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Early summer distribution of Antarctic krill sexual development in the Scotia-Weddell region: a multivariate approach*

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Summary. The sexual Development of antarctic krill was studied during the EPOS leg 2 cruise (November 1988–January 1989) in the seasonally ice covered Scotia-Weddell Confluence area. Multiple Correspondence Analysis (MCA) was used to elucidate the general trends of variation of biological (body-size, molt stage) and environmental data (geographical position, sea-ice extension, sampling time) associated with female development. In November, female krill from the ice covered area (Weddell Sea) had a juvenile ovary, while pelagic female krill from Scotia Sea were in advanced previtellogenesis, and one third of them had already spawned. The successive samples from the Confluence illustrated a rapid advance of sexual development during the whole period. Both observation of live krill maintained on board and the MCA confirmed the general trend of distribution of sexual development in relation with size structure and environmental factors. Previtellogenesis occurs in relation with the ice-edge, while vitellogenesis is performed in short cycles in the summer pelagic habitat. The degree of sexual development attained by krill samples (measured by the sexual development index, SDI) is then a good indicator of the biological activity of the krill population and of its impact on the pelagic ecosystem.

Introduction

Euphausia superba is one of the most successful species in the Antarctic marine ecosystem (Quetin and Ross 1991). It is generally considered to be pelagic, but is also abundant under the sea-ice in winter (O'Brien 1987; Marschall 1988; Stretch et al. 1988; Siegel et al. 1990). Krill is believed to have developed characteristics that foster the success of the species in an environment dominated by extreme seasonal changes, such as winter-over mechanisms for survival in the ice habitat (see Quetin and Ross 1991 for review), as well as multiple spawning and release of several batches of eggs during each reproductive season (Denys and McWhinnie 1982; Ross and Quetin 1983; Cuzin-Roudy 1987b). The spawning season lasts from November to April (Everson 1977; Ikeda 1985) with a peak in January–February and with year to year variations in spawning levels (Brinton et al. 1986). The maintenance of the large biomass of the species, and the success of the reproductive effort, which occurs in combination with somatic growth during the limited Antarctic summer, raises a question concerning the availability of sufficient food resources during the same period. Consequently, there is some speculation whether the enhanced nutritional demand in summer can be satisfied by the low phytoplankton biomass and primary production of Antarctic waters in general (El Sayed 1988). This apparent contradiction is considered to be a primary reason for the association of krill abundance with highly productive areas such as the ice edge zone (Daly and Macaulay 1988) and frontal structures like the Scotia-Weddell Confluence (Hempel 1985, 1987). A study of krill from the sampling program of Leg 2 of the European *Polarstern* Study (EPOS Leg 2), conducted in this region in early summer, was expected to produce the best data to investigate how krill manage to adapt from a winter under-ice life style to a pelagic life style in summer. Such adaptation should correspond to important changes in the physiology of the animal.

The Atlantic sector of the Antarctic Ocean is known for its high abundance of krill and the region of the Scotia-Weddell Confluence is a major spawning site for this species in summer (Marr 1962). Various factors have been proposed as being responsible for krill's uneven spatial distribution and concentration in certain zones; especially physical factors such as circumpolar currents, seasonal advance and retreat of pack-ice, frontal structures and eddies (Priddle et al. 1988). Among biological factors linked to the prevalent swarming behavior of krill, for example migration in the search of food and predator avoidance (O'Brien 1987), molting, mating and spawning are certainly important in triggering krill aggregation (see

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Miller and Hampton 1989 for review). These factors all exhibit pronounced variation throughout the year.

The period covered by EPOS Leg 2 corresponded to the seasonal ice retreat followed by the establishment of summer hydrographic conditions in the Confluence region. The hydrological structure described by Cederlöf et al. (1989) during this period clearly delimited the frontal system of the Confluence separating the eastward Circumpolar Current and the northern branch of the Weddell gyre. Scotia Sea waters were found in the north and Weddell Sea waters in the southern part of the study area.

Siegel et al. (1990) have studied the distribution pattern and demography of krill during EPOS Leg 1 and Leg 2. Using cluster analysis of size frequency distributions and maturity stages (Makarov and Denys 1982), they reported that the clear spatial separation in 3 groups described during Leg 1 (spring situation) was less pronounced during Leg 2 (early summer). The combination of changes in the different physical factors at a period of intense biological activity for krill (growth and reproduction) are likely to have an important influence on krill distribution (Everson 1977). Therefore, we have followed changes in population structure due to sexual development in early summer, in an attempt to improve current understanding of functional relationships between biological factors such as sexual development and egg production and environmental factors.

The principal aim of EPOS Leg 2 was to elucidate the role of the various factors governing production and changes in biomass and community structure in Antarctic waters. Reproduction is the most energetically demanding physiological process and represents the highest level of biological activity in krill's life cycle (Cuzin-Roudy and Schalk 1989, Schalk 1990). As a result, it is logical to assume that during reproductive development krill are most likely to have the maximum impact on the other components of the pelagic food web. Heavy selective grazing by krill could lead to significant reduction of biomass and shift in species composition in the pelagic community.

