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Filtration rate and ingestion rate of Antarctic krill measured *in vitro* and *in situ*

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Abstract During the summer cruise of R/V 'JIDI' in 1992/1993 filtration rate and ingestion rate of Antarctic krill *Euphausia superba* Dana were measured by constant volume incubation, flow-through incubation and gut fluorescence method in the southern part of the Scotia Sea and in the Prydz Bay region. The mean filtration rate obtained by different methods ranged form 189. 8 to 459. 6 ml/h for sub-adult (60. 6～205. 4 mg dry weight), and 253. 6～902. 1 ml/h for adult (166. 8～598. 7 mg dry weight). No significant methodological effect was found on measurement of filtration rate if suitable experimental conditions were used with special respect to animal density and vessel volume. Within the range of food concentration (0. 44～3. 05 μg chl-*a*/l) of our experiments. The ingestion rate increased with the increasing of food concentration, while the filtration rate didn't increase. The ingestion rate obtained by gut fluorescence method had no correlation with ambient chlorophyll *a* concentration.

Key words filtration rate, ingestion rate, Antarctic krill, Southern Ocean.

1 Introduction

The filtration rate and ingestion rate are important parameters of feeding of Antarctic krill, *Euphausia superba* Dana, the most important species in the marine ecosystem of the Southern Ocean. They had been studied by many authors in laboratory by using constant volume incubation method (Kato *et al.*, 1979; Antezana *et al.*, 1982; Boyd, 1982; Kato *et al.*, 1982; Murano *et al.*, 1982; Meyer and El-Sayed, 1983; Morris *et al.*, 1983; Boyd *et al.*, 1984; Morris, 1984; Schnack, 1985). The main problem of this method is the refiltration of sea water by krill incubated in a vessel of small volume for long time. In order to lessen the effect of refiltration on the measurement of filtration rate, a flow-through technique which often used with bivalves has been used by Antezana *et al.* (1982) and Morris (1984). Up to recently, many data on filtration and ingestion rate by different methods, including constant volume incubation, flow-through technique, and estimation from energetic and physiological data, have been published, but the estimations vary greatly (Morris, 1984), up to 2 to 3 orders of magnitude.

Many authors have pointed out that it is unsuitable to estimate ingestion rate of copepods in field by using parameters obtained from laboratory conditions, which are quite different from the real field conditions. And some disadvantages in laboratory ex-

periments, such as the stress of capture, confinement of experiment vessel, domestication caused by lengthy incubation, may provide unreliable results (Roman and Rublee, 1980; Head, 1986; Wang and Conover, 1986). So it is important to measure grazing rate under natural conditions by using *in situ* techniques. "Gut fluorescence" method introduced by Mackas and Bohrer (1976) has been widely used to estimate ingestion rate of copepods *in situ*. But no data on ingestion rate of *Euphausia superba* by this method have been reported until now.

This paper presents some results on filtration rates and ingestion rates obtained by constant volume incubation, flow-through technique, and gut fluorescence method conducted during austral summer of 1992/1993 on R/V 'JIDI'. Methodological effects on filtration rate and ingestion rate are also discussed.

2 Material and method

2.1 In vitro filtration rate and ingestion rate experiments

Constant volume incubation — Live krill used in this experiment were from three samples. Sample 1 was obtained at about 58°34′W, 62°19′S on Dec. 15, 1992 by conical net vertical hauling. Sample 2 was collected at 58°01′W, 62°57′S on Dec. 24, 1992 by HSPS (High-Speed Plankton Sampler) oblique trawling. Sample 3 was from HSPS tow at 63°00′E, 64°15′S on Jan. 13, 1993. Krill were kept and acclimated in 500-litre plastic tanks on deck for about 2 days before experiments. Animals were prefed with algae obtained by filtering surface seaswater through 20 μm mesh. For individual krill incubation, 5-litre glass beaker was chosen as experimental chambers. Ambient surface seawater or seawater added with algae which was obtained by filtering surface seawater through 20 μm mesh were supplied in different concentrations. In each experiment, 6 experiment beakers and 1 control (the same but no animal) were incubated at 1±0.2℃ in dark for about 6～8 hours. For mass incubations, 5 individuals were placed into 7-litre and 25-litre beakers filled with surface seawater enriched by 20 μm mesh filtering. In each experiment, 2 experiment chambers and 1 control were incubated on deck at about 1±0.5℃ in dark for about 6～8 hours.

At the beginning and end of experiment, 200 ml water samples were filtered through Whatman GF/F filters and extracted in 90% acetone for 24～48 hours. Fluorescence was measured with a Turner-Designs fluorometer. Body length (from tip of rostrum to tip of telson) and dry weight of each animal was measured after experiments. The details of these experiments conditions were shown in Table 1.

