



Effect of salinity and temperature on the biochemical parameters and ionic content of the shrimp *Macrobrachium nipponense* (De Hann, 1849)

Marwa Ali Majeed Al- Obaidi^{1*} and Manal Mohammed Akbar¹

Biology Department – College of Education of pure science – University of Basrah –
Basrah – Iraq

*Corresponding Author: pgs.marwa.ali@uobasrah.edu.iq

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ABSTRACT

This study 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 to the control group, all metrics increased significantly ($p < 0.05$), although biochemical parameters decreased significantly ($p < 0.05$). The results also showed a negative relationship between temperature, salinity, total protein, glucose, K, Na, ALT, and AST, while a positive relationship with Urea, Cl, Ca, and Cholestrol.

INTRODUCTION

The species *M. nipponense* was considered an alien species to our inland water. and showed for the first time in Shatt Al-Arab by **Salman (2006)** and it's entered Iraqi water by drifting with water masses coming from Al-Karon River to Shatt Al-Arab, and to inland water (**Al Maliki, 2016; Al-Maliki,2017**).

This genus *Macrobrachium* includes 200 species most of which live in fresh water, and some spend part or most of their life in brackish Waters, so the larval stage tolerates wide ranges of changes in salinity so that they migrate from brackish water to fresh water, and

feed on different animals and plants parts (New, 2002). Understanding the ideal conditions for raising this species, which may alter during growth, is crucial since temperature and salinity are major variables influencing the physiology and osmoregulation of prawns.

Researchers set out to determine the effect of temperature and salinity on biochemical parameters, stress enzymes and ionic contents of *M. nipponense*.

MATERIALS AND METHODS

Experiment Design:

Al-Mashab, a district of Shatt Al-Arab in the northern Basra province of southern Iraq, was the site of the prawn sample (*M. nipponense*). Samples were transported directly to the laboratory to acclimated for 5 days. Shrimp of (65-85) mm in length were maintained in a Recycling Aquarium System (RAS). It consists of 17 plastic containers, each of 60 L with dechlorinated water (24 °C, 2-7 ppt, 6.0 O₂ mg/L, pH 6-7), and fed daily with a special diet (Protein, Carbohydrate, Fat) to prepared the shrimp for the experiment. After acclimation, shrimp was transferred from (RAS) to 25 aquariums of 10 L. In order to avoid osmotic shock, the salinity was changed gradually until the experiment of four different concentrations of salinity (1, 5, 10, and 15) ppt and four temperatures (20, 25, 30, and 35) °C was completed. The addition of Chinese salt obtained the salinity concentration. A total of 16 groups of 25 shrimp per group, in addition to the control were chosen at random (N=500). Once adjustments to desired salinity and temperature were completed, all aquariums were aerated. Dissolved oxygen, salinity, and temperature were measured daily, and the shrimp fed, feces, and unconsumed food were removed from the aquarium daily. The shrimp remain for ten days (Buckle *et al.*, 2006). Biochemical parameter: Shrimp of each combination of salinity and temperature concentration after ten days were used to collect the hemolymph sample from the Hepatopancreatic gland by using a pipet puncher in the abdominal membrane stored at 18 °C, and this hemolymph will measure many parameters. Barham and Trinder (1972) described the glucose oxidase technique as a means to quantify glucose concentration. Dumas *et al.* (1981) assessed cholesterol levels and total protein using commercial colorimetric kits. Our stress enzyme AST and ALT measurements were based on the work of Reitman and Frankle (1957).

The spectrophotometer was used to read the sample at a wavelength of 550 nm. Urea concentration was measured by acting of urenase at a wavelength of 600nm (Tietz, 2006). Also, the electrolyte concentration (Cl, Ca, Na, and K) was measured by a chemical autoanalyzer.

Statistical analysis

The program (SPSS) and Complete Randomized Design (CRD) were used to analyze the results statistically. Tow-Way Anova was used, and the value of the least significant difference (LSD) was extracted at the probability level $p \leq 0.05$ to compare the arithmetic means of the coefficients.

