

# Spring and autumn reproduction of *Calanus sinicus* in the Yellow Sea

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**ABSTRACT:** The fecundity, hatching success and naupliar survival of *Calanus sinicus* were studied in the Yellow Sea during research cruises in April and October 2006, with emphasis on the regulation of reproduction. During both cruises, the egg production rate (EPR) showed large spatial variations (0 to 25.4 eggs female<sup>-1</sup> d<sup>-1</sup>), generally coinciding with the food availability. In April, the abundant phytoplankton and ciliates in the study area supported active reproduction, which would probably initiate the annual population development. In October, females remained immature in the Yellow Sea Cold Water Mass, likely due to the unfavorable environment (poor food and low temperature). However, reproduction and recruitment were high in the neritic region, which may explain the local population recovery in late autumn. Hatching success varied markedly among stations in April (4 to 85%), whereas it was high overall in the neritic region during October (>90%). Based on the potential recruitment rates, the spring recruitment would be more important for the annual population dynamics. Female gonad maturity, body size and lipid reserve were examined in relation to fecundity. Regression analyses suggested that the reproductive index (defined as the proportion of females with mature gonads) could be an indicator for the EPR of *C. sinicus*. Among the regulating factors, external food (ciliates and phytoplankton) seems essential for reproduction, whereas inner lipid reserve may mainly serve metabolic needs. Moreover, fecundity is positively related to body size but independent of temperature, which might exert indirect influences on reproduction.

**KEY WORDS:** *Calanus sinicus* · Reproduction · Gonad maturity · Food limitation · Yellow Sea

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## INTRODUCTION

The copepod *Calanus sinicus* may account for about 80% of the total zooplankton biomass in the Yellow Sea (Chen 1964), linking primary production to higher trophic levels (Meng 2003). Given its ecological importance, it was set as one of the target species in the China-GLOBEC program (Sun 2005). *C. sinicus* exhibits a unique life cycle associating with the hydrographic feature of the Yellow Sea, which is a marginal sea (average depth: 44 m) of the northwest Pacific that experiences seasonal thermal stratification. The *C. sinicus* population starts to develop from

March to April and reaches maximal abundance by June. Then the Yellow Sea Cold Water Mass (YSCWM) gradually develops in the bottom waters of the continental shelf of the Yellow Sea, characterized by the low temperatures in the bottom waters and very high water temperature above it (Su & Weng 1994). Following the hydrographic change, *C. sinicus* maintains its major population in the YSCWM under diapause over the summer (Pu et al. 2004), with extremely low population abundance in the nearshore region due to the unfavorable high temperature. When the stratification weakens in late autumn to winter, *C. sinicus* emerges from the YSCWM and

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gradually distributes itself across the Yellow Sea, thus completing the annual cycle (Sun 2005).

Several studies have reported the reproductive characteristics of *Calanus sinicus* in the Yellow Sea. Spring and autumn are 2 critical periods for the recruitment of *C. sinicus* (Zhang 2003, Sun 2005). While fecundity is low in March but high in May and June (Zhang et al. 2005), there are few data showing whether active reproduction begins in April when the spring phytoplankton bloom usually occurs and *C. sinicus* population starts rising in the coastal waters. It is necessary to know the connections among the food availability, reproduction and population dynamics of *C. sinicus* in early spring. On the other hand, Zhang et al. (2007) reported that reproduction of *C. sinicus* nearly ceases in the YSCWM during summer, but it remains unclear whether this continues to late autumn when the stratification weakens and *C. sinicus* is thought to terminate dormancy (Su & Weng 1994, Sun 2005). In the meantime, the summer-declined population would gradually recover in nearshore areas (Wang & Zuo 2004, Sun 2005). We are not sure whether this process is mainly driven by the local recruitment or advection from the central region where the population is larger.

Many factors can influence the *in situ* egg production of marine copepods (Bunker & Hirst 2004). (1) The energy required by reproduction may come from 2 nutritional sources; the external food supply usually has primary influence on fecundity (e.g. Uye & Murase 1997, Niehoff 2000, Runge et al. 2006), whereas the inner lipid reserve may have varying roles during reproduction of different species (Lee et al. 2006, Niehoff 2007, Jónasdóttir et al. 2008). In the Yellow Sea, *Calanus sinicus* can accumulate lipid in the oil sac; however, previous studies have focused on the effect of food (Uye & Murase 1997, Zhang et al. 2005, Huo et al. 2008), leaving the role of lipid reserve during reproduction unknown. (2) Other factors such as temperature, body size, age and feeding history can also influence reproduction either directly or indirectly (Hirche et al. 1997, Rey et al. 1999, Campbell & Head 2000, Rey-Rassat et al. 2002, Bunker & Hirst 2004, Castellani & Altunbaş 2006). In the field, the effects of these factors can be complicated due to the interactions among them, which may be clarified by multivariate statistical analysis (Gislason 2005, Castellani & Altunbaş 2006). To our knowledge, the influences of these factors on reproduction of *C. sinicus* have only been studied sporadically. (3) Knowledge on gonad development, which can give us a mechanistic understanding of copepod reproduction (Niehoff 2007), is still lacking in *C. sinicus*. To sum up, a comprehensive understanding of the regulation on reproduction of *C. sinicus* is required.

In this study, we conducted 2 surveys during April and October to November, measuring the fecundity, hatch-

ing success and naupliar survival of *Calanus sinicus* to reveal the recruitment in relation to population dynamics. We focused especially on the gonad maturity of *C. sinicus*, trying to elucidate how its reproduction is regulated by a set of environmental and intrinsic factors.

## MATERIALS AND METHODS

We conducted 2 cruises in the southern Yellow Sea during 10 to 30 April and 18 October to 4 November 2006. At each station, hydrography, chlorophyll *a* (chl *a*) concentration, ciliate abundance, as well as population abundance and structure were examined. Measurements of the reproductive rates and female body features were made at some selected stations (Fig. 1).

