Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(4): 1085 – 1095 (2024)
www.ejabf.journals.ekb.eg

Effect of Salinity and Temperature on the Biochemical Parameters and Ionic Content of the Shrimp *Macrobrachium nipponense* (De Hann, 1849)

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ARTICLE INFO
Article History:
Received: July 7, 2024
Accepted: July 31, 2024
Online: Aug. 4, 2024

Keywords:
Biochemical parameters,
Stress enzymes,
Ionic content,
Macrobrachium nipponense

ABSTRACT
This study was set out to quantify biochemical parameters in freshwater prawns (*M. nipponense*) raised in four different salinity levels and temperature ranges (20, 25, 30, and 35°C) as well as stress enzymes (ALT and AST), ionic content (Cl, K, Na, and Ca), and osmotic pressure. The Shatt al-Arab River in the Al-Mashab region of southern Iraq was the site of the *M. nipponense* sample collection. This study used a recycling aquarium system (RAS) with sixty-liter plastic containers for each subject. In comparison with the control group, all metrics increased significantly ($P< 0.05$), although biochemical parameters decreased significantly ($P< 0.05$). In addition, a negative relationship was detected between temperature, salinity, total protein, glucose, K, Na, ALT, and AST and a positive relationship with urea, Cl, Ca, and cholestrol.

INTRODUCTION

The species *Macrobrachium nipponense* was first reported as an alien species in the Shatt al-Arab by **Salman (2006)** and was introduced into Iraqi waters through water masses from the Al-Karon River **(Al Maliki, 2016; Al-Maliki, 2017)**. This genus, which includes approximately 200 species, mostly inhabits freshwater environments, though some species can tolerate brackish water. The larval stages of *M. nipponense* are adaptable to varying salinities, allowing them to migrate between brackish and freshwater environments and feed on various aquatic organisms and plant matter **(New, 2002)**. Understanding the optimal conditions for cultivating this species is important, as temperature and salinity are key factors influencing its physiology and osmoregulation.

Researchers are investigating how temperature and salinity affect the biochemical parameters, stress enzymes, and ionic content of *M. nipponense*.

MATERIALS AND METHODS

Experiment design

In the district of Al-Mashab, Shatt al-Arab, northern Basra province, southern Iraq, *M. nipponense* prawns were sampled and directly transported to the laboratory for a 5-day acclimation period. Shrimp measuring 65- 85mm in length were kept in a recycling aquarium system (RAS) composed of 17 plastic containers, each with a 60L capacity. These containers were filled with dechlorinated water maintained at 24°C, with salinity ranging from 2- 7ppt, oxygen levels of 6.0mg/ L, and a pH of 6- 7. The shrimp samples were daily fed a specialized diet containing proteins, carbohydrates, and fats to prepare them for experimentation.

After acclimation, the shrimp were moved to 25 separate 10L aquariums. To prevent osmotic shock, salinity levels were gradually adjusted. The experiment involved four different salinity concentrations (1, 5, 10, and 15ppt) and four temperatures (20, 25, 30, and 35°C). Salinity was adjusted using Chinese salt, and a total of 16 experimental groups, each containing 25 shrimp, plus a control group, were randomly selected (N=500). Once the desired salinity and temperature were established, all aquariums were aerated. Dissolved oxygen, salinity, and temperature were daily monitored. The shrimps were fed, and waste such as feces and unconsumed food was daily removed. The shrimps were observed for ten days **(Buckle *et al.*, 2006)**.

For biochemical analysis, hemolymph samples were collected from the hepatopancreatic gland using a pipet puncher after ten days and were stored at 18°C. Glucose concentration was quantified using the glucose oxidase technique **(Barham & Trinder, 1972)**. Cholesterol levels and total protein were measured using commercial colorimetric kits **(Doumas *et al.*, 1981)**. Stress enzyme levels (AST and ALT) were assessed based on the method of **Reitman and Frankel (1957)**. Urea concentration was determined using urenase at a wavelength of 600nm **(Tietz, 2006)**. Electrolyte concentrations (Cl, Ca, Na, and K) were measured with a chemical autoanalyzer.

Statistical analysis

Statistical analysis was performed using the SPSS program and complete randomized design (CRD). Two-way ANOVA was applied, and the least significant difference (LSD) was determined at a probability level of $P \leq 0.05$ to compare the arithmetic means of the coefficients.

