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Effect of temperature stress on the survival of juvenile Chinese mitten crab (*Eriocheir sinensis*)

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Abstract

Eriocheir sinensis, which is an important aquaculture species, belongs to the class Crustacea. To discuss the temperature tolerance of *E. sinensis*, the survival rates of juvenile crabs of *E. sinensis* were determined in this paper using a series of temperature settings (normal temperature, 25°C, 30°C, 32°C, 34°C, 35°C, 36°C, 38°C, 40 °C). The results indicated that except the normal temperature level, mortality rate almost reached 100% at other temperatures. The time of death of all crabs at 30℃, 35℃, and 40℃ was different. All the crabs died in only 10 minutes at 40℃, while they died after 3 days and 7 days at 35 ℃ and 30℃, respectively. In addition to normal temperature conditions, minimum survival time of the juvenile crabs of *E. sinensis* at other temperature condition was no more than 24 h. Before the 8th day, the death rate at 30℃ and normal temperature was different, change of turning slope for concentration of NH_3-N and TN was contrary. Under normal temperature, the behavior of abdomen extension was not observed in juvenile crabs but the percentage time of abdomen extension was relatively long at 25℃. This research studied the influence of temperature stress on the survival of juvenile crabs of *E. sinensis* to provide information reference for the production and transportation of juvenile crab of *E. sinensis*.

Keywords: *Eriocheir sinensis*, Temperature stress, Survival, Behavior response

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Introduction

The Chinese mitten crab (*Eriocheir sinensis*) is considered to be one of the specific aquaculture species in China, which belongs to Crustacea, Decapoda, Crapsida, *Eriocheir* (Pan, 2002). Normally Chinese mitten crab were cultured for two years, in the first year it is cultured from crab larvae to button size, and in the other year it is cultured to market size in ponds, reservoirs or other waters fields. So the juvenile crabs of *E. sinensis* are also known as buttony crab. Chinese mitten crabs can survive in various environments, such as estuarine waters, freshwater lakes and ponds. In addition to China, the Chinese mitten crab is also distributed in Europe and the Americas (Zou and Si-Fa, 2002). Chinese mitten crabs are widely distributed in the Yangtze River, Liaohe, Oujiang and other waters in China, of which Yangtze River is the most excellent quality. Due to its high nutritional value and market demand, Chinese mitten crab has increasingly become a promising commercial species. The aquaculture industry has been rapidly developing in many parts with Chinese mitten crab aquaculture as a pillar industry of aquaculture (Wang *et al*., 2016).

Average water temperature in the middle and lower Yangtze River is 31-35℃ in summer and 0-4℃ in winter. Crab grow from early March to early November, for up to about 240 days. This temperature range is very suitable for the growth of Chinese mitten crabs (Zhang, 2000). With the global climate change, extreme weather is becoming more and more frequent. To understand the survival mechanism of economic species under extreme weather, such as temperature, is particularly important. Temperature is one of the most important environmental factors affecting crabs, which can affect neuromuscular activity, respiration, blood circulation and lipid metabolism and other physiological activities (Aardt, 1993). The relationship between North America dungeness crab (*Cancer magister*) (Kondzela and Shirley, 1993; Sulkin and Mckeen, 1996), Red king crab (*Kamchatka cancer*) in the North Pacific (Shirley and Shirley 1989; Kittaka and Onoda 2002), North American blue crab (*Callinectes sapidus*) (Leffler, 1972; Fisher, 1999), Mud crab (*Scylla serrata*) (Hamasaki, 2003; Ruscoe *et al*., 2004), Distant sea crab (*Portunus pelagicus*) (Bryars and Havenhand, 2006) and temperature has been reported in a large number of literature. While, at present there is little research on temperature tolerance of Chinese mitten crab, (Bo Huang *et al*., 2001) focused on the effects of temperature on the *E. sinensis* larval growth and development. The results suggested that the survival rate increases with temperature.

In order to know more details on the biology of Chinese mitten crab and provide references for aquaculture to ensure the sustainable development of Chinese mitten crab industry, the juvenile crab was employed as the research object to study the impact of high temperature stress on its survival.

Materials and methods

254 juvenile crabs of *E. sinensis*, with

an average weight of 6-10 g, were captured from Dongting Lake region in China, where the crab culture was common and the crab was the main economic resource. Sampling was done in April, when water temperature in Dongting Lake is 15-23 °C. Normal body color, lively and healthy individuals were chosen and reared in freshwater lake (112.37°E , 29.07°N) to adapt to laboratory conditions.

