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RNA CONTENT OF THE ANTARCTIC KRILL (*EUPHAUSIA SUPERBA* DANA), AN ESTIMATOR OF NATURAL GROWTH RATE

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Abstract: The ribonucleic acid (RNA) content of the krill was measured on specimens collected during the KAIYO-MARU cruise to the Wilkes Land, Antactica, January 1980. Of 41 specimens analysed, the RNA content varied from 2.5 to 8.2% of body protein (mean: 5.1%). A higher RNA content was seen in smaller specimens, and the relationship was $\log Y = -0.34 \log X + 1.27$, where Y is the RNA content as percent of protein, and X is the body size as mg protein. Combining this equation with the RNA-growth rate equation of BÅMSTEDT and SKJOLDAL (Limnol. Oceanogr., **25**, 304, 1980), the growth rate of the krill was estimated to be 0.17–0.19 mm day^{-1} for specimens greater than 30 mm body length. Assuming a favorable feeding period of 6 months in a year for this animal, post-larvae could grow to the final size adults in 201 days. Validity of these results depends on the RNA-growth rate equation. In the light of possible species-specific variation in the RNA-growth rate relationship, this relationship needs to be established for the Antarctic krill in future laboratory experiments.

1. Introduction

An early attempt in estimating the growth rate of the Antarctic krill (*Euphausia superba*) was based on the length of life span; *i.e.* average growth rate is calculated from final body size divided by the length of life span (*cf.*, MAUCHLINE and FISHER, 1969). Modal size analysis suggested that the krill live two to four years (RUUD, 1932; FRASER, 1936; BARGMANN, 1945; IVANOV, 1970; MACKINTOSH, 1972), computer analysis of a mixed normal distribution model suggested 5 to more than 6–7 years (SIEGEL, 1986; ROSENBERG *et al.*, 1986), age-pigment determination indicated 6 years (ETTERSHANK, 1983, 1985), laboratory growth experiment indicated 8–11 years (IKEDA, 1985), and so on.

Added to these conflicting results of estimated life span, the problem in estimating the growth rate of the krill in the Southern Ocean is confounded by its seasonality. Active growth of the krill is expected during the Antarctic summer when phytoplankton are abundant, but a significant slowdown of growth, even a negative growth, may be the case during the Antarctic winter when phytoplankton growth ceases. A recent study suggests that maximum growth of the krill is limited to only 3 months a year in the Southern Ocean (ROSENBERG *et al.*, 1986).

The present study aims at establishing the ribonucleic acid (RNA) content of the krill during their growth season (austral summer) in an attempt to estimate their growth rate. RNA is a necessary precursor to protein synthesis and has been used as an

estimator of growth rate of marine invertebrates (SUTCLIFFE, 1965, 1970; DAGG and LITTLEPAGE, 1972; SKJOLDAL and BÅMSTEDT, 1976; BÅMSTEDT and SKJOLDAL, 1980). BUCKLEY (1984) has successfully used the RNA : DNA ratio to predict the growth rate of larval fishes in the sea.

2. Materials and Methods

2.1. *Krill*

During the KAIYO-MARU cruise to off Wilkes Land, Antarctica, in mid January–early February 1980, samplings were made with a rectangular net (3×3 m mouth, 5.6 mm mesh aperture) fitted with an open-closing cod-end (FOXTON, 1963). Sampling sites were located between 64°43′–65°01′S and 100°22′–119°57′E, and specimens were collected from swarms found at 0–40 m depth. When the net arrived on deck, specimens (juveniles and adults) were quickly sorted out, immediately frozen at −50°C and transported to the Australian Institute of Marine Science (AIMS) where the following analyses were made.

