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Effects of salinity, carbonate alkalinity, and pH on physiological indicators of nutrition transporter for potential habitat restoration of amphipod *Eogammarus possjeticus**

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Abstract The effects of three environmental factors, salinity, carbonate alkalinity, and pH, on the survival, feeding, and respiratory metabolism of *Eogammarus possjeticus* (Amphipoda: Gammaridae) were investigated experimentally. The results show that *E. possjeticus* could tolerate a broad salinity range. The 24-h lowest median lethal salinity was 2.70, and the highest was 47.33. The 24-h median lethal alkalinity and pH were 23.05 mmol/L and 9.91, respectively; both values decreased gradually with time. Different values of salinity, carbonate alkalinity, and pH resulted in significant differences in the cumulative mortality ($P<0.05$). The ingestion rate and feed absorption efficiency were significantly affected by the coupling of the three environmental factors ($P<0.05$). With increases in carbonate alkalinity, salinity, and pH, both ingestion rate and feed absorption efficiency exhibited a downward trend, indicating a decline in feeding ability under high salinity and more alkaline water conditions. The coupling of salinity, carbonate alkalinity, and pH also had a significant effect on respiration and excretion ($P<0.05$). The oxygen consumption rate increased first and then decreased with increasing carbonate alkalinity. Under the same carbonate alkalinity values, the oxygen consumption rate increased with increasing salinity. Under the same carbonate alkalinity and salinity, the oxygen consumption rate initially increased and then decreased with increasing pH. The O:N ratio first increased and then decreased with increasing carbonate alkalinity. When carbonate alkalinity was less than 6 mmol/L, the O:N ratio increased with increasing salinity and decreased with increasing pH. The results demonstrate that changes in salinity, carbonate alkalinity, and pH had a measurable impact on the osmotic pressure equilibrium in *E. possjeticus* and affected the energy supply mode (i.e. ratio of metabolic substrate).

Keyword: *Eogammarus possjeticus*; saline and alkaline water; survival; feeding; respiratory metabolism

1 INTRODUCTION

Vast areas of saline and alkaline waters have become a common environmental problem globally. High salinity, carbonate alkalinity, and pH are considered three major stressors that affect the survival, growth, and reproduction of aquatic animals in saline and alkaline waters (Lin et al., 2013). Water bodies that are both saline and alkaline have poor buffering capacity because of an unstable proportion

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of major ions, including CO_3^{2-} and HCO_3^-. Highly alkaline waters have relatively low CO_2 tension, which reduces the blood CO_2 tension in aquatic animals and thus can cause persistent alkalosis (Yao et al., 2016). Because of these characteristics, most saline and alkaline land remains unexploited. However, with the continued shrinking of available inland water resources, many countries have now begun to study how to effectively use saline and alkaline waters. One important approach is to set up aquaculture in these waters using plants and aquatic animals that are saline and alkaline tolerant.

China is rich in saline and alkaline water resources, covering an area of approximately 45.87 million hectares (Lin et al., 2013). The reasons for the abundance of saline and alkaline water resources may be due to climate warming, sea-level rise, drought and precipitation reduction, and human activities (such as agricultural irrigation), etc. (Yao et al., 2010). To date, several aquatic animals, including *Fenneropenaeus chinensis*, *Penaeus vannamei*, *Exopalaemon carinicauda*, and *Acipenser baeri*, have been cultured successfully using saline and alkaline waters (Fang et al., 1999; Zhou et al., 2007; Huang et al., 2010; Jaffer et al., 2020). To prevent intensive aquaculture from causing adverse effects, such as disease and environmental pollution, we need to shift the focus toward aquaculture infrastructure that enhances the disease resistance of cultured species and improves the aquatic environment. Specifically, live food is added during the culture process to enhance the disease resistance of aquatic animals and improve the environment (Xue et al., 2018).

Amphipods are one of the most diverse and quantitatively dominant groups of organisms in the benthic macrofauna (Cunha et al., 1997; Dauby et al., 2001). Because amphipods are rich in nutrients, such as proteins, fats, and carbohydrates, they are a high-quality natural food for aquatic animals, including fish and crustaceans (Xue et al., 2013; Baeza-Rojano et al., 2014). Additionally, amphipods play a key role in decomposition and remineralization processes in benthic ecosystems (Sainte-Marie, 1992), and have a potential role in remediating the benthic environment within aquaculture systems (Ananthi et al., 2011). With the gradual development of aquatic animal culture in saline and alkaline waters, the demand for live food in the culturing process has increased accordingly. Therefore, it is necessary to carry out research into the physioecological responses of food organisms to saline and alkaline waters.

In this study, *Eogammarus possjeticus*, a predominant species of amphipods in marine aquaculture ponds in Shandong Peninsula (China), was used for survival, feeding, and respiratory metabolism analyses under different salinity, carbonate alkalinity, and pH conditions. The aim of this study was to describe the physiological response of amphipods to saline and alkaline waters. The results provide useful data to describe the mechanisms of salinity and alkalinity tolerance in aquatic animals, and they guide the rational development and utilization of saline and alkaline water resources.