Female krill were staged for sexual development according to the key given by Cuzin-Roudy and Amsler (1991). Each sample was then characterized by the average stage of development or sexual development index (SDI), which expresses the sexual condition of the krill population at the time and place of sampling. Variations in the krill's SDI across the studied area should then reflect either the presence of different krill populations or, more likely, differences in the timing of sexual development of members of the same population, subject to different environmental and nutritional histories.

Beside stages of sexual development, data on body-size and molt stage were recorded from the different samples. Environmental conditions were monitored by geographical position, ice condition and time of the samplings. The latter parameter measured seasonal advance. The hypothesis is made that these conditions are most likely to induce the physiological changes that correspond to active reproduction and consequently, the population structure and distribution of krill in the area during early summer.

Multivariate methods are widely used to analyze such complex structures of ecological data (de Leeuw 1987). Multiple Correspondence Analysis (MCA) is concerned with the analysis of a multivariate sample of qualitative (categorical) rather than quantitative variables (Gower 1987) and is the appropriate tool to study the relationships between sets of variables which are either quantitative and categorized or qualitative and coded (Legendre and Legendre 1983). The method is used here to investigate the pattern of relationships between krill biological and environmental factors. This analysis should enable the general trends in variation of the different descriptors and eventual links between them to be characterized. Such trends and links should highlight factors responsible for the distribution of the krill population in the study area at the onset of the reproductive effort, and improve our understanding of krill's adaptative strategies in general.

Materials and methods

Krill sampling

Krill studied here were obtained from the stations shown in Fig. 1. A stern-operated open RMT 1+8 was used in ice-free areas. Oblique tows were made in the upper 100, 300 and 400 m of the water column. An ORI net with a mesh size of 500 μm, converted into a vertical net by adding a cod-end line and connecting a 35 kg weight to the collector, and a Bongo net (total mouth area: 1.5 m^2, same mesh size) were used from a beam in ice covered areas. Duplicate hauls were made from 400 m to the surface with a hauling speed of 1 m/s (Cuzin-Roudy and Schalk 1989). Juvenile krill were sampled with a hand net from the side of an ice-floe on one occasion (station 156).

A total of 107 samples were taken along two survey transects, 49°W and 47°W, between 57° and 62°S, during the period 26 Nov. 1988 to 4 Jan. 1989. *Euphausia superba* was present in 30% of the vertical hauls and in 56% of the RMT trawls. Twenty three samples were used in this study; twenty samples from EPOS leg 2 (with two small samples from stations 177, and 178 pooled together); three RMT 1+8 samples from EPOS leg 1 (6–15 Nov. 1988) from the same area were added to the study (samples 122, 127 and 141) (Fig. 1).

Krill were sorted from the samples and initially fixed in 10% buffered formalin. They were later transferred to 70% ethanol with 1% glycerin.

Krill maintenance on board

When krill were abundant in the samples (over 50 specimens), the most lively animals were immediately transferred to 50 l tanks maintained in a cold room (0°+0.5°C) and the rest of the sample fixed in Formalin. The water in the tanks was changed daily. Fifty to 100 live specimens were kept free in a tank, or isolated singly or in pairs in perforated transparent plastic boxes (270 ml and 1.5 l, respectively). They were fed daily with sampled phytoplankton when available, and with frozen pieces of ice containing ice-algae ("brown-ice") for juvenile krill collected among ice floes. The held animals were observed daily for survival, behavior, molting, mating and spawning. Body-size, molt stage and stage of sexual development were determined at the end of the experiment and specimens fixed for comparison with the corresponding sample. Krill were maintained live on board for up to 34 days.

Biological variables

Two descriptors were measured on a total of 506 krill from 23 samples:

