 31	20.0	6	1.0 ± 0.4
14b	0–50	9	13.6 ± 17	11.1	6	1.6 ± 0.8
Transect 2 on-shelf						
42	0–50	14	18.2 ± 15	14.3	6	9.6 ± 1.9
44	0–50	18	14.2 ± 18	18.8	6	8.6 ± 3.8
49	0–50	20	16.2 ± 14	10.5	6	4.1 ± 2.1
50	0–50	15	27.6 ± 26	7.7	6	10.1 ± 4.4
Transect 2 off-shelf						
54	0–50	12	1.8 ± 6	81.8	3	4.3 ± 1.3
	50–150	–	–	–	4	4.1 ± 0.7
55	0–50	15	24.8 ± 22	7.1	6	8.5 ± 1.9
	50–100	–	–	–	6	3.7 ± 2.0
	100–200	–	–	–	3	2.7 ± 0.4
	200–500	–	–	–	1[b]	1.7
	500–700	–	–	–	1[b]	0.2

[a]Each replicate is a pooled sample of 5 females.
[b]Mean of 2 females.

C. finmarchicus were highest of all stations (235 m^{-3}), largely a consequence of the presence of nauplii and CI, which alone contributed >70% of the total *C. finmarchicus* at this station. Bearing in mind that the youngest developmental stages were most likely undersampled, this pattern is presumably even more significant.

The depth profile from stratified WP-2 net hauls at station 55 (Figure 4) showed a dominance of overwintering individuals (CIV–adults) in both deep water (500–700 m) and surface water (0–50 and 50–100 m). Nauplii–CIII were dominant at the two intermediate depth intervals.

Female *C. finmarchicus* from surface waters (0–50 m) stations off the shelf, both north (stations 14, 14a, and 14b; Mann–Whitney *U*-test $p < 0.026$, $n = 64$) and south (stations 54 and 55; Mann–Whitney *U*-test $p < 0.004$, $n = 63$) of the shelf, exhibited a significantly higher WE content than females on the shelf (Figure 5). Females on the shelf had a low WE content (<2 μg female^{-1}).

At selected stations (11, 14, 14a, 49, and 54), depth-stratified copepod samples were collected in addition to the sample from the surface layer (0–50 m). At station 14a, where the depth profile extended down to 500 m, the WE content of females increased with increasing depth (Table 2). At stations on the shelf, however, there was no increase in lipid content with depth, although the individual variation in WE content within stations was high (100–340%).

At T_1, there was significantly more TAG (two-way ANOVA $p < 0.026$, $n = 64$) in females collected on the shelf than in females collected off the shelf. There was no significant difference between on- and off-shelf stations along T_2 (two-way ANOVA $p < 0.432$, $n = 63$). At all stations except 14a, the TAG content in females from the surface (0–50 m) was higher than the WE content (Table 2). The TAG levels were generally high (10–40 μg female^{-1}; Figure 6). No correlation was observed between water depth at which the females resided and their TAG content on station 14a, or generally between lipid content and prosome length.

Discussion

The mixing of off-shelf water with on-shelf water is most pronounced on the southwestern shelf towards the Faroe Bank Channel (Gaard and Hansen, 2000). Salinity and temperature data confirmed this pattern during the April 2003 cruise. The front between AW and Shelf Water was on the shelf (~150-m bottom depth) along both transects. However, there was a more distinct separation of on- and off-shelf water masses north of the Faroe Islands, where advection of AW onto the shelf was less. Neither the water column on the shelf nor the water masses surrounding the shelf were stratified.

The higher temperature (>1°C) and lower salinity (~0.1) on the southwestern transect (T_2) indicated the partly different origin of the water masses, the northern waters seeming to be mixed with some EICW flowing from the northwest.