Filtration rate and ingestion rate in the constant volume experiments were calculated according to Frost (1972).

Flow-through incubation — Krill used for these experiments were from Sample 2. Five krills were placed in a 7-litre "experiment chamber". Sea water came from a 25-litre tank (supplying tank) filled with algae-enriched seawater, and then passed through the "experiment chamber". Water flowing out off the "experiment chamber" was collected by a collecting tank. Water flow was controlled in a constant rate (about 3500 ml/h).

Table 1. Summary of experimental conditions for constant volume incubation

	Material	Experiment time and internal	Incubation density	Initial food concentration (μg • chl a/l)	Dry weight (mg)
Exp. 1	Sample 1	Dec. 15, 1992 1515～1915	1 ind. /5l	0.79	25.4～210.5
Exp. 2	Sample 1	Dec. 16,1992 0230～0830	1 ind. /5l	2.12	25.4～210.5
Exp. 3	Sample 2	Dec. 26, 1992 1140～0740	1 ind. /5l	0.51	45.0～299.8
Exp. 4	Sample 2	Dec. 26～27,1992 2100～0500	1 ind. /5l	0.62	45.0～299.8
Exp. 5	Sample 2	Dec. 28,1992 0200～0945	1 ind. /5l	1.28	45.0～299.8
Exp. 6	Sample 2	Dec. 26～27,1992 2250～0610	5 ind. /7l	1.16	60.6～79.7
Exp. 7	Sample 3	Jan. 18～19,1993 2215～0435	5 ind. /25l	3.10, 3.47	226.0～272.0

Exp. 1～5: single individual incubation exp. ;Exp. 5～6:mass incubation exp. ; Exp. 7: flow-through exp.

The experiments were conducted in dark for about 7 hours at 1±1℃. At the beginning and end of each experiment chlorophyll *a* concentration in supplying tank and in collecting tank were measured. And we assumed that the algae growth rates in collecting tank were the same as in supplying tank. The phytoplankton consumed by krill per unit time can be calculated by the formula given by Hildreth and Crisp (1976),i. e. ,

$$F \cdot C_0 = (RC_1 - RC_2)/N$$

or

$$F = \frac{RC_1 - RC_2}{C_0 \cdot N},$$

where R is water flow rate through experiment chamber (ml • h^{-1}), C_0 is the phytoplankton concentration around the krill (ng chl-a • ml^{-1}), C_1 and C_2 are phytoplankton concentrations in inflow and outflow water separately (ng chl-a • ml^{-1}), N is the number of krill and F is filtration rate (ml • ind.$^{-1}$ • h^{-1}).

We use the average value of C_1 and C_2 as C_0, and then:

$$F = \frac{2R(C_1 - C_2)}{N(C_1 + C_2)}$$

and

$$I = FCo = \frac{RC_1 - RC_2}{N}$$

where I is ingestion rate of krill (ng chl-a • ind.$^{-1}$ • h^{-1})

2. 2 In situ filtration rate and ingestion rate measurement

Stomach and gut evacuation experiment—130 active individuals were collected from Sample 2 after acclimated and prefed for 27 hours. Krills were rinsed with filtered seawater (GF/F twice) and then transferred into four 15-litre plastic carboys containing filtered seawater. The carboys were kept in dark under temperature of 1 ± 0.2℃. During the experiment subsamples of 10 individuals were taken at intervals of 15～60 minutes for as long as 7 hours. The subsamples were frozen immediately and kept at −25℃ for later stomach and gut pigment analysis. 10 individuals were incubated for 24 hours for determining the background of stomach and gut fluorescence. Faecal pellet produced by krill during incubation were moved out by pipette.

Chlorophyll and phaeopigment in stomach and gut were determined by the method mentioned above. The equation of $G_t-G'=(G_0-G')e^{-rt}$ was used to obtain the value of evacuation rate by regression analysis, where G_0 is the initial gut pigment content, G_t is the gut pigment content at time t, and r is the evacuation rate. G' was the background value of stomach and gut fluorescence.

Stomach and gut pigment content measurement—10 to 30 freshly caught krill from 19 net samples were frozen immediately for later analysis. Table 2 shows the details of the sampling data. After returning to laboratory, the pigment content in stomach and gut were analysed. Body length (mm, from the tip of rostrum to the tip of telson), wet weight (mg), and maturity stage (Makarov and Denys, 1980) were recorded. The stomach and gut were dissected out as fast as possible in dim light. Dissected stomach and gut were ground separately and chlorophyll and phaeopigment in stomach and gut were extracted by 90% acetone in dark at −25℃ for 48 hours. Samples were centrifuged at 2000 rpm. for 10 minutes and pigment levels were analysed by the fluorescence method (Yentsen and Menzel, 1963) determined on the Turner 10 fluoremeter.