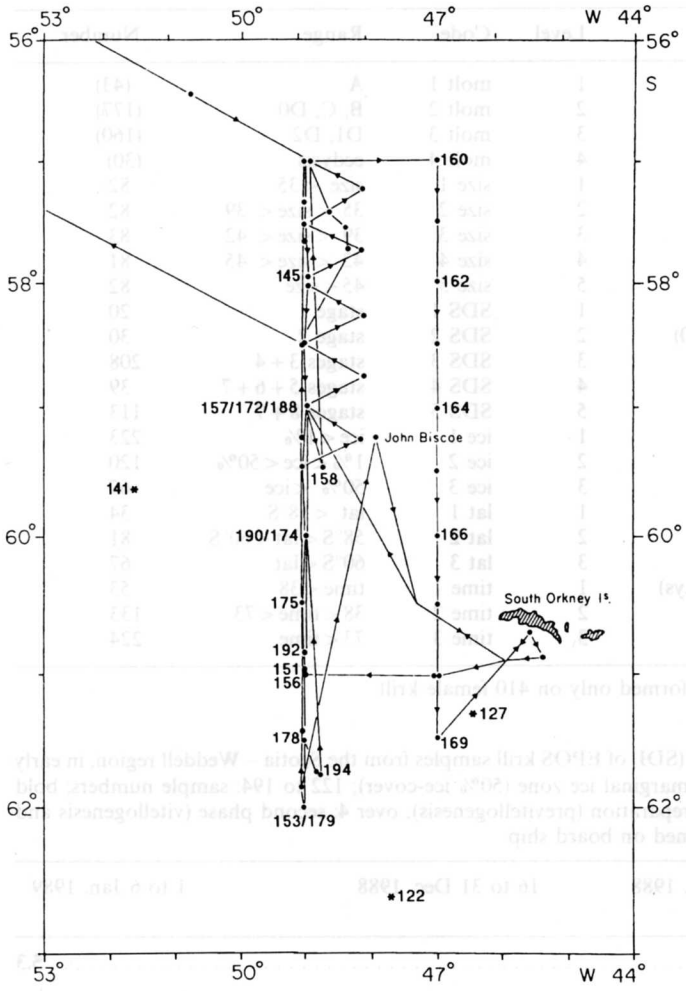

Fig. 1. Station map during EPOS, Leg 2 (from Anonymous 1989). The numbers indicate stations from which krill were obtained for the present study. *Stars* the 3 stations sampled during EPOS, Leg 1

Body size (size) was measured with a digital calliper, from the tip of the rostrum to the end of the uropods (Standard Length 1, Mauchline 1981).

Sexual differentiation was expressed as follows (Cuzin-Roudy 1987a): 1) Juvenile: no developed secondary sexual characters; 2) Subadults: developing secondary sexual characters (petasmata and ampullae for males; thelycum for females); 3) Adults: fully developed secondary sexual characters.

For adult female krill, the stages of *sexual development* (stages 1 to 10) were determined for all female specimens in the different samples (or for 50 females when samples were large). The key and the squash technique for the ovary given by Cuzin-Roudy and Amsler (1991) were used. Makarov and Denys (1980) staging method was also used for comparison.

For each sample, an average stage, or *Sexual Development Index* (SDI) was calculated from the relative frequencies (F_r) of female krill in each developmental stage (sds) according to:

$$\text{SDI} = \text{sum of } (F_r \times \text{sds})$$

The SDI, which expresses the degree of reproductive development attained by the population at the place and time of the sampling, was calculated for 20 EPOS Leg 2 samples and for 3 samples from Leg 1. The results were used to map the biological activity of krill in the area, during the study period.

A third biological descriptor, *molt stage*, was established for 410 female krill (from a total of 506) by microscopic observation of the antennal scale. Molt staging was done on live krill on board ship when possible, but more generally was undertaken on fixed samples. The molt staging system used by Cuzin-Roudy (1987b) after Buchholz (1982), was reduced to 4 stages that can be recognized in formalin fixed krill. Namely, *molt 1*- early postmolt (stage A), with soft cuticle; *molt 2*- postmolt and intermolt (stages B, C and D0), with no extensive retraction of the epidermis under the cuticle; *molt 3*- premolt (D1, D2) with obvious retraction; *molt 4*- ecdysis (D3 and ecdysis in progress), with thin old cuticle already detached (Table 1).

Supplementary variables

Data concerning sea-ice conditions, the structure of the water masses, the frontal zone and their variations during the study period, were given in Hempel et al. (1989). The position of the front (F) that marks the northern limit of the Confluence, and of the zone corresponding to 50% ice-cover (MIZ) are given as a frame for the distribution of the SDI in Table 2.

Three variables were used to characterize the environment of the different samples. *Latitude* in degrees, for the geographical position; *time* in half-day units, to express the succession of the samples in the advancing season; and *sea ice condition*, expressed as the percentage of ice coverage (Franeker 1989).

Chlorophyll-*a* concentrations were available for 16 krill stations (Anonymous 1989). An eventual correlation between Chlorophyll-*a* level and SDI values was checked using Spearman's rank coefficient of correlation (Siegel 1956). The correlation ($r_s = 0.39$) was not significantly different from 0 ($P \leq 0.05$). Consequently, the Chlorophyll-*a* data were not included in the analysis.

Multiple correspondence analysis (MCA) of female data

MCA was used here to disclose the complex data set for female krill. MCA is an ordination method concerned with multivariate sampling of categorical variables. Such techniques are widely used for the analysis of ecological data (Gower 1987). MCA describes parsimoniously the total inertia of a multidimensional set of data with fewer dimensions, or axes, which then represent the best integration of the information contained in the data. Among the inertia methods, MCA is concerned with contingency tables of categorical variables and uses Chi-square distances.

The indicator matrix of data. The original data set comprised matrices of data from 410 krill specimens described by 6 variables, or 506 krill described by 5 variables, when molt stage was not considered.