RESULTS

1- Effect of salinity and temperature on biochemical parameters

Fig. (1) illustrates the variations in biochemical parameters of *M. nipponense* across different salinity and temperature conditions. The highest average values for total protein, cholesterol, urea, and glucose in 1ppt salinity were observed at 20°C, with values of 6.0, 29.44, 22.56, and 76.36mmol/ L, respectively. In contrast, at 5ppt salinity, the peak values were higher, with values 6.0, 60.99, 34.36, and 65.8mmol/ L at 20°C.

For salinity levels of 10ppt, the highest average biochemical parameters were noted at temperatures of 30 and 35°C, with values of 4.5, 37.75, 25.03, and 63.63mmol/ L. At 15ppt salinity, the highest values were 4.76, 28.8, 36.83, and 43.30mmol/ L at 20°C.

The results indicate a negative relationship between salinity and temperature with all biochemical parameters ($P < 0.05$), except for cholesterol and urea, which exhibited a positive relationship. Compared to the control group, there was a significant decline in all biochemical parameters at a level of ($P < 0.05$).

Table (1) summarizes the correlation of biochemical indicators (glucose, cholesterol, urea, and total protein) with temperature and salinity during the experimental period for *M. nipponense*.

Table 1. the correlation of indicators of biological compounds with environmental factors in river shrimp *M. nipponense*

Biochemical parameter	**Salinity**		**Temperature**	
	r	**Sig**	**R**	**Sig**
Total protein	-0.221	0.118	-0.252	0.075
Cholesterol	0.069	0.631	-0.256	0.07
Urea	0.091	0.526	-0.347	0.013
Glucose	-0.293	0.037	-0.027	0.852

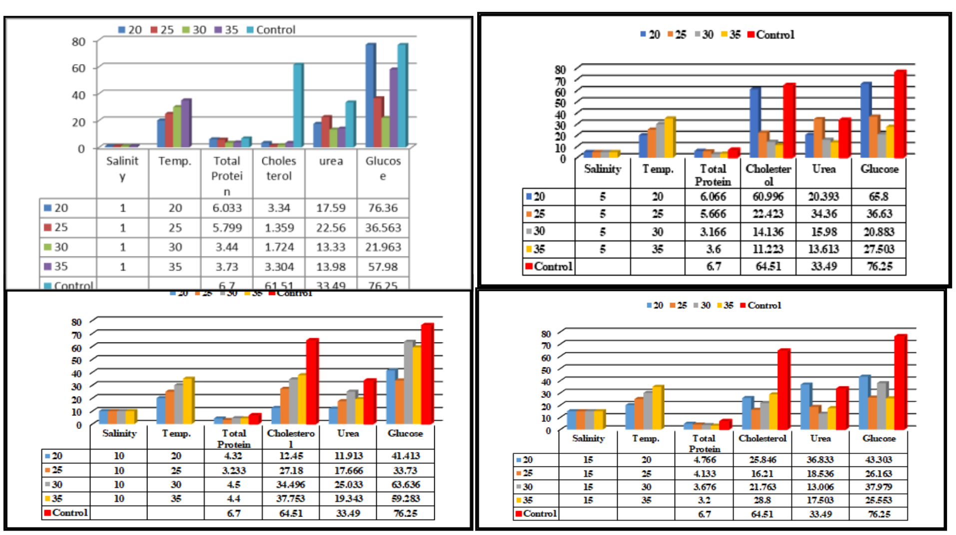

Fig. 1. Effect of salinity and temperature on biochemical parameters in hemolymph of *M. nipponense*

2- Effect of salinity and temperature on stress enzymes (ALT & AST)

Fig. (2) illustrates the impact of salinity and temperature on stress enzymes ALT and AST in *M. nipponense*. The data show that the enzyme levels were significantly higher ($P < 0.05$) compared to the control group, with values of 337.6U/ L for ALT and 268.6 U/L for AST. The highest recorded levels of the ALT enzyme were 477.13, 575.36, 566.75, and 392.06U/ L, corresponding to shrimp exposed to salinities of 1, 5, 10, and 15ppt, respectively. Similarly, the highest levels of the AST enzyme were 367.83, 596.86, 511.33, and 458.36U/ L under the same salinity conditions.

Moreover, the results revealed an inverse relationship between both stress enzymes, ALT and AST, and the levels of salinity & temperature.

Table (2) presents the correlation of enzyme indicators (AST and ALT) with temperature and salinity during the experimental period for *M. nipponense*.