A total of 354 speciman from 19 sampling sites were analysed. Ingestion rate was calculated from $I = rG_0$, and filtration rate was calculated from $F = I/\bar{C}$, where $\bar{C}$ was the ambient chlorophyll *a* concentration.

3 Results

3. 1 Filtration rates and ingestion rates obtained from different experimental methods

Filtration rates and ingestion rates, as well as weight specific filtration rates and ingestion rates of different groups obtained by different methods were listed in Table 2.

In constant volume single incubation experiments, krill were classified into three groups by dry weight (DW): juvenile (DW = 43.1 ± 2.5 mg), sub-adult (DW = 74.1 ± 4.3 mg) and adult (DW = 253.8 ± 14.0 mg). The average values of filtration rate (FR, ml • ind^{-1} • h^{-1}), ingestion rate (IR, ng chl-a • ind^{-1} • h^{-1}), weight specific filtration rate (WSFR, ml • mg DW^{-1} • h^{-1}), and weight-specific ingestion rate (WSIR,

ng chl-a • mg DW^{-1} • h^{-1}) of all the three groups were 258.3±25.0, 225.1±34.1, 3.6±0.4, and 3.9±0.7 separately.

Table 2. List of filtration rates, ingestion rates, weight-specific filtration rates and ingestion rates obtained from different experimental methods, as well as body dry weights and food concentration

Methods	DW M±SE	FC M	FR M±SE	WSFR M±SE	IR M±SE	WSIR M±SE
A	43.1±2.5	1.07	243.4±32.3	5.7±0.7	256.3	6.5±1.6
	(25.4~49.4)	(0.46~2.15)	(95.4~415.5)	(2.1~8.5)	(68.5~571.5)	(1.5~16.9)
	74.1±4.3	1.02	189.8±28.8	2.7±0.5	216.5±53.8	3.1±0.8
	(60.6~111.9)	(0.47~2.15)	(72.9~463.0)	(0.9~7.1)	(45.2~845.6)	(0.6~12.9)
	253.8±14.0	0.82	253.6±14.0	1.7±0.3	412.6±59.3	1.4±0.4
	(210.5~299.8)	(0.44~1.83)	(198.5~679.1)	(0.7~2.7)	(111.9~832.6)	(0.4~4.0)
	Average		258.3±25.1	3.6±0.4	255.1±34.4	3.9±0.7
B	249.5±11.7	2.78	389.9±71.5	1.6±0.2	1062.6±91.0	4.3±0.1
	(226.1~272.8)	(2.50~3.05)	(131.9~461.4)	(1.4~1.7)	(971.6~1153.5)	(4.2~4.3)
C	68.5±2.8	1.03	84.0±10.8	1.3±0.2	85.8±7.1	1.3±0.1
	(65.7~69.2)	(0.99~1.07)	(73.2~94.8)	(1.1~1.4)	(78.1~93.4)	(1.2~1.4)
D	67.5±3.2	0.94	290.2±22.2	4.1±0.9	307.9±32.6	4.3±1.0
	(66.8~68.1)	(0.92~0.95)	(268.0~312.4)	(3.2~4.9)	(275.3~340.4)	(3.3~5.3)
E	168.8±8.6	0.28	459.6±51.4	3.0±0.3	180.6±19.24	1.3±0.1
	(44.4~205.4)	(0.15~0.52)	(36.0~2559.9)	(0.2~15.1)	(35.4~902.0)	(0.2~10.5)
	274.3±6.1	0.45	902.1±53.9	1.7±0.1	464.4±28.3	3.4±0.2
	(166.8~598.7)	(0.15~1.67)	(34.5~5880.6)	(0.1~16.8)	(36.5~3040.3)	(0.1~24.1)
	Average		784.6±43.1	3.3±0.2	389.7±22.5	1.6±0.1

A: single incubation in constant volume; B: mass incubation in constant volume of 5 krill • 25 l^{-1} density; C: mass incubation in constant volume of 5 krill • 7 l^{-1} density; D: flow-through method; E: gut fluorescence method. DW = body dry weight (mg); FC = initial food concentration (μg chl-a • l^{-1}); FR = filtration rate (ml • ind.$^{-1}$ • h^{-1}); WSFR = weight-specific filtration rate (ml • mgDW^{-1} • h^{-1}); IR = ingestion rate (ng chl-a • ind^{-1} • h^{-1}); WSIR = weight specific ingestion rate (ng chl-a • mgDW^{-1} • h^{-1}); M = mean; SE = standard error; data in parentheses are ranges.