The original variables were either quantitative (body-size, latitude, time) or qualitative and coded (molt- or sexual development stages, ice condition). The quantitative variables were disjuncted and coded to transform them into categorical variables, as shown in Table 1. Body sizes (size) were regrouped in 5 levels, each containing a similar number of specimens. Stages of sexual development (Cuzin-Roudy and Amsler 1991) were regrouped in five levels, corresponding to the developmental phases of the ovary – gametogenesis (SDS 1); oogenesis (SDS 2); the two phases of oocyte growth and yolk accumulation that are previtellogenesis (SDS 3) and vitellogenesis (SDS 4); spawning and postspawned (SDS 5). Molt stages were the 4 stages defined from formalin fixed samples. Data for ice-coverage, latitude and time were grouped in three levels each.

The contingency table for MCA (Table 3). A multiple contingency table, or "Burt matrix" was built by crossing the different levels (or modalities) of the *n* variables. A contingency table is symmetrical and

Table 1. Coding of female krill data into categorical variables

Variable	Level	Code	Range	Number
Molt stage (7)[a]	1	molt 1	A	(43)
	2	molt 2	B, C, D0	(177)
	3	molt 3	D1, D2	(160)
	4	molt 4	ecdysis	(30)
Body-size (mm)	1	size 1	size <35	82
	2	size 2	$35<$size<39	82
	3	size 3	$39<$size<42	83
	4	size 4	$42<$size<45	81
	5	size 5	$45<$size	82
Stages of sexual development (10)	1	SDS 1	stage 1	20
	2	SDS 2	stage 2	30
	3	SDS 3	stages 3+4	208
	4	SDS 4	stages 5+6+7	39
	5	SDS 5	stages 8+9	113
Ice coverage (%)	1	ice 1	ice $<1\%$	223
	2	ice 2	$1\%<$ice$<50\%$	120
	3	ice 3	$50\%<$ice	67
Latitude (degrees)	1	lat 1	lat $<58°$S	34
	2	lat 2	$58°$S$<$lat$<60°$S	81
	3	lat 3	$60°$S$<$lat	67
Sampling time (half-days)	1	time 1	time <38	53
	2	time 2	$38<$time<73	133
	3	time 3	$73<$time	224

[a] Molt staging was performed only on 410 female krill

Table 2. Space and time distribution of the Sexual Development Index (SDI) of EPOS krill samples from the Scotia – Weddell region, in early summer. F: front limiting the Confluence from the Scotia Sea; MIZ: marginal ice zone (50% ice-cover); 122 to 194: sample numbers; bold numbers: SDI (increasing from 1: juvenile; 3 to 4: first phase of egg preparation (previtellogenesis); over 4: second phase (vitellogenesis and spawning); italic numbers: SDI obtained for krill subsamples maintained on board ship

Time / Latitude	6 to 15/11/88	15 to 30 Nov. 1988	1 to 15 Dec. 1988	16 to 31 Dec. 1988	1 to 6 Jan. 1989
57°S			160/**3.9**		**5.3**
57°30S		F			
58°S		145/**5.1**	162/**6.5**		**7.1**
58°30S			F		
59°S			157/**5.5** 5.7 F	172/**4.6** 188/**5.1**	**5.2**
			164/**6.2**		**6.9**
59°30S	141/**2.8**		158/**5.2**	**5.9**	
60°S	MIZ		166/**3.9**		**5.0**
				174/**5.2** 190/**6.4**	
60°30S		MIZ	MIZ	175/**5.1**	
61°S		151/**1.4**	156/**1.0**	MIZ	**1.4**
61°30S	127/**3.5**			169, 177, 178, 179, 192, 194/**1.4**	
62°S	122/**2.3**	153/**2.2**			

analogous to a correlation matrix, as it displays the two by two occurrences of the variables (Gower 1987). Table 2 shows the Burt matrix obtained with $n=6$ variables and 410 female krill. The n diagonal blocks show the totals for rows and columns (e.g., 208 female krill were scored SDS 3). The (n^2-n) off-diagonal blocks of the Burt matrix display the numbers of common occurrence of the categorical variables two by two (e.g., 96 female krill were SDS 3 and molt 2). Each block is then equivalent to a 2-way contingency table. An MCA was run on the complete Burt table using a MSDOS computer and BIOMECO computer program.

The biological variables (body-size, molt- and sexual development stages) were considered as "active" and used for the calculation of the factorial axes. The other variables, which are descriptive of the environment, were projected as "supplementary" variables into the new space defined by the axes. The informative value of an "active" modality was expressed both by its contribution to the axes and planes and by its correlations with them. Links between the different variables were illustrated by the pattern of their appearance on the two-dimensional diagrams produced by the better defined planes.

A MCA was run first on the contingency table built with 6 variables, including molt – stage, for 410 female krill (Table 3). A second MCA was run next for the set of 506 krill with 5 variables (molt stages excluded), in order to check the robustness of the method.

Results

In situ female development

The average developmental stage of the different samples, or SDI, are mapped in Table 2, with time of sampling and latitude.