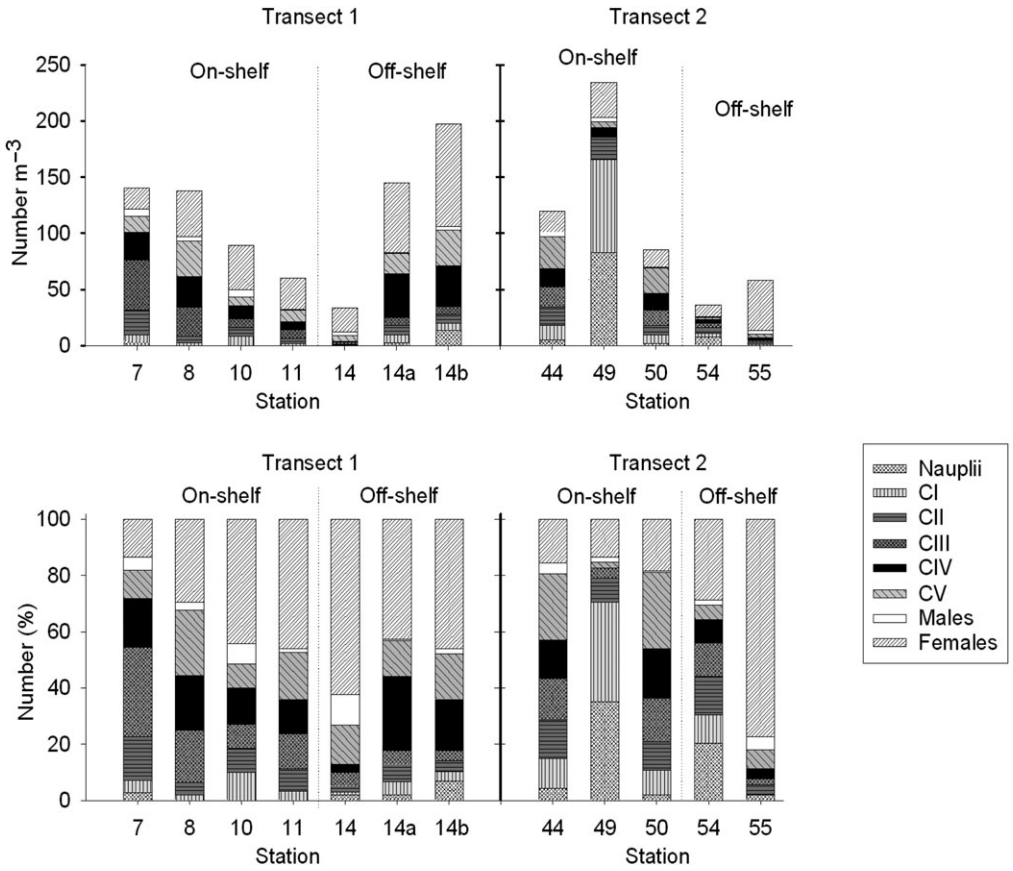

Figure 3. (Upper panel) Abundance and development stage composition of *Calanus finmarchicus* along the two transects on the Faroe Shelf. (Lower panel) Relative development stage composition at the different stations along the two transects on the shelf. Dashed lines indicate separation between on- and off-shelf water masses.

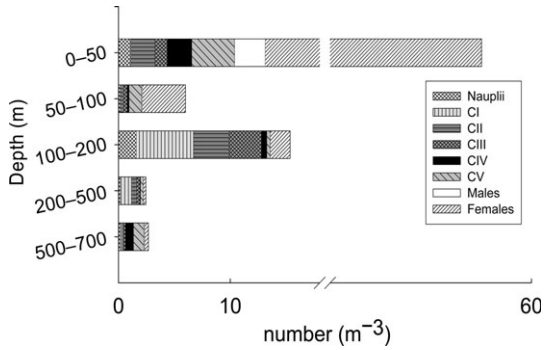

Figure 4. Vertical profile of development stage composition of *Calanus finmarchicus* at off-shelf station 55 on transect T_2.

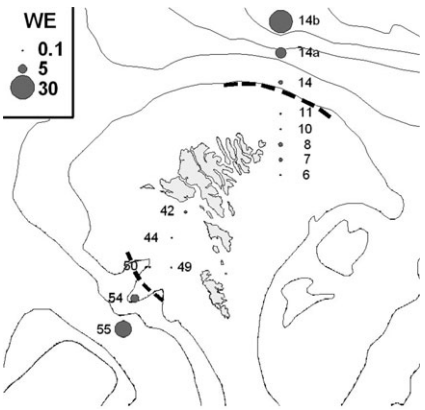

Figure 5. WE content of *Calanus finmarchicus* (μg female^{-1}) from water depths of 0–50 m. Dashed lines indicate separation between on- and off-shelf water masses.

The phytoplankton community on and around the Faroe Shelf was at a pre-bloom stage. Phytoplankton biomass was in general low (<1 μg Chl *a* l^{-1}), with a tendency for higher phytoplankton biomasses on the southwestern than on the northeastern part of the shelf. Gaard (1996b) reported higher phytoplankton biomasses north of the Faroe Islands, and suggested that the Faroe Shelf spring bloom would commence in this area. Our data could not confirm this statement.