In constant volume mass incubation experiments with krill density of 5 ind. in 7 litres krill were all sub-adults with mean dry weight of 68.5±2.8 mg. The average FR, IR, WSFR and WSIR were 84.0±10.8, 85.8±7.1, 1.25±0.2 and 1.3±0.1 separately. For those of 5 ind. in 5 litres experiments, the average IR, FR, WSFR and WSIR were 389.9±71.5, 1062.6±91.0, 1.6±0.2 and 4.3±0.1 separately. Krill used in those experiments were adult with mean dry weight of 249.5±11.7 mg. For experiments of flow-through incubation, krill were all sub-adults with dry weight of 67.5 ±3.2 mg. The average FR, IR, WSFR and WSIR were 290.2±22.2, 307.9±32.6, 4.1±0.9 and 4.3±1.0 separately. Krill used for stomach and gut fluorescence analysis were sub-adults (DW = 168.8±8.6 mg) and adults (274.3±6.1 mg). 4 juveniles were not included. The average FR, IR, WSFR and WSIR were 784.6±43.1, 389.7±22.5, 3.3±0.2, 1.6±0.1 separately.

3.2 *ANOVA analysis for methodological differences*

In order to see if there is any difference in filtration rate and ingestion rate by differ-

ent methods, data of filtration rates and ingestion rates come from weight-matched krill in different experimental methods were used for single-factor Analysis of Variation (ANOVA). The statistical results were list in Table 3. For filtration rate, the differences between most of the results were not statistically significant , except that between 5 individuals in 7 liters density mass incubation of constant volume and flow-through method (ANOVA: $F[1,2]= 69.760$, $P = 0.014$). For ingestion rate the difference between them was also statistically significant (ANOVA: $F[1, 2] = 44.12$, $P = 0.0219$). The difference between single incubation and 5 ind. in 25 liters mass incubation (ANONA: $F[1,7]=15.64$, $P=0.0055$), and that between single incubation and fluorescent method (ANOVA: $F[1,265]=12.20$, $P=0.0006$) were statistically significant.

Table 3. One-way ANOVA analysis of the difference of filtration rate and ingestion rate by different experimental methods

		df	F-ratio	P	Level 1 M±SE	Level 2 M±SE
A via B	FR	1,7	0	0.9880	391.91±64.14 (7)	389.92±71.42 (2)
	IR *	1,7	15.64	0.0055	328.40±92.38(7)	1062.38±90.95(2)
A via C	FR	1,14	1.525	0.2372	176.67±27.54(14)	84.00±10.77(2)
	IR	1,14	0.654	0.4438	211.98±57.61(14)	85.88±7.52(2)
Avia D	FR	1,16	2.891	0.1085	165.09±25.25(16)	290.20±22.20(2)
	IR	1,16	0.559	0.4734	196.22±51.32(16)	307.85±32.55(2)
A via E	FR	1,265	0.266	0.6199	259.55±25.82(34)	286.55±19.61(233)
	IR *	1,265	12.202	0.0006	257.54±35.32(34)	657.69±43.39(233)
B via E	FR	1,60	0.039	0.8461	389.95±71.45(2)	445.33±50.73(60)
	IR	1,60	0.024	0.8795	1062.55±90.95(2)	980.60±96.15(60)
C via D	FR *	1,2	69.760	0.0140	84.00±10.80(2)	290.20±22.20(2)
	IR *	1,2	44.120	0.0219	85.75±7.65(2)	307.85±32.55(2)
C via E	FR	1,10	0.500	0.5029	84.00±10.80(2)	264.00±109.55(10)
	IR	1,10	1.200	0.2991	85.75±7.65(2)	478.00±154.46(10)
D via E	FR	1,10	0.011	0.9214	290.20±22.20(2)	264.29±109.55(10)
	IR	1,10	0.18	0.6845	326.40±14.00(2)	478.86±154.46(10)

FR=filtration rate (ml • ind^{-1} • h^{-1}), IR=ingestion rate (ng chl-a • ind^{-1} • h^{-1}), P=significant level, M=mean, SE=standard error. A~E: the same as in Table 2; data in parentheses are the number of samples; * indicates statistacally significant.

3.3 Filtration rate and ingestion rate in relation to food concentration

The filtration rate and ingestion rate were plotted against food concentration in Fig. 1 for incubation experiments and Fig. 2 for stomach and gut fluorescence method. In incubation experiments, filtration rate increased slightly with the increasing of food concentration, but the correlation between filtration rate and food concentration was not significant ($R=0.097$, $F[1,37]= 0.35$; Fig. 1A). The correlation between ingestion rate and food concentration was positive significant ($R=0.803$, $F[1,37]=67.20$, Fig. 1B).

