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The variability in abundance of eugregarines living in the Antarctic krill

Kunio T. Takahashi[1*†], So Kawaguchi[2], Masaki Kobayashi[1] and Tatsuki Toda[1]

[1] *Faculty of Engineering, Soka University, 1–236, Tangi-cho, Hachioji-shi, Tokyo 192-8577 (*kuni@nipr.ac.jp)*
[2] *Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia*

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Abstract: The variability in abundance of eugregarines associated with the Antarctic krill *Euphausia superba* was studied using samples collected from the vicinity of the Antarctic Peninsula. Body length, maturity stage and moult stage with respect to variation in eugregarines infection in krill were examined. Body length was significantly correlated with abundance of eugregarines. The interaction between moult stage and maturity stage was statistically analyzed by ANCOVA with body length as the covariate. The analysis of moult stage did not show a significant effect on abundance of eugregarines while the interaction between maturity stage and body length showed significance. Larger krill have more eugregarines than smaller krill at the same maturity stage. Body length appears to be the most important factor determining the abundance of eugregarines.

key words: eugregarine, parasite, Antarctic krill, body length, ANCOVA

Introduction

Gregarines (Phylum Apicomplexa, Class Sporozoa) are common internal parasites of many invertebrates (Zuk, 1987; Théodoridès, 1989). These groups of parasites have been reported from various species of crustaceans; amphipods, copepods, decapods, euphausiids (Théodoridès and Desportes, 1975; Kulka and Coney, 1984), and shrimps (Lightner, 1993; Jones *et al*., 1994). *Cephaloidophora pacifica* Avdeev (Order Eugregarinida) was formally described from *Euphausia superba* collected in the east Pacific and Indian Ocean sectors of the Antarctic Ocean (Avdeev, 1985). Moreover, Kobayashi *et al*. (unpublished data) found *C. pacifica* in krill from several stations, vicinity of the Antarctic Peninsula, Syowa Station and Indian and Pacific sectors. Therefore, *C. pacifica* can be considered as common parasites of the Antarctic krill, and are distributed widely throughout the Antarctic Ocean.

Gregarines occurring in very high numbers in the gut of shrimps can cause growth reduction and possibly mortality of their host (Lightner, 1993). In krill, their infestation may be pathogenic because of the close relationship between the level of eugregarine infestation and the number of clots in the diverticula observed by Avdeev and Vagin

† Present address: Department of Polar Science, The Graduate University for Advanced Studies, National Institute of Polar Research, Kaga 1-chome, Itabashi-ku, Tokyo 173-8515.

(1987). Therefore, the high level of infestation would cause a considerable negative impact on krill. However, the cause of the variability in abundance of eugregarines in krill has not been established.

The objectives of the present study are to examine the distribution pattern of the parasite in the vicinity of the Antarctic Peninsula during the austral summer, and to statisitically analyze three possible biological factors; body length, maturity stage and moult stage with respect to variation in eugregarine infection in krill. The area around the Antarctic Peninsula is especially known as one of the best fishing grounds for Antarctic krill (Ichii, 1990; Kawaguchi and Satake, 1994).

Materials and methods

Samples of krill were collected at ten stations with a KYMT net (*Kaiyo-maru* mid water trawl: mouth area, 9 m^2; mesh size, 3.4 mm) (National Research Institute of Far Seas Fisheries, 1995) by the R/V *Kaiyo-maru* of the Japan Fisheries Agency during the 7th Antarctic Expedition from October 1994 to March 1995, on two transects near the South Shetland Islands and Elephant Island both located north of the Antarctic Peninsula (Fig. 1). The net was towed obliquely from an approximate depth of 200 m to the surface at a speed of between 2 and 3 knots. Further sampling information is shown in Table 1. Immediately after collection, samples from each haul were preserved in 10% buffered formalin in seawater.