The generally low gut fluorescence content of female *C. finmarchicus* reflected the pre-bloom situation on the Faroe Shelf. Along T_1, the gut fluorescence levels were low (1–4 ng Chl equivalents female^{-1}), indicating that the copepods had low phytoplankton intake. *Calanus finmarchicus* from T_2 had significantly greater gut fluorescence than those from T_1, although the values were still low. Irigoien *et al.* (2000) refer to similar low pre-bloom levels of gut fluorescence from *C. finmarchicus* caught south of Iceland (0.1–0.8 ng Chl equivalents individual^{-1}), and from adult females caught at Weathership M in the Norwegian Sea (0.7–0.9 ng Chl equivalents female^{-1}; see also Irigoien *et al.*,

Table 2. Mean ± s.d. body size and lipid-class content of female *Calanus finmarchicus* from the two transects on the Faroe Shelf.

Station	Depth (m)	*n*	Prosome length (μm) ± s.d.	Lipid class (μg female^{-1}) ± s.d.		
				WE	TAG	Phospholipid
Transect 1 on-shelf						
6	0–50	2	2 525 ± 40	0.0 ± 0.0	39.7 ± 50.8	16.8 ± 2.4
7	0–50	6	2 443 ± 130	1.0 ± 1.7	33.4 ± 44.1	7.3 ± 3.2
8	0–50	9	2 665 ± 180	1.2 ± 3.2	23.0 ± 50.8	10.4 ± 8.1
10	0–50	9	2 567 ± 90	0.1 ± 0.1	12.5 ± 11.0	11.4 ± 9.6
11	0–50	11	2 568 ± 130	0.1 ± 0.3	20.5 ± 37.2	11.5 ± 6.9
	50–100	11	2 591 ± 70	<0.1 ± 0.0	2.7 ± 2.3	13.3 ± 9.7
Transect 1 off-shelf						
14	0–50	9	2 538 ± 160	1.3 ± 2.3	2.5 ± 2.3	12.6 ± 4.8
	50–100	10	2 530 ± 110	0.7 ± 1.4	2.7 ± 1.0	16.3 ± 6.4
14a	0–50	11	2 705 ± 130	7.2 ± 15.1	6.3 ± 8.2	16.6 ± 8.1
	50–100	10	2 620 ± 80	11.5 ± 16.3	16.1 ± 18.5	15.6 ± 13.3
	100–200	10	2 755 ± 160	12.9 ± 17.8	5.0 ± 5.7	24.0 ± 21.8
	250–500	8	2 706 ± 180	26.7 ± 40.5	16.7 ± 31.1	10.9 ± 5.8
14b	0–50	7	2 800 ± 190	28.0 ± 59.5	35.2 ± 52.0	18.4 ± 11.2
Transect 2 on-shelf						
42	0–50	11	2 400 ± 150	0.5 ± 1.7	11.0 ± 10.0	12.5 ± 6.6
44	0–50	13	2 554 ± 120	0.1 ± 0.1	25.2 ± 22.5	10.4 ± 3.6
50	0–50	7	2 493 ± 160	0.2 ± 0.3	21.3 ± 18.3	14.1 ± 5.1
49	0–50	11	2 405 ± 260	0.4 ± 1.1	18.2 ± 27.9	12.4 ± 9.2
	50–200	13	2 500 ± 220	0.1 ± 0.1	24.0 ± 34.4	10.9 ± 7.9
Transect 2 off-shelf						
54	0–50	12	2 492 ± 150	4.7 ± 14.9	21.9 ± 25.6	23.8 ± 21.1
	50–150	10	2 470 ± 150	0.5 ± 0.9	20.2 ± 19.3	12.9 ± 4.7
55	0–50	21	2 450 ± 150	15.8 ± 18.3	27.1 ± 41.9	16.7 ± 11.6
On-shelf	0–50	79	2 515 ± 170	0.4 ± 1.4	20.6 ± 29.6	11.5 ± 6.6
Off-shelf	0–50	60	2 562 ± 190	11.9 ± 25.2	20.4 ± 34.1	17.7 ± 12.9

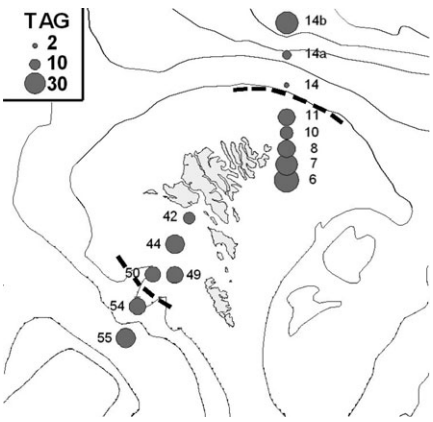

Figure 6. Content of TAG in *Calanus finmarchicus* (μg female^{-1}) from water depths of 0–50 m. Dashed lines indicate separation between on- and off-shelf water masses.