2 MATERIAL AND METHOD

2.1 Collection and cultivation of amphipods

The individuals of amphipod *E. possjeticus* were collected from Laizhou Bay (37°03′N–37°10′N, 119°29′E–119°30′E) in April 2018 using a suction sampler and shifted through a 2-mm meshed sieve. The animal samples were then transported to the laboratory for three hours in three sealed plastic bags, filled with one-third seawater (10 L) and two-thirds of the pure oxygen. Large numbers of healthy and vigorous individuals of *E. possjeticus* (the mean values and standard deviations of body size and body weight were 1.1±0.1 cm and 12.1±2.8 mg, respectively) were selected for temporary cultivation under natural seawater conditions in the laboratory. Eight corresponding incubators (60 cm long, 45 cm wide and 40 cm high) filled with 60 L of seawater were set up. In total, 1 500–2 000 individuals were added to each incubator. During the period of temporary cultivation, the environmental parameters of seawater were measured by the multi-parameter environmental monitoring system (YSI6600, YSI, USA) every day. The seawater temperature was 10–13°C, the salinity was 29–32 practical salinity units, the pH was 8.0–8.2, and the dissolved oxygen was 6.5–7.1 mg/L. The amphipods were cultured with the photoperiod of 12 h L:12 h D. The seawater was renewed every day and the organisms were fed with fresh macroalgae (*Enteromorpha* sp.) once a day. The fresh macroalgae were collected from the seaside of Fushan Bay (36°02′N–36°03′N, 120°21′E–120°22′E) in April 2018. The wet weight of the macroalgae was at 150%–200% of the amphipod's total wet body weight. The amphipods were allowed to acclimatize to the cultivation conditions for one week before the experiments.

2.2 Experimental design and sampling

2.2.1 Cumulative mortality and median lethal dose

Three tests were conducted (salinity, carbonate alkalinity, and pH). The cumulative mortalities under different salinities (1, 5, 10, 15, 20, 25, 30-control, 35, 40, 45, 50, and 55), carbonate alkalinities (3-control, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, and 45 mmol/L) and pH values (8.0-control, 8.5, 9.0, 9.5, 8.0, 10.0, and 10.5) were studied. The seawater pumped from the seacoast of Fushan Bay was shipped to the laboratory and adjusted to the desired salinities by adding crude sea-salt or mixing with aerated tap water. The carbonate alkalinity of seawater was adjusted by adding sodium bicarbonate (Macklin Ltd., Shanghai, China), and the pH values were maintained for the experimental requirement through the use of HCl (0.1 mol/L) and NaOH (0.1 mol/L). During the experiments, the temperature was kept at (16.5±1.5)°C, and other conditions were the same as temporary cultivation.

Experiments were conducted using 2-L plastic tanks filled with 1 L of continuously aerated water with the above salinity, carbonate alkalinity, and pH values. The amphipods were randomly selected from temporary incubators and transferred directly to the experimental tanks using a plastic pipette. The mortality rate and the median lethal dose at the time points of 24, 48, 72, and 96 h were calculated. Each test tank had 30 individuals. The control and experimental groups were tested in triplicate.

The calculation of median lethal dose:

Cumulative mortality data were used to estimate median lethal dose (LD_{50}) and their respective confidence intervals (95%) at different experimental levels, using the improved Trimmed Spearman Karber method (Huang and Xu, 2017). The formula was as follows:

$$LD_{50}=\log^{-1}[X_m-i(\sum p-0.5)],$$

where i is the group distance, that is, the logarithm difference of two adjacent groups of processing conditions; X_m is the logarithm of the maximum processing condition; p is for each treatment group mortality (mortality is expressed in decimal); Σp is for the sum of each treatment group of mortality.

Calculation of 95% confidence interval of LD_{50} was based on the following equation:

$$S_{x50}=i\sqrt{\sum\frac{pq}{n}},$$

$$LD_{50}(95\%)=\log^{-1}(\log LD_{50}\pm1.96\times S_{x50}),$$

where S_{x50} is the standard error of $\log LD_{50}$; q is survival rate of all treatment groups, q=1–p; n is the number of amphipods in each group.

In this paper, L_{Sal50}, L_{Ca50}, and L_{pH50} were respectively used to represent the LD_{50} of salinity, carbonate alkalinity, and pH.

2.2.2 Determination of ingestion, respiration, and excretion

In order to study the coupling response of *E. possjeticus* on carbonate alkalinity, pH, and salinity, 36 treatments including four carbonate alkalinities (3-control, 6, 9, and 12 mmol/L), three pH values (8.0-control, 8.5 and 9.0) and three salinities (20, 30-control and 40) were tested to determine the ingestion and respiratory excretion of *E. possjeticus*.