RESULTS

Effect of salinity and temperature on Biochemical Parameters:

Figure 1. showed changes in biochemical parameters in different salinity and temperature. So, it showed that the highest average values in total protein, cholesterol, urea, and glucose in 1 ppt were (6.0, 29.44, 22.56, and 76.36) mmol/L in (20) °C, respectively. In comparison, the highest values in 5 ppt were (6.0, 60.99, 34.36 and 65.8) mmol/L in (20) °C respectively. While, The higher average biochemical parameters values at temperatures 10 ppt with 30 °C and 35 °C were (4.5, 37.75, 25.03, and 63.63) mmol/L, respectively. In comparison, at 15 ppt, the high values were (4.76, 28.8, 36.83, and 43.30) mmol/L at 20 °C respectively. The results also showed negative relationship between salinity and temperature with all biochemical parameters in level ($p < 0.05$) except cholesterol and urea have a positive relationship.

In comparison to the control group, all biochemical parameters showed a significant decline at level ($p < 0.05$) in the values.

Table 1. shows the correlation of indicators of biochemical compounds in the eastern river shrimp *M. nipponense* (glucose, cholesterol, urea, and total protein) with temperature and salinity during the experimental period.

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

biochemical parameters	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

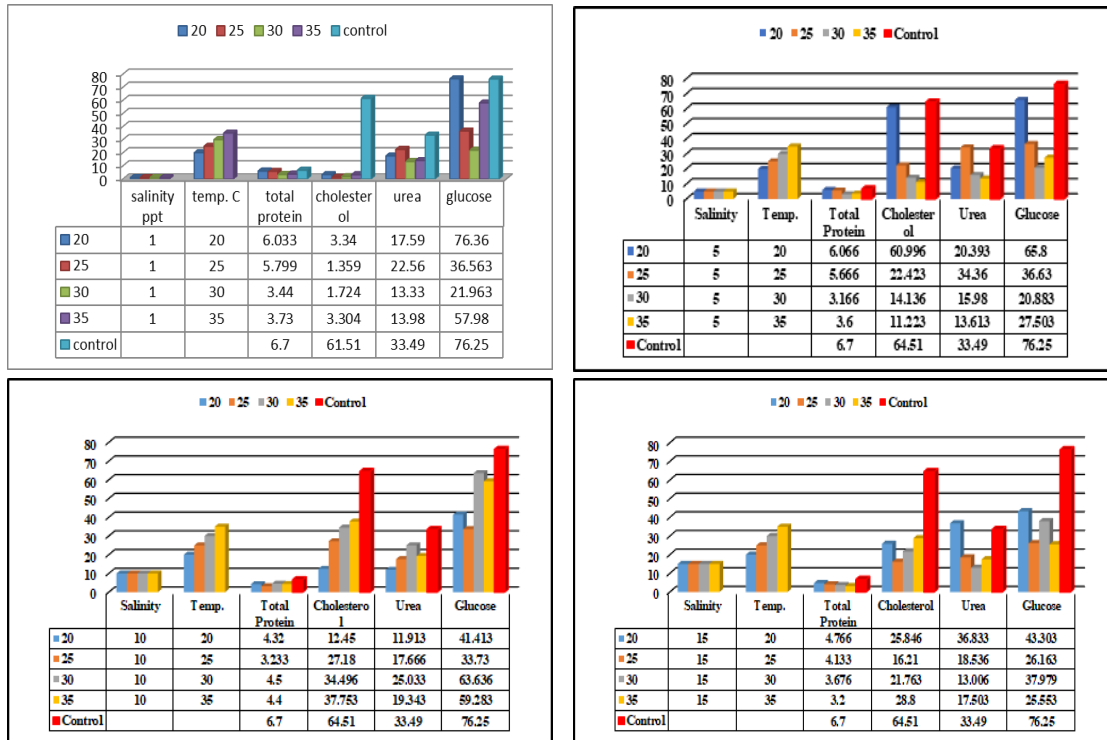


Fig. 1. Effect of salinity and temperature on Biochemical Parameters in hemolymph of *M. nipponense*.