Table 3. Contingency table (Burt matrix) built for the Multiple Correspondence Analysis of biological and supplementary descriptors of the female krill development and distribution in the Scotia-Weddell area in early summer. Half of the symetrical table is presented. The diagonal blocks give the total number of occurrences (bold numbers) for the different levels of the categorical variables. Each off-diagonal block is a two-way contengency table

Variable		Molt				Size					SDS					Ice			Latitude			Time		
	Level	1	2	3	4	1	2	3	4	5	1	2	3	4	5	1	2	3	1	2	3	1	2	3
Molt	1	**43**	0	0	0																			
	2	0	**177**	0	0																			
	3	0	0	**160**	0																			
	4	0	0	0	**30**																			
Size	1	4	24	49	5	**82**	0	0	0	0														
	2	8	45	24	5	0	**82**	0	0	0														
	3	12	41	25	5	0	0	**83**	0	0														
	4	13	35	23	10	0	0	0	**81**	0														
	5	6	32	39	5	0	0	0	0	**82**														
SDS	1	2	7	11	0	20	0	0	0	0	**20**	0	0	0	0									
	2	1	8	19	2	20	6	4	0	0	0	**30**	0	0	0									
	3	25	96	72	15	31	57	56	41	23	0	0	**208**	0	0									
	4	6	12	17	4	0	0	4	10	25	0	0	0	**39**	0									
	5	9	54	41	9	11	19	19	30	34	0	0	0	0	**113**									
Ice	1	*24*	*110*	*74*	*15*	*13*	*38*	*58*	*61*	*53*	*5*	*0*	*121*	*16*	*81*	***223***	*0*	*0*						
	2	*15*	*49*	*45*	*11*	*24*	*29*	*20*	*18*	*29*	*4*	*12*	*56*	*23*	*25*	*0*	***120***	*0*						
	3	*4*	*18*	*41*	*4*	*45*	*15*	*5*	*2*	*0*	*11*	*18*	*31*	*0*	*7*	*0*	*0*	***67***						
Latitude	1	*2*	*19*	*12*	*1*	*0*	*4*	*11*	*9*	*10*	*0*	*0*	*27*	*0*	*7*	*34*	*0*	*0*	***34***	*0*	*0*			
	2	*31*	*106*	*77*	*19*	*23*	*38*	*50*	*59*	*63*	*9*	*2*	*105*	*35*	*82*	*152*	*81*	*0*	*0*	***233***	*0*			
	3	*10*	*52*	*71*	*10*	*59*	*40*	*22*	*13*	*9*	*11*	*28*	*76*	*4*	*24*	*37*	*39*	*67*	*0*	*0*	***143***			
Time	1	*3*	*14*	*31*	*5*	*29*	*15*	*5*	*3*	*1*	*1*	*13*	*33*	*0*	*6*	*0*	*5*	*48*	*0*	*5*	48	***53***	*0*	*0*
	2	*17*	*57*	*48*	*11*	*27*	*34*	*23*	*22*	*27*	*14*	*4*	*62*	*22*	*31*	*45*	*76*	*12*	*0*	*121*	*12*	*0*	***133***	*0*
	3	*23*	*106*	*81*	*14*	*26*	*33*	*55*	*56*	*54*	*5*	*13*	*113*	*17*	*76*	*178*	*39*	*7*	*34*	*107*	*83*	*0*	*0*	***224***

During the whole period, juvenile female krill (size range 18.8–30.0 mm body-length) sampled from the ice covered area showed an ovarian development limited to germinal zones filled with oogonia 1 and 2 (stage 1).

In November, female krill (body-length: 25.5 to 46.0 mm) sampled in the ice of the Weddell Sea waters showed various development of the thelycum and were subadult or adult. About one third of the adult female krill presented ovaries filled with developing oocytes in early previtellogenesis. The SDI of the samples from the ice remained lower than 4 (Table 2). Only half the males were adult and produced spermatophores.

As early as late November, most pelagic adult female krill sampled from the Scotia Sea (north of the Confluence) had large ovaries in advanced previtellogenesis, and about 30% of them had already spawned. Seventy percent of the males were sexually active. In December, samples from the same area showed that up to 60% of females had spawned at least once. The SDI of these samples were over 4, indicating that part of the females were in vitellogenesis.

Samples from the Confluence taken on the successive transects showed a rapid increase in the degree of sexual development with advancing time. The previtellogenetic phase was dominant when the Confluence was still influenced by the MIZ. Later on, in December, when MIZ had retreated further South, many females were in vitellogenesis and spawning, and the SDI of the samples were higher than 5 (Table 2).

If we consider globally all subadult and adult female krill (size range 29.0 to 59.0 mm body size) sampled in open water during the whole period, there was an overlap of size range between juvenile, subadult and adult krill indicating that sexual development does not start at a precise body-size. Stage 4 (late previtellogenesis) was the most frequent developmental stage of the ovary, and accounted for 50% of the total adult female krill (400). Pooled with stage 8 (a cyclical return to a previtellogenetic ovary after spawning), stages 4+8 accounted for 82% of females. These results suggest that previtellogenesis is a long process compared to vitellogenesis.

