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LIPOLYSIS *POST MORTEM* IN NORTH ATLANTIC KRILL

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Abstract—1. Three common species of North Atlantic krill, *Meganyctiphanes norvegica* (M. Sars), *Thysanoessa inermis* (Krøyer) and *T. raschii* (M. Sars), have been stored at 0°C *post mortem*, and the lipolytic activity followed by measuring changes in the lipid composition during storage.

2. Both phosphoglycerides and triacylglycerols were subjected to extensive hydrolysis with the formation of free fatty acids in all krill species examined, whereas wax esters, constituting a considerable proportion of the lipids in the *Thysanoessa* species, were not hydrolysed at all.

3. In *M. norvegica* the triacylglycerols and phosphoglycerides were hydrolysed at similar rates, whereas in *T. inermis* and *T. raschii* the phosphoglycerides were hydrolysed most rapidly.

4. For all krill species examined, the rate of production of free fatty acids was nearly constant during the initial phase of storage, and subsequently declined on prolonged storage.

5. At the end of the storage period of 16–24 days, the free fatty acids constituted about 35% of the total lipid in *M. norvegica*, and about 50% in the *Thysanoessa* species.

6. The rate of production of free fatty acids was about the same in all the three species of krill and seemed to be independent of the total lipid content.

INTRODUCTION

Recent studies on Antarctic krill have demonstrated that both phosphoglycerides and triacylglycerols serve as depot lipids in *Euphausia superba*, whereas *E. crystallorophias* deposits wax esters, triacylglycerols and phosphoglycerides (Ellingsen and Mohr, 1981; Ellingsen, 1982). Furthermore, Ellingsen (1982) has shown that storage of samples of krill *post mortem* resulted in extensive degradation of triacylglycerols and phosphoglycerides with the formation of free fatty acids, thus suggesting that these species of Antarctic krill contain significant levels of active lipases and phospholipases.

The work by Ellingsen (1982) formed the basis of a program for examination of the chemical composition and *post mortem* changes in three common species of North Atlantic krill: *M. norvegica*, *T. inermis* and *T. raschii*. The lipid composition of North Atlantic krill has been examined in detail by Sargent and Falk-Petersen (1981) and Saether *et al.* (1985). Saether *et al.* (1985) have provided clear evidence that triacylglycerols are the only depot lipids in *M. norvegica*, whereas phosphoglycerides, wax esters and triacylglycerols serve as depot lipids in *T. inermis* and *T. raschii*. The *post mortem* changes in the lipid of these species have not as yet been studied. The present communication provides data relating to the *post mortem* changes in the lipids of these species of North Atlantic krill.

MATERIALS AND METHODS

The krill used in the present studies were caught in Balsfjorden and Ullsfjorden in Northern Norway during the period 23 February–21 April 1982. Details of the catches are [illegible] been described in detail by Saether *et al.* (1985). On board the vessel 20–50 ml samples of whole and homogenized krill were distributed into 100 ml plastic bottles with screw caps within 15–30 min of catching, and stored as described below. Krill were also frozen in CO_2–ice within 5–15 min of catching and stored at −80°C for no longer than 1 year before being used experimentally. A closer examination of the different *Thysanoessa* species was carried out in the laboratory. Blocks of krill were thawed and sorted according to species at 0°C within 4–6 hr of thawing. Each specimen was subsequently refrozen on CO_2–ice after sorting. Krill belonging to the same species and haul were pooled and homogenized in the frozen state, using an Ultra-Turrax TP 18-10 (Janke & Kunkel KG). Samples of 2–5 g of the homogenates were distributed into 10 ml glass tubes with screw caps.

The samples of both fresh and frozen/thawed krill were stored in an ice-water bath, and individual samples removed as a function of time, and stored at −80°C until analysed. The entire content of each sample was homogenized and used in the analysis. The lipids were extracted with chloroform–methanol–water according to Hardy and Keay (1972). The main classes of lipid were separated by thin layer chromatography in accordance with Ellingsen (1982), as described by Saether *et al.* (1985). The dry weight of each sample was determined by drying 1–2 g of homogenate for 24 hr at 105°C.