2.2. *RNA analysis*

The storage period of the krill specimens was 20–36 days prior to analysis. Frozen specimens were weighed individually (*i.e.* wet weight) and homogenized in 10–40 m*l* of cold distilled water, depending on the size of the specimens, together with a GF/C glass fiber filter. The GF/C filter was used to facilitate better trituration of specimens. Of the homogenate thus obtained, 0.1 m*l* was used for protein determination by the method of LOWRY *et al.* (1951), and 0.8 m*l* for RNA measurements by the method of DAGG and LITTLEPAGE (1972). DAGG and LITTLEPAGE's method is a modification of the Schmidt-Thaunhauser method, based on the absorbance at 260 nm of the acid-soluble, alkali-hydrolysed fraction of the homogenates. Yeast RNA was used as a standard. Determination of both protein and RNA was made in duplicate. Appropriate dilution with distilled water was made for some protein samples of which spectrophotometric readings exceeded 1.5.

The relationship between protein (W_p, mg) and wet weight (W_{ww}, mg) was $W_{ww} = 60.0 + 8.9 W_p$ ($r = 0.989$, $n = 41$). For juvenile and adult krill, wet weight may be converted to body length (L) using the equation $\log W_{ww} = -3.23 + 3.70 \log L$ (*cf.*, IKEDA, 1984).

3. Results and Discussion

3.1. *RNA*

A total of 41 specimens were analysed, ranging in size from 90 to 1793 mg wet weight or from 6 to 192 mg protein, and including 9 gravid females (Fig. 1). The RNA content ranged from 2.5% to 8.2% of protein (mean: 5.1%). Since data points of the 9 gravid females did not deviate appreciably from the others (juveniles and adults), all 41 data were pooled for the following analysis. Smaller krill contained greater amount of RNA, and the relationship with body size (W_p) was expressed by GM regression (*cf.*, RICKER, 1973),

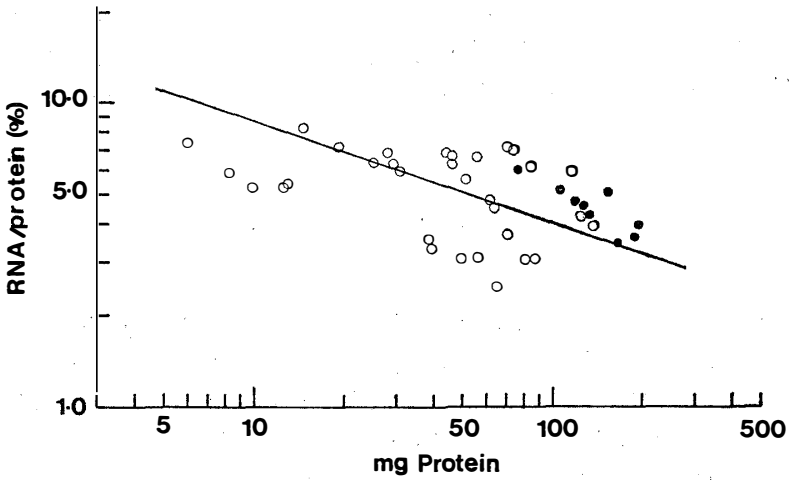

Fig. 1. The relationship between the RNA content (% of protein) and the body size (mg protein) in juvenile and adult krill (Euphausia superba). *Data points of gravid females are shown by closed circles. Superimposed GM regression line is; $Y=1.27-0.34X$ ($r=-0.440$, $df=39$, $p<0.01$). 95% confidence limits of the slope are −0.23 to −0.49.*

$$\log(\%\mathrm{RNA})=1.27-0.34\log W_{\mathrm{p}}\ (r=-0.440,\ df=39,\ p<0.01), \qquad (1)$$

where %RNA is the RNA content as percent of protein.