Three samples taken at intervals at the same spot in the Confluence were comparable enough to allow a rough estimate of the timing of the vitellogenetic process, assuming that they were taken from the same population. Between December 5 and 29 (samples 157, 172 and 188), a progression in ovarian development was apparent. In the first sample, preparation of the first spawn was going on and 58% of the females were spawning for the first time. In the second and the third sample, females were back to stage 4 (63% and 85% respectively) and were preparing a second and a third spawn, respectively, allowing 10 to 12 days for the duration of a vitellogenetic cycle between two successive spawns.

Live krill observations

Live krill were maintained on board for direct observations, in an attempt to estimate the timing of the successive phases of development.

Juvenile krill from the ice were kept on board for 34 days, with melting brown ice as food. These survived well, molted and showed ovarian development: at the end of the experiment about 50% of the female specimens had developed young oocytes from the oogonia 2 and had attained stage 2. None developed secondary sexual characters nor started previtellogenesis. It is concluded that the formation of oocytes (oogenesis) was possible with ice algae as food and that it occurred before adult differentiation.

Krill molted in the tanks, during night rather than day. We observed on adult females kept with males that molting was rapidly followed by mating. Spermatophores lost during the molting process were already present on the thelycum of recently molted females, with soft new cuticle (stage A).

Several live female krill with a swollen thorax and blue gray ovary (III D of Makarov and Denys, and our stage 7) were isolated. They spawned in the tanks in the next 3 to 5 days and showed then a reduced ovary. On a squash, these ovaries contained more or less abundant previtellogenetic (ocl) and vitellogenetic oocytes (oc2) beside a few residual mature ones (oc4) from the recent spawning. They were actually stage 8.

In the tanks, adult female krill from the Scotia Sea passed through the vitellogenetic stages and spawned. At the end of the experiment, after 24 days, up to 89% of the females from the December samples were in stages 6, 7 and 8, i.e. in active vitellogenesis, egg maturation and spawning.

Krill from the Confluence followed, with some delay, the same pattern of ovarian sequences as krill from the Scotia Sea. The SDI increased, and 60% of the females had spawned at the end of the experiment.

SDI values attained by the subsamples maintained on board are given in Table 2 and compared with the SDI at time of sampling.

These observations were taken as a confirmation that the previtellogenetic oocytes develop in synchrony but, enter vitellogenesis in successive batches, thereby resulting in successive spawns. Up to 3 distinct batches of oocytes at different stages of development, but never more, were observed simultaneously in female krill ovaries.

The vitellogenesis of one batch is a rapid process. Live female krill maintained individually were followed from stage 5 to stage 8 and took 10 to 13 days for a process of vitellogenesis.

No recovery of ovaries left with young oocytes after a complete spawning was observed during the on board study. It is concluded that vitellogenesis could be maintained even with poor food, while on board conditions permitted neither the initiation nor the completion of previtellogenesis. Previtellogenesis appeared then to be more immediately food dependant than vitellogenesis.

Results of MCA

The first and second axes accounted for 23% and 17% of the total variance, respectively. Figure 2 presents the results of the analysis of the Burt matrix. The pattern of the

