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Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian sector of the Antarctic Ocean: influence of geographical location, sexual maturity stage and distribution among organs

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ABSTRACT: Lipid content and lipid class composition of *Euphausia superba* were studied at different levels for populations and individuals sampled in the Indian sector of the Antarctic Ocean. Strong site-to-site variability was recorded which could only partially be related to sex or development stage differences. Three groups of stations could be differentiated. Northern stations were characterized by 'high lipid-high triglyceride' content, western and eastern locations by 'high lipid-high phosphatidyl choline' content and southern areas by 'low lipid-high phosphatidyl ethanolamine/glycolipid' content. Such variability was likely related to advected populations having spent variable lengths of time in the area studied. Lipid content and class among organs were studied in 5 body fractions: abdomen, stomach, digestive gland, gonad and fat body. In absolute terms, the highest concentrations were observed in the ovaries of mature females and the abdomens of the other stages. In relative terms (% dry weight), the digestive gland displayed the highest level, except in mature females. Distribution varied with stages, with low triglyceride levels in abdomen tissues of most stages and in the fat body and stomach fractions of subadults. High triglyceride levels were recorded in the other fractions for post spawning females and males, as well as in the fat body fraction for mature females and in subadult gonads. A reverse pattern was observed for the relative content of phosphatidyl choline. Phosphatidyl ethanolamine showed maximum values in the abdomen and the gonad. Glycolipid percentages were maximum in the abdomen, suggesting a structural role. The roles of the different lipid classes are discussed with respect to the function of the organ.

KEY WORDS: Krill · Lipids · Spatial heterogeneity · Maturity stage organs

INTRODUCTION

The role of lipids in Antarctic krill has been the concern of several papers in relation to reproduction (Clark 1980, 1984, Kolokowska 1991, Pond et al. 1995, Virtue et al. 1996), energy storage for overwintering (Quetin & Ross 1991, Hagen et al. 1996) and trophic interactions (Bottino 1974, Reinhardt & Van Vleet 1986, Virtue et al. 1993a, b). Krill accumulate lipids mainly as triacylglycerols during the spring and summer when phytoplankton are abundant (Clarke 1984, Hagen et al. 1996).

Neutral lipids are utilized whenever energy levels exceed food intake. In krill the 2 major energy utilizing events are summer reproduction and winter survival under low phytoplankton conditions. Krill store significant amounts of lipids (Clarke 1984, Hagen et al. 1996), although most studies have concluded that the concentrations are not sufficient to meet the energy requirements during the winter, when food supply is low (Quetin & Ross 1991, Quetin et al. 1994). However, the contribution of lipids to the overall survival strategy of

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krill appears quite significant (Hagen et al. 1996) and, during the summer period, is channeled mainly into reproductive output.

Krill reproduction takes place over several spawning phases (Cuzin-Roudy 1987, Quetin & Ross 1991); hence, their reproductive output seems directly related to the richness of the food supply (Ross & Quetin 1986). Yolk accumulation is characterized by high concentrations of neutral lipids and high levels of triacylglycerols in ovaries of mature females (Clarke 1980, Hagen 1988, Pond et al. 1995). Males do not seem to accumulate neutral lipids in relation to spermatophore production, but various reports have suggested that the energy cost of frequent remating may be high, though distributed differently over time (Virtue et al. 1996). The resulting time integral may represent a significant depletion in lipid content, as reported by Pond et al. (1995).

Contrary to the high levels found in large calanoid copepods, wax esters are not present in krill lipids, and reliance on triacylglycerols as a storage moiety is now well established (Clarke 1980, Hagen 1988, Mayzaud 1997). Reports by Ellingsen (1982), Saether et al. (1986), Hagen (1988), and Hagen et al. (1996) suggested that polar lipids, and more specifically phosphatidyl choline (PC), may also serve as storage lipids. The involvement of cell structural components as an energy source has been reported for PC as a source of essential polyunsaturated fatty acids in fish for egg and larval development (see Fraser et al. 1988). This differentiation in the actual role of the lipid classes could provide additional resources during times of increased energy demands.