2 weeks later, 200 juvenile crabs of *E. sinensis* were divided randomly into five groups (n=40 for each group, half male and half female), and transferred to five water tanks (80 cm×58 cm×40 cm) for rearing. The following temperatures were tested: 25°C, 30°C, 35°C, 40°C, normal temperature (<25°C) as the control group. Weight, carapace length, carapace width and height were measured for each crab before experiment (Montu *et al.*, 1996). Pebbles, at a diameter of 2 cm, were placed at the bottom of the tanks serving as a crab refuge. The depth of the water was set at 30 cm, which was in accordance with field culture environment. 5 g food was fed at 22:00 every day during the experiment. At the same time, an oxygen pump worked 8 hours every day to supply dissolved oxygen. Water temperature was measured every day for normal temperature level and dead crabs were also recorded for all levels. At the same time, pH, ammonia nitrogen (NH_3-N), total nitrogen (TN) and total phosphorus (TP) were also measured every three days.

In order to determine the threshold of juvenile crabs of *E. sinensis* to temperature precisely, an experiment with fewer intervals of temperature was designed. Glass aquaria (44 cm×28 cm ×28cm) with paving pebbles at the bottom and 24 cm of water column was used. The following temperature were tested: normal temperature, 25℃, 30℃, 32℃, 34℃, 35℃, 36℃, 38℃, 40℃. Three female crabs and three male crabs were reared in each temperature level. Death time of every crab was recorded for all the levels. Besides the death time, behavior response to temperature was explored. The behavior of the juvenile crabs of *E. sinensis* under different temperatures was recorded with a Panasonic video camera (HDC-HS900) after 5 min for adaption. Behavioral indicators, locomotor activity, movement of mouthparts, cleaning of antennae, antennule retraction, flicking of antennae, percentage time of abdomen, eyestalk extension movement, and percentage time of closure behavior were distinguished (McGaw and McMahon., 1999). The behavior number or sustained time of above behaviors were counted from the video. Because the living time was different, statistic was only carried out 1 min.

Temperature control device (Kedibo WK-SM3) was used in the experient to ensure the temperatures were as expected. Dissolved oxygen was measured with a portable dissolved oxygen device (Shanghai Leici JPB-607A). pH was measured with a portable pH device (Shanghai Leici PHS-3E). NH_3-N, TN, TP were checked by standard method (Wei *et al.*, 2008).

An independent sample test was employed to analyze the role of sex and temperature in the survival process. Temperature control study using model behavior analysis.

Result

Morphology of juvenile crabs of Eriocheir sinensis

The morphological indexes of all experimental crabs were counted. The mean body weights of the crabs were measured to be 8.543±2.555 g for females and 9.304±2.977 g for males, the difference was significant ($p<0.05$). The average carapace length of female crabs was 2.409±0.270 cm, the average carapace width of female crabs was 2.617±0.284 cm, and the average height at withers of female crabs was 1.262±0.144 cm; the average carapace length of male crabs was 2.465±0.265 cm, the average carapace width of male crabs was 2.617±0.284 cm, and the average height at withers of male crabs was 1.269±0.162 cm. There were no significant differences in mean carapace length, width and height between the females and the males ($p>0.05$). (Table 1).

Table 1: Morphology of juvenile of *Eriocheir sinensis.*

	Carapace length(cm) Mean±SD	**Carapace width(cm) Mean±SD**	**Carapace height(cm) Mean±SD**	**Weight(g) Mean±SD**
Females	2.409±0.270	2.617±0.284	1.262±0.144	8.543±2.555
Males	2.465±0.265	2.628±0.291	1.269±0.162	9.304±2.977
T-test	$p>0.05$	$p>0.05$	$p>0.05$	$p<0.05$*

* indicates significant difference ($p<0.05$).