The experiment was initiated after 3 days of an adaptation period. Before each feeding, uneaten food was removed and faeces were syphoned out from each tank. Each feeding group was fed with algae (1g wet weight). Faeces that accumulated at the bottom of the tanks after 24 h of feeding were syphoned into large beakers and uneaten algae were carefully removed with forceps. Each test tank had 30 individuals. The control and experimental groups were tested in triplicate. Calculation formula of feeding index was as follows:

$$\text{IR }(\%)=\text{DW}/\text{WW}\times100,$$

$$\text{AR }(\%)=(\text{DW}-\text{DW}_f)/\text{DW}\times100,$$

where IR is ingestion rate, DW is dry weight of feed eaten, WW is wet weight of amphipods; AR is absorption rate, DW_f is dry weight of faeces.

Respiration was calculated by comparing the dissolved oxygen content of closed 1-L bottles filled with seawater and containing 30 individuals to that in a reference bottle with no animals, after 4-h incubation in each test. Ammonia excretion was measured at the end of the respiratory experiments. No food was provided during the respiration and excretion tests. The oxygen consumption rate [OR, mg/(g·h)] and ammonia excretion rate [NR, mg/(g·h)] were calculated based on the changes in the initial and final dissolved oxygen and ammonia nitrogen concentrations. The calculation formula was as follows:

$$\text{OR}=[(D_0-D_t)\times V]/(W\times t),$$

$$\text{NR}=[(N_t-N_0)\times V]/(W\times t),$$

$$\text{O:N}=(\text{OR}/16)/(\text{NR}/14),$$

where D_0 and D_t are the dissolved oxygen content of

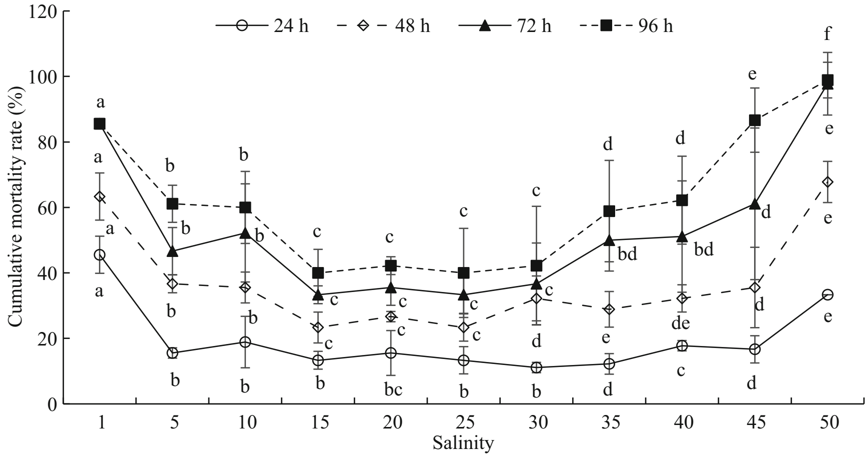

Fig.1 The cumulative mortality rates of *E. possjeticus* under different salinities

Different lowercase letters indicate significant differences (*P*<0.05) among different treatments, the same as below.

the reference bottle with no animals and the experimental group respectively, N_0 and N_t are the ammonia nitrogen concentrations in water of the reference bottle with no animals and the experimental group (mg/L). V is the breathing bottle volume (L), W is the body weight of the individuals (g fresh weight), t is experiment duration (h) and O:N is the ratio of oxygen consumption rate and ammonia excretion rate.

Dissolved oxygen was determined by a multi-parameter handheld monitor (SmarTROLL-MP, In-Situ, USA), and ammonia nitrogen was tested by sodium hypobromate oxidation (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and Standardization Administration of the People's Republic of China, 2008).

2.3 Data analyses

Statistical analyses were carried out using the Excel and IBM SPSS statistic software. Obtained data were analyzed by means of one-way ANOVA with repeated measures. When significant main effects were observed, Tukey test was used for post hoc analysis. A probability of $P<0.05$ was chosen as the significant level in all analyses.

3 RESULT

3.1 Cumulative mortality and median lethal dose

3.1.1 Salinity

The cumulative mortality of *E. possjeticus* under different salinity conditions is shown in Fig.1. Following a sudden rise or fall in salinity, cumulative mortality increased gradually. Relatively high mortality was observed when salinity was <5 or >45. With unchanged salinity, cumulative mortality increased after longer periods of time. L_{Sal50} values at various time points and corresponding 95% confidence intervals were obtained using a modified Karber's method (Table 1). With elapsed time, there were gradual increases in the L_{Sal50l} (24 h=2.70; 48 h=5.75; 72 h=7.59; 96 h=10.67), while L_{Sal50h} decreased gradually (24 h=47.33; 48 h=41.78; 72 h=35.41; 96 h=23.00). One-way ANOVA revealed a significant effect of salinity on the mortality of *E. possjeticus* ($P<0.05$).