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

Figure 2. Shows the effect of salinity and temperatures on stress enzymes ALT and AST. The data revealed that the values of enzymes “were higher (p<0.05) than the control group” (337.6 and 268.6) U/L. The highest levels of ALT enzyme were (477.13, 575.36, 566.75, and 392.06) U/L. In contrast, the highest levels of AST enzyme were (367.83, 596.86, 511.33, and 458.36) U/L for the shrimp *M. nipponense*, which was exposed to salinity (1, 5, 10, and 15) ppt, respectively. Also, the results showed an inverse relationship between both stress enzymes ALT and AST and salinity and temperature groups.

Table 2. shows the correlation of enzyme indicators in the eastern river shrimp *M. nipponense*, represented by AST and ALT, with temperature and salinity during the experimental period.

Table 2. Shows the Correlation of stress enzyme indicators with environmental factors in river shrimp.

Enzymes	Salinity		Temperature	
	R	Sig	R	sig
AST	-0.173	0.224	-0.301	0.032
ALT	-0.311	0.026	-0.495	0.000

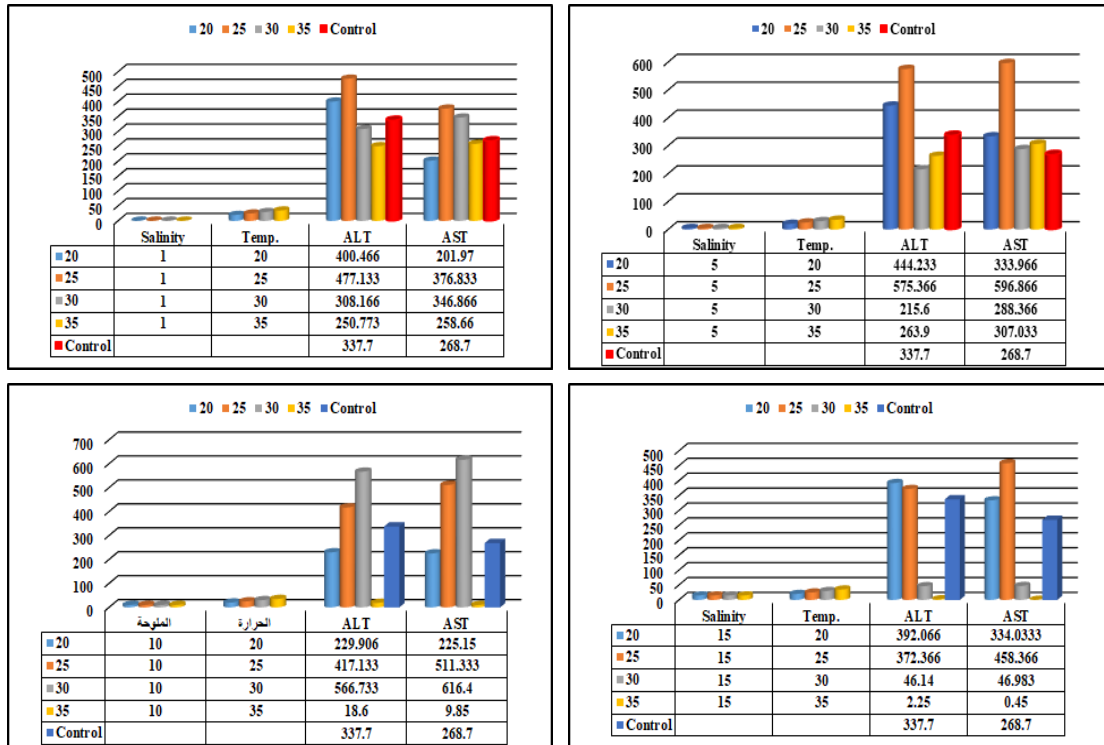


Fig. 2. Effect of salinity and temperature on the ALT & AST levels in the hemolymph of *M. nipponense*.