One hundred individual krill were randomly selected from each sample for mea-

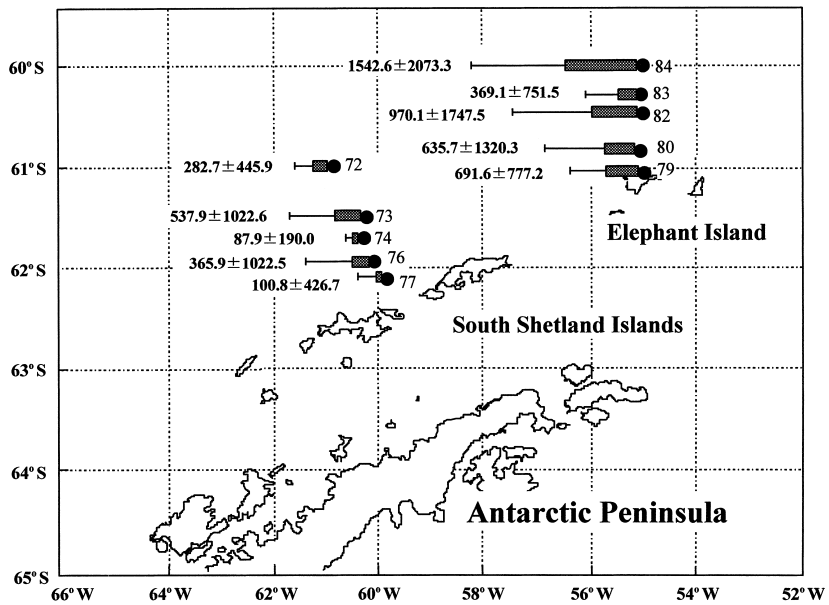

Fig. 1. Sampling locations (●) and intensity of eugregarines. Bars indicate weighted average intensity for different maturiry stage (±SD) for each station.

Table 1. Sampling information for KYMT net tows during this study.

Station	Sampling date	Sampling time (LT)	Position		Sampling depth (m)	Catch / Tow (g)	Biomass (g / 1000m^3)
72	15 Jan 95	16:08	60° 59' 7"S	61° 12' 3"W	200	690	33.0
73	15 Jan 95	22:30	61° 29' 9"S	60° 35' 7"W	204	1370	98.7
74	16 Jan 95	04:12	61° 45' 3"S	60° 22' 3"W	200	1130	46.0
76	16 Jan 95	07:55	61° 55' 0"S	60° 04' 7"W	218	15000	478.0
77	16 Jan 95	21:31	62° 03' 4"S	59° 59' 6"W	180	270	14.1
84	18 Jan 95	03:06	60° 00' 0"S	54° 57' 3"W	200	580	29.9
83	18 Jan 95	09:01	60° 15' 9"S	54° 59' 9"W	200	430	26.6
82	18 Jan 95	12:45	62° 30' 6"S	55° 01' 0"W	200	430	22.5
80	18 Jan 95	20:51	60° 52' 4"S	55° 01' 2"W	200	700	42.5
79	19 Jan 95	01:11	61° 00' 0"S	54° 58' 7"W	200	110	5.8

surement of body length, determination of maturity stage and moult stage. Body length was measured from the anterior margin of the orbit to the tip of the telson in 1 mm size classes (Siegel, 1982; Miller, 1983). Maturity stages of krill were identified according to the classification of Makarov and Denys (1981). Moult stages were determined by observing the uropod (Buchholz, 1982; Nicol and Stolp, 1990).

The digestive tract of krill was removed with forceps under a stereomicroscope. For samples of maturity stages containing less than 10 krill, all krill were dissected, while for samples containing more than 10 krill, 10 specimens were randomly selected and dissected (Table 2). Digestive tracts were torn into small parts with forceps in a zooplankton counting chamber, and eugregarines were counted using an inverted microscope. Two motile stages of the eugregarine, immature and mature gamonts as defined by Avdeev (1987) and also referred to as trophozoite (Levine, 1971), were counted.

Statistical analyses were carried out using the Statistical Package of the Social Science (SPSS), version 8.0 (SPSS Inc., Chicago). For comparison of variation in the abundance of eugregarines, the data was transformed using a $\log_{10}$ (number of eugregarines $+1$) function to reduce the bias of very high numbers. Goodness of fit was tested by the Chi-square test at a 5% level of significance. Variability in abundance of eugregarines was evaluated using an analysis of covariance (ANCOVA).