1998). Gut fluorescence decreased with increasing water depth (Table 2), but there was no significant difference in this parameter between on- and off-shelf stations. The low phytoplankton grazing by *C. finmarchicus* northeast of the Faroe Islands indicated that it had only been present in the surface waters for a short period or that phytoplankton concentrations there were low.

Female secondary production, measured as the daily egg production, was significantly higher on the shelf than off it. The rates of egg production were high for a pre-bloom situation (18.3 eggs female^{-1} d^{-1}). Gaard (2000) observed markedly lower egg production rates at the same time of the year on the central shelf (<3.1 eggs female^{-1} d^{-1}) than over the northwestern shelf (>10 eggs female^{-1} d^{-1}). This low rate of egg production on the central shelf was clear until the end of May, when the spring bloom created the basis for higher rates of egg production (Gaard, 2000). However, Richardson *et al.* (1999) observed comparably high rates of egg production (5–25 eggs female^{-1} d^{-1}) in the Faroe–Shetland Channel with equally low Chl *a* concentrations (0.2 μg chlorophyll *a* l^{-1}), and other studies have found a short increase in egg production on the Faroe Shelf around the end of April, well before the expected increase in egg production related to the spring bloom (Gaard, 2000; Debes *et al.*, 2005; Debes and Eliasen, 2006). This periodic increase in the rate of egg production cannot be explained by Chl *a* concentrations alone. Irigoien *et al.* (1998) reported that the ingestion of Chl *a* and ciliates could account for just 23% of the total energy required for egg production in a pre-bloom situation in the Norwegian Sea. The protozooplankton concentration on the Faroe Shelf was too low (1.4 mg C m^{-3}) to fuel egg production by *C. finmarchicus* in a similar pre-bloom situation (Debes *et al.*, 2005). Hence, the

high rates of egg production and the low food concentrations we found suggest that *C. finmarchicus* commences reproduction using stored energy resources soon after emergence in the surface waters, as suggested by Richardson *et al.* (1999).

The *Calanus* stage composition on the stations along T_1 (north) was skewed towards advanced overwintering copepodite stages (CIV and CV) and adult males and females, indicating that *C. finmarchicus* outside the shelf were still emerging from overwintering habitats and that reproduction had only recently been initiated. On T_1, the overwintered stages and especially females dominated at all stations (73–90%), except at station 7 (45%). The proportion of individuals belonging to G_1 (nauplii–CIII) increased further onto the shelf. In contrast, at all stations along T_2, nauplii–CIII constituted a higher fraction of the population (36–83%), except for station 55 outside the shelf, where just 8% belonged to G_1. This indicates that *C. finmarchicus* had resided on the Faroe Shelf for some time and was reproducing. Several studies have observed a significant abundance of G_1 before the spring bloom (in late April) on the Faroe Shelf (e.g. Gaard, 2000; Debes *et al.*, 2005; Debes and Eliasen, 2006).

Emergence from diapause depths and reproduction by *C. finmarchicus* north of the Faroe Islands were delayed compared with the situation south of the Faroe Islands, probably because of the $\geq 1^{\circ}$C temperature differences. Gaard and Nattestad (2002) noted that reproduction from AW north of the Faroe Islands was initiated 1 month earlier than from EICW north of the islands. The proportion of copepodites (CI–CIII) was significantly higher in AW (80% of the population) than in EICW (7%).

In this study, the stage composition reflects a delay in ascendance and reproduction of *C. finmarchicus* northeast of the Faroe Islands, compared with the situation over the southwestern shelf. The same difference was observed by Gaard (1996a), who found that emergence from overwintering, reproduction, and migration back to diapause depths in EICW was delayed compared with the same situation in AW.

The WE content of *C. finmarchicus* females was in general low in spring around the Faroe Islands (Jónasdóttir, 1999; Richardson *et al.*, 1999), but similar to the levels reported for other shelf areas (Miller *et al.*, 1998) and in shallow areas in the North Sea (Kattner and Krause, 1989).

