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The scheduling of spawning with the moult cycle in Northern krill (Crustacea: Euphausiacea): a strategy for allocating lipids to reproduction

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Summary

Euphausiids moult and grow throughout their life, which implies sharing of resources between growth and reproduction for adult krill. In the Northern krill, *Meganyctiphanes norvegica* (M. Sars), female krill produce eggs cyclically. Spawning moult cycles alternate with vitellogenic moult cycles for lipid yolk accumulation. Histology shows that lipids are associated with the R cells of the digestive gland in both sexes, with the yolk platelets of mature oocytes and with the fat body cell membranes and blood lacunae in reproducing females. Mature female krill can have a total lipid content twice as high as males, mostly due to accumulation in the ovary, the fat body and the haemolymph. In contrast, in males, as well as in non-reproducing females, the highest percentage of lipids is found in the digestive gland and the haemolymph. In *Meganyctiphanes norvegica*, the most abundant lipid fractions are polar lipids and triglycerides, the latter being relatively low in reproducing female gonad and fat body. Triglycerides are believed to be a pure energy source and polar lipids are essential for membrane development in embryos. The fatty acid content and composition of the triglyceride and polar lipid fractions in females are different from males, related to both reproductive and dietary processes. Higher levels of polyunsaturated fatty acids (PUFA) in the polar lipid fraction were found in reproductive females. During the non-reproductive season, the converse was found, indicating the specific role PUFA and other fatty acids play in growth and egg production. Adaptive processes linked to reproduction were studied comparatively in three populations of the Northern krill — Clyde Sea (W, Scotland), Kattegat (E, Denmark), Ligurian Sea (Mediterranean) — all differing considerably in climatic and trophic conditions. Such adjustments in lipid synthesis and storage are viewed as reproductive strategies developed by the Northern krill in response to different environmental conditions.

Key words: *Meganyctiphanes norvegica*, Northern krill, lipids, reproductive strategies

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Introduction

Meganyctiphanes norvegica (M. Sars) is abundant in the North Atlantic and marginal seas of Europe, including the Mediterranean, and plays an important role in the pelagic ecosystem. This species both feeds on phytoplankton and copepods and represents a major diet for baleen whales, pelagic fish and other carnivorous species.

Euphausiids moult as reproducing adults (Mauchline and Fisher, 1969). Therefore, they have to balance resources between growth (through moulting) and egg production during their whole adult life. The reproductive pattern of *M. norvegica* is characterized by multiple spawnings of numerous small eggs produced during a limited season of reproduction for two successive years at least (Mauchline and Fisher, 1969). Rapid development results in numerous long-lived planktotrophic pelagic larvae, thus favouring dispersion by surface oceanic currents and gene flow between populations. Egg production coincides with the season when food is abundant and available to the offspring (Cuzin-Roudy, 1993). Periodic spawnings by individual female krill result in continuous egg production throughout the reproductive season. Egg maturation and release appears to be linked to the crucial events of the moult cycle (Cuzin-Roudy and Buchholz, 1999). The mature ovarian egg mass is released during premoult of a spawning moult cycle in one or two spawnings. The first spawning occurs just after apolysis, at D0 stage, the second follows at D1 of the moult cycle. At moult (ecdysis) the ovary still contains developing oocytes which are ready for lipidic yolk accumulation (vitellogenesis), thus forming another batch of eggs. In consequence, the maximum lipid accumulation should be attained by "ready-to-spawn" females at late moult stage C, preceding apolysis and egg release. Moult stage C is known to be a resting stage for epidermal activity and the storage phase for organic reserves of the crustacean moult cycle (Skinner, 1985).

A comparative study of three populations of *M. norvegica* adapted to contrasting environments, i.e., the Clyde Sea (W, Scotland), the Kattegat (E, Denmark) and the Ligurian Sea (N-W, Mediterranean), has shown that the moult and spawning cycle duration was influenced by temperature (Cuzin-Roudy and Buchholz, 1999). An intermoult period (IMP) as short as 7 days was found for an *in situ* temperature of 13 °C in the Ligurian Sea. A similar IMP occurred in the Kattegat population under warm summer conditions, but was 16 days at 4 °C in spring.

Lipid content and composition vary with season (Buchholz and Prado-Fiedler, 1987; Falk-Petersen 1981; Saether et al., 1986), and likely also with location and the reproductive status. The analysis of both polar lipid and triacylglycerol fatty acid composition can be used to identify food web interactions and provides insight into the physiological status of an organism (Mayzaud et al., 1976; Virtue et al., 1993; Graeve et al., 1994). The relative importance of triglycerides, the principal lipids for energy storage, and polar lipids, the main structural lipid components, were studied in different krill tissues and organs in relation to reproduction at the three locations. The purpose was to gain an insight into the functional role and adaptive value of these lipid components in reproducing and non-reproducing male and female krill from populations adapted to different environments.