Mortality rates of the juvenile crabs of E. sinensis at different temperatures

Normal temperature fluctuated between 20℃ ~ 25℃ during the experiental period and was lower than the lowest temperature level, 25℃ (Fig. 1). Combined with the control temperature level, there was a successive temperature gradient to explore relationship between survival and temperature of juvenile crabs of *E. sinensis*. With the increase of temperature, from normal temperature, to 25℃, 30℃, 35℃ and 40℃, mortality rate of crabs increased (Fig. 2). Except in the normal temperature level, mortality rate almost reached 100% for other temperatures. Time of death of all crabs at 30℃, 35℃, 40℃ was different. At 40℃ all crabs died in only 10 minutes, while they died in 3 days and 7 days at 35 ℃ and 30℃, respectively. There was an obvious tendency that high temperature meant the crabs would die in a shorter time. In accordance with the above tendency, mortality rate of crab reached 92.5% under 25℃ on the 16th day. When all the crabs almost died for all control temperature levels, 50% of crabs in the normal temperature level, survived. Besides the mortality rate, there was also a huge difference in the death process. The death happened in such a short time that the mortality rate suddenly rose and reached 100% for the high temperature level, such as 30 ℃ or higher temperature. The crabs died gradually at temperature of 25℃

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and normal temperature level. Basing on the above analysis, temperature played an important role in the development of juvenile crabs of *E. sinensis*. 25℃ may be the threshold temperature for juvenile crabs of *E. sinensis*.

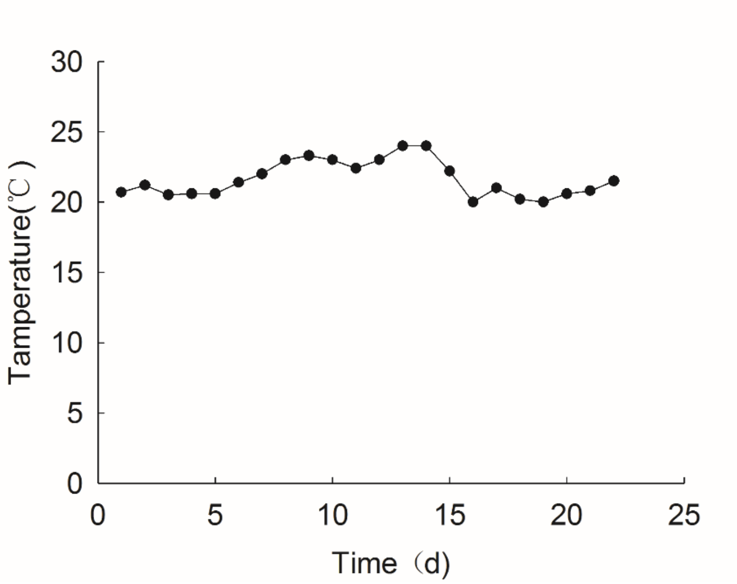

Figure 1: Fluctuations of normal temperature during the experience.

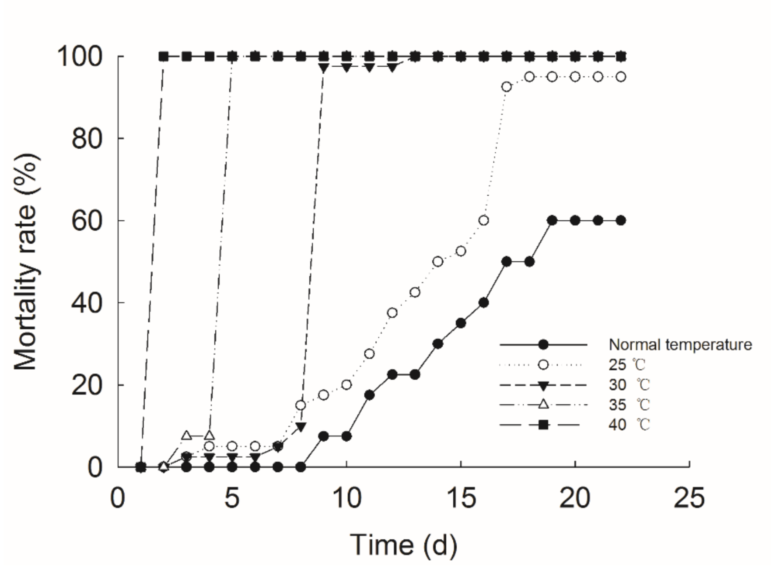

Figure 2: Mortality rate of juvenile *of Eriocheir sinensis* under different temperatures.

