SOME OBSERVATIONS ON KRILL (*Euphausia superba* Dana) MAINTAINED ALIVE IN THE LABORATORY

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ABSTRACT. Specimens from three swarms of adolescent krill, *Euphausia superba* Dana, were maintained in a laboratory for several months. Fresh weight, dry weight and ash weight interrelationships were examined in whole krill and cast moults. Moulting occurred at 13–14 day intervals during summer, becoming less frequent towards winter when moulting ceased altogether. Although kept singly, individual krill from the same swarm tended to moult together. Cast moults represented a loss of 10 per cent of the animal's total mineral salts, but, apart from chitin, losses of organic material were small.

THE trivial name krill comes from an old Norwegian whaling term for whale food, and is often applied more or less indiscriminately to any swarming crustacean which may form the food of baleen whales. In particular, it is frequently applied to euphausiids, of which five species occur regularly in waters south of the Antarctic Convergence (John, 1936; Mauchline and Fisher, 1969). In this paper the term krill is restricted to the large Antarctic euphausiid *Euphausia superba* Dana.

E. superba forms the principal food of many whales, seals, penguins and fish, and is an important organism in the Antarctic food web (Knox, 1970). Working from material collected by the Discovery Investigations, Marr (1962) and Mackintosh (1972) have largely elucidated the life cycle of krill and the effect of latitude and water temperature on growth rate, but data on the physiology and biochemistry of krill are preliminary and scattered.

Mackintosh (1967) maintained a small number of krill in glass jars at South Georgia and presented preliminary data for moulting frequency. He tentatively confirmed that moulting is either less frequent or ceases altogether during winter, a suggestion originally advanced by Marr (1962). Raymont and others (1971) and Ferguson and Raymont (1974) have analysed the gross biochemical composition of deep-frozen and freeze-dried samples of krill, and Srinivasagam and others (1971) determined the composition of free and protein-bound amino acids in whole krill. The lipid component has been examined in detail by several authors. Bottino (1973) found wax esters present in the related *E. crystallorophias* but not in *E. superba*, and the fatty acids have been examined by a number of workers (Nonaka and Koizumi, 1964; Hansen, 1969; Pierce and others, 1969; Hansen and Meiklen, 1970; Sidhu and others, 1970; Van der Veen and others, 1971; Bottino, 1972).

The data presented here are the results of observations on a group of krill maintained alive in a laboratory at King Edward Point, South Georgia (lat. 54° 17′ S., long. 36° 30′ W.), during the summer of 1970–71.

MATERIALS AND METHODS

Specimens of *E. superba* were obtained from swarms which appeared in King Edward Cove on 1 December 1970, 1 January 1971 and 2 March 1971. Krill were captured with a small hand net and transferred to either 7 or 45 l. polyethylene buckets containing fresh sea-water. Single krill were kept in the small buckets, but up to 30 were maintained in the large bins. To maintain environmental temperature in the buckets they were placed in a depth of 6 in. of flowing sea-water. Water in the buckets was changed at approximately weekly intervals and the green colour of the stomachs suggested that the krill were successfully feeding on diatoms contained in the sea-water. Although some krill died very soon after capture, 75 per cent survived more than 1 month and 60 per cent passed through four or more ecdyses. However, in all cases where a consecutive series of cast moults was obtained from an individual krill, the dry weights of these moults steadily decreased. Death invariably occurred during or shortly after moulting, which always took place at night (as recorded for *E. pacifica* by Komaki (1966), Lasker (1966) and Fowler and others (1971)).

At least once every 24 hr. the temperature of the water in the buckets was measured and any moults or corpses removed. Fresh moults were collected, blotted dry with a filter paper,

weighed, dried for 24 hr. in an oven at 60° C and weighed again. The dried moults were then pooled to provide sufficient material and analysed for either ash or lipid and carbohydrate content. Insufficient material was obtained to analyse for protein or chitin.

Ash content was determined after ignition for 24 hr. in a muffle furnace at 550° C. This temperature was sufficient for complete ignition, but not high enough to cause significant loss of potassium (Grove and others, 1961).

Lipid was extracted from the dried moults by the method of Bligh and Dyer (1959). After phase separation the lipid weight was determined by evaporating the lower (chloroform) layer in a tarred flask. The upper (methanol–water) phase and the interfacial fluff were each refluxed with 10 per cent aqueous trichloracetic acid for 1 hr. and the carbohydrates determined by the phenol/sulphuric acid method of Dubois and others (1956), using glucose as standard.

