Summer reproduction of the planktonic copepod *Calanus sinicus* in the Yellow Sea: influences of high surface temperature and cold bottom water

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We have observed that *Calanus sinicus* retreated from neritic areas in the Yellow Sea and concentrated in the Yellow Sea Cold Bottom Water (YSCBW) area in summer. To investigate the summer reproductive strategy of *C. sinicus* in this situation, effects of high temperature on reproduction and hatching, as well as geographical variation of in situ egg production rate, were studied by onboard incubation in August 2001. Diel vertical migration (DVM) of females was investigated within and outside the YSCBW, respectively. Onboard incubation at 27°C (i.e. surface temperature) resulted in lower fecundities than that at 9.8 and 12.8°C (i.e. bottom temperature inside and outside the YSCBW) together with decreased hatching rates and increased naupliar malformation. Egg production was more active at stations outside the YSCBW than inside, where chlorophyll-a concentration was also relatively low. Females inside the YSCBW underwent DVM although they rarely entered the surface layer, but DVM was not observed outside the YSCBW. We conclude that surface temperature in summer has deleterious effects on *C. sinicus* egg production and hatching, and that it cannot reproduce successfully over the whole area. Inside the YSCBW, egg production is depressed by low food availability, while females outside suffer from high temperatures because of strong vertical mixing.

**INTRODUCTION**

*Calanus sinicus* is an ecologically important copepod species found in shelf waters around China, Japan and Korea. It is the main food source of many commercially important planktivorous fishes, such as anchovy and mackerel (Uye *et al.*, 1999; Meng, 2001). Although *C. sinicus* is found throughout the continental shelf waters of China, its seasonal and geographical distribution is distinctly defined by water temperature. Its occurrence in waters of the Taiwan Strait and South China Sea is limited to winter and spring (Lin and Li, 1984; Hwang and Wong, 2005), and in the Yellow and East China Seas, it is almost completely absent from neritic areas in summer and concentrated in central part of the Yellow Sea, known as the Yellow Sea Cold Bottom Water (YSCBW) (Chen, 1964; Wang *et al.*, 2003).

Surface temperature in the Yellow Sea in summer is higher than 23°C, which is the suggested upper thermal limit of *C. sinicus* (Uye, 1988). Some other studies have indicated that *C. sinicus* thrives between 10 and 20°C (Li, 1963; Huang and Zheng, 1986), and negative effects of high temperature have been investigated in different developmental stages of this species. In the laboratory, mortality of adult *C. sinicus* increases dramatically when incubation temperatures exceed 23°C (Huang and Zheng, 1986). During onboard incubation, surface...
temperature in the Yellow Sea in summer was found to be lethal to the fifth copepodite stage (CV) regardless of food availability (Pu et al., 2004a). However, possible negative effects of high temperature on reproduction of C. sinicus have not been investigated.

Many copepod species have evolved diapause mechanisms to overcome unfavorable environments. However, rather than dormancy, active feeding has been observed in adults and CVs of C. sinicus by feeding and enzyme activity measurements (Han et al., 2002; Li et al., 2004). Therefore, the summer reproductive strategy was investigated in onboard incubations. Our particular objectives are (i) to elucidate in situ reproduction of C. sinicus in summer, (ii) to examine effects of high temperature on egg production and hatching, and (iii) to evaluate the possibility of such effects in the natural environment of the YSCBW.

METHOD

Study area

In this survey, three transects, including a main transect (stations 1-1 to 1-9) across the expected center of YSCBW and two additional transects (stations 2-1 to 2-4 and 3-2 to 3-5) across the south boundary area (Fig. 1), were investigated during 11-24 August 2001, when surface temperatures in the Southern Yellow Sea are at the annual maximum. The main transect was covered twice during the survey. In addition to studies at grid stations, 24 h continuous observations were conducted at two of these stations (stations 1-7 and 2-3), respectively, inside and outside YSCBW.

Environmental conditions

At grid stations, vertical temperature and salinity profiles were measured with a CTD (Seabird 19 plus). Chlorophyll-a concentration in different layers (0, 10, 20, 30, 40 and bottom) was measured with a Turner Designs Fluorometer after 500 mL seawater was filtered on GF/F filters and extracted with 90% acetone. At anchor stations, CTD observations were carried out every 3 h to measure diel changes in thermal structure.