Following the guidelines in Bush *et al*. (1997), the term "prevalence", as used in this paper, is defined as the number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species. The term "intensity" is defined as the number of individuals of a particular parasite species in a single infected host.

Results and discussion

Eugregarines were found in the digestive tract of 376 of 383 specimens of *E. superba* examined (prevalence $=98.2\%$). Intensity ranged from 0 to 8505 individuals

Table 2. Individual numbers of krill dissected at each maturity stage. Numbers in parenthesis indicate the total numbers of krill selected in sampling stations.

Station	Juvenile	Male					Female					Total
	I	II A1	II A2	II A3	III A	III B	II B	III A	III BC	III D	III E	
South Shetland Islands transect												
72	0	0	0	0	0	10	0	0	10	10	0	30
						(72)			(13)	(15)		(100)
73	0	0	2	0	1	10	0	1	10	10	0	34
			(2)		(1)	(36)		(1)	(31)	(29)		(100)
74	0	0	1	4	0	10	0	2	10	10	0	37
			(1)	(4)		(58)		(2)	(10)	(25)		(100)
76	1	0	9	7	2	10	2	10	10	10	0	61
	(1)		(9)	(7)	(2)	(15)	(2)	(19)	(29)	(16)		(100)
77	7	1	10	5	2	3	10	10	10	0	0	58
	(7)	(1)	(17)	(5)	(2)	(3)	(23)	(19)	(23)			(100)
Elephant Island transect												
84	0	0	0	0	1	10	0	0	1	10	0	22
					(1)	(56)			(1)	(42)		(100)
83	0	0	2	0	4	10	0	0	0	10	2	28
			(2)		(4)	(57)				(35)	(2)	(100)
82	0	1	1	1	3	10	0	0	10	10	0	36
		(1)	(1)	(1)	(3)	(19)			(30)	(44)		(100)
80	1	0	1	1	0	10	0	0	10	10	0	33
	(1)		(1)	(1)		(28)			(30)	(39)		(100)
79	10	1	2	1	3	10	2	2	3	10	0	44
	(40)	(1)	(2)	(1)	(3)	(36)	(2)	(2)	(3)	(10)		(100)
Total	19	3	28	19	16	93	14	25	74	90	2	383
	(49)	(3)	(35)	(19)	(16)	(380)	(27)	(43)	(170)	(255)	(2)	(1000)

(Mean±SD: 493.2±1099.1). Weighted average intensity for different maturity stage for all stations on the South Shetland Islands transect ranged from 86.7 to 577.3 individuals/krill, while that of stations along the Elephant Island transect ranged from 514.0 to 1485.1 inds/krill (Fig. 1). Intensity of the eugregarine was higher on the Elephant Island transect (801.2±1392.1) than on the South Shetland Islands transect (264.5±741.9) (Fig. 2). These results suggested that the interindividual intensity on the Elephant Island transect was highly variable as compared to its on the South Shetland Islands transect. The frequency distribution of parasites per krill showed a standard deviation greater than the mean, suggesting a highly aggregated distribution (Fig. 2). This is a typical distribution pattern found in parasites living in marine species (Pennycuick, 1971; Boxshall, 1974; Jakob, 1987).

Body length of krill was larger in the offshore stations than in inshore stations on both transects (Fig. 3). The largest krill were found at offshore stations 74, 83 and 84 (57.1 mm), while the smallest individual was found at inshore station 79 (16.5 mm). In this region, adult krill move from coastal waters to the open sea for spawning in spring/summer, and return to coastal waters in autumn (Siegel, 1988). Sub-adult and juvenile krill, however, stay close to the coast throughout the year (Siegel, 1988). The increase of mean body length on both transects toward the offshore region would thus seem to be due to migration of krill during summer. Mean body length of krill from South Shetland