There were clear differences in non-polar lipid content in relation to the frontal system, both south and north of the shelf. The front was most distinct north of the shelf (Figure 2), which was reflected in the elevated WE content of *C. finmarchicus* (Figure 5). Females on the shelf had very low WE levels (<2 μg WE female^{-1}), and this was also reflected in their high rate of egg production. Females from off-shelf stations (14, 14a, 14b, 54, and 55) had significantly higher levels of WE (11.9 ± 25.2 μg WE female^{-1}) than females on the shelf (0.4 ± 1.4), indicating that they had emerged in the surface waters recently, and tended to have a lesser rate of egg production. This was further supported from the depth profile at station 14a (Table 2), where the WE content of females increased with water depth. This suggested that females that had not yet ascended had not initiated egg production.

The WE content of *C. finmarchicus* females from the upper 50 m at station 14b, outside the northern part of the shelf, was 28 μg female^{-1}. Females from 250–500 m at station 14a had a similar WE content (27 μg female^{-1}), indicating that the females in the upper 50 m had emerged recently in surface waters. The high individual variation in WE content of female *C. finmarchicus* can be explained by differences in the timing of the migration from overwintering depths and the initiation of reproduction (Båmstedt, 1988; Kattner and Krause, 1989). High individual variation in WEs of *C. finmarchicus* was also reported by Webster *et al.* (2006), especially during summer.

Our findings agree with the observations of Richardson *et al.* (1999) from the Faroe–Shetland Channel and northern North Sea, where females from the upper 100 m in January–March (1994 and 1995) had significantly higher WE content off the shelf than on it. The difference in mean WE content was 25 μg female^{-1}, sufficient for the female to produce ~100 eggs, if all the WE were used for egg production (Richardson *et al.*, 1999).

The relatively high TAG levels (18 μg female^{-1}) indicate that *C. finmarchicus* was either grazing on phytoplankton or, because our grazing index and the food availability data suggest limited activity, more likely had mobilized WE to TAG, the main storage lipid in the eggs. No significant differences in TAG content were found between on- and off-shelf populations.

Female *C. finmarchicus* have high WE content immediately after ascendance and before the initiation of reproduction in early spring. By mobilizing stored WEs, significant egg production can be initiated before the phytoplankton spring bloom. Stored WEs are utilized for gonad development and egg production. WE content decreases from the time females initiate egg production, because all excess dietary energy is directed towards continued egg production, so there is no synthesis and storage of WEs in adult females (Hygum *et al.*, 2000).

When performing analysis of storage lipids in spring, at the time when females are ascending from diapause depths, great individual variations in lipid content can be expected (Båmstedt, 1988). Lipid-class analysis of individual *C. finmarchicus* can provide information on the timing of the life cycle and hence indicate when reproduction of different subpopulations takes place. High variations in WE content most likely reflect differences in residence time in the surface layers. From our lipid-class analyses, it seems that most females had either a high or a very low WE content. Intermediate WE contents were rarely found.

Significant individual variability in lipid content, feeding activity, and reproduction has been reported earlier (Båmstedt, 1988), and might be explained as population plasticity in the timing of major copepod life cycle events such as vertical migration, grazing activity, and initiation of reproduction.

In this study, we observed considerable individual variation in egg production (Table 1) and lipid content (Table 2), both within stations and between stations on- and off the shelf. Båmstedt (1988) reported that individual variability in gonad length, as a measure of reproductive potential, increased from winter to autumn. Individual variability increased also in spring, even if a major fraction of the females had relatively well developed gonads. Lipid content in individual and in pooled samples of adult calanoid copepod females is also highly variable, with little correlation with size (Båmstedt, 1988, and references therein).

For future studies, it could be useful to analyse whether females with high WE contents had fully developed and matured ovaries. Gaard (2000) reported from a study on the central Faroe Shelf that the ovaries of *C. finmarchicus* develop and mature from April to mid-June and that ~50% of females collected in late April had fully developed gonads. Moreover, there could be value in trying to correlate the WE content of individual females with the total number of eggs produced following ascendance. This would

indicate the extent of mobilization of the WEs allocated to reproduction.

In conclusion, individual *C. finmarchicus* female gut fluorescence, development stage composition, and female storage lipid levels of subpopulations confirm our hypothesis that two patterns can be identified along the two transects crossing the Faroe Shelf. Stations off the shelf are dominated by *C. finmarchicus* only recently ascended to the surface waters. At stations on the shelf, *C. finmarchicus* tend to have been in the surface water masses for some time. Finally, reproduction of the *C. finmarchicus* population on the northern transect T_1 was delayed compared with the situation for the population inhabiting the southern transect T_2.

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