The ingestion rate increased with the increasing of food concentration. In stomach and gut fluorescence experiments, an opposite result was detected. There was no significant correlation between filtration rate and ambient chlorophyll concentration ($R=0.255$, $F[1,342]=23.68$, Fig. 2A). The filtration rate even decrease slightly with the increasing of chlorophyll concentration. The correlation between ingestion rate and ambient chlorophyll concentration was not significant ($R=0.052$, $F[1,342]=0.93$, Fig. 2B).

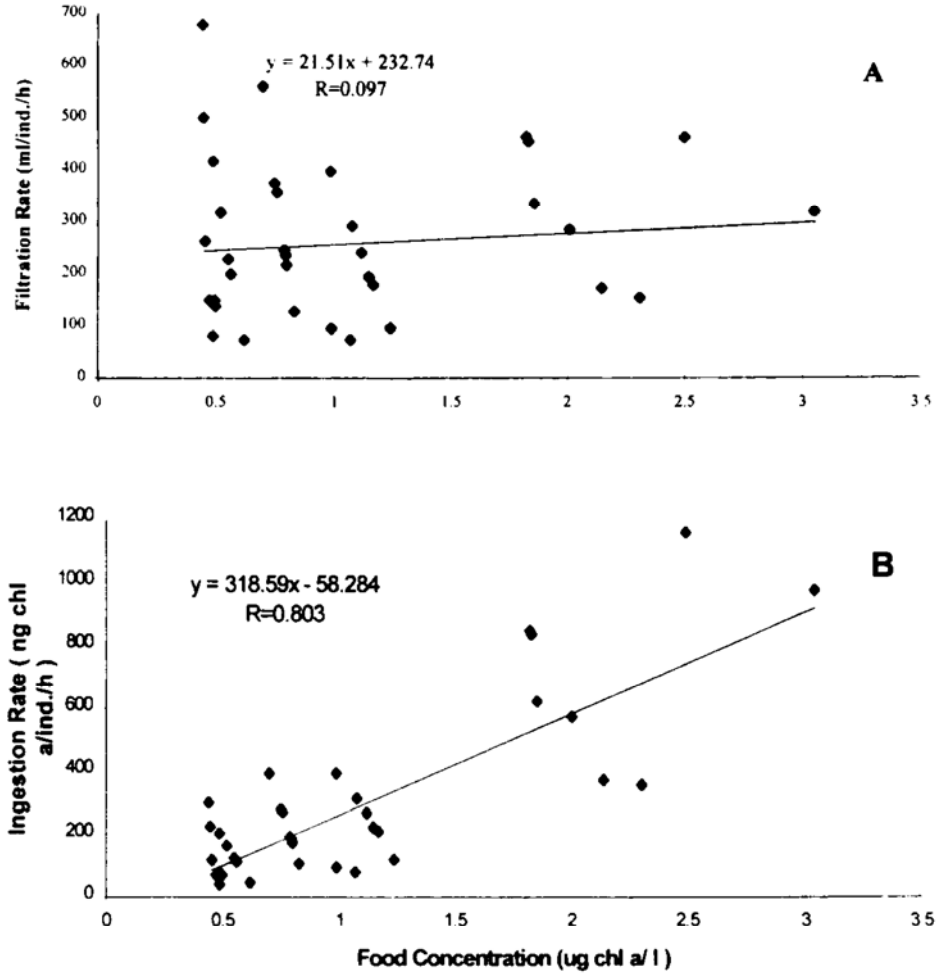

Fig. 1. Filtration rate(A) and ingestion rate (B) in relation to food concentration obtained from incubation experiment.

3.4 Stomach and gut evacuation rate

The weight-specific pigment contents in stomach and gut of krills taken at different intervals in the evacuation experiment were plotted against incubation time in Fig. 3. It was shown that the pigment content declined exponentially after transferred to filtered seawater. A equation of $Gt=3.71e^{0.427t}$ is obtained by nonlinear regression analysis ($R=0.9680$, $F[1,10]=866.47$). The evacuation rate r equal to 0.427 h^{-1}. T, the gut passage time or turnover time is the inverse of r, $T = 1/r = 2.34$ h.