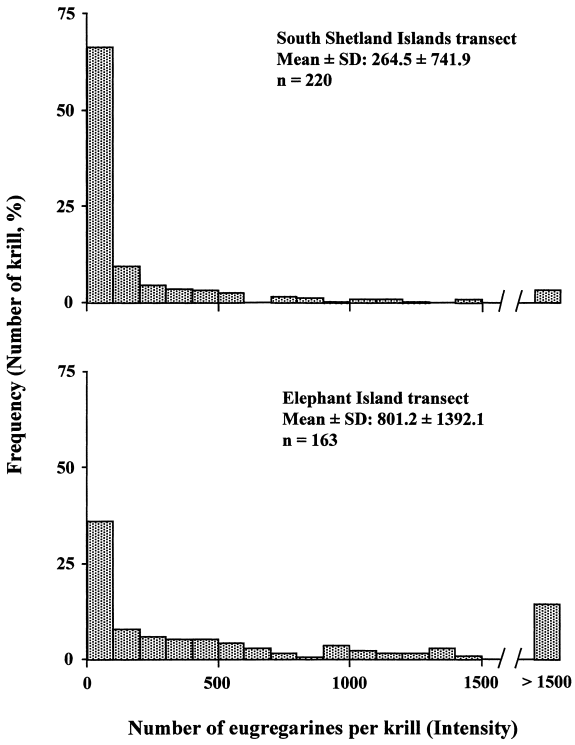

Fig. 2. Frequency distribution of number of eugregarines per krill (Intensity) on both transects.

Islands transect and Elephant Island transect did not differ, being 46.9±4.0 and 46.5± 6.9 mm, respectively. However, the standard deviation for mean body length for both transects varied by 2.9, with larger variations in population from near Elephant Island than in the South Shetland Islands population. These results suggest that krill on the Elephant Island transect had a wider range of body length as compared to krill on the South Shetland Islands transect.

The intensity increased significantly with host body length on both transects (Fig. 4, $p < 0.01$). The slope of the regression was three times greater in the Elephant Island transect (80.3) than in the South Shetland Islands transect (26.5). This may be explained by both the wider range of krill body length and the highly variable of parasite in the samples from the Elephant Island transect.

These positive correlation suggest that body length could have a significant effect on abundance of eugregarines, because the krill, which can take up a lot of food from the surrounding seawater, can increase their body mass. However, it may also raise a risk, such as enhancing infestation of parasitic eugregarines. Consequently, the intensity of the parasite may increase in well-grown krill. As another possible reason, habitat of eugregarines extends as the gut volume increases with increase in body length. As the host grows, eugregarines may accumulate in the mid-gut gland and intestine epithelial cells. In fact, they have been observed within the intestine epithelium layers of the

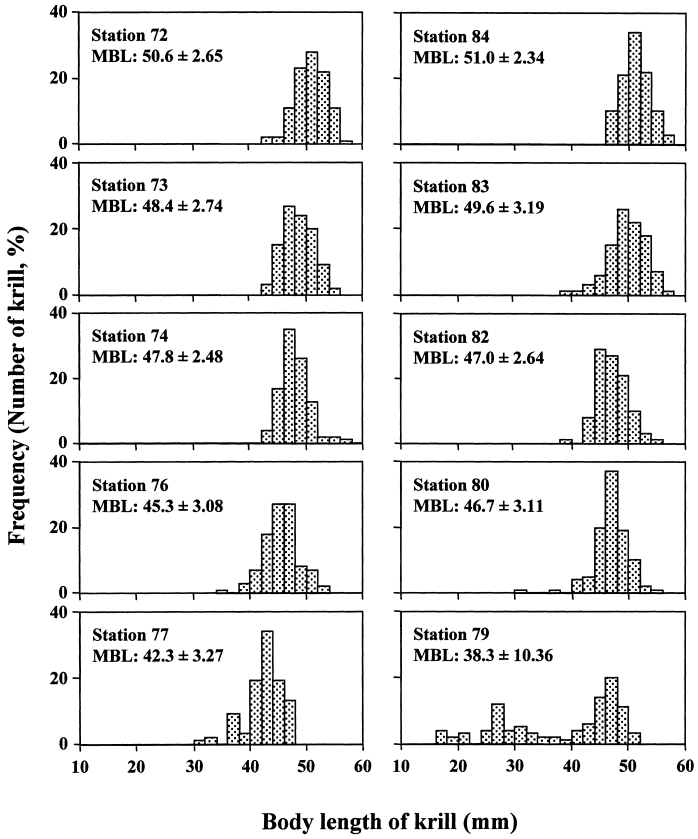

Fig. 3. Frequency distributions of body length of krill from ten stations. MBL: Mean Body Length (mean±SD).

mid-gut (Avdeev, 1987).