An extensive study on the RNA content of various zooplankton species was made in Korsfjorden, Norway, and Kosterfjorden, Sweden (SKJOLDAL and BÅMSTEDT, 1976; BÅMSTEDT and SKJOLDAL, 1980; BÅMSTEDT, 1983). Their results demonstrated greater RNA contents in smaller species, both interspecifically and intraspecifically. Within the same species, the RNA content was also found to vary with seasons. Higher RNA values usually corresponded to actively growing or breeding seasons of the animals. For euphausiids, the seasonal peaks were 17–19 μg RNA mg dry weight^{-1} (or 3.3–3.8% RNA, assuming dry weight = 2 × protein) in *Meganyctiphanes norvegica* and 53 μg RNA mg dry weight^{-1} (or 11% RNA) in *Thysanoessa* sp. The sizes of *M. norvegica* and *Thysanoessa* sp. were 28 mg and 2 mg dry weight, respectively. Using the same dry weight-protein conversion factor, and the %RNA-protein relationship established in this study, %RNA of these Norwegian krill are predicted to be 7.7%RNA (range of 95% confi dencelimits: 6.7–9.4%RNA) for *M. norvegica* and 19%RNA (range of 95% confidence limits: 12–35%RNA) for *Thysanoessa* sp. Thus, predicted %RNA values are higher than directly converted ones. The reason why the Antarctic krill contains higher RNA than the Norwegian krill is not known, but it could possibly be related to differences in physiology of animals and environmental conditions of their habitats.

3.2. Summer growth rate

Analyzing a large body of data on natural growth rates and RNA contents of marine pelagic animals, BÅMSTEDT and SKJOLDAL (1980) established a general relationship between these two parameters;

$$k\times 10^{3}=0.795\ (\mathrm{RNA}), \qquad (2)$$

where k is growth rate (d^{-1}) from exponential growth model, and (RNA) is μg RNA mg dry weight^{-1}. Using a conversion factor of dry weight $=2\times$ protein, eq. (2) can be re-written as

$$k\times 10^3=3.98\ (\%\mathrm{RNA}), \qquad (3)$$

where (%RNA) is the unit used in this study. Combining eq. (3) with eq. (1), we obtain,

$$k\times 10^3=74.3W_p-0.337. \qquad (4)$$

k thus estimated on the krill weighing 1–200 mg protein varies from 0.0740 to 0.0125, showing a rapid decrease with increasing size (Fig. 2). From the relationships between protein-wet weight and wet weight-body length, these k values based on protein are equivalent to daily increments of body length of from 0.063 to 0.186 mm. Calculations on various sizes of the krill indicated that daily increment of body length is rather stable (0.17–0.19 mm) for the krill having body length >30 mm.

Summer growth rates of juvenile and adult krill with 20–40 mm body length reported in the literature are summarized in Table 1. Among the rates based on field samplings, the lowest one of 0.063 mm day^{-1} by KANDA *et al.* (1982) was from the increase of mean size of the krill sampled from the same krill swarms which were traced for 2 weeks in the water off Enderby Land, Antarctica. CLARKE and MORRIS (1983) gave the highest rate, 0.33 mm day^{-1}, which was from 6 consecutive samplings of a krill