RESULTS AND DISCUSSION

The *post mortem* changes in the lipid constituents of three common species of North Atlantic krill, *M. norvegica*, *T. inermis* and *T. raschii*, were examined at 0°C. The studies were based on five catches of krill from fjords in northern Norway over a period of 2 months (February–April). Based on measurements of length, a majority of the krill examined seemed to be [illegible]

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Table 1. Data on the krill catches

Haul No.	Date	Locality	Krill species	Total lipid content (% of dry weight)
21	23.02.82	Svartnes in Balsfjorden	*T. inermis*	35.3
			T. raschii	27.6
			unsorted *Thysanoessa**	33.1
23	23.03.82	Ulsfjorden	*M. norvegica*	26.0
24	23.03.82	Svartnes in Balsfjorden	*T. inermis*	—
			T. raschii	—
			unsorted *Thysanoessa*†	25.5
27	20.04.82	Ulsfjorden	*M. norvegica*	22.7
28	21.04.82	Svartnes in Balsfjorden	*T. inermis*	14.9
			T. raschii	11.4
			unsorted *Thysanoessa*†	13.9

*Mixture of 70% *T. inermis* and 30% *T. raschii*.
†Mixture of 80% *T. inermis* and 20% *T. raschii*.

In *M. norvegica* both triacylglycerols and phosphoglycerides were hydrolysed with the formation of free fatty acids *post mortem*, suggesting that both lipases and phospholipases were active during storage. Judging from the data, the hydrolysis of triacylglycerols and phosphoglycerides proceeded at similar rates. The rate of release of fatty acids gradually decreased during storage in both catches examined, and at the end of the storage period, free fatty acids accounted for about 35% of the total lipid (Fig. 1). The rate of release of free fatty acids, on a dry weight basis, was also similar in the two catches (Fig. 2).

In the *Thysanoessa* species the increase in the free fatty acid content *post mortem* was primarily due to extensive hydrolysis of phosphoglycerides, but the hydrolysis of triacylglycerols was also significant (Fig. 3).

The rate of free fatty acid production was about the same in krill having widely different total lipid contents (13.9–33% dry wt), thus suggesting that the rate of lipid hydrolysis was independent of the total lipid content. (Table 1 and Fig. 2). At the end of the storage period free fatty acids constituted about 50% of the total lipid in the *Thysanoessa* species, which is significantly higher than in *M. norvegica*. A com-

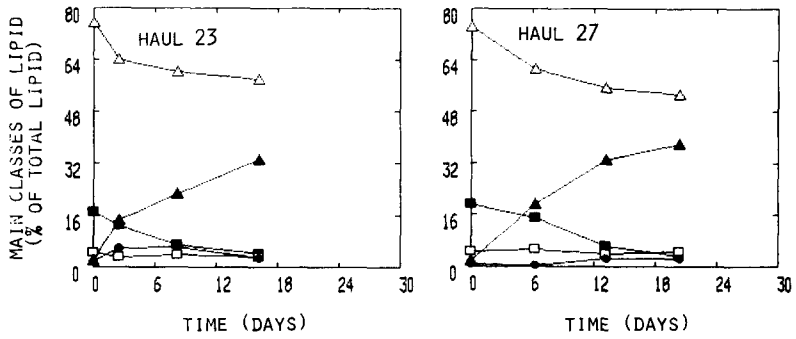

Fig. 1. Content of main classes of lipid during storage of *M. norvegica* (haul 23 and 27) at 0°C *post mortem*. Wax esters: ●, triacylglycerols: △, free fatty acids: ▲, cholesterol + diacylglycerols: □, phosphoglycerides: ■.