Since abundance of eugregarines is likely to be correlated with body length of krill, the interaction between moult stage and maturity stage was analyzed by ANCOVA with body length as the covariate.

Moult stages were grouped into three divisions; immediately post-moult (A in Buchholz, 1982), between moults (B–C), and immediately pre-moult (D) (Fig. 5). At every station, krill showed a low frequency of stage A. The majority of krill samples were in stages B–C (25–55%) and D (30–70%). Frequency trends from the inshore to offshore stations were not observed. Mean body length of krill at each moult stages showed little variation between 45.4 (A) to 46.6 (D) mm, while intensity of eugregarines ranged from 288.0 (B–C) to 820.2 (A) inds/krill among the all samples (Table 3).

Statistical analyses revealed that the moult stage did not have a significant effect on abundance of eugregarines ($p=0.089$, Table 4). Krill moult the integument and cuticle in the digestive tract. When moulting, the stomach and the hind-gut covered with cuticle

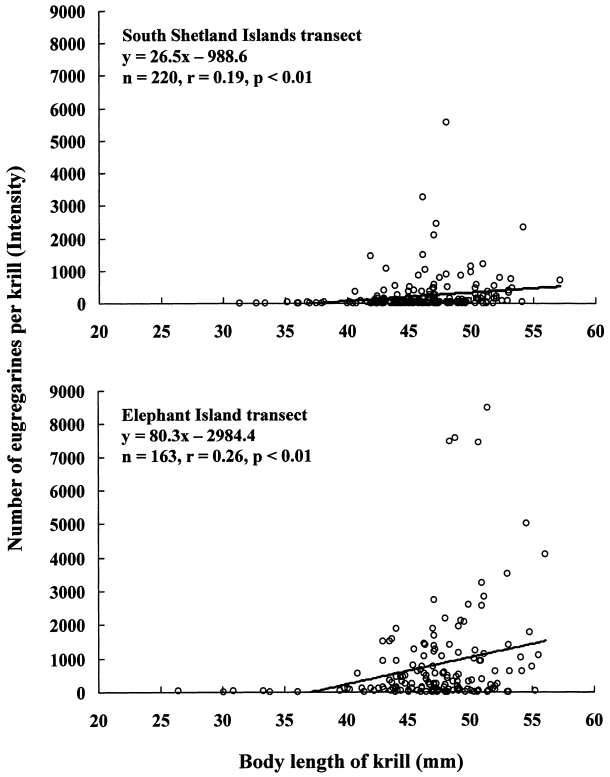

Fig. 4. Relationship between body length of krill and number of ergregarines per krill (Intensity) on both transects. The equation and fitted line of a linear regression are also shown.

are discarded in the digestive tract (Ikeda *et al*., 1984). Therefore, during moulting there is a possible discharge of eugregaines living in the stomach and the hind-gut to the outer environment. However, the results of statistical analysis in the present study suggested that eugregarines can avoid being discharged during moulting. This result concurs with the previous study of Takahashi *et al*. (2003). They reported that eugregarines at immediately pre-moult stage of the krill decreased significantly in the posterior of the hid-gut, and increased in the anterior of the hid-gut. Thus, as a strategy for avoiding discharge to the outer environment by moulting, the gamont stage, with its high motility, may move to a safety zone where no shedding occurs (mid-gut).