Table 2. The correlation of stress enzyme indicators with environmental factors in river shrimp

Enzyme	Salinity		Temperature	
	r	Sig	r	sig
AST	-0.173	0.224	-0.01	0.032
ALT	-0.311	0.026	-0.495	0.000

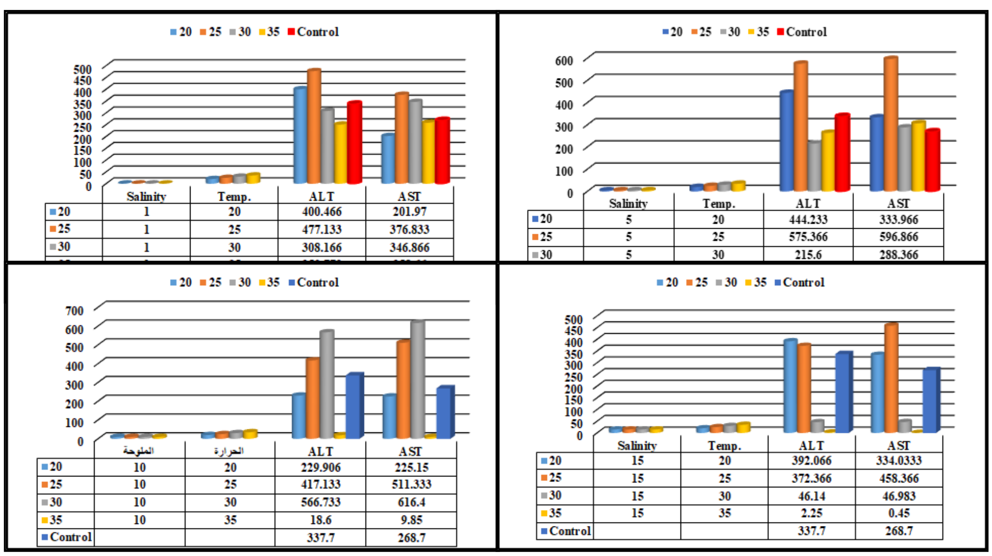

Fig. 2. Effect of salinity and temperature on the ALT & AST levels in the hemolymph of *M. nipponense*

3. Effect of salinity and temperature on ionic content of hemolymph in *M. nipponense*

Fig. (3) illustrates the ionic content (Cl, K, Na, and Ca) in the hemolymph of *M. nipponense* exposed to various salinities and temperatures. The highest ionic values were recorded at 1ppt salinity with 204.3, 8.5, 196, and 39.18mmol/ L for Cl, K, Na, and Ca, respectively, at 25°C. For 5ppt salinity, the highest values were 223.3, 9.4, 210.3, and 44.0mmol/ L at 20 and 30°C. At 10ppt salinity, the highest ion values were 180.5, 6.05, 156.6, and 47.02mmol/ L at 35°C. At 15ppt salinity, the highest values for all ions (except Ca) were observed at 25°C, with readings of 243.3, 8.5, 221.6, and 53.7mmol/ L, while Ca was higher at 35°C.

The data also showed a positive correlation between temperature and salinity with Ca and Cl. Conversely, K and Na demonstrated an inverse relationship. Compared to the control group, there was a significant reduction in total ionic content ($P < 0.05$).

Table (3) provides the correlation of ionic contents (calcium, sodium, potassium, and chlorine) with temperature and salinity during the experimental period for *M. nipponense*.

Table 3. The correlation of ionic contents in the eastern river shrimp *M. nipponense*

Ionic	Salinity		Temperature	
	r	Sig	r	Sig
Calcium	0.45	0.001	0.11	0.424
Sodium	-0.00	0.952	-0.02	0.852
Potassium	-0.15	0.289	0.00	0.969
Chloride	0.06	0.647	0.14	0.302