Survival time of the juvenile crabs of E. sinensis at different temperatures

There was a more direct evidence to show that at higher temperature, survival time was shorter. In addition to normal temperature condition, minimum survival time of the juvenile crabs of *E. sinensis* at other temperature conditions were no more than 24 h. Maximum survival time of the juvenile crabs of *E. sinensis* at normal temperature, 25℃, 30℃, 32℃, 34℃, 35℃, 36℃, 38℃, 40℃ were 555 h, 423 h, 170.50 h, 168 h, 0.21h, 75h, 36h, 0.3h, 0.1h, respectively. Mean of survival time were 394.50±130.60 h, 281.44±103.41 h, 163.34±26.96 h, 84.28±91.71 h, 3.86±4.71 h,

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72.05±13.18 h, 23.67±6.08 h, 0.09±0.10 h, 0.08±0.03 h, respectively at the series of temperature gradient. Although there was a peak at 35°C, 36°C, the tendency would not be changed. The tolerance of individuals was variable and the less test-crabs led to the result.

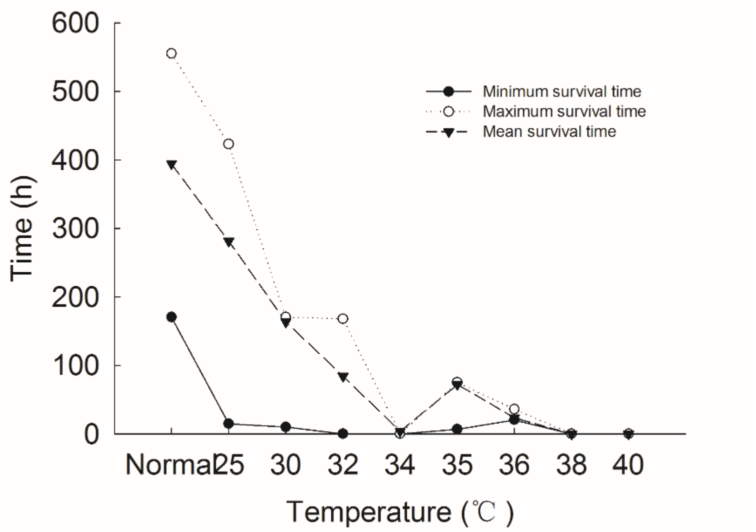

Figure 3: Survival time of juvenile *of Eriocheir sinensis* at different temperatures.

Change of water quality factors

Because crabs died in short time at temperatures of 35°C and 40°C, only change of water quality at normal temperature, 25°C (threshold temperature) and 30°C are shown (Fig. 3). The concentration of NH_3-N for the above temperature levels increased, although the gradient for different levels were variable (Fig. 4A). The regular pattern of concentration of TN was different from NH_3-N. For the first four days of the experiment, the concentration of TN increased quickly and reached about 6 mg L^{-1}, after which the concentration of TN fluctuated at 0.6 mg L^{-1} (Fig. 4B). pH of water was alkalescence and ranged from 7.15 to 7.62 (Fig. 4C). Similar to the concentration of NH_3-N, concentration of TP was also growing and gradient of normal temperature, 25°C and 30°C was similar (Fig. 4D).

Combined with the mortality rate of different temperature levels, the 8^{th} day was an important node for 30°C because all crabs died and number of dead crabs was more than the other days. There was no jumping point for normal temperature and 25°C. Focusing on the change of water quality, especially the key node, mechanism might be explored. TP should not be the key factor to affect crab survival because there were no signification differences for different temperature levels. A common feature for NH_3-N and TN have been gotten, that the concentrations of both at 30°C were higher than normal temperature and 25°C level. After 100% death, the concentration of NH_3-N and TN at 30°C decreased. A steep increase of pH also revealed the clue, that there might be a close relationship between both NH_3-N and TN and crab survival. The

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concentrations of NH_3-N and TN were not the highest nor the lowest, so there was no threshold value for Chinese mitten crab, but there was accumulation effect. Before the 8th day, the death rate at 30℃ and normal temperature were different, change of turning slopes for concentrations of NH_3-N and TN were contrary. The concentration of NH_3-N and TN at 30℃ rose first and then declined. The concentration of NH_3-N and TN at normal temperature always rose, but the gradient was different. No matter the death rate of crabs or concentrations of NH_3-N and TN at 25℃ was intergrade. Basing on the above analysis, change in concentration of NH_3-N and TN might affect survival of the juvenile crabs of *E. sinensis*.