**Physical and food environment.** Temperature and salinity profiles were acquired by a CTD (Seabird Electronics, SBE 25). For chl *a* measurements, 500 ml of seawater was collected at a series of depths (0, 5, 10, 20, 30, 50, and 70 m, depending on the water depth) and GF/F filtered. Samples were then stored at  $-20^{\circ}\text{C}$  in the dark for 2 d before pigment extraction in 90% acetone for 24 h. The chl *a* concentration was measured with a Turner Designs fluorometer. The mean chl *a* concentration was calculated by dividing the integrated values throughout the whole water column at stations in April and the neritic stations in October. At stations inside the YSCWM in October, given that *Calanus sinicus* inhabits waters beneath the thermocline to avoid high temperature (Wang & Zuo 2004), we calculated the mean chl *a* of the respective layers rather than the whole water column.

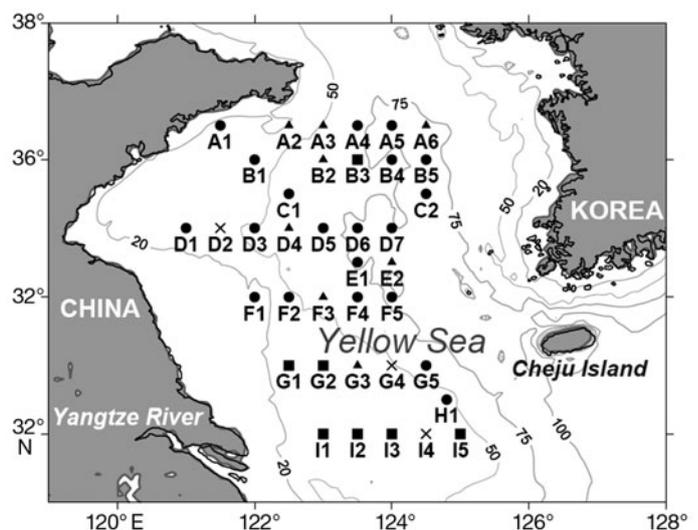


Fig. 1. Study area. Stations with egg production measurements in both cruises (●), only in April (■), only in October (▲) and stations without egg production measurement (×) are shown. Contour lines indicate isobaths (m)

At the same layers of the chl *a* samples, 1 l ciliate samples were collected and preserved with 1% acid Lugol's iodine solution in plastic bottles. The total abundance of ciliates was examined under an Olympus CKX41 inverted microscope at 100× magnification after pre-concentration by serial settling methods. We calculated the mean ciliate abundance following the same method for chl *a*.

**Sampling and egg production experiment.** To determine the population abundance and structure, copepods were collected by a 500 µm mesh zooplankton net (mouth opening 0.5 m<sup>2</sup>) and preserved in 5% formalin seawater solution. All tows were carried out vertically from 4 m above the bottom to the sea surface. *Calanus sinicus* was enumerated by developmental stages back in the laboratory. Our plankton net was too coarse for quantitatively collecting C1 to C3 (body width: <~480 µm; without the appendages), so we only present the abundance of the later stages (C4 to C6: body width >~600 µm).

For egg production rate (EPR) measurements, additional vertical hauls were taken to catch live females. After capture, samples were immediately diluted into a 20 l bucket filled with *in situ* seawater. Then 25 to 50 healthy females of *Calanus sinicus* were sorted with a wide-mouthed pipette into either plastic cylinders (volume: 350 ml; with false bottom of 330 µm mesh size to prevent cannibalism; 5 females per cylinder) or 6 well tissue culture plates (12 ml each well; 1 female per well). We added 70 µm filtered *in situ* seawater as the culture water. All cultures were placed under ambient temperature. For the bottle culture, eggs were collected and counted after 24 h, whereas for the multi-well incubation, to minimize the influence of cannibalism, eggs were checked every 4 to 6 h and removed after counting during the 24 h incubation period. We found no significant difference of the EPR values between the 2 incubation methods in 20 parallel experiments (Wilcoxon signed ranks test,  $p > 0.1$ ), similar to Niehoff et al. (1999). We found that cannibalism was negligible in both methods.

For hatching success (HS) measurements, at each station more than 80 eggs (originated from at least 12 females) were gently transferred by a pipette into petri dishes filled with 0.45 µm filtered seawater under ambient temperature. Hatched nauplii were counted every 12 h until hatching was complete. In October, we also measured naupliar survival (NS) by incubating the nauplii until Stage N3. HS was calculated as the proportion of nauplii to the total eggs at the start of the incubation, whereas NS was calculated as the proportion of the final N3s to the initial eggs.

**Female body features.** At each station with egg production experiments, prosome length (PL), gonad development and oil sac volume of more than 30 (usu-

ally >40) females were examined under a dissecting microscope using formalin-preserved samples. Gonad development stages were determined following the procedures of Niehoff & Runge (2003). Four stages were classified: GS1, GS2 and GS3 were defined as immature females with increasing maturity, while GS4 represented mature females ready to spawn. The reproductive index (RI) was defined as the proportion of GS4 females. Measured laterally, oil sac volume (OSV) was calculated by the following equation:  $OSV = (1/6) \pi L W^2$  (Svetlichny et al. 2006), where L and W are the length and maximal width of oil sac, respectively.

**Statistical analysis.** All statistical analyses were performed using SPSS software (v.13.0). Mann-Whitney *U*-tests were used to compare the regional and seasonal differences. Normality and homogeneity were examined by applying Kolmogorov-Smirnov and Bartlett tests, respectively. Before regression analyses, the EPR data was  $\log(EPR + 1)$  transformed for normalization. Given the vertical mixing of the water column in April and the near-bottom distribution pattern of *Calanus sinicus* in October (Wang & Zuo 2004), bottom temperature was adopted to represent the habitat temperature. To examine the relationships between fecundity and either external factors (temperature, chl *a* concentration and ciliate abundance) or internal factors (PL, RI and OSV), we performed both univariate regressions (simple linear regressions and non-linear regressions when necessary) and multiple stepwise linear regressions.

## RESULTS

### Physical and food environment

In April, both bottom and surface temperature gradually increased southward, ranging from 4.9°C to 13.4°C (Fig. 2A,B). The water column was well mixed over the study area (Fig. 2C). The mean chl *a* concentration and ciliate abundance were generally higher in the north of transact E than in the south (Mann-Whitney *U*-tests,  $p < 0.01$  in both parameters). In the northeast, a diatom bloom (mostly *Thalassiosira pacifica*) had developed, with surface chl *a* reaching to a maximum of 15 mg m<sup>-3</sup> at Stn B5. In most parts of the southern region, chl *a* was relatively low (mostly <0.8 mg m<sup>-3</sup>), particularly in the southeast (Fig. 2D). The ciliate abundance was also lowest in the southeast but peaked in the northwest (Fig. 2E).