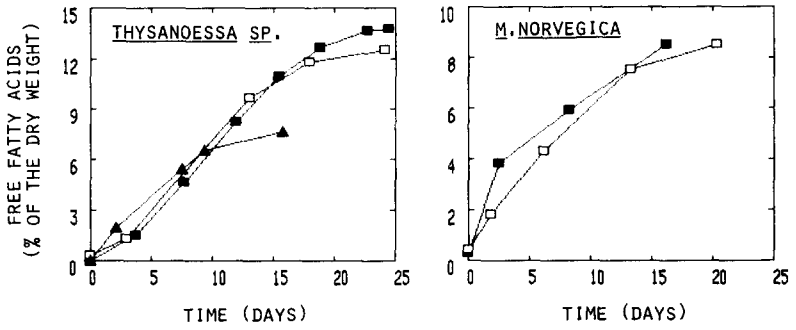

Fig. 2. Content of free fatty acids in different hauls of *Thysanoessa* species (haul 21: ■, 24: □ and 28: ▲) and in *M. norvegica* (haul 23: ■ and 27: □) stored at 0°C *post mortem*.

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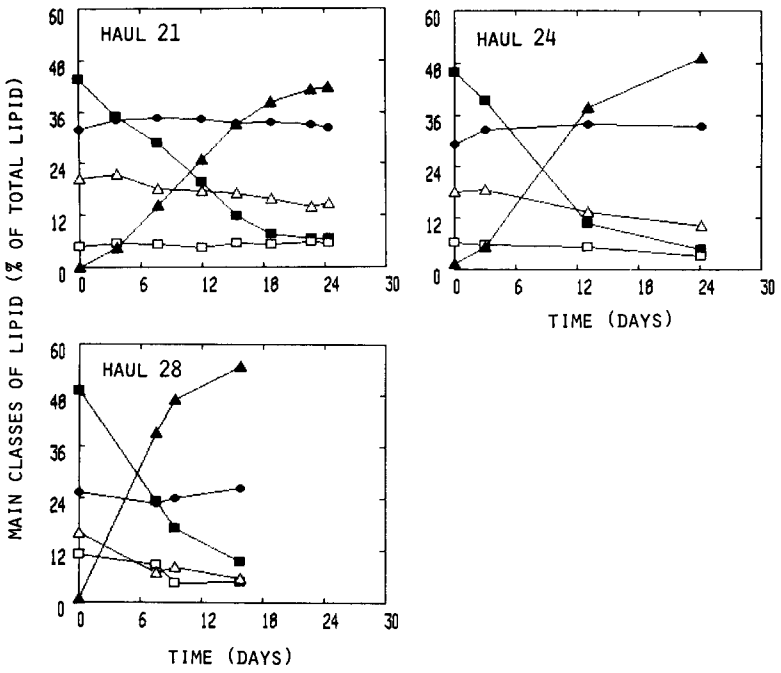

Fig. 3. Content of main classes of lipid during storage of *Thysanoessa* species (haul 21, 24 and 28) at 0°C *post mortem*. Wax esters: ●, triacylglycerols: △, free fatty acids: ▲, cholesterol + diacylglycerols: □, phosphoglycerides: ■.

parison of the rates of hydrolysis in North Atlantic and Antarctic krill reveals that the rates observed in *M. norvegica*, the *Thysanoessa* species and *E. crystallorophias* are nearly the same, and higher than that detected in *E. superba* (Fig. 4). The content of wax esters and cholesterol varied little during storage of the North Atlantic krill species *post mortem*, suggesting that there is no significant formation of cholesteryl esters during the first week of storage (Fig. 3). The contents of wax esters and cholesterol were likewise constant during the first week of storage of *E. crystallorophias* (Ellingsen, 1982). Esterification of cholesterol has been observed during storage of fish *post mortem* (Lovern *et al.*, 1959).