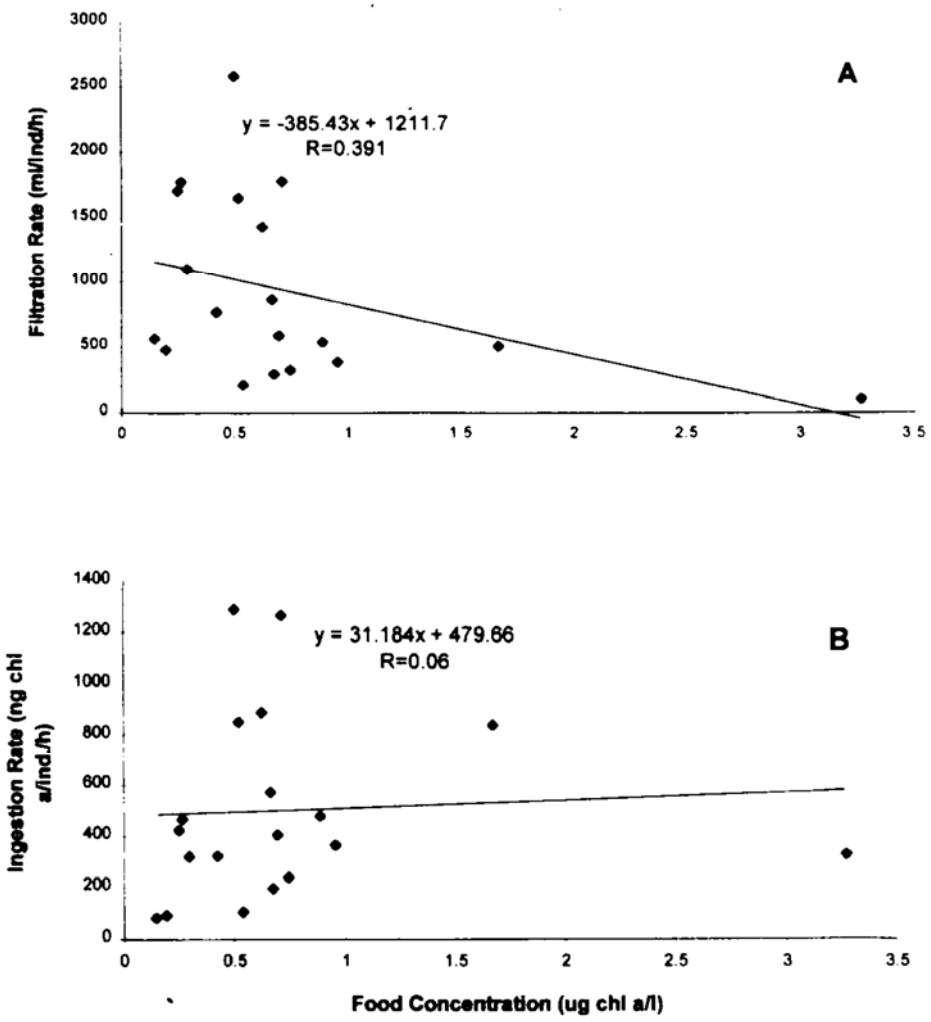

Fig. 2. Filtration rate(A) and ingestion rate (B) in relation to food concentration obtained from stomach and gut fluorescence method.

4 Discussion

The literatures on studies of filtration rate and ingestion rate of Antarctic krill provide us numerous but confused data. This may be due to the different methods used by different authors in their experiments. The former workers determine the filtration rate and ingestion rate by using direct methods. That means most of the experiments are conducted in constant volume under different conditions (type and concentration of food , volume of container, incubation intervals and so on). Filtration rate and ingestion rate are calculated based on the changes of food concentration (chlorophyll *a* or food particles) at the beginning and end of incubation. For this method refiltration is a big problem, which would cause an underestimation of filtration rate and ingestion rate. The smaller the container and the longer the incubation time, the more serious the refiltration problem. Williams (1982) has summarised the limitations of the assumptions used in constant volume experiments. Clarke and Mirris (1983) have discussed the limitations of this technique when dealing with a relative large, active, pelagic crustacean such as *E. super-*