In October, the YSCWM dominated the central part of the survey region (Fig. 3). According to Weng & Wang (1982), we adopted the 10°C bottom isotherm as the boundary of the YSCWM, which was characterized by

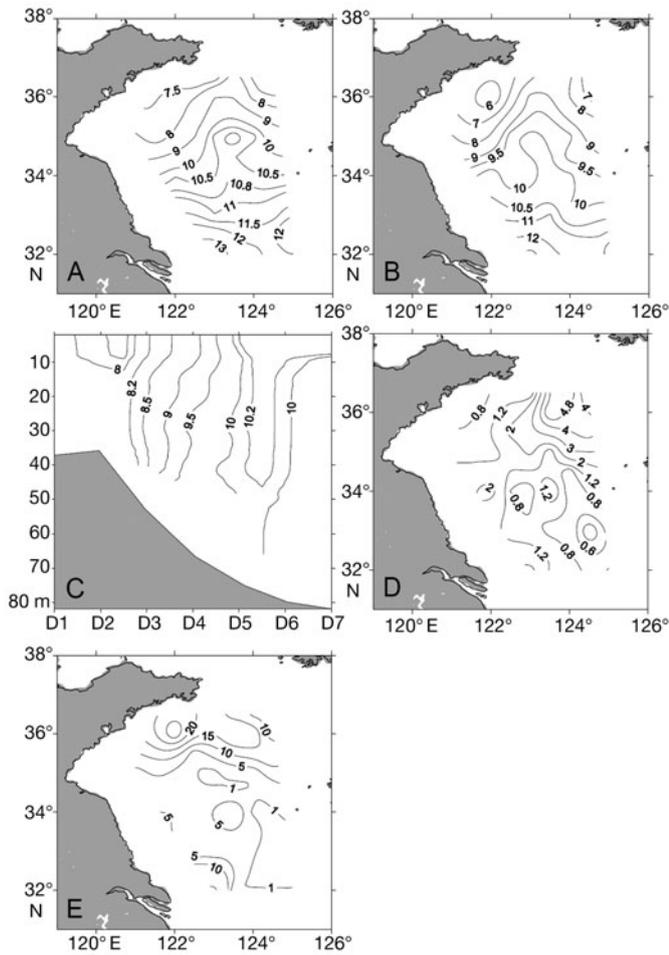


Fig. 2. Distribution of (A) surface temperature ( $^{\circ}\text{C}$ ), (B) bottom temperature ( $^{\circ}\text{C}$ ), (D) mean chl *a* concentration ( $\text{mg m}^{-3}$ ) and (E) mean ciliate abundance ( $10^2 \text{ ind. l}^{-1}$ ) in April 2006. The vertical temperature profile along Transect D ( $35^{\circ}\text{N}$ ; see Fig. 1) is shown in (C), with the x-axis indicating stations and the y-axis representing water depths (m)

thermal stratification (Fig. 3C), poor food conditions (chl *a*  $< 0.3 \text{ mg m}^{-3}$ , Fig. 3D; ciliate abundance  $< 500 \text{ ind. l}^{-1}$ , Fig. 3E), and relatively high salinity ( $> 33$ ; data not shown). By contrast, waters outside the YSCWM exhibited mixing or less stratified feature, together with lower salinity and better food supply (Fig. 3D,E).

#### Population abundance and structure

The abundance and structure of the later stage *Calanus sinicus* are shown in Fig. 4. In April, the population had a relatively homogeneous distribution over the survey region, peaking at neritic Stn D2, reaching  $6.6 \times 10^4 \text{ ind. m}^{-2}$  (Fig. 4A). In October, except at Stn A1, the population abundance was rather low in

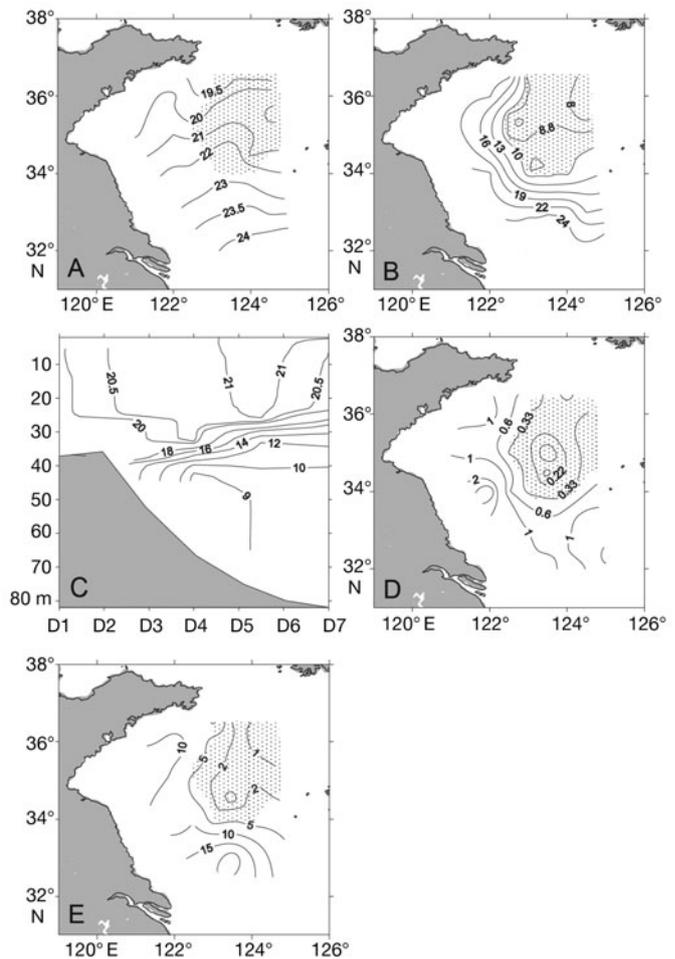


Fig. 3. Distribution of (A) surface temperature ( $^{\circ}\text{C}$ ), (B) bottom temperature ( $^{\circ}\text{C}$ ), (D) mean chl *a* concentration ( $\text{mg m}^{-3}$ ) and (E) mean ciliate abundance ( $10^2 \text{ ind. l}^{-1}$ ) in October 2006. The vertical temperature profile along Transect D ( $35^{\circ}\text{N}$ ; see Fig. 1) is shown in (C), with the x-axis indicating stations and the y-axis representing water depths (m). The shaded areas in (A), (B), (D) and (E) indicate the territory of the Yellow Sea Cold Water Mass

the neritic region (mean  $569 \text{ ind. m}^{-2}$ ), compared with that in the domain of the YSCWM (mean  $7671 \text{ ind. m}^{-2}$ ; Fig. 4B). The copepodite Stage V (C5) dominated the population inside the YSCWM (mostly  $\sim 50$  to  $80\%$ ), whereas the neritic population mainly consisted of females (usually  $> 50\%$ ).