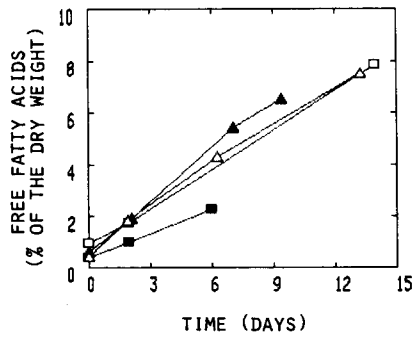

Fig. 4. Content of free fatty acids during *post mortem* storage at 0°C of *M. norvegica* (haul 27: △), *Thysanoessa* species (haul 28: ▲) and Antarctic krill species, examined by

The studies of the *post mortem* changes in the *Thysanoessa* species on board the research vessel were based on catches containing 70–80% *T. inermis* and 20–30% *T. raschii*. A separation of *T. inermis* and *T. raschii* required microscopy of each individual, which was not possible on board the vessel, due to priority given to other experiments. However, frozen krill from haul 28 were thawed in the laboratory, sorted according to species, homogenized and stored at 0°C for a closer examination of lipolytic activity in *T. inermis* and *T. raschii*. The results indicate a similar pattern of lipid degradation in the two species (Fig. 5). Furthermore, the rate of release of free fatty acids was also nearly the same in the two species (Fig. 6).

It is of interest to consider the lipolytic activity in relation to the lipid class composition of the different species of krill. In *M. norvegica* triacylglycerols are the only depot lipids, whereas in *T. inermis* and *T. raschii* phosphoglycerides, wax esters and triacylglycerols serve as depot lipids (Saether *et al.*, 1985). *E. crystallorophias* also deposits phosphoglycerides, wax esters and triacylglycerols, whilst *E. superba* uses only phosphoglycerides and triacylglycerols as depot lipids (Ellingsen, 1982). A systematic comparison of the different species of krill reveal no correlation between the relative proportion of the main classes of lipid, and the rate at which the lipids are hydrolysed. It appears, therefore, that the lipolytic activity in each species depends on other factors, such as the level of lipases and phospholipases in the krill, the extent of emulsification of the substrates and the degree of contact between enzymes and substrates. Studies of different species of

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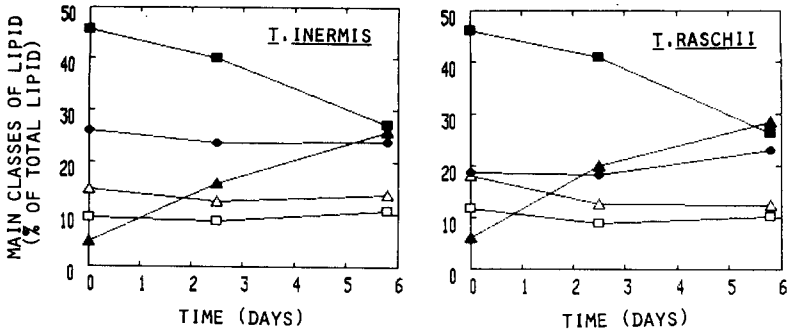

Fig. 5. Content of main classes of lipid during storage of homogenized, frozen and thawed *T. inermis* and *T. raschii* (haul 28) at 0°C. Wax esters: ●, triacylglycerols: △, free fatty acids: ▲, cholesterol + diacylglycerols: □, phosphoglycerides: ■.

the lipase- and phospholipase activity and the content of triacylglycerols and phosphoglycerides (Olley *et al.*, 1962; Shono and Toyomizu, 1973; Nair *et al.*, 1978).

As pointed out above, phosphoglycerides were subjected to more extensive hydrolysis than triacylglycerols in the *Thysanoessa* species (Figs 3 and 5). A similar observation was made in the study of the lipid degradation in the Antarctic krill *E. superba* and *E. crystallorophias* (Ellingsen, 1982), whereas in *M. norvegica* the lipase activity was of the same order as the phospholipase activity (Fig. 1). It has been proposed that the more extensive degradation of phosphoglycerides than triacylglycerols in Antarctic krill species may be due to the more hydrophilic character of the phosphoglycerides, and hence a better enzyme to substrate contact (Ellingsen, 1982). The same may apply to North Atlantic *Thysanoessa* species. Triacylglycerols are more hydrophobic, and may as a result, require more effective emulsification for hydrolysis to take place. Wax esters are even less polar than triacylglycerols, and inadequate emulsification and/or a low enzyme level may explain why the wax esters were not subjected to *post mortem* hydrolysis neither in the *Thysanoessa* species nor in *E. crystallorophias* (present results and Ellingsen, 1982) The triacylglycerols in *M. norvegica* were subjected to much more extensive hydrolysis than those of the *Thysanoessa* species, which may be due to a higher lipase content, and/or better emulsification and, hence, a better enzyme-substrate contact.

