# Estimation of some hemolymph components in the swimming blue crab *Portunus segnis* and the invasive crab *Eriocheir hepuensis* in the Shatt al-Arab and northeastern Arabian Gulf, Iraq

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**Abstract**

Seasonal fluctuations in the concentrations of the enzymes alanine transaminase ALT and aspartae aminotransferase AST in males of *Portunus segnis* reached 28.5, with the lowest values being 12.2. The greatest concentrations of the AST enzyme were 24.3, with the lowest concentrations being 15.3. Females had the highest ALT enzyme values of 27.9 and the lowest readings of 12.3. The highest values for AST enzyme concentrations were 17.8 and the lowest values were 8.2, and the highest values for invasive crab males *Eriocheir hepuensis* were 64.2 and the lowest values were 22.2; the highest values for AST enzyme concentrations were 69 and the lowest concentrations were 28.2, while the highest values for ALT enzyme concentrations were 62.3 and the lowest values were 25.3. The highest AST values were 67.9, while the lowest readings were 22.4. In terms of seasonal changes in total protein, glucose, and cholesterol concentrations in male blue crabs, the highest and lowest total protein concentrations were 7.5 and 4.7, respectively, while the highest and lowest glucose concentrations were 2.9 and 1.2, and the highest and lowest cholesterol concentrations were 1.9 and 0.2, respectively, while in females it reached 1.9 and 0.2. The greatest and lowest total protein concentrations were 5 and 2.6, respectively, whereas the highest and lowest glucose concentrations were 2.3 and 1.4, and the highest and lowest cholesterol concentrations were 0.9 and 0.3. The highest and lowest concentrations of total protein, glucose, and cholesterol in the invasive crab males were 1.4 and 2.9, respectively, as a result of seasonal fluctuations in total protein, glucose, and cholesterol. The highest and lowest glucose concentrations were 9.7 and 5.9, respectively, while the highest and lowest cholesterol readings were 1.8 and 1.0. Females had the greatest and lowest total protein concentrations of 2.7 and 1.4, respectively, whereas the highest and lowest glucose concentrations were 7.0 and 3.0, respectively, and the highest and lowest cholesterol concentrations were 1.9 and 1.3.

**Keywords:** ALT , AST , total protein, glucose, cholesterol

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## Introduction

Branchyura are crustaceans that belong to the order decapoda. There are approximately 6793 species spread across the world's oceans, and there are several species of them, including fresh and marine water, especially in tropical and subtropical regions, and it is an important food source for humans. Shrimps and crabs represent different genera of marine decapods (Sternberg and Cumberlidge, 2001). *P. segnis*, the swimming blue crab, thrives in saline water and prefers muddy and rocky places along beaches, island shores, and some rivers. Adults can also be found in tidal areas, coral reefs, and mangroves, whereas juveniles prefer estuary environments (Corsini-Foka and Kondylatos, 2004). The swimming blue crab enters the estuary water for feeding, protection, and growth, as well as to complete its life cycle, which is mostly dependent on the estuary water, which it uses as an environment for larvae and juveniles' growth and development (Richmond et al; 2002). Adults of *E. hepuensis* can survive in temperatures ranging from 4 to 32° C, especially in winter at 5° C in estuarine waters and at freezing temperatures of 0° C in coastal environments. Because E. hepuensis is one of the animals with a wide salt tolerance euryhline, the first larval stage (zoea1) and the larval stage (zoea2) of the hatched larvae occur when the salinity is between 10-25, and the transformation of the larvae from the third stage occurs when the salinity is between 10-25. (zoea3 to the stage 3). The fifth (zoea5) is found in more saline coastal waters, where it develops into juveniles that migrate to rivers (Anger, 1991). Naser et al (2012) recorded the invasive species *E. hepuensis* in subtropical parts of western Asia (Iraq) and northern Iran (Naderloo, 2014), but its presence in hot tropical regions is limited. The threat that invasive species bring to biology and the ecosystem is what makes them dangerous. In 2021, Yasser et al added another marine crabs record from the north west of the Arabian Gulf, *P. segnis*.

The purpose of this study is to quantify some hemolymph properties by measuring some enzymes and basic components of lymph and comparing them to those of other species in various environment

## Materials and Methods

Between December 2016 and November 2017, samples of swimming *P. segnis* and *E. hepuensis* were obtained seasonaly using trawling nets in various sites of the Shatt al-Arab and northwest of the Arabian Gulf. The samples were kept refrigerated until they arrived at the laboratory.

The fluid (hemolymph) of each animal was withdrawn with a Syringe needle, transferred to a clean 1 ml Abendrov, centrifuged for 15 minutes, and the samples were maintained at a temperature of (-2) C until measurement.