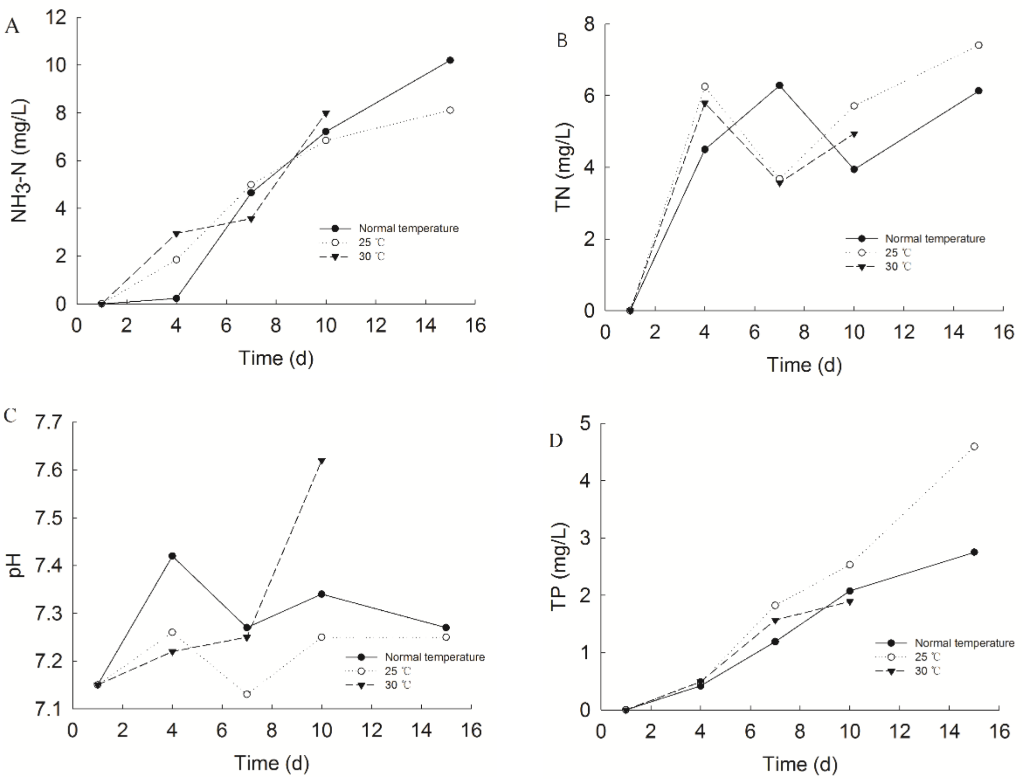

Figure 4: Water quality factor changes at different temperatures.

Behavioral responses to temperature change

The *E. sinensis* died quickly in conditions when temperature were over 25℃, so this paper only collects the statistics of behavior activities of *E. sinensis* under normal temperature or below 25℃ (Table 2). No matter under normal temperature or below 25℃, there were no significant differences between the behaviors of female and male crabs ($p>0.05$). The movement of mouth parts and flicking of antennae were the two main behaviors of juvenile crabs under two temperature gradients. Under different temperatures, there was no significant difference between the frequency of movement of mouth parts of female and male crabs ($p>0.05$); there was no significant difference between the frequencies of flicking of antennae for female crabs under different temperatures. However, the frequency of flicking of antennae for male crabs was 100.67±2.52 under normal temperature and it was 77.33±2.08 at 25℃. The frequency of flicking of antennae under normal temperature was significantly higher than that at 25℃ ($p < 0.01$). Under normal temperature, the behavior of abdomen extension was not observed in juvenile crabs but the percentage time of abdomen extension was relatively long at 25℃. The percentage time of abdomen extension was 27.67±6.81 seconds within one minute for female crabs and it was 41.33±9.87 seconds within one minute for male crabs. Significant differences could be observed in terms of the percentage time of abdomen extension under two temperature conditions between female crabs and male crabs ($p < 0.05$). The frequency of locomotor activity, cleaning of antennae, antennule retraction and eyestalk movement was low and the difference was not obvious under two temperature gradients ($p > 0.05$). Closure reaction was not observed during the observation.

Table 2: Behavioral comparison between *Eriocheir sinensis* under different temperature.