Materials and Methods

Three populations of *M. norvegica* were sampled during summer and winter cruises in three locations, as described in Table 1, using an Isaacs–Kidd Midwater Trawl (I–K MT) or a Multiple Opening Closing Net and Environmental Sensing System (MOCNESS) (Wiebe et al., 1976) with 2 mm mesh nets.

Adult krill were immediately sorted from the catches, staged alive for sex and sexual development stage (SDS), measured for body length (BL), frozen in liquid nitrogen and stored at −80 °C. For the February 1995 Ligurian sample, haemolymph was extracted with a Hamilton syringe (50 µl) from live krill and frozen separately. Five specimens of similar sex, BL and SDS were pooled for each haemolymph sample.

Female krill were staged for sexual development according to Cuzin-Roudy (1993) and Cuzin-Roudy and Buchholz (1999) using six stages (SDS) based on the ovarian structure:

- SDS 1: resting ovary, with principal germinal zone and young oocytes
- SDS 2: primary oocytes in previtellogenesis (glycoproteic yolk accumulation)
- SDS 3: vitellogenesis (lipidic yolk accumulation) for a batch of oocytes
- SDS 4: "ready-to-spawn" mature batch of oocytes
- SDS 5_1, 5_2: partially (5_1), or completely "post-spawn" krill (5_2)
- SDS 6: ovary in oosorption.

In the three populations, SDS 2 is characteristic for the onset of the reproductive period, SDS 3–5 for full spawning activity, and SDS 6 for the ovarian regression when the reproductive period is over.

Table 1. Cruise names, sampling locations, dates and corresponding reproductive status for the krill *Meganyctiphanes norvegica*

Clyde Sea, W Scotland		Kattegat, E Denmark		Ligurian Sea, Mediterranean				
Scotex I	Scotex II	Kattex I	Kattex II	Pre-LIGEX	BIOMEG	LIGEX I	BIOMEG, LIGEX II	
07/96	02/97	07/96	03/97	05/94	03/92 02/95	04/96	09/92	09/97
Repro.	Non-repro.	Repro.	Early repro.	Repro. ended	Repro.	Repro.	Non-repro.	

Repro, reproductive period.

Sub-samples of live specimens from the different catches were fixed for histology in Baker's fluid (Cuzin-Roudy, 1993). Paraffin serial sections of the cephalothorax were stained with Sudan black B and nuclear Fast red to reveal lipid accumulation.

In the laboratory frozen specimens were quickly dissected using the method for Antarctic krill of Mayzaud et al. (1998). The abdomen was separated from the cephalothorax. Next, the digestive gland was extirpated as a whole and the thorax was cut into a dorsal part with the gonad and a ventral one with the fat body (Cuzin-Roudy, 1993).

Lipid extraction of all body fractions was conducted according to the method of Bligh and Dyer (1959). Lipid content was estimated according to Barnes and Blackstock (1973). Lipid classes were quantified after chromatographic separation coupled with FID detection on a Iatroscan Mark III TH 10 (Ackman, 1981). Neutral lipids were developed in hexane, diethylether, formic acid 97:3:1 (v/v). Polar lipids were separated in benzene, chloroform formic acid 50:20:1.5 (v/v) and next in methanol, chloroform, ammonium hydroxide 27.3% 50:50:5 (v/v), using the double development method of St. Angelo and James (1993).

Lipids were separated into triacylglycerol and polar lipid fractions by column chromatography on silica gel. These fractions were eluted with six volumes of chloroform and methanol respectively. Fatty acids of each fraction were treated with methanol/hydrochloric acid/chloroform under nitrogen (10:1:1 v/v/v, 100°C, 1 hr) to form fatty acid methyl esters (FAME). Gas liquid chromatographic (GLC) analyses were performed with a Perkin Elmer XL GC equipped with a J and W fused silica capillary column (SE-30), a flame ionization detector (FID) and a split injector system.

Results

Histological lipid localization

Ovaries at a "ready-to-spawn" stage (SDS4) of Ligurian krill (March 1992 and February 1995 samples) showed Sudan black stained vitelline platelets in mature oocytes (oc 4) (Fig. 1, A) next to Fast red stained young and previtellogenic oocytes. In the well developed latero-ventral fat body (Fig. 1, B), lipids appeared as thin black granules associated with the membranes of the expanded fat body cells. Blood lacunae were densely black stained, indicating another type of lipid accumulation in the haemolymph of this organ.