Geographical differences in fecundity

At grid stations, egg production rate (EPR) of C. sinicus was measured by onboard incubation, in order to investigate the geographical variation of fecundity (Range, 1985; Plourde and Range, 1993). C. sinicus were collected with a 330 μm plankton net (mouth opening: 0.5 m², length: 2 m, sealed cod-end) towed vertically from 2 m above the bottom to the surface. Specimens were placed in a 20 L bucket filled with pre-cooled water (15°C) and transported to the lab, where healthy adult females were sorted out as soon as possible. Five adult females were incubated with 70 μm filtered natural seawater in 350-mL plastic bottles with false bottoms, made of 500 μm meshes to prevent egg cannibalism (Runge and Roff, 2000). Five replicates were kept on a 12 h:12 h Light:Dark cycle in incubators, the temperature of which was preset to 18°C (accuracy: ±0.2°C), equivalent to that in the mid-thermocline. Usually, the whole procedure could be finished in 15 min, with little disturbance of the females. Eggs in the bottles were counted after the 24 h incubation.

Temperature effects on reproduction

Temperature effects were investigated by onboard incubation at anchor stations 1-9 and 2-3. C. sinicus was captured and sorted as described above. Incubators were preset to different temperatures, equivalent to those found in the surface layer (27°C) and bottom layer outside (12°C) and inside (9.8°C) YSCBW, respectively. Twenty females (5 individuals per bottle×4 replicates) were incubated at each temperature for 3 days following the same procedure as for the egg production measurements, except that eggs were counted every 4–8 h and...
incubated at the same temperature as spawned to estimate subsequent hatching. The number of eggs hatched and nauplii showing embryonic malformations were counted at the same intervals. All the individuals were incubated in 70 μm filtered natural seawater. All the incubation bottles were refilled with freshly filtered seawater after egg counting, while every 24 h the females were transferred to a new incubating bottle.

**Diel vertical migration**

At these two anchor stations, 24 h continuous observations of the diel vertical migration (DVM) of females were made every 3 h using a 57 L large volume water sampler deployed at different depths (0, 5, 10, 20, 30, 50, 75 m and 1 m above the sea floor). Specimens were filtered (35 μm sieves), preserved with 5% formalin and enumerated in the laboratory. Since the thermocline was narrow and with a strong temperature gradient, a specially designed large volume water sampler was used instead of multi-layer net, which sampled a depth range rather than precise depth. Vertical profiles of temperature and salinity were also measured every 3 h to determine diurnal variation.

**RESULTS**

**Temperature**

There was a strong thermocline along the main transect with water temperature decreasing from 27 to 9.8°C over a depth of about 10 m (i.e. 12 ~ 22 m), but the thermocline along the two additional transects was less pronounced, with water temperature decreasing gradually from the surface to the bottom at most stations. The water column was well mixed at station 3-3, with uniform water column temperatures. Although surface temperature was similar along the main transect, bottom temperatures varied from 16°C at the first station to below 10°C from station 1-3 to 1-8, and then increased to more than 12°C at station 1-9. The main transect was repeated 9 days later and the temperature conditions were similar to those during the first leg. Along the additional transects, bottom temperature was usually 16 ~ 20°C, and surface temperature from 26 to 28°C (Fig. 2). Using the 10°C isotherm as the boundary of the cold water mass (Weng and Wang, 1982), the main transect ran across the YSCBW and the two other additional transects were situated outside.

At the anchor station inside the YSCBW, thermal stratification was quite consistent, and temperature and range of the thermocline barely changed during the 24 h observations (Fig. 3). However, both the position and range of the thermocline at the other anchor station outside the YSCBW varied greatly over 24 h, indicating that vertical mixing was strong at this station.

**Chlorophyll-a concentration**

Chlorophyll-a concentration was extremely low at stations inside the YSCBW. It was <0.30 mg m⁻³ at each depth along the main transect except for station 1-1, which lies in the well-mixed neritic area. At most stations along the two additional transects, chlorophyll-a concentration was higher than 0.20 mg m⁻³, and was as high as 4.25 mg m⁻³ at station 3-2. Highest chlorophyll-a concentration was usually encountered in the surface 20 m both outside and inside the YSCBW (Fig. 4).