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

Figure 3. displays the ionic content (Cl, K, Na, and Ca) “in the hemolymph of *M. nipponense* exposed to different salinity and temperature”. The highest values were for salinity 1ppt treatment (204.3, 8.5, 196, and 39.18) mmol/L at (25) °C, respectively. In contrast, for 5 ppt, the highest values were (223.3, 9.4, 210.3, and 44.0) mmol/L at 20 and 30 °C, respectively. The results showed that at 10 ppt, the ions values were higher (180.5, 6.05, 156.6, and 47.02) mmol/L at 35 °C respectively, however at 15 ppt, the results

indicated the values of all ions were higher ($p < 0.05$) at 25 °C except Ca which is high in 35 °C (243.3, 8.5, 221.6 and 53.7) mmol/L respectively. Additionally, for Ca and Cl, the data demonstrated a positive correlation with temperature and salinity. While, for K and Na, the inverse was true. In comparison to the control group, the findings demonstrated a significant reduction in total ionic content ($p < 0.05$).

Table 3. shows the correlation of ionic contents in the eastern river shrimp *M. nipponense* (calcium, sodium, potassium, and chlorine) with temperature and salinity during the experimental period.

Table 3. shows 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

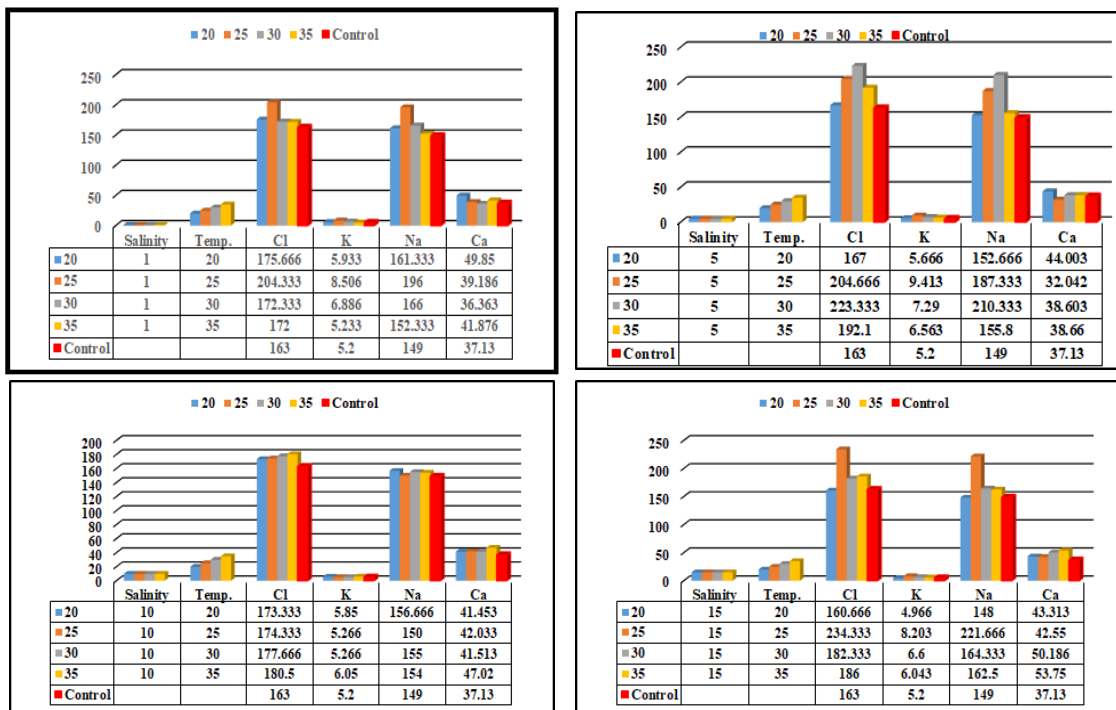


Fig. 3. Effect of salinity and temperature on ionic content in hemolymph of *M. nipponense*.