Table 1 The median lethal salinity (L_{Sal50}) and 95% confidence interval of *E. possjeticus*

Time (h)	L_{Sal50}		95% confidence interval
	L_{Sal50l} (lowest)	L_{Sal50h} (highest)	
24	2.70	47.33	1.06–49.11
48	5.75	41.78	2.34–43.65
72	7.59	35.41	3.05–37.33
96	10.67	23.00	5.18–24.76

3.1.2 Carbonate alkalinity

The cumulative mortality of *E. possjeticus* under different carbonate alkalinity levels is depicted in Fig.2. There were significant increases in the cumulative mortality ($P<0.05$) with increased carbonate alkalinity over time. L_{Ca50} values at various time points and corresponding 95% confidence intervals are provided in Table 2. With increased time, L_{Ca50} of *E. possjeticus* decreased gradually from 23.05 (24 h) to 17.14 (48 h), 10.90 (72 h), and finally 7.35 (96 h) in mmol/L.

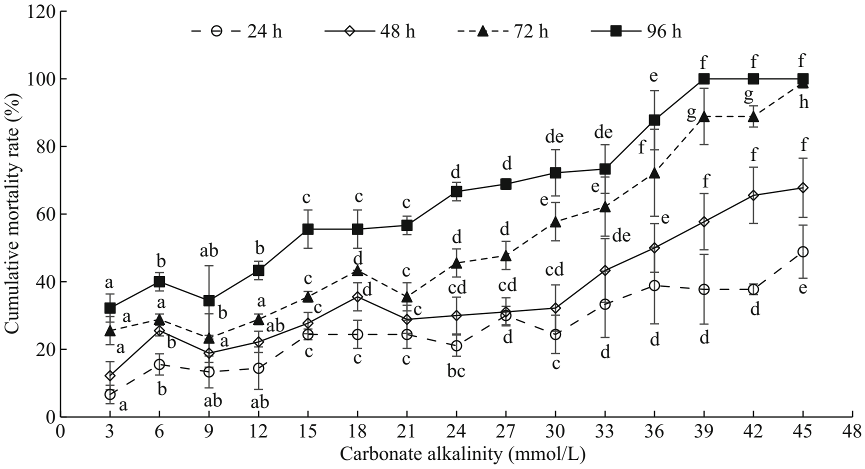

Fig.2 The cumulative mortality rates of *E. possjeticus* under different carbonate alkalinities

Table 2 The median lethal carbonate alkalinities (L_{Ca50}) and 95% confidence interval of *E. possjeticus*

Time (h)	L_{Ca50} (mmol/L)	95% confidence interval
24	23.05	20.56–25.84
48	17.14	15.18–19.36
72	10.90	9.69–12.26
96	7.35	6.57–8.22

Table 3 The median lethal pH (L_{pH50}) and 95% confidence interval of *E. possjeticus*

Time (h)	L_{pH50}	95% confidence interval
24	9.91	9.75–10.08
48	9.37	9.20–9.54
72	8.98	8.80–9.16
96	8.70	8.54–8.87

3.1.3 pH

The cumulative mortality of *E. possjeticus* under different pH conditions is shown in Fig.3. With an increase in pH over time, the cumulative mortality rate increased significantly (P<0.05). L_{pH50} values at various time points and corresponding 95% confidence intervals are summarized in Table 3. With time, L_{pH50} of *E. possjeticus* decreased gradually from 9.91 at 24 h, 9.37 (48 h), 8.98 (72 h), to 8.70 at 96 h.

3.2 Ingestion rate and feed absorption efficiency

Daily ingestion rates and feed absorption efficiencies decreased gradually with increasing carbonate alkalinity (Figs.4 & 5). Both parameters were highest at a carbonate alkalinity of 3 mmol/L and lowest at 12 mmol/L. Similarly, gradual decreases occurred in the daily ingestion rate and feed absorption efficiency with increasing salinity and constant carbonate alkalinity. In addition, both parameters exhibited a downward trend with increasing pH under the same carbonate alkalinity and salinity. Repeated ANOVA analyses revealed significant differences in the daily ingestion rate and feed absorption efficiency of *E. possjeticus* among various groups under different carbonate alkalinity, salinity, and pH levels (P<0.05).