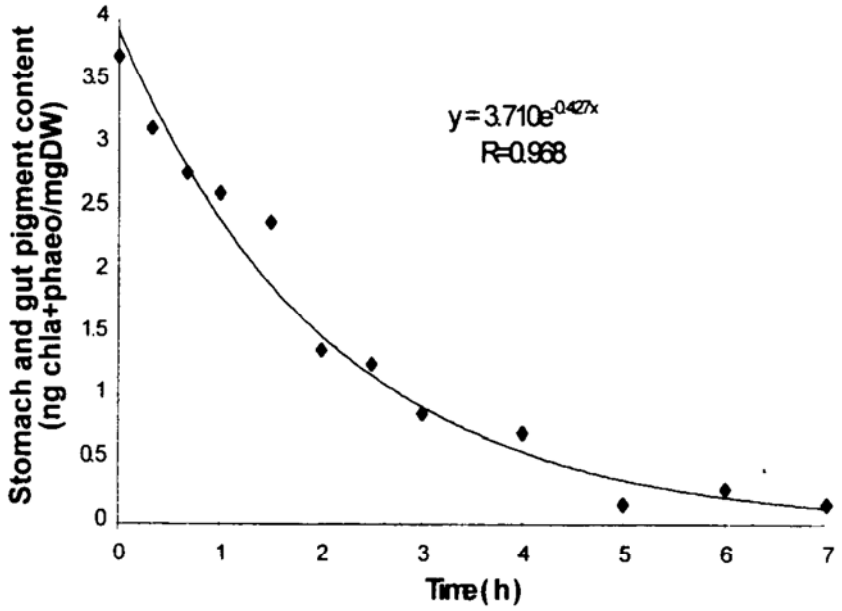

Fig. 3. Exponential decline of pigment level [(ng (chl-a+ phaeo.)/mgDW)] in stomach and gut after transferred to filtered seawater.

ba (cf. Morris, 1984).

Table 4 gives the summary of data on filtration rates and ingestion rates obtained by constant volume experiments from different authors. The differences in results among different authors are obvious, but the causes are unknown. Krill density and duration of incubation may be the main factors which influence the results of experiments. The comparatively smaller filtration rates by Kato *et al.* (1979,1982), Morris (1984) than that of this study may be caused by the comparatively small container (0.5～2 litre) and long time incubation (as long as 24 hours). In this study, the filtration rate and ingestion rate obtained from 5 ind. in 7 liters density mass incubation are distinctly smaller than that obtained from single incubations in 5 litre vessel and 5 ind. in 25 liters density mass incubations. This may be caused by the overcrowding of krill in 5 ind. in 7 liters density mass incubation. Price *et al.* (1988) showed that *E. superba* kept in 50 litre tubs greatly increased their feeding rates relative to animals feeding in much smaller volumes of water. Small incubation volume affects the results of feeding experiments also through the confinement of feeding activity , since it is often observed that the extention of "feeding basket" and swimming motion of the pleopods occur synchronously, although Boyd *et al.* (1984) have mentioned that krill may feed while hovering in the water.

Different kinds of algae used by different authors may be another reason that caused the different results of experiments. For instance, the size of food particle would have a strong influence on the efficiency of filtration . Experimentally measured filtration rate for different particle size range varied considerately (McClatchie and Boyd, 1983; Morris, 1984).

In order to lessen the effect of refiltration on measuring of feeding rate, the flow-through technique which was often used with bivalves has been used by Antezana *et al.*

(1982) and Morris (1984) to measure grazing rate of Antarctic krill. Table 5 shows the results of filtration rate and weight-specific filtration rate given by Morris (1984) and by present authors. The mean filtration rate (290. 2 ml • ind. $^{-1}$ • h^{-1} of krill with 67. 5 mg mean body dry weight) measured from flow-through experiments of this study is greater than that measured from constant volume experiments (189. 8 ml • ind. $^{-1}$ • h^{-1} of krill with 74. 1 mg DW), but smaller than that measured by Morris (1984) (947. 1 ml • ind. $^{-1}$ • h^{-1} for krill with 44. 2 mg DW).

Table 4. Summary of filtration rates and ingestion rates obtained by constant volume experiments of different authors

Source	Incubation density	Incubation time (h)	Initial chl a concentration (μg chl-a • l^{-1})	Body dry weight (mg)	Filtration rate (ml • ind^{-1} • h^{-1})	Ingestion rate (ng chl-a • ind^{-1} • h^{-1})
Kato *et al.* (1979)	0. 5l • ind^{-1}	4～7	40	3. 8	8. 3	
				11. 2	13. 1	
					79. 0	20. 3
					230. 0	135. 0
Kato *et al.* (1982)	2l • ind^{-1}	24	0. 92	125. 8	34. 7	24. 5
				280. 8	78. 5	
	40l • 30ind^{-1}	6～9	0. 92	244. 9	57. 4	
			13. 7	244. 9	164. 0	1468
Meyer and El-Sayed (1983) Morris (1984)	4. 5l • ind^{-1}	2～27	0. 18	25. 1	29. 1	
			1. 03	25. 1	70. 6	
			2. 91	29. 3	43. 9	
			0. 22	35. 9	257. 6	
			0. 30	120. 5	1. 7	
			0. 58	121. 3	33. 5	
Boyd *et al.* (1984)	4. 2l • ind^{-1}	12	10. 5	117. 8	<450	4484
Schnack (1985)	5l • 1—5ind^{-1}	8～9. 5	1. 70			
			4. 39			
Morris (1984)	2l • ind^{-1}	1～2		50	24. 6	
This study	5l • ind^{-1}	6～8		43. 1	243. 4	256. 3
				74. 1	189. 8	216. 5
				253. 8	253. 6	412. 6
	25l • 5ind^{-1}	6～8		249. 5	389. 9	1062. 6
	7l • 5ind^{-1}	6～8		68. 5	84. 0	85. 8