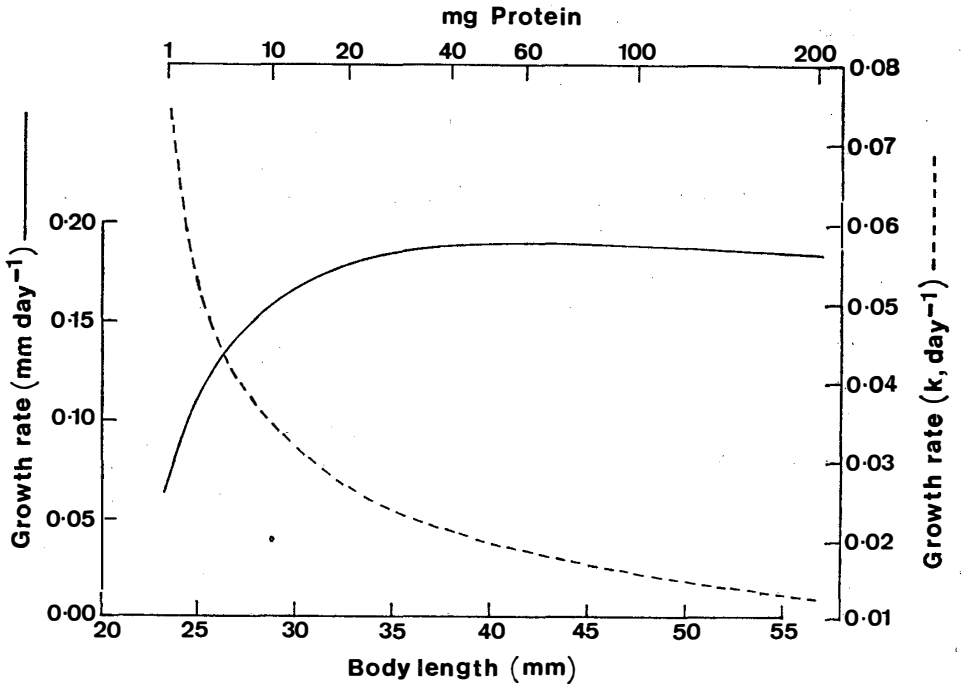

Fig. 2. Estimated growth rates of juvenile and adult krill (Euphausia superba) *from the RNA content. Growth rates were expressed on the bases of protein* (k, d^{-1}) *and body length* (*mm* d^{-1}). *See text for details.*

Table 1. Estimated summer growth rates of juvenile and adult krill (Euphausia superba, *body length=20–40 mm) in the field and observed maximum growth rates of this animal in laboratory experiments.*

Growth rate (mm day^{-1})	References
Field investigation	
0.063	KANDA *et al.* (1982)
0.33	CLARKE and MORRIS (1983)
0.105–0.179	ROSENBERG *et al.* (1986)
0.11	MCCLATCHIE (1988)
Laboratory experiment	
0.035	MCWHINNIE and DENYS (1978)
0.035	MURANO *et al.* (1979)
0.068	POLECK and DENYS (1982)
0.070	IKEDA *et al.* (1985)
0.089	IKEDA and THOMAS (1987)
0.156	IKEDA (1987)
RNA analysis	
0.19	This study

patch found near South Georgia. The 0.105–0.179 mm day^{-1} given by ROSENBERG *et al.* (1986) were derived from re-analysis by a modified SHEPHERD's method of Discovery data collected between 1928 and 1938. MCCLATCHIE (1988) used a computer program called "ELEFAN" (*cf.*, PAULY and DAVID, 1981) to analyse monthly size frequency data collected by STEPNIK (1982) over one year at Admiralty Bay, South Shetland Islands.

Laboratory experiments have been made not only at Antarctic stations (MCWHINNIE and DENYS, 1978; POLECK and DENYS, 1982), but also in Tokyo (MURANO *et al.*, 1979) and in Tasmania (IKEDA *et al.*, 1985; IKEDA and THOMAS, 1987; IKEDA, 1987). Among these laboratory data, those reported by IKEDA and his colleagues are generally higher than the others. In particular, the growth rate reported by IKEDA (1987) is the highest. It is interesting to note that IKEDA's (1987) specimens were grown from eggs in the laboratory in contrast with the use of wild specimens transported to the laboratory in other studies. While the growth data from field investigations represent growth at the population level, those from laboratory experiments are on individuals. Therefore, higher rates can be expected with the latter approach. However, variations within each approach are too large to detect this expected trend. Compared with the growth rates of the krill derived from these two approaches, the rates estimated from RNA are in general higher, except for the outstanding data of CLARKE and MORRIS (1983).

3.3. Life span

Eq. (4) can be used to estimate developmental time of the krill. Since $dW_p/dt = k \times W_p$,

$$dW_p/dt = 74.3 \times 10^{-3} W_p^{0.663}. \quad (5)$$

The integrated form of eq. (5) is

$$t=(W_{pt}^{1-0.663}-W_{p0}^{1-0.663})/0.0743(1-0.663), \quad (6)$$

where W_{p0} and W_{pt} are protein weight at time 0 and t.

Let protein of post-larva and adult of final size be 0.8 mg and 200 mg (IKEDA, 1984), respectively, then eq. (6) predicts t as 201 days. Taking into consideration the 95% confidence limits of the slope of eq. (1), the range of t was estimated as 188–217 days.