The composition of the lipids in cast moults was examined by thin-layer chromatography using the solvent system of Freeman and West (1966). Lipid bands were detected by spraying the plate with 50 per cent v/v aqueous sulphuric acid followed by heating to 100° C, and identified by comparing their R_f values with those of standard lipids.

Separate samples from each swarm were collected for measurement. The following measurements were made on the dorsal surface of straightened krill, using vernier calipers:

Total length	The distance from the rear of the eye socket to the tip of the telson.
Carapace length	The distance from the rear of the eye socket to the rear of the carapace in the dorsal mid line.
Carapace width	The greatest width of the carapace.
Uropod length	The distance from the base to the tip of the uropod, including setae.

The fresh weight of individual krill was measured after gentle blotting on a filter paper. Dry weight was measured after drying for 24 hr. in an oven at 60° C, and ash weight after ignition for 24 hr. in a muffle furnace at 550° C.

Results

Details of the samples from the three swarms are given in Table I. All three samples con-

Table I. Total length and fresh-weight data for krill samples

Date	*Total length mean±standard deviation* (mm.)	*n*	*Fresh weight mean±standard deviation* (mg.)	*n*
1 December 1970	37·0±2·9	19	n.d.	
1 January 1971	39·1±5·7	20	551·9±354·8	20
2 March 1971	27·5±3·2	29	156·9±63·2	30

n.d. Not determined.

sisted of male and female adolescent krill corresponding to stages B and C of Bargmann's (1945) classification. The mean length of the krill from the March swarm was significantly smaller than that of krill from either the December or January swarms ($P < 0{\cdot}001$). This heterogeneity in the krill population appears to be due to different hatching times and growth rates caused by variations in temperature and food availability within the distribution of *E. superba* (Mackintosh, 1972).

The relationship between total length and fresh weight is shown in Fig. 1. A straight line was fitted by the method of least squares to the logarithmically transformed data, and the

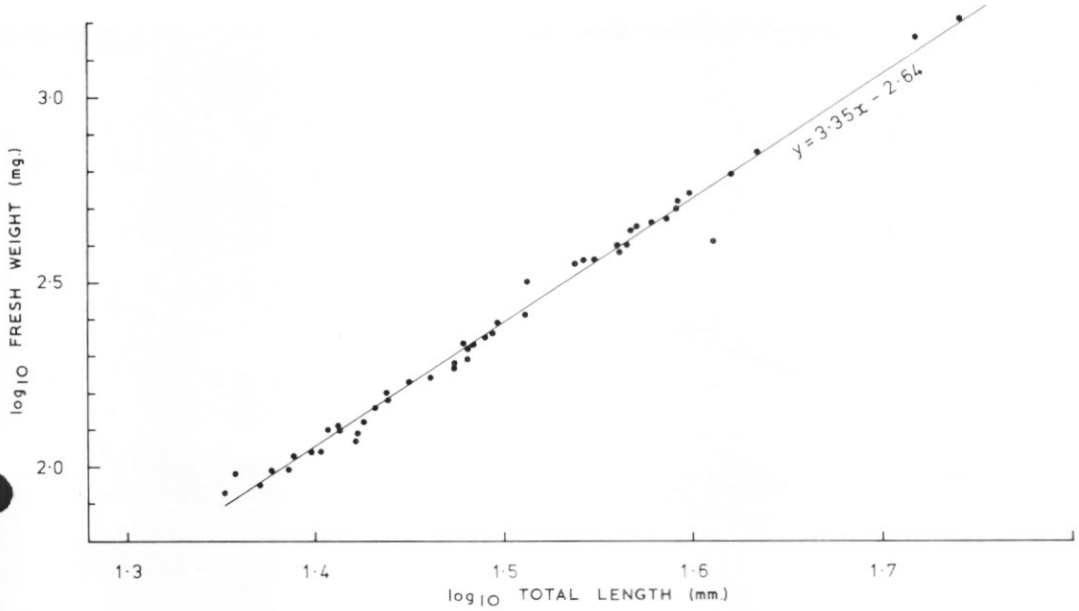

Fig. 1. Relationship between fresh weight and total length for adolescent krill of both sexes from all three swarms. The line was fitted by the method of least squares to the logarithmically transformed data. Variance about the regression line: $1{\cdot}10 \times 10^{-3}$; variance of the regression coefficient: $2{\cdot}73 \times 10^{-3}$; n: 49. This relationship is not significantly different from that of McHardy (presented by Lockyer *in* Mackintosh (1973)); t: 0·233, $0{\cdot}90 > P > 0{\cdot}80$.