The resting ovary (September 1992 and September 1997 samples) was reduced to paired dorsal sacs (Fig. 1, C) filled with young, undeveloped primary oocytes (yoc). Here, only the digestive gland stained with Sudan black indicating lipid accumulation in the R cells of the digestive gland tubules. The fat body was still well developed (Fig. 1, D), but the cells appeared empty. Here also, lipid accumulation was evident in blood lacunae.

Total lipids

Table 2 shows that no significant difference in the total body lipid content was found when mature females (SDS4) were compared with males, or with females with a regressing ovary (SDS 6). Total lipid content of cephalothorax appeared slightly higher for "ready-to-spawn" SDS 4 females than for males or females in sexual regression. However, clear significant differences were observed (Table 3) when the ovary, the fat body and the digestive gland were measured separately. Mature females (SDS 4) had almost twice the amount of lipids in the gonad than regressing females and males. No differences were seen in the total lipid content of the fat body and digestive gland, between sexes or reproductive stages.

Lipid fractions

The proportion of triglycerides and polar lipids (Table 4) was significantly different in the ovary of

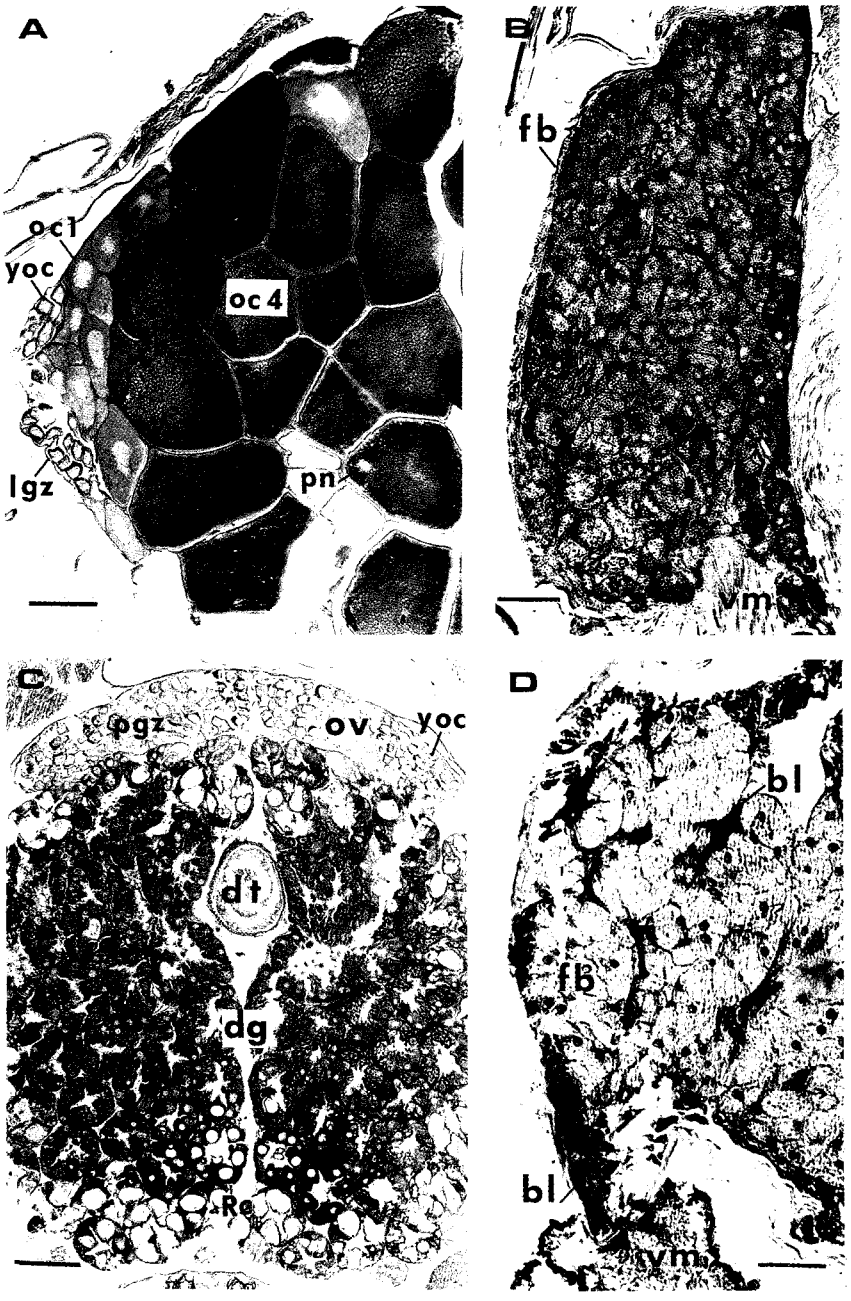

Fig. 1. *Meganyctiphanes norvegica*, Ligurian Sea. Lipid accumulation in ovary, fat body and digestive gland with Sudan B staining of histological sections. A. Ovarian lobe filled with mature oocytes (oc4) loaded with lipidic yolk, in the ovary of a "ready-to-spawn" female krill (March 1992). B. Fat body of a reproducing female krill (March 1992) showing lipid accumulation in association with the fat body cell membranes. C. Transverse section of the thorax of a female krill in sexual rest (September 1992) at the level of the digestive gland. Note the small dorsal ovary reduced to the principal germinal zones and the lipid accumulation in the digestive tubule R cells. D. Fat body of a female krill in sexual regression (May 1994). Note the empty fat body and the lipid accumulation in blood lacunae. bl, blood lacuna; dg, digestive gland; dt, digestive tract; fb, fat body; lgz, lateral germinal zone; oc1 and oc4, type 1 (previtellogenic) and type 4 (mature) oocytes; pgz, principal germinal zone; pn, peripheral nucleus; Rc, R cells; vm, ventral muscle; yoc, young oocytes. Scale bar: A, C: 125μm; B, D: 70 μm.

Table 2. *Meganyctiphanes norvegica*, Ligurian Sea. Total lipid content (% of dry weight, standard deviation in brackets) of adult Ligurian krill in reproduction (April 1997) and just after the reproductive period (May 1994)

	Total lipid content				