Our knowledge on the sites of lipid synthesis and catabolism in krill is still limited. Lipid composition has been given for 3 main body fractions, i.e. abdomen, digestive gland and mature ovaries (Clarke 1980, Saether et al. 1985, Virtue et al. 1993a), with respect to neutral lipid accumulation. Little is known on the variability of such composition with growth or sexual maturity stage. The potential role of the glyco-lipoprotein complex present in the fat body described by Cuzin-Roudy (1993) remains to be evaluated.

The objective of the present study was to evaluate, for an open-ocean krill population, the influence of exogenous and endogenous factors in the control of lipids during summer. Changes in lipid concentration and composition were investigated at 2 different levels: population and specific organs or body fractions of different growth and maturity stages.

MATERIAL AND METHODS

Sampling. *Euphausia superba* were obtained from RMT 8 oblique tows made to a depth of 100 m during 2 cruises of the RV 'Marion Dufresne' in February 1981 (FIBEX) and February 1994 (ANTARES 2). Positions of sampling stations and cruise track for the first cruise are given in Fig. 1. Samples from the second cruise were obtained at 2 stations (66° 41' S, 61° 50' E and 63° 00' S, 70° 20' E). Krill were sorted immediately after

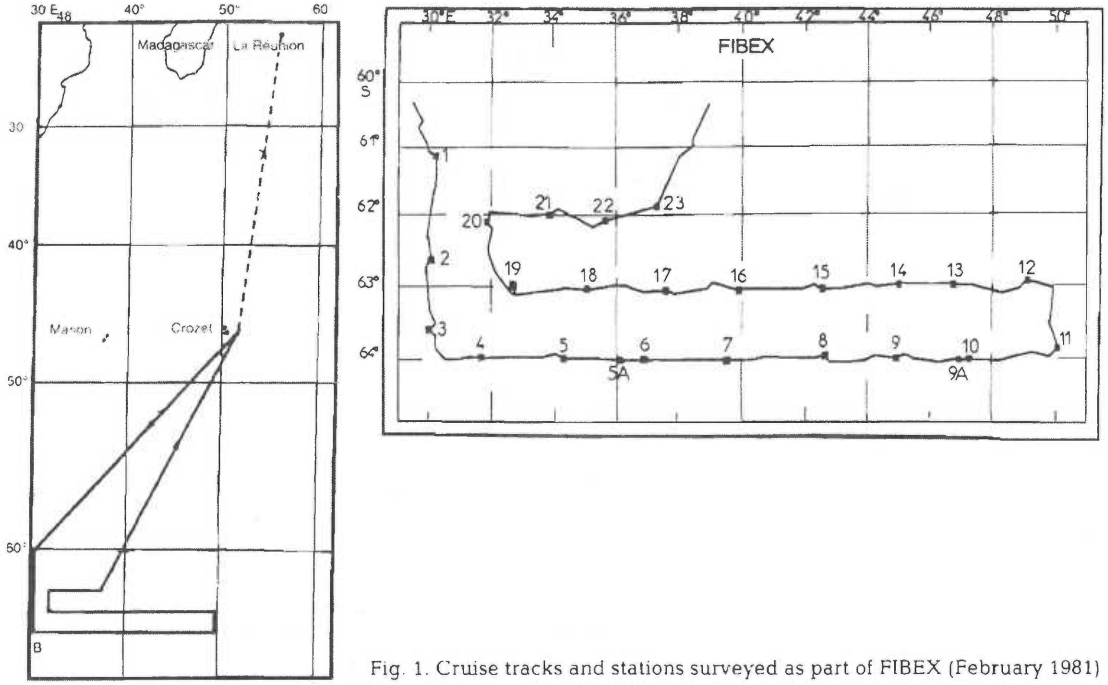

Fig. 1. Cruise tracks and stations surveyed as part of FIBEX (February 1981)