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 because of 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. nipponense* is able to thrive in environments with salinities ranging from 7 to 20 ppt.

According to Fan et al. (2023), *M. nipponense* can adapt to environmental changes and withstand salinity from 1 to 15 ppt. Wang et al. (2023) studied osmolarity, physiological metabolism, antioxidant capacity, and apoptosis in *M. rosenbergii* during 96 h 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, which changes the organism's tolerance range, and salinity can change temperature's effects.

The effect of temperature and salinity on biochemical parameters Some bioactive compounds in river shrimp (glucose, cholesterol, total protein, urea) were studied. Glucose is the main source of energy and the processes of aerobic and anaerobic respiration and fermentation in most living organisms (Boerio et al., 1991).

In the current study, glucose concentrations showed a significant difference in different salinity concentrations. The highest value was at 1 ppt at a temperature of 20°C, reaching 76.36, which agrees with a study (Wang et al., 2023). Glucose showed significant differences with different temperatures. The combined effect of different salinity concentrations with different temperatures ($P < 0.05$). There were no significant differences with salinity only, and this does not agree with what (Rahi et al., 2021) indicated in their study, which showed all five salinity treatments (0‰, 2.5‰, 5‰, 10‰, 30‰) showed a significant increase ($P < 0.05$) in glucose and serotonin levels compared to the control salinity (20‰). Cholesterol is an essential composition in the cell membrane of all animal cells. Cholesterol makes the cell membrane soft, protects the cell, and maintains its survival by changing its shape and allowing animals to move, unlike plants, which are restricted to the plant cell wall (Yeagle, 1991). The results of the current study showed that cholesterol reached its highest value at a temperature of 20°C and a salinity of 25. The results also showed that there is a non-significant inverse relationship with temperature and a non-significant positive relationship with salinity. However, there are significant differences when combined with temperature and salinity ($P < 0.05$). Proteins are characterized as biochemical indicators that are relied upon to detect the conditions to which an organism is exposed in its environment. The results showed that the highest rate of total protein level was at a salinity level of 1 & 5 ppt and a

temperature of 20°C (6.0 g/dl). This is consistent with (Khanjani *et al.*, 2020), who showed in the statistical analysis that the biochemical analysis of the shrimp body composition increases in protein and fat content at the lowest salinity level ($P < 0.05$).

The results of the current study showed that the highest percentage of urea concentration at the salinity level of 15 ppt reached 36.8. This is consistent with the study of (Chen and Chia, 1996), who reported that when crabs were exposed to nine different temperatures and salinity regimes, ammonia increased with decreasing salinity, urea increased with increasing salinity, and nitrogen secretion increased with increasing temperature. The results showed a non-significant, inverse relationship with temperature, a non-significant positive relationship with salinity, and the presence of significant differences when exposed to the combined effect of temperature and salinity.

The study also agreed with (Chung Lee and Jiann-Chu Chen 2003), who explained that measuring the levels of urea and uric acid in *Marsupenaeus japonicus* shrimp acclimated to different degrees of salinity observed an increase in urea in the liver, gills, intestines, and muscles at high salinities of 42 ppt and a decrease in low salinity of 18 ppt.

Metal ions are widely found in biological systems and participate in many important biochemical processes, such as transporting materials, converting energy, transferring information, and regulating metabolism in the bodies of living organisms (Zheng *et al.*, 2020).

There are ten or twelve metals—sodium, potassium, calcium, magnesium, iron, cobalt, zinc, nickel, vanadium, and molybdenum—required for life, and many metal ions, particularly transition metal ions, play a role in the catalysis of enzymes (According to Permyakov, 2021).