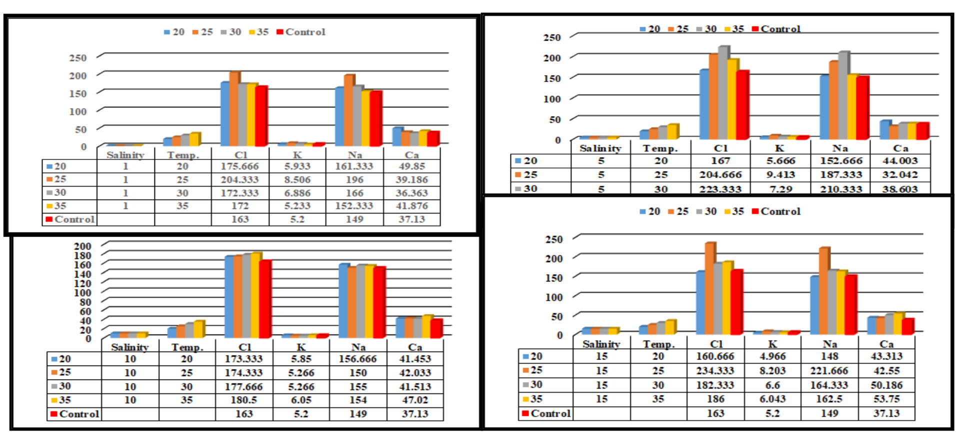

Fig. 3. Effect of salinity and temperature on ionic content in hemolymph of *M. nipponense*.

DISCUSSION

One of the most influential abiotic influences on aquatic ecosystems is water temperature, which in turn affects biological populations via temperature changes **(Bonacina *et al*., 2023)**. According to **Abdelrahman *et al*. (2019)**, differences in water temperature patterns greatly affect the development and survival of prawns in ponds and the concentration of salt due to its effects on crustacean physiological metabolism, survival, and dispersion. It is a significant environmental component. While crustaceans may survive in a variety of salt environments, they are susceptible to mortality when exposed to sudden changes in salinity. Nonetheless, *M. nippoenense* is able to thrive in environments with salinities ranging from 7 to 20ppt.

According to **Fan *et al.* (2023)**, *M. nipponense* can adapt to environmental changes and withstand salinity from 1 to 15ppt. **Wang *et al.* (2023)** studied osmolarity, physiological metabolism, antioxidant capacity, and apoptosis in *M. rosenbergii* during 96h of acute salinity stress and found that the response was ambiguous. **Guest and Durocher (1979)** examined *M. amazonicum* larval survival at 25°C with salinity between 0 and 15%. In their laboratory study, **Allan *et al*. (2006)** found that salinity affected larvae and molting levels in *Macrobrachium amazonicum* at temperatures near the upper limits of survival. Temperature and salinity are crucial to marine and brackish water life. The two elements typically interact in a complicated way because temperature may affect salinity's effects, changing the organism's tolerance range; moreover, salinity can change temperature's effects.

The study investigated several bioactive compounds in river shrimp, including glucose, cholesterol, total protein, and urea. Glucose, a primary energy source, is involved in aerobic and anaerobic respiration and fermentation in most living organisms **(Boerio *et al*., 1991)**.

In this study, glucose concentrations varied significantly with different salinity levels. The highest glucose concentration, 76.36, was observed at 1ppt salinity and a temperature of 20°C, which is consistent with the findings of **Wang *et al.* (2023)**. In addition, significant differences in glucose levels were noted with varying temperatures and the combined effect of different salinity levels and temperatures ($P < 0.05$). However, there were no significant differences observed with salinity alone, contrary to **Rahi *et al.* (2021)**, who reported significant increases ($P < 0.05$) in glucose and serotonin levels across all five salinity treatments (0, 2.5, 5, 10, 30‰) compared to the control salinity (20‰).

Cholesterol, an essential component of cell membranes in all animal cells, contributes to membrane fluidity, protection, and cellular shape. Unlike plants, which have rigid cell walls, animal cells rely on cholesterol to maintain membrane flexibility and functionality **(Yeagle, 1991)**. In this study, cholesterol levels peaked at 20°C and a salinity of 25. Additionally, the results revealed a non-significant inverse relationship with temperature and a non-significant positive relationship with salinity.

However, significant differences were observed when temperature and salinity were combined ($P< 0.05$). Proteins serve as biochemical indicators that reflect the environmental conditions to which an organism is exposed. The study found that the highest total protein levels were at salinity levels of 1 and 5ppt and a temperature of 20°C (6.0g/ dl). This result aligns with **Khanjani *et al.* (2020)**, who reported an increase in protein and fat content at the lowest salinity levels ($P< 0.05$).

For urea concentration, the highest percentage, 36.8, was recorded at a salinity level of 15ppt. This finding match that of **Chen and Chia (1996)**, who observed that urea levels increased with salinity, and ammonia levels increased with decreasing salinity, while nitrogen secretion increased with temperature. The current study showed a non-significant inverse relationship with temperature, a non-significant positive relationship with salinity, and significant differences when both temperature and salinity were combined.