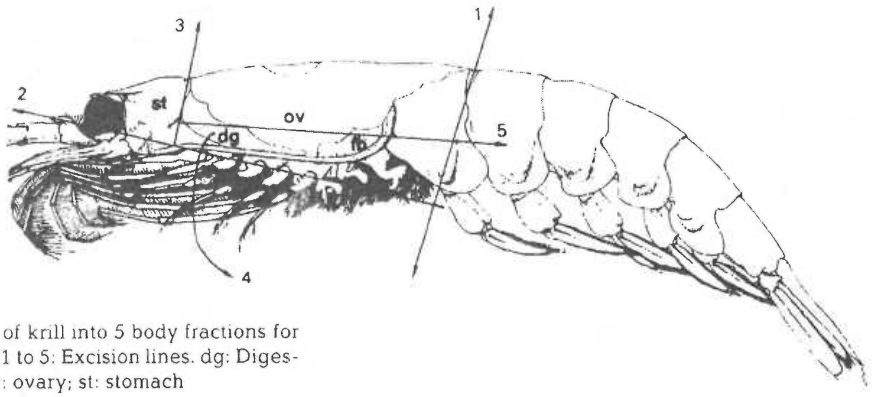

Fig. 2. *Euphausia superba*. Dissection of krill into 5 body fractions for the separation of the principal organs. 1 to 5: Excision lines. dg: Digestive gland; fb: fat body; ov: ovary; st: stomach

capture, rinsed with distilled water and deep frozen (−80°C). They were stored at −70°C under nitrogen and transported to France within 4 mo.

Additional samples were collected at different depths for particulate chlorophyll and proteins as descriptors of the trophic environment. Protocols and detailed results can be found in Mayzaud et al. (1985).

Stage determination. For population study (FIBEX), at each station a subset of 20 individual krill were identified to development or sexual stage, measured (BL: standard length 1; Mauchline 1980), weighed (wet weight: WW) and extracted. The rest of the sample was weighed and that part was treated as representative of the whole population. Female were separated into 'maturing' and 'post spawn' categories. Maturing females were recorded as IIIC (Makarov & Denys 1980).

For the study of specific stages and organs, frozen krill were scored individually for sex, sexual development and maturity (Makarov & Denys 1980, Cuzin-Roudy & Amsler 1991) and measured for body length (BL) while thawing on an ice cold plate under a microscope. Mature female krill (BL = 44.52 to 58.79 mm) with a swollen thorax were staged IIID and SDS 7 (Fig. 2). Spent female krill (BL = 43.52 to 55.70 mm) had a small ovary and contracted and irregular lobes. The thoracic cavity was mainly filled with hemolymph. They were scored as SDS 9 rather than IIIA, in order to take into account the ovarian regression which occurs normally at the end of the reproductive season (Cuzin-Roudy 1987, Cuzin-Roudy & Amsler 1991). Among the 10 male krill dissected (BL = 36.75 to 60.52 mm), 4 were mature and 6 had empty ampullae and were scored 'post mature'. Immature young adults (BL = 38.35 to 47.04 mm) will be referred to as subadults.

Separation of main organs. The krill were dissected while thawing to separate either organs or body fractions containing a main organ. During the dissection the specimens were placed on pre-weighted/pre-extracted filter paper (Whatman 42 extracted in chloroform:methanol 2:1), which collected the fluids originating from each step. Five fractions were separated (Fig. 2): (1) The *abdomen* fraction was obtained from excision lines 1 and 2 and contained mostly muscle and cuticle from the abdomen and the various appendages. (2) The anterior fraction containing principally the *stomach* was next obtained by excision line 3. Minor components were, in decreasing order: eyes, brain ganglia, and cuticle. (3) The *digestive gland* and the digestive tract were next excised as a whole from the anterior section of the thorax (excision line 4). Whenever the digestive gland was not sufficiently cohesive, recovery was accompanied by leakage of greenish fluid, which was collected on the filter paper, extracted and combined. (4) The fraction containing the *gonad* was obtained from the dorsal part of the thorax. In mature females the thoracic cavity was overfilled with the swollen ovary and excision along line 5 could not be made without damaging it. The resulting leakage of fluid was collected on the filter paper, extracted and combined. (5) The last fraction contained the *fat body*, the conjunctive tissue which fills the ventral part of the thoracic cavity and comes in to contact with the ovary (Cuzin-Roudy 1993). A very minor component was the nervous tissue and cuticle.