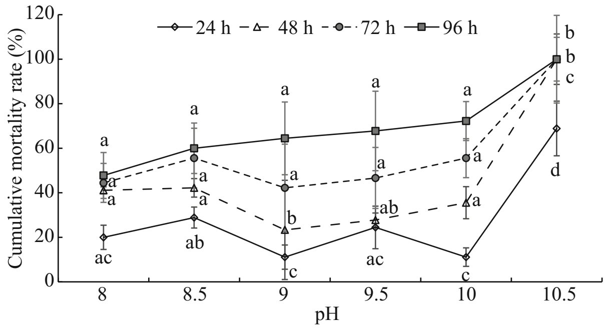

Fig.3 The cumulative mortality rates of *E. possjeticus* under different pH

3.3 Oxygen consumption and ammonia excretion rates, and the O:N ratio

With increasing carbonate alkalinity, oxygen consumption rates first increased and then decreased (Fig.6). Among various salinity and pH conditions, oxygen consumption rates were highest at a carbonate alkalinity of 6 mmol/L and lowest at 12 mmol/L. Oxygen consumption rates exhibited an upward trend with increasing salinity under the same carbonate alkalinity. In contrast, it first increased and then decreased with increasing pH under the same carbonate alkalinity and salinity. Ammonia excretion rates tended to increase with increasing pH when carbonate alkalinity was <6 mmol/L. Little variation

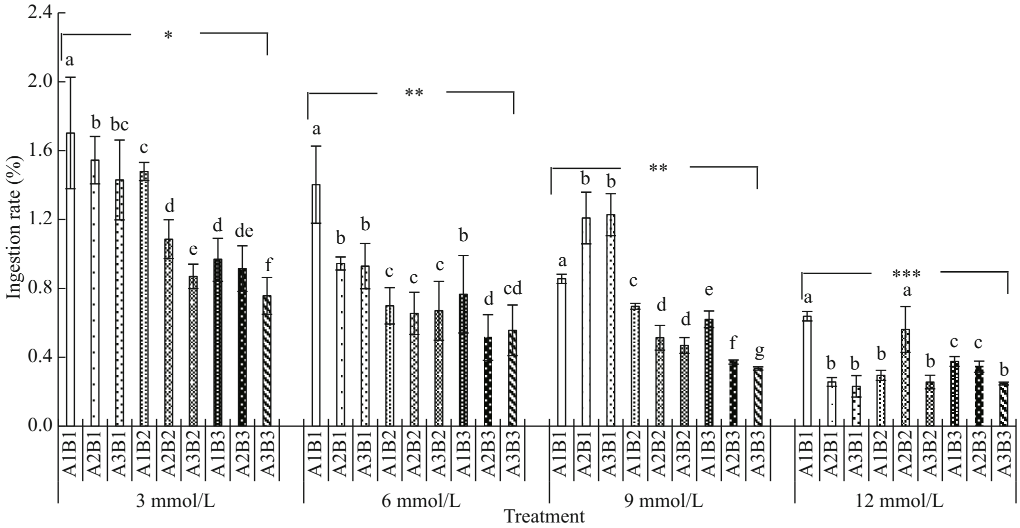

Fig.4 The ingestion rate of *E. possjeticus* at different salinities, carbonate alkalinities, and pH

A1, A2, and A3 represent pH 8.0, 8.5, and 9.0 respectively. B1, B2, and B3 represent salinity of 20, 30, and 40 respectively. The carbonate alkalinity was 3, 6, 9, and 12 mmol/L respectively. Different letters indicate significant differences among groups under the same carbonate alkalinity, and different asterisks indicate significant differences among groups of carbonate alkalinity.

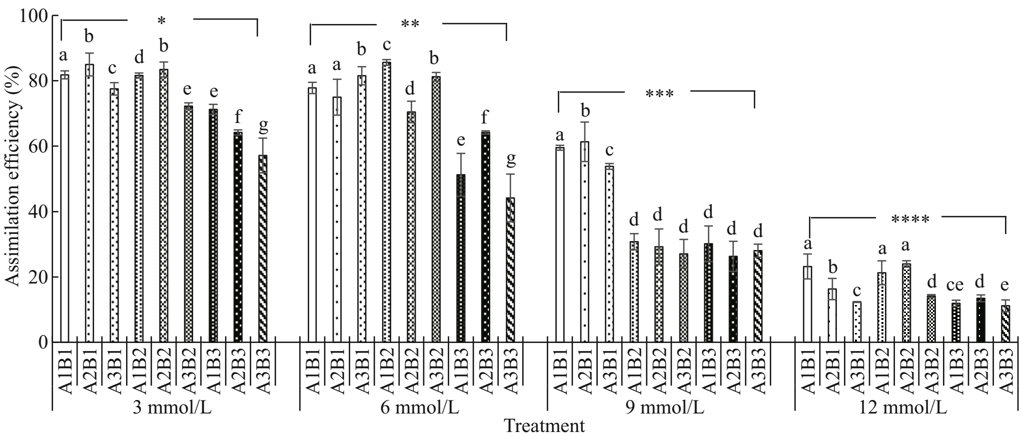

Fig.5 The assimilation efficiency of *E. possjeticus* at different salinities, carbonate alkalinities, and pH