relationship is not significantly different from that obtained by McHardy for freshly thawed deep-frozen specimens (data presented by Lockyer *in* Mackintosh (1973).) This relationship has also been investigated by Nemoto (1959) and Burukovsky (1967) using fresh krill, and also by Heyerdahl (1932) using krill sampled from whale stomachs. However, these latter authors all expressed their data as a series of averaged points and so comparison with this study is not possible. Lockyer (*in* Mackintosh, 1973) presented a factor for converting volume data (measured on formalin- and alcohol-preserved specimens) to weight data (measured on freshly thawed deep-frozen specimens), and Mauchline (1967) analysed the volume and weight characteristics of formalin-preserved specimens of many species of euphausiid, including krill. However, comparison of data from fresh material with data from formalin-preserved specimens is of little value since this preservative causes weight changes (Howmiller, 1972). The relationships between dry weight and fresh weight, and between ash weight and dry weight are shown in Figs. 2 and 3. These results from fresh samples fall within the range of values reported for frozen samples by Ferguson and Raymont (1974). The relationships between the other measured variables are given in Table II.

TABLE II. MEASUREMENTS OF KRILL

	Mean	*Standard deviation*	*n*
Ratio, carapace length/total length	0·296	0·013	64
Ratio, uropod length/total length	0·205	0·010	63
Ratio, carapace width/carapace length	0·361	0·025	62

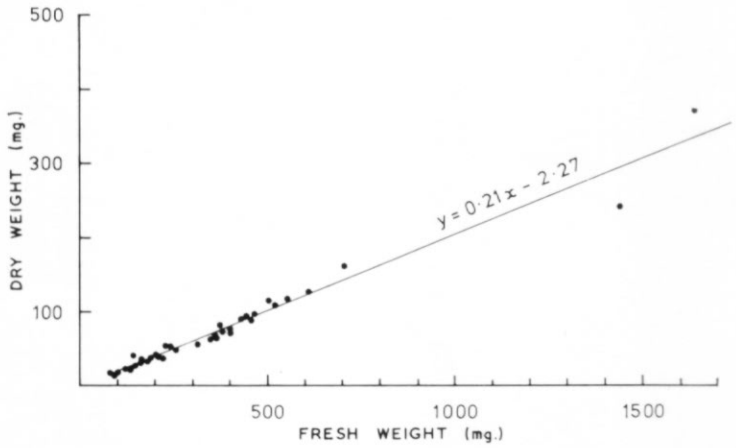

Fig. 2. Relationship between dry weight and fresh weight for adolescent krill of both sexes from all three swarms. The line was fitted by the method of least squares. Variance about the regression line: 120·9; variance of the regression coefficient: $2{\cdot}81 \times 10^{-5}$; n: 47.

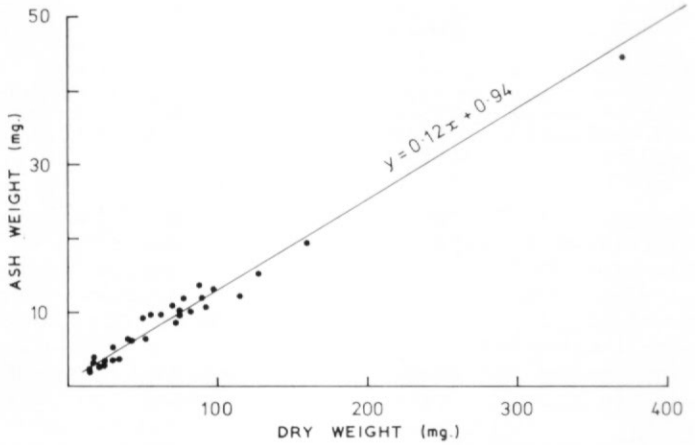

Fig. 3. Relationship between ash weight and dry weight for adolescent krill of both sexes from all three swarms. The line was fitted by the method of least squares. Variance about the regression line: 1·55; variance of the regression coefficient: $1{\cdot}16 \times 10^{-6}$; n: 31.

The results obtained for moulting frequency largely confirm the pattern observed by Mackintosh (1967). During summer the krill moulted at 13–14 day intervals, and this interval increased towards winter. The relationship between intermoult period and time was different for each swarm (Fig. 4). The increase in intermoult period was associated with a fall in temperature of the water in the containers, and moulting ceased altogether in those krill surviving into winter. An increase in intermoult period with decreasing temperature has been reported for *E. pacifica*, and this relationship appeared to be independent of whether or not the euphausiids were fed (Lasker, 1966; Fowler and others, 1971).