The wet weight of the 5 organs or body fractions was recorded, and additional specimens were dissected the same way to obtain wet weight/dry weight ratios. In this case dry weight was obtained after oven drying at 60°C to constant weight.

Lipid extraction and determination. Entire krill were placed frozen on crushed ice and brought to 0°C. Size (BL) and fresh weight (WW) were measured before lipid extraction, according to the method of Bligh & Dyer (1959). Either the extracted lipids were weighed in tarred vials or their concentration was estimated according to Barnes & Blackstock (1973) but with *Euphausia superba* lipids as standards instead of cholesterol. Both determinations yielded similar values. The lipid extracts were then placed under nitrogen at −70°C until analysis. Body fractions were extracted immediately after dissection using the same protocol.

Lipid classes were quantified after chromatographic separation coupled with FID detection on a Iatroscan Mark III TH 10 (Ackman 1981). Total lipid extracts were applied to chromarods SIII using microcapillaries (1 µl) and analyzed in duplicate. Neutral lipids were separated using a double development procedure with the following solvent systems: n-hexane:benzene:formic acid 80:20:0.5 (by volume) followed by n-hexane:diethylether:formic acid 97:3:0.5 (v/v). Glycolipids were separated according to Hirayama & Morita (1980) with chloroform:ethyl acetate:acetone:methanol:acetic acid:H_2O 60:12:15:16:3:3 (v/v). Phospholipids were separated with chloroform:methanol:H_2O 65:35:4 (v/v). Individual calibration of rods was achieved with commercial standards according to Ackman (1981). Using the solvent system indicated, all listed lipid classes, and in particular free fatty acids, were separated and accounted for.

To validate the Iatroscan separation and identification, neutral and polar lipids were further isolated on a preparative scale by column chromatography on silica gel (Bio-Sil HA, minus 325 mesh). The neutral lipid fraction was eluted with 6 column volumes of chloroform, the acetone mobile compounds were eluted with 4 volumes of acetone and the phospholipids were eluted with 6 volumes of methanol. All operations took place under nitrogen. Each fraction collected was further separated by thin-layer chromatography (TLC) on pre-coated silica gel plates (Analtech, Uniplate) and developed with hexane:diethylether:acetic acid 80:20:1.5 (v/v) for neutral lipids, or chloroform:methanol:aqueous ammonia 85:30:1 (v/v) for glycolipids, and chloroform:methanol:acetic acid:H_2O 25:15:4:2 (v/v) for polar lipids. Lipid classes were visualized using dichlorofluorescein and identification was achieved by comparison with standard mixtures. Specific detection of phospholipids and glycolipids by TLC was made with molybdenum blue and diphenylamine reagents (Stahl 1969). Each of these lipid classes was then applied to chromarods SIII and developed as previously to confirm both identification and retention times of the peaks recorded with total extract.

No attempt was made to hydrogenate the samples (Shantha & Ackman 1990) because no evidence of subfractionation effects due to the variety of fatty acids could be detected. As recently shown by Miller et al. (1998), the use of commercial standards resulted in an underestimate of the actual concentration of triglycerides but without changes in the shape of the FID response.

Data analysis. The allometric relations for the different maturity stages ($WW = a\,BL^b$) were computed after log-log transformation and model I regression (Sokal & Rohlf 1981).