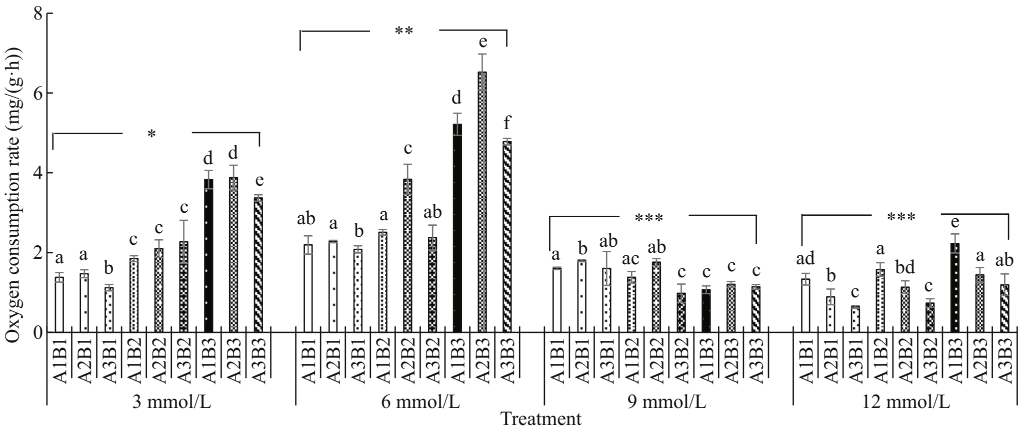

Fig.6 The oxygen consumption rate of *E. possjeticus* at different salinities, carbonate alkalinities, and pH

was observed in ammonia excretion rates under various salinity and pH conditions when carbonate alkalinity was greater than 9 mmol/L (Fig.7). The O:N ratios initially increased and then decreased with increasing carbonate alkalinity. Under a carbonate alkalinity of <6 mmol/L, the O:N ratios first increased

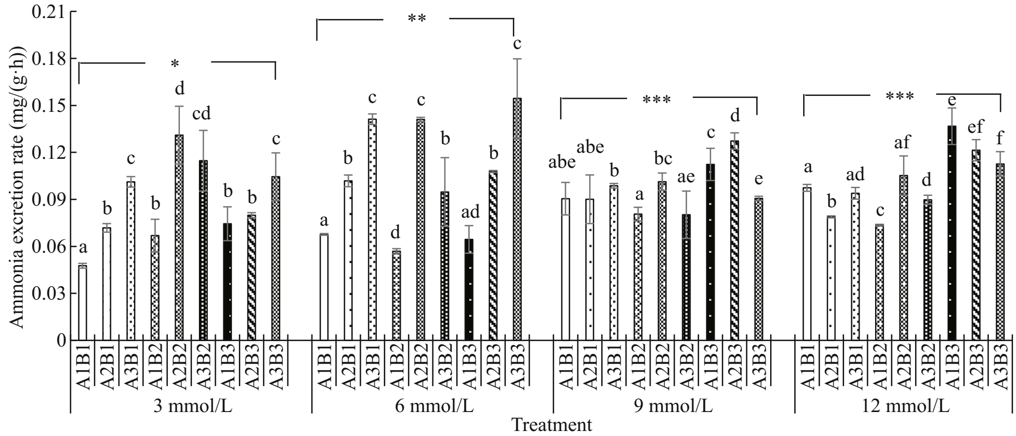

Fig.7 The ammonia excretion rate of *E. possjeticus* at different salinities, carbonate alkalinities, and pH

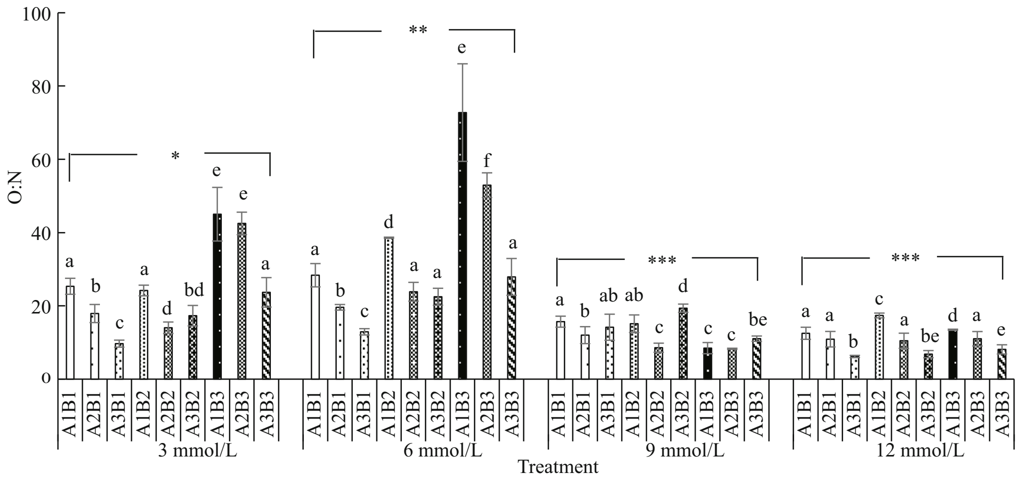

Fig.8 The O:N ratio of *E. possjeticus* at different salinities, carbonate alkalinities, and pH