**Geographical variation of salinity and EPR**

Average salinity at each station increased gradually offshore, from 30.95 to 34.38 psu, but surface salinity at some offshore stations was as low as 28.77, possibly due to recent rainfall. Inside the YSCBW where bottom temperature was 8.7 to 10°C, EPR was comparatively low, from 0.4 to 1.5 eggs female⁻¹ day⁻¹ (first leg) or nil (second leg) at most stations. Somewhat higher rates, up to 5.7 eggs female⁻¹ day⁻¹ (station 2-1) were measured at most stations outside the YSCBW (Fig. 5), despite the salinity gradient.

**Impacts of high temperature**

During continuous incubation at 27°C, egg production reached a maximum within 24 h but spawning ceased during the following 48 h. At 12 and 9.8°C, egg production lasted continued for almost three days and attained maxima in the second day (Fig. 6). In total, spawning output in these 3 days was only 4.1 eggs female⁻¹ at 27°C but increased to 8.1 and 9.0 eggs female⁻¹ at 12 and 9.8°C, respectively. Naupliar percentage malformation was on average 6.1% at 27°C, higher than 5.0% at both 12 and 18°C and 2.4% at 9.8°C (Table I). Mean hatching success declined from 92.3% at 9.8 and 12°C to 63.7% at 27°C. The variability (SD) of both malformation and hatching success (from 25% to 85%) was also higher at 27°C than at 12 and 9.8°C. It is uncertain whether or not nauplii hatched at 27°C can moult and develop successfully because only microscopically observed malformations were recorded and subsequent development was not followed.

Three types of embryonic malformation were visible in hatching experiments (Fig. 7). Some nauplii with normal bodies had missing appendages, and others developed
asymmetric bodies that made identification difficult. In others, where embryonic development had ceased before hatching was complete, nauplii had not elongated and identification of meta- and urosome was difficult.

**Diel vertical migration**

At station 1–7 inside the YSCBW, CVs were much more abundant than females, and most of them (>90% total) remained in the bottom layer throughout the day (Fig. 8). Although females concentrated in bottom layers only during daytime and migrated upwards during the night, they seldom moved above the thermocline. At station 2–3 outside the YSCBW, females were more abundant than CVs, particularly in the 20–30 m middle layers, but none undertook DVM.

### DISCUSSION

Our results indicate that high surface temperature in summer is deleterious to both fecundity and hatching of *C. sinicus*. In previous studies, it was established that ultra-violet B radiation and diatom toxins can induce embryonic malformation in both *C. sinicus* and *C. helgolandicus* (Poulet et al., 1995; Uye, 1996; Naganuma et al., 1997; Lacuna and Uye, 2000, 2001), and the malformations observed in our study are similar to those associated with high UVB radiation doses or diatom toxins. This suggests that high temperatures might have damaging impacts on reproduction or somehow impair the health of *C. sinicus*. The reproduction rate of calanoid copepods has been observed to increase with temperature only within their optimal range, and to decrease beyond that range (Escribano et al., 1996;
Uriarte et al., 1998). Surface temperature in this study may be not only beyond ‘the optimal thermal range’, but also beyond thermal tolerance.

In fact, natural conditions are much more complicated, and influences of high temperature are also variable. At first, besides temperature, egg production can be affected by some other environmental factors. Since the YSCBW is characterized by low bottom temperature and low chlorophyll-a concentration, EPR within can be reduced by low food availability. In this study, average chlorophyll-a concentration varied from 0.09 to 0.19 mg m$^{-3}$ inside the YSCBW, even lower than that in winter in this area. At stations outside, chlorophyll-a concentration along the two additional transects was comparatively high, varying from 0.12 to 1.83 mg m$^{-3}$. Secondly, since it was thermally stratified inside the YSCBW, both females and eggs can thereby avoid the high surface temperature by reducing DVM. In previous studies, copepod species have been observed to change their seasonal and DVM pattern according to different environmental conditions (Huntley and Brooks, 1982; Carter and Goudie, 1986).

Although the deleterious effects of high temperature cannot be extrapolated directly to natural population, our findings suggest that C. sinicus has little chance of reproducing successfully either inside and outside the YSCBW area. This conclusion is supported by the low abundance of eggs and nauplii during simultaneous sampling (Pu et al., 2004a). Inside the YSCBW, fecundity is restricted by low food availability, and eggs produced beneath the warm surface layer could suffer from low temperature. Risks of being preyed on in water column and after reaching the bottom mud will increase, because of prolonged hatching time in cold

Fig. 3. Vertical thermal structure during 24 h observations at stations 1-7 inside and 2-3 outside the Yellow Sea Cold Bottom Water.