Sodium, potassium, calcium, and chloride ions were all a part of this investigation. According to Niu *et al.* (2023), calcium ions are crucial for several vital cellular processes. They are among the most prevalent mineral ions in nature. Calcium is a signaling molecule in animal tissues and fluids that regulates almost every critical cellular process (Carafoli and Krebs, 2016).

The results showed that the highest level of calcium ion at a temperature of 35°C and a salinity level of 15 parts per thousand reached 20.1 mmol/L, as well as the level of salinity of 1 part per thousand at a temperature of 20°C, which amounted to 49.85 mmol/L in the hemolymph of the treated group when compared with the control group. The results showed that there was a non-significant negative relationship with temperature and a negative moderate relationship with salinity when exposed to higher temperature and salinity together ($P < 0.05$).

Jaffer *et al.* (2020) suggested that total calcium levels are maintained regardless of salinity levels because calcium may be unrelated to communication regulation mechanisms in the white shrimp *L. vannamei*.

Sodium and Potassium To sustain bodily fluid equilibrium and neuron and muscle function, living organisms need alkaline metal ions Na^+ and K^+ . Potassium ions are prevalent within cells, and sodium ions outside (Permyakov, 2021). The hemolymph potassium ion of the group treated at 25°C and 15 parts per thousand salinity reached 8.20 mmol/L. At 25°C and 5 parts per thousand, it reached 9.4 mmol/L compared to the control group.

Results indicate no significant link between temperature and salinity but substantial differences ($P < 0.05$) when considered together. As the most prevalent anion in extracellular fluid, chloride is vital for digestion, muscular action, bodily fluid management, and acid-base balance. Chloride is crucial to electrical neutrality (Bohn and de Morais, 2016).

The group treated at 25°C and 15 ppt salinity had the greatest chlorine ion rate in the hemolymph, 234.33 mmol/L, and at 30°C and 5 ppt, 223.3 mmol/L. Jiann-Chu and Jun-Len (1994) found that salinity raises chlorine concentration while temperature reduces it. The research found a positive correlation between chloride ions and temperature and salinity, with significant differences seen when combined ($P < 0.05$).

The current study showed the osmolarity of the hemolymph of *M. nipponense* increased (411.5 mol/L) with an increase of salinity (15ppt) and decrease in temperature (20 °C) that results agree with the study of Buckle *et al.* (2006) on *Lipopenaous vannamei* at different temperature and salinity. Liver and pancreas tissues are used to know the effect of a stressful environment on an organism and to show changes in the internal structure in the early stage of environmental stress.

The present research found that enzyme (AST) concentration was maximum at 10 ppt salinity and 30°C and 5 ppt salinity and 20°C. Yang *et al.* (2020) found that liver and pancreatic enzymes work at 24°C. Significantly greater than other temps ($P < 0.05$). Although temperature and salinity have a non-significant negative connection, the combined impact of varied temperatures and salinities results in significant variations ($P < 0.05$). Temperature and salinity affect the ALT enzyme. The liver and pancreas aid crustaceans in secretion, molting, metabolism, and energy storage. The present investigation found that enzyme concentration was maximum at 5 ppt salinity at 25°C and 10 ppt at 30°C. Results indicate a substantial negative association with temperature, a non-significant inverse relationship with salinity, and significant differences when combined ($P < 0.05$).

CONCLUSION

The study showed the effect of salinity and temperature groups on the biochemical parameters (Total protein, Urea, Cholesterol and Glucose) and Ionic content (Cl, K, Na and Ca) and stress enzymes ALT and AST in addition to Osmolarity of *M. nipponense* which reared in four temperatures (20, 25, 30 and 35) C and four salinity (1, 5, 10 and 15) ppt.

All biochemical parameters were decrease compared to control group, while increase in ALT & AST compared to control group.

The results showed also negative relationship between temperature, salinity and total protein, glucose, K, Na and ALT and AST however a positive relationship with Urea , Cl , Ca and Cholesterol.

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