#### Female body features

In April, female body size (PL) generally decreased southward, with a wide range (2.30 to 2.72 mm; Table 1). In October, females developed a significantly smaller body with relatively narrow variation (2.08 to 2.27 mm; Table 1) and there was no significant difference of PL between females inside and outside the

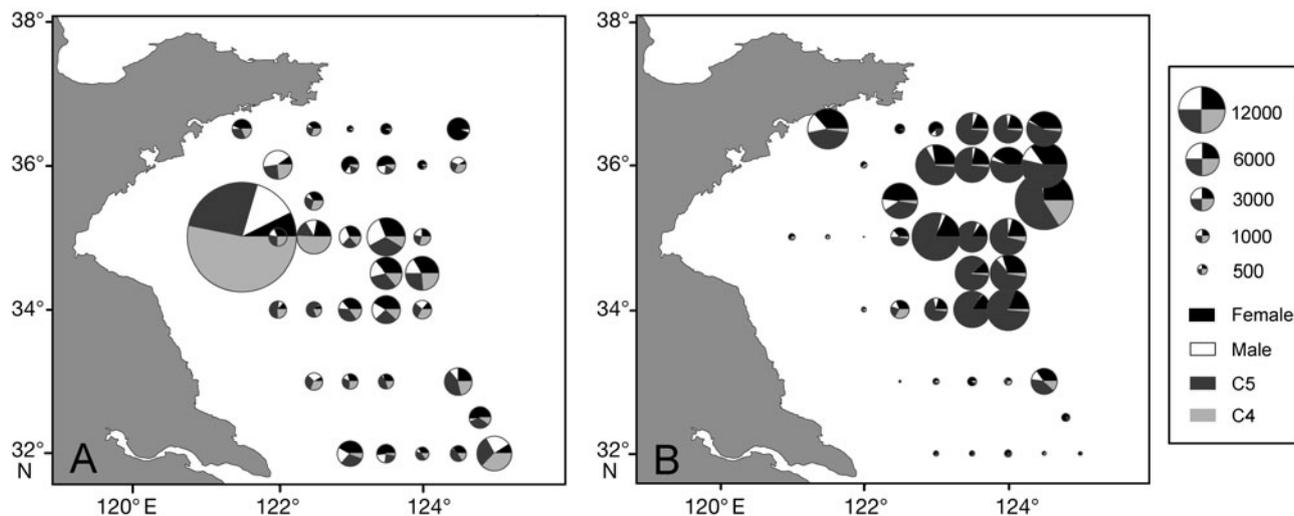


Fig. 4. *Calanus sinicus*. Population abundance (ind.  $m^{-2}$ ) and structure in (A) April and (B) October 2006. Shading in pies indicates different development stages and sex, and proportions are shown as the percentage of the pie area. Pie size: abundance (ind.  $m^{-2}$ ); population abundance increases linearly with the pie area at each station

YSCWM (Mann-Whitney  $U$ -test,  $p > 0.1$ ). A negative relationship between PL and temperature was found (simple regression,  $r^2 = 0.17$ ,  $p < 0.05$ ), in agreement with Uye (1988).

In April, most females from north of Stns E1 and E2 were mature (RI  $> 80\%$ ), whereas less than 50% females were mature in most areas south of Stns E1 and E2 (Table 1). In October, females remained immature inside the YSCWM except at Stn B5 where the RI was only 5%. In contrast, except for Stn A1, mature females could be found at all stations outside the YSCWM, although the RI varied greatly (9 to 90%; Table 1). Combining the data from the 2 cruises, there were significant relationships between the RI and food availabilities (chl  $a$ : logarithmic regression,  $r^2 = 0.54$ ,  $p < 0.001$ ; ciliates: linear regression,  $r^2 = 0.16$ ,  $p < 0.05$ ).

In April, lipid storage volume (represented by OSV) was higher north of Stns E1 and E2 than in the south (Mann-Whitney  $U$ -test,  $p < 0.01$ ; Table 1), probably a result of the body size difference. In October, there was no significant difference of OSV between females inside and outside the YSCWM (Mann-Whitney  $U$ -test,  $p > 0.1$ ; Table 1). Females collected in spring generally accumulated greater amount of lipids than the ones collected in autumn (Mann-Whitney  $U$ -test,  $p < 0.001$ ).

### Egg production

During both cruises, the EPR displayed large spatial variations (April: 0 to 25.4 eggs female $^{-1}$  d $^{-1}$ ; October: 0 to 21.5 eggs female $^{-1}$  d $^{-1}$ ). In April, the EPR was gener-

ally higher north of Stns E1 and E2 than in the south (Mann-Whitney  $U$ -test,  $p < 0.01$ ) and they were extremely low ( $< 1$  eggs female $^{-1}$  d $^{-1}$ ; Fig. 5A) in the southeast. This pattern was generally coincident with the food availability, with the exception of the bloom area, where fecundities were only moderate (2.7 to 13.8 eggs female $^{-1}$  d $^{-1}$ ). In October, females spawned actively in the neritic region with an average EPR of 9.8 eggs female $^{-1}$  d $^{-1}$ , whereas there was no egg production inside the YSCWM (Fig. 5B). Over the survey region, fecundities were generally higher in April than in October (Mann-Whitney  $U$ -test,  $p < 0.05$ ).