Fig. 4. Vertical distribution of chlorophyll-a concentrations (mg m$^{-3}$) at stations along the three transects.

Fig. 5. C. sinicus. EPR, Salinity (S) and bottom temperatures (BT) at each station. (Error bars show SD of EPR, but minimum and maximum value of salinity).
environments (Uye, 2000). Outside the YSCBW, the cold refuge for *C. sinicus* was eliminated at most stations on the two external transects, and DVM of females was interrupted. The evident vertical mixing during the diurnal cycle may change the surrounding temperature of *C. sinicus* without resulting in any adaptation. Carried passively into the high-temperature surface layer, females and eggs spawned will encounter high mortality. The same circumstances also happened to other developmental stages (Pu et al., 2004b), and they together resulted in decline of *C. sinicus* population size outside YSCBW (Wang et al., 2003). Vertical mixing along the two additional transects might result from invasion of the Kuroshio Current in a northwestward

Fig. 6. *C. sinicus*. Continuous egg production of 20 females incubated at different temperatures, 27, 12 and 9.8°C. (Error bars show SD).

![Continuous egg production of 20 females incubated at different temperatures, 27, 12 and 9.8°C. (Error bars show SD).](https://academic.oup.com/plankt/article-abstract/29/2/179/1555809)

**Table I:** *C. sinicus*. Variation of hatching success and malformation rate when incubated at different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>27°C</th>
<th>12°C</th>
<th>9.8°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount of eggs in 3 days</td>
<td>150</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Hatching percentage (% ± SD)</td>
<td>63.7 ± 24.1</td>
<td>92.3 ± 0.4</td>
<td>92.3 ± 8.6</td>
</tr>
<tr>
<td>Malformation rate (% ± SD)</td>
<td>6.1 ± 3.8</td>
<td>5.0 ± 2.1</td>
<td>2.4 ± 0.4</td>
</tr>
</tbody>
</table>

Diurnal type of embryonic malformation observed in experiments (A) Asymmetric; (B) Lack of appendages; (C) Halt at a certain phase.

Fig. 7. *C. sinicus*. Different types of embryonic malformation observed in experiments (A) Asymmetric; (B) Lack of appendages; (C) Halt at a certain phase. 

![Different types of embryonic malformation observed in experiments (A) Asymmetric; (B) Lack of appendages; (C) Halt at a certain phase.](https://academic.oup.com/plankt/article-abstract/29/2/179/1555809)
direction, and that from stations 1-1 to 1-2 most probably caused by tidal currents (Tang, 1997), whereas a stable vertical thermal structure was a feature of the YSCBW (Weng and Wang, 1982).

*Calanus sinicus* in the Yellow Sea produced normal eggs at all stations, and even when sediments were inspected no resting eggs were found (Zhang, unpublished results). This suggests that a special reproductive strategy, other than diapause, is adopted by females for over-summering. In this study, reduced DVM can protect females from high surface temperature, but it is valid only inside the YSCBW. In previous studies, not only EPR, but also prosome length and dry weight of *C. sinicus*, were found to be lowest from August to October, when water temperature increases beyond the upper thermal limit (Zhang et al., 2005). During this period, fewer eggs were matured in one clutch, and the spawning interval was evidently prolonged (Zhang et al., 2005). The low spawning frequency is a possible reason for the nil egg production during onboard incubation.

In answer to our original questions, *C. sinicus* spawned at different stations in summer, but EPR varied geographically in relation to the location of the YSCBW. During onboard incubation, high surface temperature in summer in the Yellow Sea is definitely deleterious to reproduction and hatching of *C. sinicus*. It is suggested that the natural population cannot reproduce successfully in the whole area investigated, not only because of the high surface temperature, but also low chlorophyll-a concentration inside the YSCBW and physical mixing outside. We suggest that the importance of the YSCBW is not in its ability to allow females to reproduce, but the fact that the cold bottom water acts as a refuge during summer for survival of adults and immature stages (mainly CVs) until the following season, i.e. when the water temperature becomes optimal again. Together with low bottom temperature, stable vertical thermal structure is another prerequisite for over-summering strategy of *C. sinicus*.

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