with increasing salinity but then decreased with further increasing pH. Under a carbonate alkalinity >6 mmol/L, various salinity and pH conditions yielded relatively low O:N ratios and showed little variation (Fig.8). Repeated ANOVA analyses revealed significant differences in the oxygen consumption and ammonia excretion rates as well as the O:N ratios of *E. possjeticus* among various groups under different carbonate alkalinity, salinity, and pH levels ($P<0.05$).

4 DISCUSSION

4.1 Salinity, carbonate alkalinity, and pH tolerance

The effects of salinity on amphipods are highly diverse and species specific (Normant and Lamprecht, 2006). For example, the biomass of *Gammarus chevreuxi* is closely linked to salinity (Subida et al., 2005), whereas *G. locusta* demonstrates good salinity tolerance with its survival and growth not affected significantly within a certain range (Neuparth et al, 2002). In the present study, the L_{Sal50l} at 24 h of *E. possjeticus* was 2.70 and the corresponding L_{Sal50h} was 47.33, indicating a broad salinity tolerance range for this species. The survival strategy of amphipods is *r*-selection (natural populations commonly kept at low densities by density-independent mortality should evolve high intrinsic rate of growth, but be unable to have superior performance at high population densities) (Mueller and Ayala, 1981); strong fecundity and high biomass albeit with high mortality (Duffy and Hay, 2000). In addition to the increased cumulative mortality of *E. possjeticus* in various treatment groups, the mortality of the control seawater group also increased with time. Thus, the 95% confidence interval of the L_{Sal50} values for *E. possjeticus* at various time points might be an underestimation.

The carbonate alkalinity in aquaculture water is positively significant within a certain range to the survival and growth of aquatic animals, but it likely has adverse effects beyond a certain limit (Zhao et al., 2016). Yang et al. (2004) reported L_{Ca50} values for juvenile *Penaeus vannamei* of 14.29 (24 h), 12.55

(48 h), and 12.01 mmol/L (96 h) under pH values of 7.50–8.72. Similar to prior studies, carbonate alkalinity was found to be positively correlated with the mortality of *E. possjeticus*. In other words, the mortality of *E. possjeticus* increased with increasing carbonate alkalinity. The L_{Ca50} at 24 h of *E. possjeticus* was 23.05 mmol/L under pH values of 7.98–8.35. With time, the cumulative mortality of *E. possjeticus* increased such that at 48 h, 72 h, and 96 h, the L_{Ca50} values changed to 17.14, 10.90, and 7.35 mmol/L, respectively. These results demonstrate that *E. possjeticus* has a higher tolerance to carbonate alkalinity than *P. vannamei* over a short period. However, because of species differences, such as survival strategy, the mortality of *E. possjeticus* increases with time and its tolerance to carbonate alkalinity declines considerably relative to *P. vannamei*.

Another important ecological factor affecting the survival of cultured organisms is pH. Aquatic organisms all have a pH tolerance range and an optimal range. When pH in the external environment deviates outside the optimal range, it will affect the normal physiological activities of aquatic organisms and can even cause death (Wu et al., 2012). Our results found that greater deviations of water pH from the control group led to higher mortality rates for *E. possjeticus*; as time elapsed, the cumulative mortality of *E. possjeticus* also increased gradually. These observations are consistent with previous findings from crustaceans, such as *Palaemonetes sinensis* (Wang et al., 2002; Jiang et al., 2017). This study obtained 24 h, 48 h, 72 h, and 96 h L_{pH50} values for *E. possjeticus* of 9.91, 9.37, 8.98, and 8.70, respectively. *E. possjeticus* exhibits a relatively low tolerance to pH compared with shrimp, such as *P. sinensis* (96 h L_{pH50}=10.52) (Jiang et al., 2017). This low pH tolerance is closely associated with its species characteristics, suggesting that *E. possjeticus* is more sensitive to environmental change.