Combining the data from both cruises, we performed the simple regression analyses (Table 2). The relationship between EPR and RI was very close, and it remained true when examined separately in each cruise, particularly in October (simple regression,  $r^2 = 0.70$ ,  $p < 0.001$ ). Following RI, the food items (ciliate abundance and chl  $a$  concentration) showed significant and positive effects on fecundity. EPR was positively related to PL, whereas it was independent of either temperature or OSV (Table 2). To remove the body-size effect, we calculated the relative OSV by dividing OSV by PL $^3$ . The relationship between the EPR and the relative OSV remained insignificant (simple regression,  $r^2 = 0.10$ ,  $p > 0.05$ ).

To clarify the effects of the environmental and intrinsic variables, we performed stepwise multiple linear regressions, without incorporating the parameter 'RI'. Two models were established (Table 3). Food items showed primary importance in reproduction. In Model 1, only ciliate abundance was included, explaining 37% of the variance of log (EPR + 1). Chl  $a$

Table 1. *Calanus sinicus*. Female body features: prosome length (PL; mm, mean  $\pm$  SD), reproductive index (RI, proportion of females with mature gonad) and oil sac volume (OSV;  $10^{-2}$  mm<sup>3</sup>, mean  $\pm$  SE). ‘-’ indicates no data is available. Data from stations in the YSCWM in October are in bold

Stn	PL (mm)		RI (%)		OSV ( $10^{-2}$ mm <sup>3</sup> )	
	Apr	Oct	Apr	Oct	Apr	Oct
A1	2.72 $\pm$ 0.12	2.09 $\pm$ 0.07	86	0	2.41 $\pm$ 0.33	0.00
A2	-	2.10 $\pm$ 0.09	-	10	-	0.03 $\pm$ 0.02
A3	-	2.24 $\pm$ 0.09	-	76	-	0.00
A4	2.33 $\pm$ 0.16	<b>2.16 <math>\pm</math> 0.09</b>	97	<b>0</b>	0.00	<b>1.53 <math>\pm</math> 0.25</b>
A5	-	<b>2.20 <math>\pm</math> 0.17</b>	-	<b>0</b>	-	<b>0.68 <math>\pm</math> 0.18</b>
A6	-	<b>2.25 <math>\pm</math> 0.11</b>	-	<b>0</b>	-	<b>1.07 <math>\pm</math> 0.22</b>
B1	2.59 $\pm$ 0.15	2.10 $\pm$ 0.08	97	34	0.76 $\pm$ 0.25	0.01 $\pm$ 0.01
B2	-	<b>2.22 <math>\pm</math> 0.14</b>	-	<b>0</b>	-	<b>0.92 <math>\pm</math> 0.21</b>
B3	2.43 $\pm$ 0.23	-	88	-	1.04 $\pm$ 0.53	-
B4	2.32 $\pm$ 0.11	<b>2.20 <math>\pm</math> 0.14</b>	91	<b>0</b>	0.60 $\pm$ 0.46	<b>0.32 <math>\pm</math> 0.13</b>
B5	2.37 $\pm$ 0.17	<b>2.23 <math>\pm</math> 0.09</b>	97	<b>5</b>	0.45 $\pm$ 0.33	<b>0.55 <math>\pm</math> 0.13</b>
C1	2.58 $\pm$ 0.18	<b>2.22 <math>\pm</math> 0.09</b>	91	<b>0</b>	1.28 $\pm$ 0.24	<b>0.60 <math>\pm</math> 0.16</b>
C2	2.52 $\pm$ 0.24	-	75	-	0.46 $\pm$ 0.16	0.00
D1	2.52 $\pm$ 0.16	2.08 $\pm$ 0.06	50	90	0.90 $\pm$ 0.21	0.06 $\pm$ 0.05
D3	2.56 $\pm$ 0.23	-	91	-	2.15 $\pm$ 0.59	0.00
D4	-	2.22 $\pm$ 0.16	-	28	-	0.35 $\pm$ 0.09
D5	2.52 $\pm$ 0.11	<b>2.15 <math>\pm</math> 0.13</b>	86	<b>0</b>	0.47 $\pm$ 0.18	<b>0.29 <math>\pm</math> 0.11</b>
D6	2.56 $\pm$ 0.11	<b>2.21 <math>\pm</math> 0.15</b>	79	<b>0</b>	0.81 $\pm$ 0.30	<b>0.16 <math>\pm</math> 0.07</b>
D7	2.52 $\pm$ 0.2	<b>2.23 <math>\pm</math> 0.12</b>	56	<b>0</b>	1.39 $\pm$ 0.35	<b>0.17 <math>\pm</math> 0.05</b>
E1	2.57 $\pm$ 0.13	<b>2.19 <math>\pm</math> 0.12</b>	67	<b>0</b>	0.46 $\pm$ 0.14	<b>0.15 <math>\pm</math> 0.12</b>
E2	-	<b>2.14 <math>\pm</math> 0.12</b>	-	<b>0</b>	-	<b>0.11 <math>\pm</math> 0.11</b>
F1	2.62 $\pm$ 0.15	2.19 $\pm$ 0.10	66	67	1.39 $\pm$ 0.34	0.79 $\pm$ 0.39
F2	2.59 $\pm$ 0.17	2.27 $\pm$ 0.07	19	82	3.33 $\pm$ 0.61	1.46 $\pm$ 0.25
F3	-	<b>2.18 <math>\pm</math> 0.13</b>	-	<b>0</b>	-	<b>0.40 <math>\pm</math> 0.14</b>
F4	2.41 $\pm$ 0.11	<b>2.20 <math>\pm</math> 0.11</b>	94	<b>0</b>	0.14 $\pm$ 0.12	<b>0.29 <math>\pm</math> 0.15</b>
F5	2.33 $\pm$ 0.08	<b>2.21 <math>\pm</math> 0.11</b>	81	<b>0</b>	0.03 $\pm$ 0.03	<b>0.06 <math>\pm</math> 0.04</b>
G1	2.45 $\pm$ 0.22	-	43	-	0.22 $\pm$ 0.22	-
G2	2.32 $\pm$ 0.16	-	46	-	0.00	-
G3	-	2.26 $\pm$ 0.13	-	10	-	0.89 $\pm$ 0.27
G5	2.30 $\pm$ 0.14	2.26 $\pm$ 0.08	22	36	0.00	2.18 $\pm$ 0.29
H1	2.35 $\pm$ 0.09	2.21 $\pm$ 0.08	26	9	0.00	1.01 $\pm$ 0.29
I1	2.37 $\pm$ 0.13	-	59	-	0.45 $\pm$ 0.18	-
I2	2.32 $\pm$ 0.09	-	27	-	0.03 $\pm$ 0.01	-
I3	2.32 $\pm$ 0.10	-	39	-	0.00	-
I5	2.36 $\pm$ 0.12	-	31	-	0.19 $\pm$ 0.13	-
Average	2.46	2.19	65	17	0.73	0.50
Range	2.30–2.72	2.08–2.27	19–97	0–90	0–3.33	0–2.18

concentration was added into Model 2, which explained 45% of the variability.