Since the krill eggs spawned during the austral summer in the Antarctic waters can develop to the last larval stage (furcilia VI stage; *ca.* 10 mm body length) in 127 days (IKEDA, 1984), the krill of the last furcilia stage could grow to the final size (60 mm body length) by the end of the second summer or early third summer, assuming periods of 6 months of non-growth and 6 months of growth in a year. Thus, the estimate of the growth rate based on the RNA content is close to the 2–3 years life span hypothesis (see "Introduction"). It should be noted that the validity of this conclusion depends on the RNA-growth rate equation of BÅMSTEDT and SKJOLDAL (1980) which has been applied to the present calculation. As pointed out by BÅMSTEDT and SKJOLDAL (1980), the RNA-growth rate relationship can be highly species-specific, thereby its application, not to mixed species assemblages, but to a single species may cause a significant bias. Because laboratory culture of the krill has become feasible (IKEDA, 1987), the species-specific RNA-growth rate relationship for the krill can be established using laboratory grown krill of various sizes. With such a krill specific RNA-growth rate equation, RNA data of wild krill can favorably be converted to their growth rates. While the present study concerned only with summer specimens, specimens from other seasons, particularly from winter, are needed for our better understanding of the krill growth over a one year cycle. HOPKINS *et al.* (1984) demonstrated that the euphausiids (*Thysanoessa inermis* and *T. raschi*) in a sub-arctic fjord do shrink during food impoverished winter conditions.

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References

BÅMSTEDT, U. (1983): RNA concentration in zooplankton; Seasonal variation in boreal species. Mar. Ecol. Prog. Ser., **11**, 291–297.

BÅMSTEDT, U. and SKJOLDAL, H. R. (1980): RNA concentration of zooplankton; Relationship with size and growth. Limnol. Oceanogr., **25**, 304–316.

BARGMANN, H. E. (1945): The development and life history of adolescent and adult krill, *Euphausia superba*. Discovery Rep., **23**, 103–176.

BUCKLEY, L. J. (1984): RNA-DNA ratio; An index of larval fish growth in the sea. Mar. Biol., **80**, 291–298.

CLARKE, A. and MORRIS, D. (1983): Towards an energy budget of krill; The physiology and biochemistry of *Euphausia superba* DANA. Polar Biol., **2**, 69–86.

DAGG, M. J. and LITTLEPAGE, J. L. (1972): Relationships between growth rate and RNA, DNA, protein and dry weight in *Artemia salina* and *Euchaeta elongata*. Mar. Biol., **17**, 162–170.

ETTERSHANK, G. (1983): Age structure and cyclical annual size change in the Antarctic krill, *Euphausia superba* DANA. Polar Biol., **2**, 189–193.

ETTERSHANK, G. (1985): Population age structure in males and juveniles of the Antarctic krill, *Euphausia superba* DANA. Polar Biol., **4**, 199–201.

FOXTON, P. (1963): An automatic opening-closing device for large plankton nets and mid-water trawls. J. Mar. Biol. Assoc. U.K., **43**, 295–308.

FRASER, F. C. (1936): On the development and distribution of the young stages of krill (*Euphausia superba*). Discovery Rep., **14**, 1–192.

HOPKINS, C. C. E., TANDE, K. S., GRONVIK, S. and SARGENT, J. R. (1984): Ecological investigations of the zooplankton community of Balsfjorden, northern Norway; An analysis of growth and overwintering tactics in relation to niche and environment in *Metridia longa* (LUBBOCK), *Calanus finmarchicus* (GUNNERUS), *Thysanoessa inermis* (KROYER) and *T. raschi* (M. SARS). J. Exp. Mar. Biol. Ecol., **82**, 77–99.

IKEDA, T. (1984): Sequences in metabolic rates and elemental composition (C, N, P) during the development of *Euphausia superba* DANA, and estimated food requirements during its life span. J. Crust. Biol., **4** (Spec. No. 1), 273–284.

IKEDA, T. (1985): Life history of Antarctic krill *Euphausia superba*; A new look from an experimental approach. Bull. Mar. Sci., **37**, 599–608.