One- and two-factor variance analyses were performed for total lipids as well as lipid classes. Multiple comparisons of means were achieved using the Tukey test procedure. Comparison of linear regression equations was achieved by covariance analysis. All the above procedures were carried out according to Sokal & Rohlf (1981). Systat 7.0 statistical package was used for all bivariate tests (Wilkinson 1996).

Principal component analysis (PCA) was performed after arcsine transformation to normalize percentage data. Details on the method and means of interpretation are given in Mayzaud et al. (1989). To facilitate the representation of the factor scores structure, an ascending hierarchical clustering method was used (Lebart et al. 1995) to group those observations which displayed maximum similitude and to produce a number of classes best represented in a dendrogram.

Correspondence analysis (Benzecri 1969, Gower 1987) was performed on a data matrix transformed to relative frequencies and scaled so that each row (or column) can be viewed as a row (or column) of conditional probability distribution. Distances between profiles were computed with χ^2 metrics. This distance gives symmetry to the 2 sets of data so that each factorial axis of the cloud of variables corresponds to a factorial axis of the cloud of observations. Thus, it was possible to represent simultaneously descriptors and observations on the plane defined by the factorial axes. Interpretation and representation followed that described above for PCA.

Computation of multivariate tests was made using the SPAD 3.0 software (Lebart et al. 1995).

RESULTS

Size, weight and lipid relationships

The size, wet weight and lipid content of the 4 categories of krill collected during the FIBEX cruise are summarized in Table 1. Subadults displayed the smallest size and weight of the 4 groups, while males and females showed similar size ranges (31 to 44 mm) but slightly different wet weight distribution. Mean values were not statistically different (*t*-test, $p < 0.05$) but ranges suggested a trend towards maturing females and males being heavier (0.57 to 1.28 mg and 0.4 to 1.21 mg, respectively) compared to post spawn females. Lipid content relative to wet weight suggested maximum accumulation in maturing females (3.3%). Males tended to show minimum lipid content although the high variability in this case prevented statistical significance.

The wet weight (WW) relationships with size or lipid content were established for each stage present in the samples collected, i.e. subadults, males and females. The log-log regressions between size and weight

Table 1. *Euphausia superba*. Range and mean values of krill size, wet weight and total lipid content (% wet weight) of specific maturity stages of individuals collected during FIBEX. n = number of individuals analyzed

Stage	n	Size range (mm)	Mean size ± SD	Wet weight range (mg)	Mean wet weight ± SD	Total lipid range (% wet wt)	Mean total lipid ± SD
Subadults	33	23–35	29.7 ± 3.1	0.20–0.59	0.37 ± 0.12	1.1–4.7	2.9 ± 0.9
Males	54	31–43	37.1 ± 2.6	0.40–1.21	0.74 ± 0.18	0.7–5.0	2.2 ± 0.9
Maturing females	14	35–44	39.3 ± 2.6	0.57–1.28	0.93 ± 0.22	1.6–4.8	3.3 ± 0.8
Post spawn females	20	35–42	38.1 ± 1.8	0.53–0.96	0.74 ± 0.11	1.1–4.8	2.8 ± 1.2

(Fig. 3) appeared to be similar for all 3 stages (slope: $F_{2,118} = 2.07$; intercept: $F_{2,120} = 0.26$) corresponding to an overall regression equation of:

$$\log WW = -0.08 + 3.12 \log BL$$
$$(r = 0.967,\ F_{1,124} = 1779,\ p < 0.0001)$$

Relationships between WW and total lipids or phospholipids (Fig. 3) were all significant ($p < 0.008$). The regressions for male individuals showed slopes or intercepts different from the other 2 stages. Males and females appeared to accumulate lipids and phospholipids at a similar rate (slope: $F_{1,85} = 0.272$) but with a lower intensity (intercept: $F_{1,86} = 14.25$) for a given size in males. Triglycerides illustrated a different pattern of changes with only 2 significant regressions, those for subadults (n = 33, r = 0.673) and females (n = 33, r = 0.556), which showed a significant difference in intercept ($F_{1,66} = 5.54$) but not in slope ($F_{1,65} = 0.04$). In these 2 stages, triglyc-