	N	Sample date	Total body	Cephalothorax	Abdomen
SDS 4:					
Females	12	April 1996	21 (5)	32 (9)	10 (1.5)
Males	12	Idem	23 (5)	23 (5)	8 (1.5)
SDS 6:					
Females	10	May 1994	16 (2)	25 (4)	8 (1)
Males	10	Idem	18 (3)	29 (10)	8 (1.5)

SDS 4, "ready-to-spawn" females; SDS 6, "spent" females.

Table 3. *Meganyctiphanes norvegica*, Ligurian Sea. Total lipid content (% dry weight, standard deviation in brackets) of the principal thoracic organs in mature (SDS 4) and regressing (SDS 6) female krill

	Total lipid content				
	N	Sample date	Ovary	Fat body	Digestive gland
Females:					
SDS 4	12	April 1996	43 (11)	19 (7)	58 (21)
SDS 6	10	May 1994	16 (4)	13 (2)	52 (10)
Males	10	Idem	24 (10)	14 (2)	44 (19)

Table 4. *Meganyctiphanes norvegica*, Ligurian Sea. Triglycerides and polar lipids content (% of dry weight, standard deviation in brackets) of the ovary, the fat body (FB) and the digestive gland (DG) of mature (SDS 4) and regressing (SDS 6) female krill

	Females (SDS 4), April 1996 (n=12)			Females (SDS 6), May 1994 (n=10)		
	Ovary	FB	DG	Ovary	FB	DG
Triglycerides	22 (6)	28 (9)	64 (11)	48 (3)	40 (9)	71 (12)
Polar lipids	65 (5)	59 (8)	28 (8)	43 (3)	46 (5)	24 (10)

Table 5. *Meganyctiphanes norvegica*, Ligurian Sea. Total lipid content ($\mu g/\mu l$) and lipid classes (% of total lipid content) of the haemolymph in reproductive male and female Ligurian krill (February 1995) (standard deviation in parentheses)

	Reproductive krill			
	Males (n=10)		Females (n=10)	
Haemolymph lipid content, $\mu g/\mu l$	99	(9)	162	(20)
Lipid classes, %:				
Triglycerides	10	(0.3)	24	(4)
Polar lipids	62	(28)	60	(5)

Table 6. *Meganyctiphanes norvegica*. Sum of the fatty acid components (%) of the triacylglyceride and polar lipid fractions in krill from the Clyde, the Kattegat and Ligurian Seas

	SCOTEX I Repro		SCOTEX II Non-repro		KATTEX I Repro		KATTEX II Early repro		LIGEX I Repro		LIGEX II Non-repro	
	M	F	M	F	M	F	M	F	M	F	M	F
Tri:												
Saturated	27	31	22	25	31	31	28	28	34	40	34	31
Mono	38	44	38	37	37	47	46	49	24	24	24	31
Poly	34	25	39	37	30	20	25	22	40	35	40	37
Polar:												
Saturated	27	22	22	22	26	21	22	24	28	23	22	22
Mono	25	20	25	26	21	17	19	18	19	15	19	20
Poly	47	58	53	51	53	62	57	57	53	61	59	57

Repro, reproduction; M, male; F, female; Tri, triacylglyceride fatty acids; Polar, polar lipid fatty acids; Mono, mono-unsaturated; Poly, polyunsaturated.

mature females "ready to spawn" (SDS 4) compared with "spent" females (SDS 6). Polar lipids were higher than triglycerides in the ovary and fat body of SDS 4 females, but no difference was found between these lipid classes in the case of SDS 6 females. Triglycerides were predominant in the digestive gland of female krill at both stages.