#### Hatching success and naupliar survival

The HS varied largely over the survey region in April (4 to 85%; Fig. 5C). In October, however, most eggs hatched successfully (90 to 100%; Fig. 5D) and survived to N3 (NS > 85%; data not shown) in the neritic region, where reproduction was active. There was no significant relationship between HS and any of the environmental or internal factors measured in the present study.

## DISCUSSION

### Reproduction and recruitment

The magnitudes of egg production in April were comparable to the high fecundities in late spring (Zhang 2003), indicating active reproduction began then, which agrees with Chen (1964), who inferred the same result from population data. The food supply in the northern part of the study area initiated the active reproduction, which may further explain the population increase in early spring observed by Sun (2005). Although fecundity generally coincided with food conditions, the EPR was only moderate in the diatom bloom area, where ciliate was also relatively abundant (Fig. 5A). The population there seemed to be entering the active reproducing phase, judging by the high proportion of mature females (Table 1). In this case, it is likely that fecundity was limited by nutritional deficiency due to the dominance of mono diatom species (Pond et al. 1996, Jónasdóttir et al. 2002), as laboratory experiments have demonstrated that mixed diets could sustain higher EPR than some single diatom diets in *Calanus sinicus* (Li et al. 2006).

During the autumn cruise, the population features (population aggregation and C5 dominance) and the inactive reproduction of *Calanus sinicus* in the YSCWM were similar to conditions in summer (Wang et al. 2003, Li et al. 2004, Zhang et al. 2007), suggesting the impact of the thermal stratification would at least continue to early November.

This confirms that the YSCWM provides a shelter for *C. sinicus* to survive rather than stimulating reproduction (Wang & Zuo 2004, Zhang et al. 2007). On the other hand, fecundities outside the YSCWM were higher than those in summer (Zhang 2003), probably a response to the more favorable food and temperature conditions (Fig. 3). Compared with the unsuccessful summer recruitment (Zhang et al. 2007), the relatively high EPR, HS and NS in the nearshore area implied the high potential of population increase, which could also be confirmed by the high proportion of N1 to C3 (>~80% of total abundance) collected by a finer zooplankton net (Huo et al. 2008). We infer that the recovery of the nearshore population in late autumn (Wang & Zuo 2004, Sun 2005)

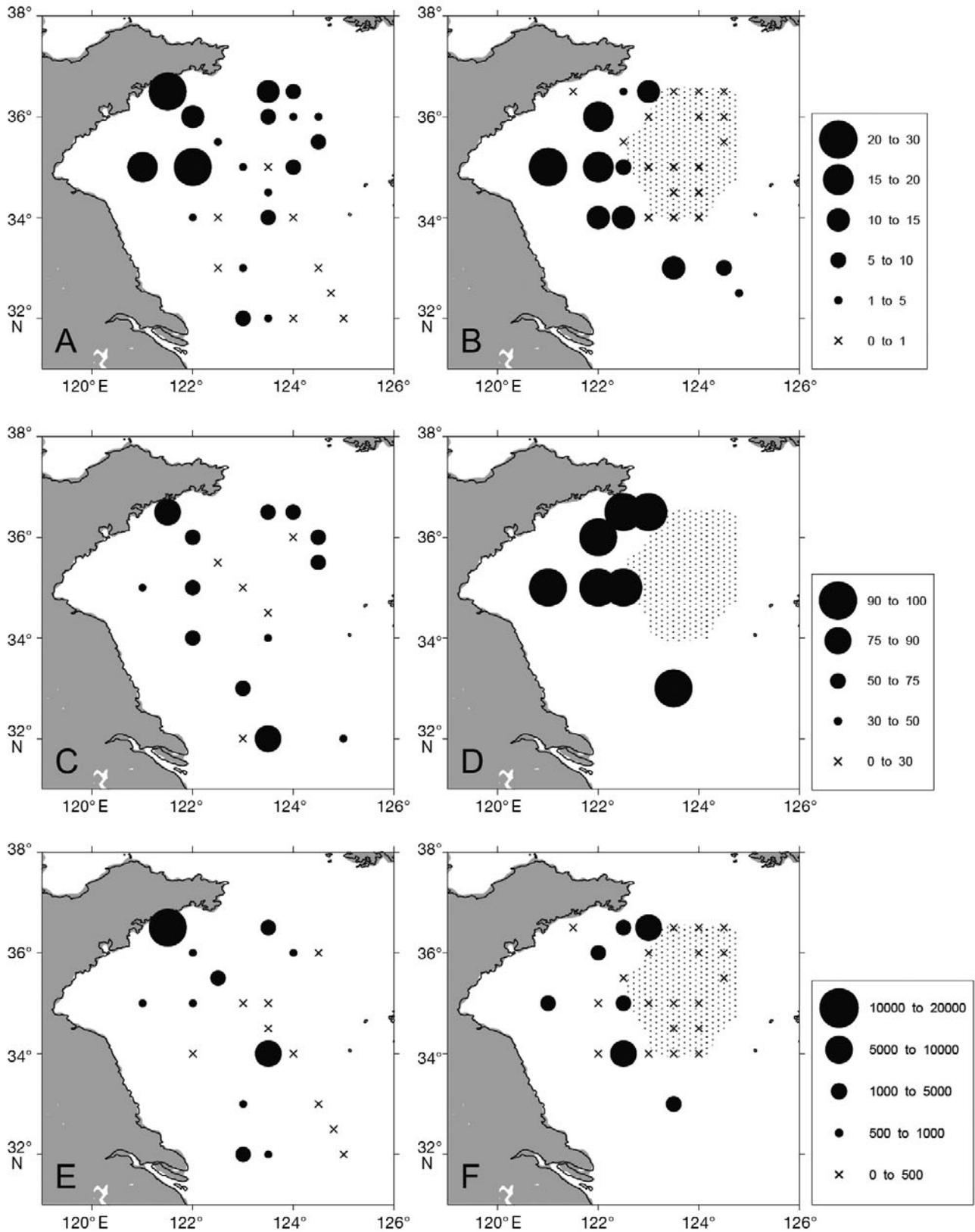


Fig. 5. *Calanus sinicus*. (A,B) Egg production rate (eggs female<sup>-1</sup> d<sup>-1</sup>), (C,D) egg hatching success (%) and (E,F) potential recruitment rate (PRR, nauplii m<sup>-2</sup> d<sup>-1</sup>) during April and October 2006, respectively. Shaded areas in B, D and F indicate Yellow Sea Cold Water Mass