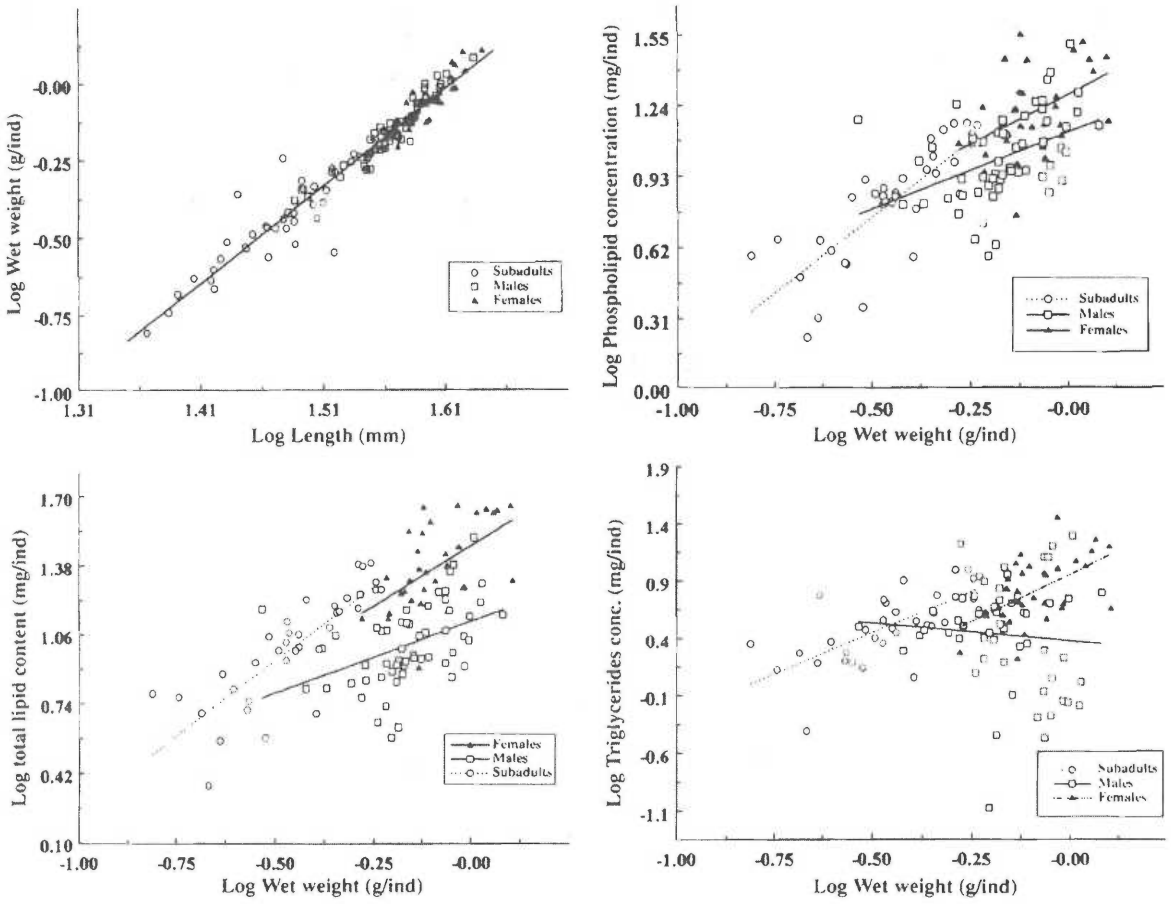

Fig. 3. *Euphausia superba*. Log-log regressions, for the 3 major developmental stages, between wet weight and body length and between wet weight and total lipid, phospholipids and triglycerides content

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