Haemolymph lipid content was considered separately for krill sampled during the early reproductive period (Table 5). Levels of circulating lipids were obviously higher in females than in males. Polar lipids were proportionally higher than triglycerides in both sexes.

Fatty acids

Table 6 summarizes the results of the fatty acids study. High levels of polyunsaturated fatty acids (PUFA) were noticeable, particularly in the polar lipid (PL) fractions [47–62% of the total PL fatty acids, with levels in the triglycerides (TG) fractions ranging from 20–40% of the total TG fatty acids]. PUFA levels of the TG fractions seemed lower in females compared with males in actively reproducing krill (SCOTEX I, KATTEX I and LIGEX I). Conversely, PUFA levels of the PL fractions were higher in reproductive female krill compared with males. There were no differences in PUFA levels between male and female in non-reproductive krill (SCOTEX II and LIGEX II) nor at the onset of reproductive period (KATTEX II). Levels of saturated fatty acids also differed between reproductive and non-reproductive krill in the polar lipid fraction only. There were no other apparent differences in levels of other component fatty acids.

Discussion

Reproducing female krill accumulate lipids in eggs which are released periodically during a limited period of the year. A previous comparative study of the spawning cycle (Cuzin-Roudy and Buchholz, 1999) has shown that the accumulation period of mature eggs in the ovary is short. Eggs are rapidly released: stage SDS4 lasted less than 6 h at 12–13 °C and less than 12 h at 4–5 °C in experiments at the different locations, and is therefore not abundant in net samples. In the present study we found that reproducing females had much higher levels of circulating lipids than males, which can be related to a rapid turn-over of lipid accumulation in yolk. Vitellogenesis is arrested during final maturation (meiosis) and release of an egg batch, i.e., during premoult of the spawning moult cycle. After moult (ecdysis), as soon as all the mature eggs have been released, vitellogenesis recommences and lipid yolk is accumulated in a new egg batch (Cuzin-Roudy, 1993; Cuzin-Roudy and Buchholz, 1999). As premoult duration varies with temperature, vitellogenesis is arrested during a shorter time at 12–13 °C (4 days) than at 4–5 °C (6 days).

In *M. norvegica*, high levels of triglycerides were found in the digestive gland, while polar lipid levels were higher, and in similar proportions, in the active ovary and fat body. This confirms the histological similarity observed for lipid depots in both organs. This is taken as an indication of a functional relationship between the fat body and the ovary concerning lipid metabolism in reproducing females.

As triglycerides are principally a storage lipid for energy reserves, levels can be influenced by location

and season. Polar lipids are structural components and basic levels of these compounds have to be maintained for regular metabolic function. These levels are maintained either through lipid ingestion, the synthesis of new lipids, or the catabolism of carbohydrates. In crustaceans, ingested neutral lipid (triglycerides) may be enzymatically cleaved and converted to phospholipids in the digestive gland for transport to various tissues by the haemolymph (Chang and O'Connor, 1983). Hence levels of the triacylglycerol fatty acids can vary substantially in response to the need of pol r lipid anabolism.

M. norvegica appeared characterized by high levels of PUFA. During the non-reproductive season, there was no significant differences in fatty acid composition between male and female krill. However, levels of the PUFA in the polar lipid fraction varied substantially between male and female krill during the reproductive season. Higher levels of PUFA in the polar lipid fraction were found in reproductive female krill compared with male and female krill in the non-reproductive season. Conversely, lower levels of PUFA in the triglyceride fraction were found in reproductive female krill compared with male and female krill in the non-reproductive season. Most of the PUFA was comprised of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid, which are predominantly membrane lipids (Ackman et al., 1970). Vitellogenic krill appear to be preferentially channelling PUFA to the polar fraction. Oocyte development requires additional membrane structural components for egg yolk platelets and embryo cell membranes synthesis. Additional PUFA were also associated with the development of the fat body tissue found in reproductive female krill (Cuzin-Roudy, 1993), indicating that this tissue may play an important role in yolk synthesis.

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