Plotting the occurrence of moulting as a series of cumulative percentage frequency curves for each swarm separately demonstrated that, although kept singly, members of the same swarm tended to moult together (Fig. 5). Fowler and others (1971) noted that *E. pacifica* tended to moult non-randomly shortly after capture, although moulting quickly became random. This initial tendency was attributed to physiological shock induced by capture.

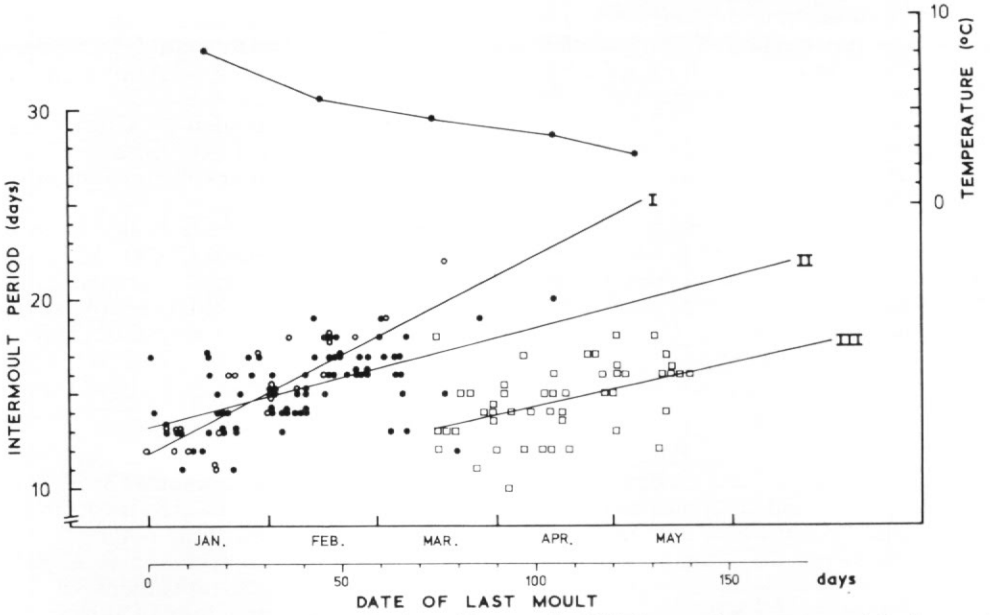

Fig. 4. Variation of intermoult period with date of last moult. Krill from each swarm were plotted separately and the regression lines were fitted by the method of least squares.

o and line I (slope: 0·10, intercept: 11·90) 1 December 1970 swarm.
● and line II (slope: 0·05, intercept: 13·15) 1 January 1971 swarm.
□ and line III (slope: 0·04, intercept: 9·96) 2 March 1971 swarm.
●————● Monthly mean temperature of sea-water in buckets.

The slopes of line I and II, and of lines I and III are significantly different ($P>0·01$). The slopes of lines II and III are not significantly different at the 5 per cent level, but the intercepts do differ significantly ($P>0·01$).

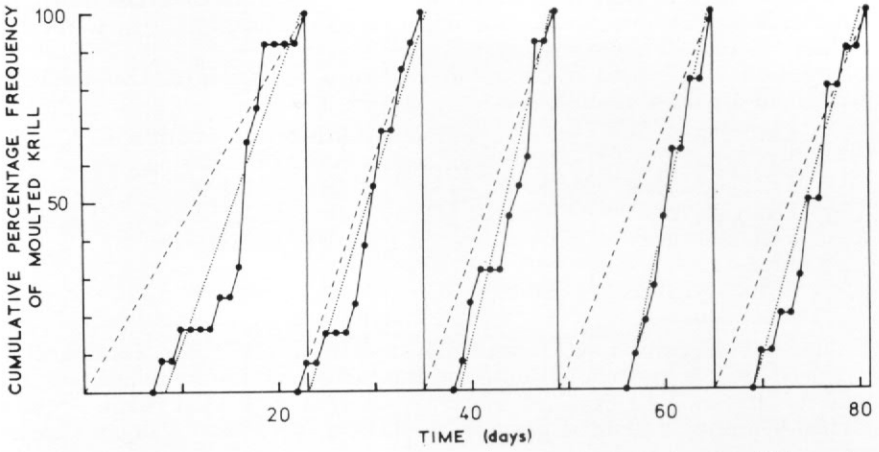

Fig. 5. Cumulative percentage frequency of krill that have moulted; plotted for 13 krill from the swarm of 2 March 1971.