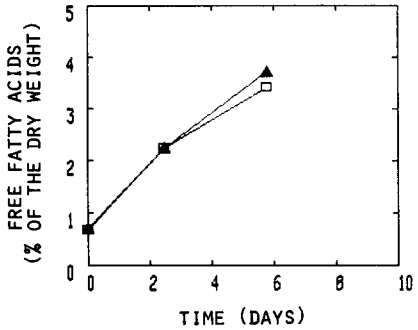

Fig. 6. Content of free fatty acids during storage of homogenized, frozen and thawed *T. inermis* (haul 28: ▲) and *T. raschii* (haul 28: □) at 0°C *post mortem*.

The lipids and lipolytic enzymes are presumably separated in the tissues of living krill. Thus, proteolytic activity is probably required to bring the lipids into contact with the lipolytic enzymes *post mortem*, as suggested by Ellingsen (1982). An extensive proteolysis *post mortem* has been established

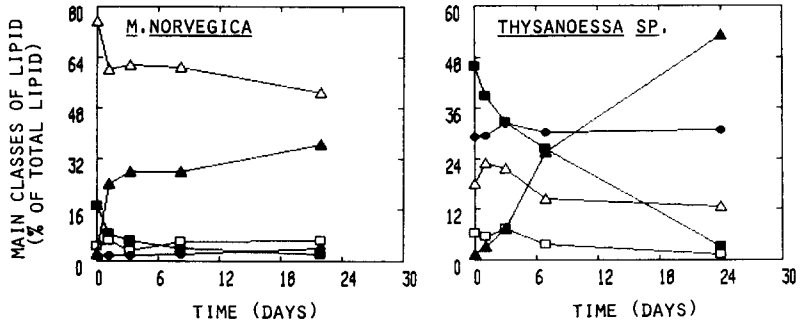

Fig. 7. Content of main classes of lipid during storage of homogenized *M. norvegica* (haul 23) and *Thysanoessa* species (haul 24) at 0°C *post mortem*. Wax esters: ●, triacylglycerols: △, free fatty acids:

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both in Antarctic krill (Ellingsen, 1982) and in North Atlantic krill (unpublished results), which may explain why lipolysis in all species of krill examined so far proceeded without any distinct, initial lag-phase (Figs 1 and 3; Ellingsen, 1982). Storage of homogenized samples of *M. norvegica* revealed a three-fold increase in the initial rate of hydrolysis of both phosphoglycerides and triacylglycerols as compared to whole krill, whereas in the *Thysanoessa* species, there were no significant differences in the rate of hydrolysis of whole- and homogenized animals (Figs 1–3, 7).

Separate studies have revealed that the bacterial activity seems to be low during the first week of storage of *M. norvegica* and *Thysanoessa* species at 0°C (unpublished results). Thus, there is reason to believe that the *post mortem* changes observed during the first week of storage are primarily due to autolytic processes. Low bacterial activity during the first week of storage *post mortem* has also been observed in Antarctic krill (Ellingsen, 1982).

In conclusion, the present work and the studies of Ellingsen (1982) have provided evidence that the hydrolysis of both phosphoglycerides and triacylglycerols is very rapid and extensive in both North Atlantic- and Antarctic krill, whereas the content of wax esters seem to be constant during *post mortem* storage of wax ester rich species. It is also concluded that the rate of production of free fatty acids, on a dry weight basis, is about the same in *M. norvegica*, *T. inermis* and *T. raschii* as in the Antarctic krill *E. crystallorophias*, examined by Ellingsen (1982).

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