Table 2. *Calanus sinicus*. Regressions of log-transformed egg production rate (EPR + 1) versus RI (reproductive index, proportion of females with mature gonad), mean ciliate abundance (ind. l<sup>-1</sup>), chl *a* concentration (mg m<sup>-3</sup>), body size (prosome length [PL], mm), temperature (°C) and oil sac volume (10<sup>-2</sup> mm<sup>3</sup>). See 'Materials and methods' for more details. Sample sizes (n), adjusted R<sup>2</sup>, and significance levels (p) are given. Regressions with p < 0.05 are marked by \*

Variable	Regression	n	Adjusted R <sup>2</sup>	p
RI (%)	log (EPR + 1) = 0.009 RI + 0.098	51	0.50	<0.001*
Ciliates	log (EPR + 1) = 0.266 ln (ciliates) - 1.038	48	0.40	<0.001*
Chl <i>a</i>	log (EPR + 1) = 0.233 ln (chl <i>a</i> ) + 0.541	50	0.25	<0.001*
Body size	log (EPR + 1) = 0.988 PL - 1.808	51	0.11	0.019*
Temperature	log (EPR + 1) = 0.028 T + 0.188	53	0.04	0.071
Oil sac volume	log (EPR + 1) = 16.817 OSV + 0.382	51	0.04	0.083

may be driven by local recruitment rather than advection from the population abundant area (the YSCWM), where individuals under Stage C4 were rare.

In contrast to Zhang et al. (2005), who recorded high HS in *Calanus sinicus*, we found that HS in April varied largely (4 to 85%; Fig. 5C), but it was not related to any of the environmental or internal parameters measured in the present study. At first, we found that most non-hatched eggs had undergone cleavages of different levels and some had even developed embryonic forms in the membranes. These findings, together with the relatively high proportions of males, indicate that the hatching failures were not due to the eggs' being unfertilized. Furthermore, we barely observed female senescence, which may also lower the egg viability (Pond et al. 1996, Jónasdóttir et al. 2002 and references therein), suggesting senescence can not explain the low HS either. Moreover, many authors have reported the inhibition of some diatom species on copepod egg viability (Poulet et al. 1994, Miralto et al. 1999, Jónasdóttir et al. 2002, Halsband-Lenk et al. 2005). However, HS does not seem to be related to the diatom concentration; it was generally moderate (50 to 75%; Fig. 5C) in the diatom bloom area, in contrast to the central region, where diatom concentration was much lower and HS was lowest (Figs. 3D & 5C), increasing the complication of this issue.

Table 3. *Calanus sinicus*. Results of stepwise multiple linear regressions with log-transformed egg production rate (EPR + 1) as the dependent variable, and ln-transformed ciliate abundance (ind. l<sup>-1</sup>) (ciliates), ln chl *a* concentration (mg m<sup>-3</sup>), prosome length (PL, mm), temperature (°C) and oil sac volume (10<sup>-2</sup> mm<sup>3</sup>) as the independent variables. Two models are established: (1) Only ciliate is included, and (2) ciliate and chl *a* are included. Sample size (n), standardized partial coefficient (Beta), significance level (p) as well as the adjusted R<sup>2</sup> are given

Model	n	Beta (p)		Adjusted R <sup>2</sup>
		Ciliates	Chl <i>a</i>	
(1) log (EPR + 1) = -0.995 + 0.256 ln (ciliates)	46	0.621 (<0.001)		0.37
(2) log (EPR + 1) = -0.734 + 0.217 ln (ciliates) + 0.143 ln (chl <i>a</i> )	46	0.525 (<0.001)	0.316 (0.008)	0.45

We estimated the potential recruitment rate (PRR) of the *Calanus sinicus* population at each station by the following equation: PRR (nauplii m<sup>-2</sup> d<sup>-1</sup>) = female abundance (ind. m<sup>-2</sup>) × EPR (eggs female<sup>-1</sup> d<sup>-1</sup>) × HS (%). In April, the recruitment could occur across most of the study area with varying rates, whereas it was restricted to the near-shore region in October (Fig. 5E,F). Over the whole survey region, the PRR in April was higher than that in October (Mann-Whitney *U*-test, p < 0.05; with average values of 1848 and 1118 nauplii m<sup>-2</sup> d<sup>-1</sup>, respectively), suggesting the spring recruitment might be more important for the annual population

dynamics. This assertion is further supported when we take into account the fact that reproduction of *C. sinicus* is longer and of greater magnitude in spring than in autumn (Zhang 2003, this study).

### Gonad maturity

Among all the examined parameters, RI was most closely related to the fecundity of *Calanus sinicus* (Table 2), agreeing with previous findings in other copepod species (e.g. Campbell & Head 2000, Ceballos et al. 2004, Ceballos & Álvarez-Marqués 2006). This indicates that the reproductive intensity of *C. sinicus* is generally dependent on the population maturity over temporal and spatial ranges in the Yellow Sea. This relationship also enables us to estimate *in situ* EPR by formalin-preserved samples in the future (Niehoff & Runge 2003). In addition, the close relationships between RI and food availability suggest external food may be essential for gonad maturation.