		Normal temperature	**25℃**	***p***
Locomotor activity(n)	Female	1.00±1.00	1.67±0.58	p>0.05
	Male	3.33±1.53	1.33±2.31	p>0.05
Movement of mouth parts(n)	Female	117±11.53	103.00±20.95	p>0.05
	Male	81.33±19.09	94.00±14.18	p>0.05
Cleaning of antennae/antennules(n)	Female	1.33±0.58	1.33±0.58	p>0.05
	Male	3.67±2.08	0.67±0.58	p>0.05
Antennule retraction(n)	Female	2.33±0.58	1.67±1.53	p>0.05
	Male	2.00±2.00	2.67±0.58	p>0.05
Flicking of antennae(s)	Female	113±18.74	84.33±14.3	p>0.05
	Male	100.67±2.52	77.33±2.08	**p<0.01****
Percentage time of abdomen extension(s)	Female	0	27.67±6.81	**p<0.05***
	Male	0	41.33±9.87	**p<0.05***
Eyestalk movement(n)	Female	1.00±1.00	1.33±1.53	p>0.05
	Male	2.67±0.58	1.33±1.16	p>0.05
Percentage time of closure behavior(s)	Female	0	0	—
	Male	0	0	—

** represents highly significant difference; * represents significant difference

Discussion

The range between 20 to 25℃ might be the suitable temperature for the growth of juvenile crabs of *E. sinensis*, which conformed to the temperature of the season in which large number of juvenile crabs of *E. sinensis* appear. If the water temperature is over 30℃, it may lead to the death of juvenile crabs, which should be noted in artificial breeding practices. From existing reports of larval cultivation of *E. sinensis*, the most suitable cultivation temperature in the experiment of Chang

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and Sang (1995) is 25℃. The appearance of juvenile crabs in Yangtze River Delta region was between April 15th to May 15th and the range of water temperature was between 20 to 25°C (Zhang 2002), which was roughly consistent with the results of this experiment.

The optimal survival temperature required by the *E. sinensis* at different growth periods is different. In the breeding season, the temperature range was 10℃ to 20℃ (Herrgesell *et al.*, 1983). The survival rate of megalopa larva was the highest between 12 to 18℃ and megalopa larva has relatively strong tolerance to low temperature. Afterwards, the optimal survival temperature gradually rises with the growth of larva (Anger, 1991). The optimal culture condition for adult *E. sinensis* was that the pH value was 6.8 and the water temperature was 28°C (Hong *et al.*, 2013). This trend conforms to the real situation where water temperature at the beginning of the breeding peak of *E. sinensis* is low but gradually rises with the progress of time.

The temperature required during the shelling period of *E. sinensis* was relatively low. Although the mechanism in this aspect remains to be further investigated, the situation in experiments and cultivation bases indicates the shelling of larva of *E. sinensis* often occurs late at night or early in the morning when the water temperature was low, which reflects the choice of low temperature of larva of *E. sinensis*.

Alkaline pH was the suitable environment for the growth of *E. sinensis* and the optimal range was 7.0 to 9.1. The respiratory activity of crustacean will be changed no matter whether it was lower of higher than this range. The ability to absorb oxygen from outside through gills will be influenced, thus affecting the oxygen consumption rate (Lin *et al.*, 2000). The pH change during the course of this experiment was within the appropriate range and thus the pH change was not the primary cause of the death of crabs in this experiment. Although the TP concentration during the course of this experiment was higher than normal aquatic water, the change node of TP concentration was not consistent with the death time pf crabs. Therefore, the high TP concentration was also not the primary cause of the death of crabs in this experiment. No fresh water was poured during the course of this experiment, so the excreta of *E. sinensis* and the residue of feed led to the increase of ammonia concentration in the water. The increase of ammonia stress concentration and the extension of stress time led to the damage of non-specific immune defense system of *E. sinensis*, thus accelerating the death of *E. sinensis* (Wang *et al.*, 2006; Hong *et al.*, 2007; Huang *et al.*, 2006). However, the initial conditions in this experiment were consistent except the temperature, so the increase of ammonia concentration was the possible cause but not the primary cause of the death acceleration of *E. sinensis*. Hong-Zhu Wang proposed that TN concentration is negatively correlated with crab yield in studying the stocking model of *E.*

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sinensis in Yangtze River basin (Wang *et al.,* 2006), which is consistent with the views of this paper.

In conclusion, temperature exerted great influence on the survival of juvenile crabs of *E. sinensis*. Obviously, temperature needs to be comprehensively considered in the production practice. Meanwhile, we should attach great significance to water quality monitoring and make adjustments to abnormal conditions. To guarantee high survival rate of juvenile crabs of *E. sinensis* in breeding, transportation and other activities, the temperature should be controlled no higher than 30℃.

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