4.2 Effects of coupled salinity, carbonate alkalinity, and pH on feeding and respiratory metabolism

Aquatic animal feeding behavior directly reflects bodily response to the external environment. For environmental changes within a certain range, such as salinity, carbonate alkalinity, and pH fluctuations, aquatic animals can alter their metabolic status via ion regulation and the activation or shutdown of the intracellular free amino acid pool, leading to a continuous increase in metabolism and ingestion rate. However, once changes in environmental conditions exceed a suitable range, the result is an inhibitory effect on the metabolism and ingestion rate (Zhang et al., 2012; Cai et al., 2017). It has been shown that feeding, growth, and feed utilization efficiency of fish are closely related to salinity and alkalinity in the aquatic environment. The optimal range of environmental salinity and alkalinity levels is beneficial for feeding, growth, and feed utilization in fish; however, when these exceed the suitable range, feeding, growth, and feed utilization efficiency all decrease. This phenomenon may be because salinity and alkalinity affect the digestive enzyme activity and energy allocation in fish (Lv et al., 2007). Feng et al. (2009) found that when salinity and pH exceeded the optimal range for *Brachionus urceus*, it significantly reduced the ingestion rate. Similarly, we observed a downward trend in the ingestion rate and feed absorption efficiency of *E. possjeticus* with increasing carbonate alkalinity, salinity, and pH. This result indicates that the feeding ability of *E. possjeticus* declined under high salinity and alkalinity conditions, which may then affect the normal growth and reproduction of this species.

Different environmental conditions can affect respiratory metabolism and the energy supply mode in aquatic animals. To adapt to aquatic environments with different salinities, diverse ion compositions, and varied pH, aquatic animals must have efficient ionic and osmotic pressure regulation mechanisms (Hwang and Lee, 2007). For example, when some marine fish species, such as *Anguilla japonica* and *Ictalurus nebulosus*, are exposed to isotonic salinity, their oxygen consumption drops to their lowest rates; deviation from isotonic salinity leads to an increase in oxygen consumption rates (He et al., 2016). Similarly, changes in the aquatic carbonate alkalinity affect osmotic pressure equilibrium in bulimia barbel *Barbus capito*, and its oxygen consumption rate increases with increasing alkalinity (Geng et al., 2017). When pH deviates from a certain range, it affects the amount and speed of dissolved oxygen that aquatic animals can acquire from the external environment (Jiang et al., 2017). When pH deviates from the optimal level, aquatic animals change their metabolic conditions to adapt to their external environment, leading to either reduction (Savant and Amte, 1995; Cao et al., 2015) or elevation (Zhang et al., 2009) in respiratory metabolism. In this study, the oxygen consumption rate of *E. possjeticus* increased with increasing salinity, whereas it initially increased and then decreased with increasing carbonate alkalinity and pH. These observations indicate that changes in salinity, carbonate alkalinity, and pH have an impact on the osmotic pressure equilibrium in *E. possjeticus*. For example, when carbonate alkalinity ≤6 mmol/L, *E. possjeticus* increased its metabolism to meet the energy demand; however, when carbonate alkalinity ≥9 mmol/L, respiratory metabolism was inhibited substantially. Moreover, under low carbonate alkalinity conditions, respiratory metabolism first increased and then decreased with increasing pH, whereas under high carbonate alkalinity conditions, respiratory metabolism gradually decreased with increasing pH. These results demonstrate that the same species may adopt different strategies to cope with various environmental stresses (Jiang et al., 2017).

O∶N ratio can reflect the ratio of protein, carbohydrate and fat metabolism of animals in a specific state, and it is an important index to reflect the energy and material utilization of animals (Ruyet et al., 2004). If energy is mainly provided by protein, the value of O∶N ratio is about 7 or less (Mayzaud, 1976). If the energy is provided mainly by protein and fat, the O∶N ratio is about 24 (Ikeda, 1974). If the energy is mainly provided by fat or carbohydrates, the O∶N ratio will be greater than 24 or even infinite (Conover and Corner, 1968). In this study, the analysis of O∶N ratios showed that coupled changes in salinity, carbonate alkalinity, and pH could affect the energy supply mode (i.e. metabolic substrate ratio) in *E. possjeticus*. The O∶N ratio was >24 under low carbonate alkalinity (≤6 mmol/L), high salinity (40), and low pH (8.0), indicating that the energy consumed by *E. possjeticus* was mainly supplied by fats. In contrast, the O∶N ratio was <24 under high carbonate alkalinity (≥9 mmol/L) as well as low carbonate alkalinity (≤6 mmol/L), low salinity (20), and high pH (9.0). This result indicates that the energy consumed by *E. possjeticus* under these conditions was supplied by proteins mainly. The evidence indicates that under different environmental conditions, crustaceans can adjust their energy supply mode to cope with environmental changes (Jiang et al., 2017). In this study, we found that the energy supply mode shifted from fats to proteins under high carbonate alkalinity and pH conditions.

5 CONCLUSION

In this study, the three environmental factors, salinity, carbonate alkalinity, and pH, showed significant effects on the survival, feeding, and respiratory metabolism of *E. possjeticus*. The results suggest that the changes in salinity, carbonate alkalinity, and pH have a measurable impact on the osmotic pressure equilibrium in *E. possjeticus* and affect the energy supply mode. This finding leads us to speculate that the population development of *E. possjeticus* would be limited in aquatic environments with high salinity, alkalinity, and pH values.

6 DATA AVAILABILITY STATEMENT

All data generated and/or analyzed during this study are available on reasonable request from the first author.

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