Food limitation may be one possible explanation for female immaturity: most females remained at Stage GS1 or GS2 inside the YSCWM (Table 1). In *Calanus* species, the development of immature stages (GS1 to GS3) can be fueled by body reserves (Hirche 1996, Niehoff & Hirche 1996), whereas the final matu-

ration to GS4 usually depends on ingested food (Plourde & Runge 1993, Hirche 1996, Niehoff 2000, Niehoff 2007). Poor food conditions are common in the YSCWM (Fig. 3D,E; Li et al. 2004, Pu et al. 2004, Zhang et al. 2007). In response, female feeding rates were extremely low there ( $<1\%$  body carbon  $d^{-1}$ ; Huo et al. 2008), which could barely meet the metabolic requirements of *C. sinicus* females (Li et al. 2004), thus there would not be sufficient external nutrition to initiate final gonad maturation. Similarly, Zhang et al. (2007) ascribed null egg production in the YSCWM during summer to food limitation, but unfortunately that study did not examine gonad development. On the other hand, we cannot exclude the possibility that immature females were experiencing dormancy, as did the immature females of *Calanus glacialis* in the Lurefjord (Niehoff & Hirche 2005), though further measurements are still required to clarify this point.

It may be economical for females in the YSCWM to remain immature instead of wasting energy on offspring who will probably risk high mortality faced with the low temperature and poor food environment (Pu et al. 2004, Wang & Zuo 2004, Zhang et al. 2007). It is probable that some of these females will survive and start maturation when the food conditions improve in early winter (Zhang 2003, S. Wang unpubl. data). Besides female immaturity, the main component of the population (C5s) remained dormant inside the YSCWM (Pu et al. 2004). These life history strategies may help *Calanus sinicus* reduce energy expenditures to maintain the population in the extreme environment during summer and autumn, waiting for improved conditions to recruit.

### Factors affecting reproduction

The regression analyses imply that microzooplankton probably serves as an important nutritional source for the reproduction of *Calanus sinicus* (Tables 2 & 3). Although *C. sinicus* mainly feeds on phytoplankton, field studies suggest it may conduct omnivorous feeding, particularly when phytoplankton food is unfavorable (Zhang et al. 2006, Sun et al. 2007). During the 2 cruises, ciliate food accounted for up to 30% of the carbon intake in females, which tended to select ciliates rather than the more abundant phytoplankton taxa (Huo et al. 2008). Compared with other food items, ciliates may have higher nutritional value and therefore enhance reproduction (Kleppel et al. 1991, Kleppel 1993, Huo et al. 2008). To fully understand the roles of microzooplankton, other components, such as heterotrophic flagellates, should be incorporated into further studies, despite their far smaller contribution to the diet of *C. sinicus* than ciliates (Huo et al. 2008).

While several studies have reported the significance of phytoplankton in reproduction of *Calanus sinicus* (Uye & Murase 1997, Zhang et al. 2005, Zhang et al. 2006), the relationship between EPR and chl *a* was not as strong as expected during this study (Table 2). Judging by particle size, the dominant phytoplankton species fell into the favorable food range for *C. sinicus* (Li et al. 2007, Huo et al. 2008), so chl *a* concentration could be considered as a proxy for potential phytoplankton food. However, the fit between fecundity and the current food supply might be affected by past feeding history and food quality (Jónasdóttir et al. 2002, Rey-Rassat et al. 2002), which, unfortunately, were not examined. Nevertheless, food items were the only factors included in the multiple regression models (Table 3), indicating their primary significance during reproduction.

The EPR of *Calanus sinicus* was independent of either absolute or relative oil storage amount (see 'Results'; Table 2). Besides, the lipid reserve generally could not initiate final gonad maturation and hence egg production of *C. sinicus* under limited food conditions; this agrees with the observations of Plourde & Runge (1993) for the spring population of *C. finmarchicus*. These findings may indicate that reproduction of *C. sinicus* is dependent on external food rather than the lipid reserve, which likely serves as a nutrient buffer for metabolic needs. According to Lee et al. (2006), *C. sinicus* seems to belong to a zooplankton group requiring sufficient ingested food for successful reproduction. However, we observed a small proportion of mature females (5%) at 1 station in the YSCWM in October (Table 1), implying that *C. sinicus* likely has certain flexibility of inner energy utilization, as reported in other species (Niehoff 2007).

The fecundity of *Calanus sinicus* was positively related to PL (Table 2), which might be mediated by the positive relationship between clutch size and PL as reported by Zhang et al. (2005). There were similar relationships in *C. finmarchicus* (Campbell & Head 2000, Runge et al. 2006). It should be noted that the body size effect is indirect and weak, particularly in October: while autumn females developed similar body sizes among stations (Table 1), they displayed distinct fecundities ranging from 0 to 21.5 eggs female<sup>-1</sup>  $d^{-1}$  (Fig. 5B).

We found the EPR of *Calanus sinicus* was independent of temperature (Table 2). Although previous laboratory research showed that higher temperature may increase EPR under saturated food conditions (Hirche et al. 1997), there is often no clear relationship between temperature and EPR during field studies, probably a result of the complex interactions among temperature and other environmental or intrinsic variables which may affect EPR (Campbell & Head 2000, Gisla-

son 2005, Runge et al. 2006). In this study, the temperature (4.9 to 24.0°C) was favorable for the reproduction of *C. sinicus* (Wang & Zuo 2004); however, the effect of temperature might be masked by factors such as food and body size. Nevertheless, temperature may exert influences on reproduction of *C. sinicus* indirectly; for example, it has a negative effect on female body size (Uye 1988, this study), which can in turn influence the clutch size and the EPR (Zhang et al. 2005). Furthermore, temperature is a regulatory factor of the metabolic rate in *C. sinicus* (Li et al. 2004), thus it can alter the energy dispensation to reproduction and other physiological requirements.

External food plays a primary role on the gonad maturation and reproduction of *Calanus sinicus*, whereas inner lipid reserve may mainly serve metabolic needs. Body size has a positive effect on fecundity, probably via the clutch size. Rather than having a direct effect, temperature may exert influences indirectly on reproduction by regulating the metabolic rates and body size.

**Acknowledgements.** We thank the captain and crew of the RV 'Beidou' for their support during sampling. We are grateful to Prof. D. Huang for providing the temperature and salinity data. We also thank the anonymous referees for their valuable comments on an earlier version of the manuscript. This research was supported by special funds from Chinese Academy of Sciences (KZCX2-YW-213), National Natural Science Foundation of China (40631008, 40576070) and National Key Basic Research Program of China (2006CB400606).

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Editorial responsibility: William Peterson,  
Newport, Oregon, USA

Submitted: March 4, 2008; Accepted: December 19, 2008  
Proofs received from author(s): March 15, 2009