For all samples analyzed, approximately 54% females, 41% males and 5% juveniles were counted. Krill were more mature in the offshore stations than in the inshore stations (Table 2). A large number of gravid females (III D in Makarov and Denys, 1981) and fully mature males (III B) were observed in the offshore stations 72 and 84. On the other hand, krill were less mature in the inshore stations, while females were still maturing (III A and III BC) and males were immature (II A1-II A3). Juveniles (I) were found at inshore stations 76, 77, 79 and 80. The maturation of krill on both transects towards the offshore region also appeared to be due to migration of krill. As the

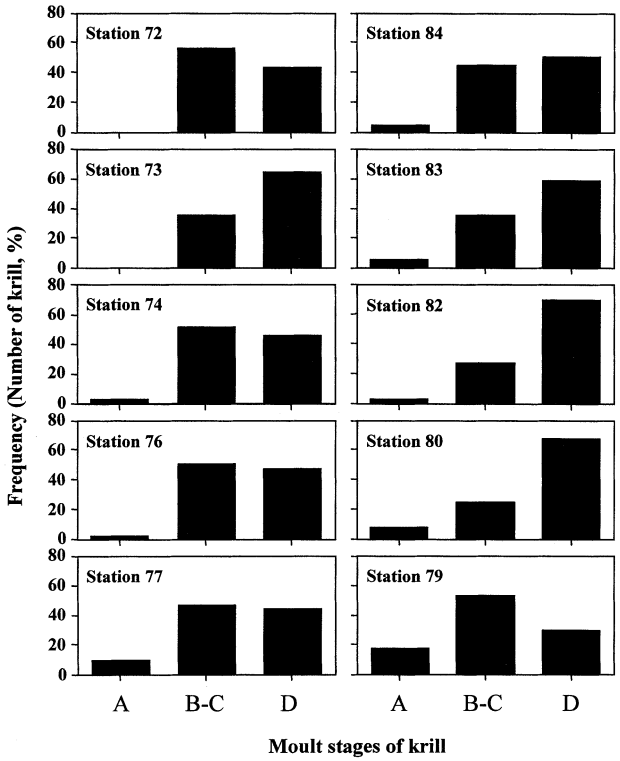

Fig. 5. Frequency distributions of moult stages of krill from ten stations. A: post-moult, B-C: between moult, D: pre-moult.

Table 3. Mean body length and intensity of eugregarines at each moult stages and maturity stages of krill.

Stage	Number of krill	Mean body length of krill (mm) ± SD	Intensity of eugregarines ± SD
Moult stage			
A (post-moult)	37	45.4 ± 5.9	820.2 ± 1458.9
B-C (between molt)	142	46.0 ± 4.8	288.0 ± 563.6
D (pre-moult)	204	46.6 ± 4.7	576.2 ± 1272.5
Maturity stage			
Juvenile (I)	19	32.6 ± 4.1	177.0 ± 314.4
Sub-Adult Male (II A1 - II A3)	50	44.4 ± 2.6	262.2 ± 631.6
Adult Male (III A - III B)	109	48.4 ± 2.7	721.2 ± 1275.1
Sub-Adult Female (II B)	14	40.3 ± 2.8	155.0 ± 356.2
Adult Female (III A - III E)	191	47.3 ± 3.7	479.8 ± 1151.9

Table 4. Interaction of moult stage and maturity stage with body length as the covariant (details of ANCOVA).

Source of variation	Degrees of freedom	Variance ratio	Significance
Moult stage (A)× Body length (C)	2	2. 437	0. 089
Maturity stage (B)× Body length (C)	10	3. 469	< 0. 001
A × B × C	14	1. 217	0. 261

host matures, mean body length was increased on both male and female (Table 3). Also, intensities of eugregarines were increased with maturity of the host among the all samples (Table 3).

The spatial distribution of eugregarines in the digestive tract of krill was not significantly different at the host's maturity stages (Takahashi *et al*., 2003). In contrast, statistical analysis of maturity stage and body length in this study showed a significant effect of maturation on eugregarinid abundance that increases with maturity of the krill ($p < 0.001$, Table 4). The results also show that larger krill have more eugregarines at the same maturity stage. Body length is thus the most important factor determining the abundance of eugregarines.

In further study, the *in situ* physiological and ecological influences of eugregarine infestation on the krill should be studied, due to the fact that krill is a key species of the Antarctic marine ecosystem.

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