The results also support **Chung and Jiann (2003)**, who found that in *Marsupenaeus japonicus* shrimp, urea levels increased in the liver, gills, intestines, and muscles at high salinities (42ppt) and decreased at low salinities (18ppt).

Metal ions play crucial roles in biological systems, participating in processes such as material transport, energy conversion, information transfer, and metabolic regulation **(Zheng *et al.*, 2020)**. Essential metals include sodium, potassium, calcium, magnesium, iron, cobalt, zinc, nickel, vanadium, and molybdenum, many of which are involved in enzyme catalysis **(Permyakov, 2021)**.

This study included sodium, potassium, calcium, and chloride ions. According to **Niu *et al.* (2023)**, calcium ions are vital for numerous cellular processes and are among the most prevalent mineral ions in nature. Calcium acts as a signaling molecule in animal tissues and fluids, regulating nearly every critical cellular process **(Carafoli & Krebs, 2016)**.

The results showed that calcium ion levels were the highest at 35°C and a salinity of 15ppt, reaching 20.1mmol/ L. Additionally, at a salinity of 1ppt and a temperature of 20°C, calcium levels in the hemolymph of the treated group were 49.85mmol/ L compared to the control group. The study found a non-significant negative relationship with temperature and a moderate negative relationship with salinity when both factors were elevated ($P< 0.05$).

Jaffer *et al.* (2020) suggested that total calcium levels in the white shrimp *L. vannamei* are maintained regardless of salinity, as calcium may not be involved in communication regulation mechanisms.
Sodium (Na^+) and potassium (K^+) ions are essential for maintaining fluid balance and supporting neuronal and muscular functions. Potassium is prevalent inside cells, while sodium is found outside **(Permyakov, 2021)**. In this study, potassium ion levels in the

hemolymph reached 8.20mmol/ L at 25°C and 15ppt salinity, and 9.4mmol/ L at 25°C and 5ppt compared to the control group. There was no significant relationship between temperature and salinity alone, but significant differences were observed when both factors were considered together ($P< 0.05$).

As the most common anion in extracellular fluid, chloride is vital for digestion, muscle function, fluid balance, and acid-base regulation. It plays a key role in maintaining electrical neutrality **(Bohn & de Morais, 2016)**. The highest chloride ion concentration in the hemolymph was observed at 25°C and 15ppt salinity (234.33mmol/ L) and at 30°C and 5ppt salinity (223.3mmol/ L). **Jiann and Jun (1994)** found that chloride concentration increases with salinity and decreases with temperature. This study found a positive correlation between chloride ions and both temperature and salinity, with significant differences observed when these factors were combined ($P< 0.05$).

The osmolarity of the hemolymph in *M. nipponense* increased to 411.5mol/ L with a salinity of 15ppt and a temperature of 20°C. These results concur with those of **Buckle *et al.* (2006)**, who studied *Lipopenaeus vannamei* under varying temperature and salinity conditions.

To assess the impact of environmental stress on internal structures, liver and pancreas tissues were examined. The study found that AST enzyme levels were at their highest at 10ppt salinity and 30°C, and also at 5ppt salinity and 20°C. **Yang *et al.* (2020)** reported that liver and pancreatic enzymes are most active at 24°C, significantly more than at other temperatures ($P< 0.05$). Although temperature and salinity alone showed a non-significant negative correlation, their combined effect resulted in significant variations ($P< 0.05$).

For the ALT enzyme, the highest concentration was found at 5ppt salinity and 25°C, and at 10ppt salinity and 30°C. The results indicate a significant negative relationship with temperature, a non-significant inverse relationship with salinity, and significant differences when temperature and salinity were combined ($P< 0.05$). The liver and pancreas play crucial roles in secretion, molting, metabolism, and energy storage in crustaceans.

CONCLUSION

The study examined the effects of salinity and temperature on various biochemical parameters (total protein, urea, cholesterol, and glucose), ionic content (Cl, K, Na, and Ca), stress enzymes (ALT and AST), and osmolarity in *M. nipponense*. The shrimp samples were reared under four temperature conditions (20, 25, 30, and 35°C) and four salinity levels (1, 5, 10, and 15ppt). The findings revealed that all biochemical parameters decreased compared to the control group, while ALT and AST levels increased. Additionally, a negative relationship was observed between temperature, salinity, and total protein, glucose, potassium (K), sodium (Na), ALT, and AST. Conversely, a positive relationship was found with urea, chloride (Cl), calcium (Ca), and cholesterol.

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