Table 5. Summary of filtration rates and weight-specific filtration rates obtained by flow-through experiments of different authors

Dry weight (mg)	Filtration rate (ml • ind. $^{-1}$ • h^{-1})	Wt. —specific filtration rate (ml • mg DW^{-1} • h^{-1})	Source
3. 5±1. 6	55. 5±20. 0	15. 9	Morris(1984)
44. 2±12. 1	947. 1±524. 9	21. 4	Morris(1984)
67. 5±3. 2	290. 2±22. 2	4. 1±0. 9	This study

In this study, it was assumed that the filtration rate of sub-adult krill would not exceed 800 ml • h^{-1}, so we provided a water flow rate of about 4000 ml • h^{-1} for the flow-through experiments. The rate of water flow used by Morris was 5000 ml • h^{-1} for a single krill which was six times greater than that used in our experiments. If the real filtration rate (actual volume of water filtered by an individual krill per unit time) of sub-adult krill was greater than 800 ml • h^{-1}, refiltration would be inevitable in this experiments. And it might be one of the reasons that we had a smaller filtration rate compared with that of Morris's. In both Morris's and our experiments, C_o, the concentration of algae around krill during incubation, was not measured. Morris used algae concentration of outflow water, C_2, instead of C_o to calculate filtration rate by using the formula of $F=R(C_1-C_2)/C_2$. In this experiments, the average of C_1 and C_2 was used to replace C_o. The filtration rate was calculated from the formula of $F = 2R(C_1-C_2)/(C_1+C_2)$. Although Hildreth and Crisp (1976) have pointed out that if C_0 can not be measured, C_2 is probably a better approximation to it when water flow rate is greater than the filtration rate. In that case the filtration rate would be overestimated. On the other hand, using the average of C_1 and C_2 as C_0 may have an underestimate . This might be another reason that our result is lower than Morris's.

Considerable literatures are devoted to argue the suitability of measuring grazing rate *in vitro*, the direct method, because they think animals under laboratory conditions may be abnormal due to artificial light, sorting, containment, over crowding, stress or damage sustained during capture. Gut fluorescence method is the commonly used method to measure zooplankton grazing rate *in situ*, especially for copepods. Although stomach and gut pigment contents of Antarctic krill have been measured by some authors (Nemoto *et al.*, 1976; Antezana *et al.*, 1982; Morris and Ricketts, 1984), there was no data on grazing rate of Antarctic krill by gut fluorescence method.

In present study evacuation rate of gut contents was obtained from only one experiment. Of course the evacuation rate of Antarctic krill would change when the food concentration, time of day and other environmental conditions changed. In recent years, there has been an increasing number of reports which have suggested that completely conversion of ingested chlorophyll into phaeopigment during passage through gut does not always occur, and a certain amount of ingested chlorophyll may be destroyed or broken down to non-fluorescent products (Wang and Conover, 1986; Conover *et al.*, 1986; Head, 1986,1992). So the hypothesis of that chlorophyll *a* is converted to phaeopigment with 100% molar efficiency in stomach and gut of *E. superba* may cause an underestimation of ingestion rate.

In present study, direct and indirect methods including constant volume single incubation, constant volume mass incubation, flow-through technique and fluorescence method were used to estimate grazing rate of Antarctic krill . As shown in Table 2, filtration rates obtained from flow through experiments are greater than those from constant volume experiments, and those obtained from *in situ* are greater than those from *in vitro*. In spite all of these, there are no statistical significant differences among filtration rates obtained from different methods, except for that between 5 individuals in 7 liters mass incubation and flow-through experiments, which may be due to over-crowding of

krill in the former experiments (Table 3). Talking about the ingestion rate which is calculated from I = FC within the range of food concentrations in my experiments, it has a positive correlation with food concentration. So the influence of experimental methods on ingestion rate can be represented by that on filtration rate. The significant differences of ingestion rate between single and mass constant volume experiments, constant volume and fluorescence method as shown in Table 3 are caused by the difference of food concentrations, because their filtration rates are almost the same. In conclusion, there is no significantly methodological effect on measurement of Antarctic krill if suitable *in vitro* experimental conditions are used with special respect to animal density and vessel volume.

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