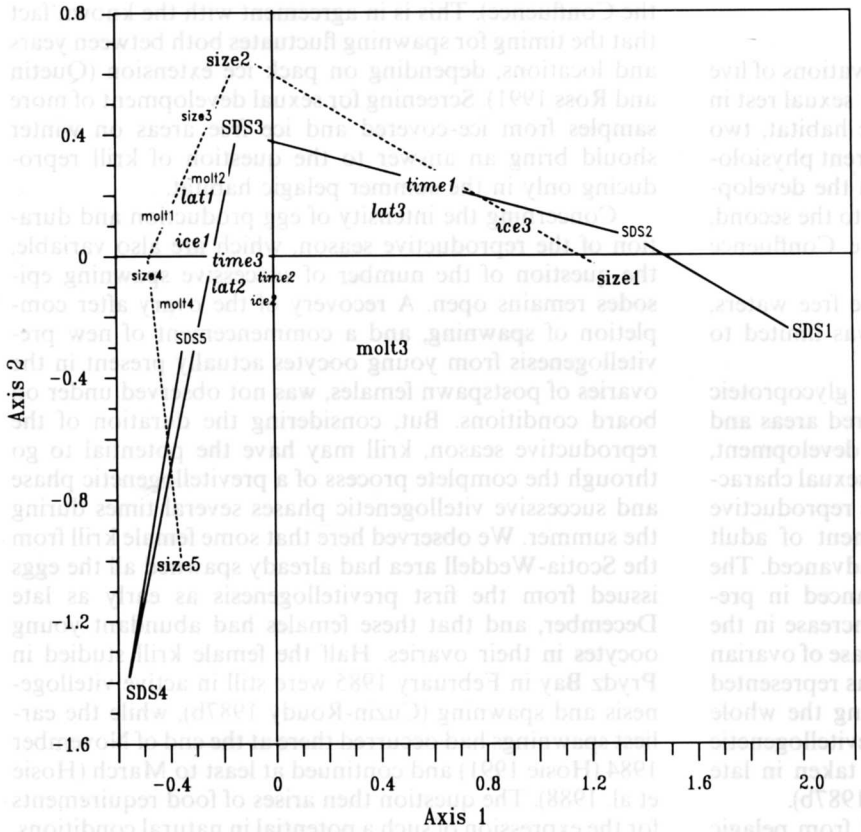

Fig. 2. Multiple correspondence analysis of female krill data from the Scotia-Weddell region in early summer. The figure shows the ordination of variables in the plane of Axis 1 and 2 (40% of the total inertia). The major trend of variation is illustrated by the lines joining sexual developmental stages (SDS 1 to 5) and increasing body-sizes (size 1 to 5). Supplementary variables are shown in italics. Codes for the variables are given in Table 1 (see text for further discussion). The size of the letters is proportional to the degree of correlation with the plane

biological characters (body-size, molt- and sexual development stage), with supplementary characters (ice-condition, latitude and sampling time), were displayed in the plane defined by the first and second axes. A general trend is evident for increasing body size and sexual development.

Biological factors. A strong link was evident between body-size and sexual development. SDS 1 (gametogenesis) tends to be associated with size 1, and in the opposite, SDS 4 (vitellogenesis and oocyte maturation) with size 5. This general trend of development showed juvenile krill, with no sexual development, to be opposite to adult krill, with all stages of egg development. Besides, SDS 3 (previtellogenesis) was linked to a whole range of sizes, while SDS 4 (vitellogenesis and oocyte maturation) appeared in large adults, which then had passed through the previous stages earlier in the season. On an other hand, SDS 5 (spawn and post-spawn) was not linked to large sizes and fell outside the general trend. We took this as confirmation that adult females of a wide range of sizes pass through this stage at the end of each spawning episode.

Molt-cycle. Molt-stage appeared independent of the other variables. Only molt 3 (premolt) and extreme sizes showed some link. Juvenile (mostly from ice) and the largest adults (mainly from the Scotia Sea) were more often in premolt. This was taken as indication of a more active physiology, which can be easily related to more active growth in juvenile krill. Concerning large adult females whose physiological effort appear more directed toward egg development, a higher frequency of premolt stages would be expected as a consequence of a lengthening of the premolt to allow time for egg maturation.

A second MCA, conducted without taking molt-cycle data into account, resulted in a similar pattern for the trends of variation of the other variables.

Additional factors. Space, time and ice variables were considered as explicative factors. Latitude was strongly associated with ice extent, especially lat. 3/ice 3, reflecting that ice was a constant for high latitudes. Ice-covered high latitudes were also strongly linked to size 1 and SDS 1, while ice 1 (no ice) and lat 1 and 2 were oppositely situated, together with adult reproducing krill. This trend reinforces the apparent dissociation of sexually immature juvenile krill, which were mostly present in the ice, and adult krill of larger sizes with maturing eggs, which were found only in the open sea. We note also that Lat 2 (Confluence) was linked to SDS 3 (previtellogenesis).

The advance in time also followed a similar trend. But, the indication that the progress in sexual development and the increase in body-size with time, inversely associated with ice cover, is somewhat biased since the sampling regime consisted of more ice stations at the beginning of the cruise. Nevertheless, time 1 and lat 3/ice 3, characteristic of the ice-covered northern Weddell Sea in early study period, are clearly associated with juvenile of small size (size 1) with sexual development limited to gametogenesis (SDS 1).

Discussion

Both the analysis of fixed samples and observations of live krill made a clear distinction between krill in sexual rest in the ice and krill reproducing in the pelagic habitat, two situations that obviously correspond to different physiological conditions. The results also illustrated the developmental steps leading from the first condition to the second, as shown by early summer changes in the Confluence region.

Juvenile krill, both from the ice and the free waters, exhibited development of the ovary that was limited to gametogenesis and oocyte formation.

Subadult and adult krill commencing glycoproteic yolk accumulation were present in ice-covered areas and the MIZ in November. This first ovarian development, that began with differentiation of secondary sexual characters, was interpreted as the first event of the reproductive season. At the same period, the development of adult female krill from the Confluence was more advanced. The ovaries were filled with oocytes well advanced in previtellogenesis, which resulted in a general increase in the size of the ovary (about 4 times). This first phase of ovarian development seems to persist in time as it was represented by 50% of the total observed females during the whole study period. Such females with large previtellogenetic ovaries were not found in pelagic samples taken in late summer 1985 in Prydz Bay (Cuzin-Roudy 1987b).

In the period of EPOS leg 2 study, krill from pelagic areas were already in the vitellogenetic phase. Lipidic yolk accumulation (vitellogenesis) commenced, for a limited number of the oocytes only, in animals whose ovaries had completed previtellogenesis. The other "ready" oocytes were concerned at later intervals, and the result was the maturation of successive batches of eggs. One vitellogenetic phase lasted 10 to 13 days for krill maintained on board and was estimated to be about 10 to 12 days for samples taken at intervals in the same area. These estimates are consistent with the duration of 2 weeks given by Denys and McWhinnie (1982), and that of 10 days proposed by Quetin and Ross (1991) for the interval between successive spawns.