IKEDA, T. (1987): Mature antarctic krill (*Euphausia superba* DANA) grown from eggs in the laboratory. J. Plankton Res., **9**, 565–569.

IKEDA, T. and THOMAS, P. (1987): Moulting interval and growth of juvenile Antarctic krill (*Euphausia superba*) fed different concentrations of the diatom *Phaeodactylum tricornutum* in the laboratory. Polar Biol., **7**, 339–343.

IKEDA, T., DIXON, P. and KIRKWOOD, J. (1985): Laboratory observations of moulting, growth and maturation in Antarctic krill (*Euphausia superba* DANA). Polar Biol., **4**, 1–8.

IVANOV, B. G. (1970): On the biology of the Antarctic krill *Euphausia superba* DANA. Mar. Biol., **7**, 340–351.

KANDA, K., TAKAGI, K. and SEKI, Y. (1982): Movement of the larger swarms of Antarctic krill *Euphausia superba* population off Enderby Land during 1976–1977. J. Tokyo Univ. Fish., **68**, 25–42.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951): Protein measurement with the Folin-Phenol reagent. J. Biol. Chem., **193**, 265–275.

MACKINTOSH, N. A. (1972): Life cycle of Antarctic krill in relation to ice and water conditions. Discovery Rep., **36**, 1–94.

MAUCHLINE, J. and FISHER, L. R. (1969): The biology of euphausiids. Adv. Mar. Biol., **7**, 1–454.

MCCLATCHIE, S. (1988): Food limited growth of *Euphausia superba* in Admiralty Bay, Antarctica. Cont. Shelf Res., **8**, 329–346.

MCWHINNIE, M. A. and DENYS, C. J. (1978): Antarctic marine living resources with special reference to krill *Euphausia superba*. Assessment of adequacy of present knowledge. Rep. No. NSF/RA 780489, Washington, D. C., National Science Foundation, 209 p.

MURANO, M., SEGAWA, S. and KATO, M. (1979): Moult and growth of the Antarctic krill in laboratory. Trans. Tokyo Univ. Fish., **3**, 99–106.

PAULY, D. and DAVID, N. (1981): ELEFAN I, a basic program for the objective extraction of growth parameters from length-frequency data. Meeresforschung, **28**, 205–211.

POLECK, T. P. and DENYS, C. J. (1982): Effect of temperature on the molting, growth and maturation of the Antarctic krill *Euphausia superba* (Crustacea: Euphausiacea) under laboratory conditions. Mar. Biol., **70**, 255–265.

RICKER, W. E. (1973): Linear regressions in fishery research. J. Fish. Res. Bd. Can., **30**, 409–434.

ROSENBERG, A. A., BEDDINGTON, J. R. and BASSON, M. (1986): Growth and longevity of krill during the first decade of pelagic whaling. Nature, **324**, 152–153.

RUUD, J. T. (1932): On the biology of Euphausiidae. Hvalrad. Skr., **2**, 1–105.

SIEGEL, V. von (1986): Investigations on the biology of antarctic krill, *Euphausia superba*, in the Bransfield Strait and adjacent areas. Mitt. Inst. Seefish., Hamburg, **38**, 1–244.

SKJOLDAL, H. R. and BÅMSTEDT, U. (1976): Studies on the deep-water pelagic community of Korsfjorden, western Norway. Adenosine phosphates and nucleic acids in *Meganyctiphanes norvegica*

(Euphausiacea) in relation to the life cycle. Sarsia, **61**, 1–13.

STEPNIK, R. (1982): All-year populational studies of the Euphausiacea (Crustacea) in the Admiralty Bay (King George Island, South Shetland Islands Antarctic). Pol. Polar Res., **3**, 49–68.

SUTCLIFFE, W. H., Jr. (1965): Growth estimates for ribonucleic acid content in some small organisms. Limnol. Oceanogr., **10** (suppl.), R253–R258.

SUTCLIFFE, W. H., Jr. (1970): Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Bd. Can., **27**, 606–609.

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