●————● Cumulative percentage frequency curves for each bout of moulting.

The average trends of these frequency curves was described by a straight line fitted to all the points on each curve by the method of least squares. The slope of each of these lines was then compared with the slope of a line representing random moulting.

····· Average trend lines for cumulative frequency curves.
– – – – – – – – Lines representing random moulting.

In four of the five cases, these lines differed in slope at the 5 per cent level of significance.

In contrast, in the data presented here for *E. superba*, synchrony increased with time. Nevertheless, it is not possible to say whether the increase in intermoult period with time and the tendency towards synchronous moulting are artefacts caused by physiological shock induced by capture, or whether they reflect the situation in oceanic swarms.

It has been remarked that a large biomass of rapidly moulting planktonic Crustacea must contribute a significant weight of organic detritus, as cast moults, to the oceanic food web (e.g. Lasker, 1964). Some measurements were therefore made to evaluate the possible nutritive value of krill moults.

The results of the biochemical analyses of krill moults are shown in Table III, and are compared with published data for whole krill (Ferguson and Raymont, 1974). Although no measurements were made, it was assumed that the bulk of the chitin + protein fraction consisted of chitin. The comparison with data for whole krill assumes that the dry weight of a cast moult was approximately 3 per cent of the dry weight of the whole krill. This value was obtained from the expression

$$\frac{100 \times \text{Mean dry weight of first moults from specimens of a given swarm}}{\text{Mean dry weight of a sample of whole krill from that swarm}}.$$

The values obtained for the January and March swarms were 2·5 and 3·1 per cent, respectively. These values are low compared with values of 10–11 per cent obtained for *E. pacifica* by Lasker (1966), and so comparisons based on a value of 10 per cent are also included in Table III. Lasker (1964) determined the nitrogen content of *E. pacifica* moults as 1·5 to 2·5 per cent of the dry weight. Assuming a similar biochemical composition in *E. pacifica* and *E. superba* moults, this would imply approximate chitin and protein contents of 34 and 12·5 per cent of the dry weight, respectively, and losses of chitin at moulting of at least 26 and 86 per cent for moult dry weights of 3 and 10 per cent of the krill dry weight, respectively. Losses of chitin at moulting may thus be significant.

The value of cast moults to the nekton or benthos will depend on the rate of decomposition and the speed at which the moults sink. The sinking speed of freshly cast moults was measured by timing their fall past a series of marks in a wide-bore glass column containing fresh sea-water at +4° C. Care was taken to minimize edge effects and to ensure that terminal velocity had been reached before timing was started. The mean sinking speed obtained for seven fresh moults was 0·83 cm./sec. (standard deviation 0·19). The rate of break-down by chitin-digesting bacteria was not measured, although four moults kept at +2° C for 14 days in fresh sea-water showed no signs of decay.

The composition of the lipid fraction was examined by thin-layer chromatography and the following lipid classes were identified:

Carotenoid	Faint, although much of this fraction may have been denatured by oven drying.
Triacylglycerol	Faint.
Diacylglycerol	Trace.
Free fatty acid	Faint.
Free sterol	Strong.
Monoacylglycerol	Trace.
Polar lipids	Strong, presumably mostly phospholipids.

This qualitative composition agrees with that of Bottino (1972) for whole krill, and presumably largely reflects the composition of the structural lipids comprising cell walls, membranes, etc. It would be expected that any purely storage lipid (such as quantities of triacylglycerol) would be mobilized prior to moulting to keep loss of organic material to a minimum.

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TABLE III. BIOCHEMICAL ANALYSES OF KRILL MOULTS

	Composition of cast krill moults (per cent of the dry weight). Mean ± standard deviation	*n*	*Approximate composition of whole krill of dry weight 100 mg. (from Ferguson and Raymont, 1974)* (mg.)	*Percentage of weight in whole krill lost in moult (assuming moult dry weight = 3 per cent whole krill dry weight)*†	*Percentage of weight in whole krill lost in moult (assuming moult dry weight = 10 per cent whole krill dry weight)*†
Ash	49	1	16	9·2	30·6
Lipid	4·2±1·4	3	30	0·4	1·4
Soluble carbohydrate	0·045±0·02	4	ca. 3·0	0·1	0·4
Insoluble carbohydrate	0·065±0·03	4			
Protein	46·7*		47	2·8	9·2
Chitin			4 (51)		

* By subtraction.
† For explanation see text.

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