The appearance of fully developed ovaries, with distinct batches of oocytes at three different states of development, found in early summer in EPOS pelagic samples, was exactly similar to ovaries of females sampled at the end of summer 1985 in Prydz Bay (Cuzin-Roudy 1987b). Such an ovarian structure seems to be typical for actively reproducing female krill.

Euphausia superba was found in large numbers in the North Eastern region of the Weddell Sea in summer (Boysen-Ennen and Piatkowski 1988), but "gravid" female krill were signaled only near the coast in the same region and were not found in the inner Weddell Sea (Siegel 1982). Krill with developed ovaries were not observed in winter pelagic samples from West of the Antarctic Peninsula (Cuzin-Roudy and Amsler 1991). In the present study, yolk accumulating and spawning female krill were not found in ice-covered regions. They occurred first in open waters (November in the Scotia Sea), and only after ice retreat in the zones seasonally covered by ice (December in the Confluence). This is in agreement with the known fact that the timing for spawning fluctuates both between years and locations, depending on pack ice extension (Quetin and Ross 1991). Screening for sexual development of more samples from ice-covered and ice free areas on winter should bring an answer to the question of krill reproducing only in the summer pelagic habitat.

Concerning the intensity of egg production and duration of the reproductive season, which are also variable, the question of the number of successive spawning episodes remains open. A recovery of the ovary after completion of spawning, and a commencement of new previtellogenesis from young oocytes actually present in the ovaries of postspawn females, was not observed under on board conditions. But, considering the duration of the reproductive season, krill may have the potential to go through the complete process of a previtellogenetic phase and successive vitellogenetic phases several times during the summer. We observed here that some female krill from the Scotia-Weddell area had already spawned all the eggs issued from the first previtellogenesis as early as late December, and that these females had abundant young oocytes in their ovaries. Half the female krill studied in Prydz Bay in February 1985 were still in active vitellogenesis and spawning (Cuzin-Roudy 1987b), while the earliest spawnings had occurred there at the end of November 1984 (Hosie 1991) and continued at least to March (Hosie et al. 1988). The question then arises of food requirements for the expression of such a potential in natural conditions.

In our experiments, vitellogenesis and egg maturation were maintained, even with poor food, while the board conditions did not permit neither the initiation nor the completion of previtellogenesis. The two phases of yolk accumulation therefore corresponded to different physiological equilibria and are likely to rely on different energy sources. This has implication for the estimation of the cost of reproduction in the energy budget of female krill. The rapid accumulation of lipids in oocytes during cyclic vitellogenesis does not seem to rely only on an increased summer food intake (Clarke and Morris 1983). The long previtellogenetic accumulation of glycoproteic yolk and the constitution of reserves of early summer might depend upon ice-algae and ice-edge blooms.

The role of the fat-body, which is well developed in previtellogenetic krill (Cuzin-Roudy, unpublished results), has certainly to be clarified, but, the quantity and quality of the food available in spring in the ice-covered habitat (ice-algae) and in the MIZ (ice-edge blooms) seem to be essential not only for the onset, but also for the maintenance of the reproductive effort. Juvenile krill have been observed feeding on ice algae (see Quetin and Ross 1991 for review), which also sustained food requiremens for juvenile gametogenesis and oogenesis in our experiments. Quetin and Ross (1991) note the probable importance of ice algae as a spring food source, and krill has been described occurring in high densities near the sea ice (Hempel 1987; Miller and Hampton 1989). The present study has shown that the physiological changes for the onset of ovarian development occurred there. It thus appears that more studies on energy budget of krill's reproductive development are necessary, as required by

Miller and Hampton (1989), especially in spring, the transition period when krill has to shift from winter sexual rest to reproductive activity.

Egg preparation and growth, are seasonal energy demanding functions in krill's life cycle. Growth parameters show a remarkable plasticity and adaptation to changing food conditions (Buchholz 1991). In the present analysis, egg maturation and molting appeared to be independent, at least for adult female krill in laboratory conditions. The relationship of molting and spawning in krill (Nicol 1989) has not yet been elucidated. Food requirements for simultaneous growth and reproduction can only be fulfilled in areas of enhanced primary production, such as the ice-edge in spring and the Confluence in summer. Consequently, grazing pressure from the actively reproducing krill population is likely to be a determinant biological factor driving the changes in the structure of the pelagic community in areas were krill are dominant. Krill accumulating yolk for reproduction offer also a rich food source to higher levels of the pelagic food web (birds, seals, whales) in early summer.

We conclude that the degree of sexual development, analysed here together with other factors and measured by a Sexual Development Index (SDI) on series of samples, is a useful indicator of the biological activity of the krill population. Determining the distribution of krill's SDI in various regions of the Antarctic Ocean should facilitate the identification of the most biologically active sectors, and help to elucidate their seasonal variability.

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