**Alanine transaminase (ALT) and Aspartae aminotransferase (AST) measurements**

The levels of ALT and AST were measured using the kit supplied by Randux - UK company. Put 0.1 ml of the serum sample in a test tube, add to it 0.5 ml of R1 reagent, mix well, and keep for 30 minutes at a temperature of 37 ° C, then add to it 0.5 ml of Reagent R2 and mix the solution well, leave it for 20 minutes at a temperature of 25°C, and then add R3 solution in an amount of 5 ml, and the absorbance of the solution was measured by a spectrophotometer after 5 minutes of mixing it well at a wavelength of 530 nm, and it was calculated AST enzyme level from the following equation: ALT, AST concentration (mmol/L) = Absorbance of the serum sample/ Standard sample absorbance* standard sample concentration.

**Cholesterol Level Measurement**

The enzymatic approach described by Tietz (1995) was used to determine the cholesterol level using a kit supplied by the Biolabo company. After adding 10 l of deionized water to the Blank Reagent tube, 10 l of R3 to the standard tube, and 10 l of serum to the serum sample tube, the tubes were placed in an incubator for 5 minutes at 37 °C or left at room temperature. After ten minutes at room tem-

perature, the total cholesterol concentration was determined using the following equation at a wavelength of 500 nm: • Total cholesterol content in mmol/L=absorbance of serum sample/absorbance of standard sample

**Total Protein Level Measurement**

To determine the concentration of total protein, the procedure described in the test kit supplied by the French company Biolabo was used. Three test tubes were labeled as follows: the sample tube, the protein standard tube, and the reagent tube. Each tube received 1 ml of Biorite R1 reagent and 20 l of deionized water. The ions were transferred to the reagent tube, 20 l of serum was added to the sample tube, and 20 l of standard reagent R2 was added to the protein standard tube. After 10 minutes at room temperature, the absorbance at a wavelength of 550 nm was measured, and the standard total protein concentration was calculated using the following equation:

Total protein content in mmol/L = serum absorbance/standard sample absorbance*.

**Glucose Level Measurement**

The glucose level was determined using a kit provided by Randux - United Kingdom. Three tubes are taken, 500 microliters of serum sample is placed in each tube, and 5 microliters of standard solution is placed in the standard tube. The tubes are shaken well, and incubated at 37 °C for 10 minutes. The absorbance is then measured at a wavelength of 500 nm using a spectrophotometer, and the glucose concentration is calculated using the following equation:

mmol/L glucose concentration = absorbance of serum sample/absorbance of standard sample*.

# Results

**Swimming blue crab *P. segnis***

The enzymes AST and ALT are measured in the lymph. Seasonal fluctuations in the concentrations of alanine transaminase ALT and aspartae aminotransferase AST are shown in Figures 1 and 2. The highest values of ALT enzyme were observed in males of the swimming blue crab *P. segnis* during spring 28.5, while the lowest values were observed during winter 12.2, while the highest values of AST enzyme concentration 24.3 were observed in summer, while the lowest concentrations 15.3 were observed in the fall. Females had the highest ALT enzyme values of 27.9 in the summer and the lowest values of 12.3 in the winter. AST concentrations reached their peak point of 17.8 in the spring and their lowest point of 8.2 in the fall.

**Total protein, glucose, and cholesterol concentrations in lymph**

Figure (3) depicts the seasonal changes in total protein, glucose, and cholesterol concentrations in the lymph of males of *P. segnis*, with the highest and lowest total protein concentrations of 7.5 and 4.7 during the fall and summer, respectively, and the highest and lowest glucose concentrations of 2.9 and 1.2 during the summer and winter, respectively. The lowest concentrations of cholesterol were 1.9 and 0.2 in the winter and summer, respectively, while the highest and lowest concentrations of total protein in females were 5 and 2.6 in the fall and summer, respectively, while the highest and lowest concentrations of glucose were 2.3 and 1.4 in the summer and winter, respectively, and the highest and lowest concentrations of cholesterol were 0.9 and 0.3 in the winter and spring (Fig. 4).

**Invasive crab *Eriocheir hepuensis***

A - Figures 5 and 6 demonstrate seasonal fluctuations in the concentrations of alanine transaminase ALT and aspartate aminotransferase AST in the lymph. The highest values in male of E. hepuensis were reported during the summer 64.2, while the lowest values were recorded during the winter 22.2, and the highest values in AST enzyme concentration were recorded during the spring 69. While females' ALT concentrations were highest in the spring and lowest in the fall, males' ALT concentrations were highest in the spring and lowest in the fall. AST concentrations reached their peak point of 67.9 in the spring and their lowest point of 22.4 in the Fall.

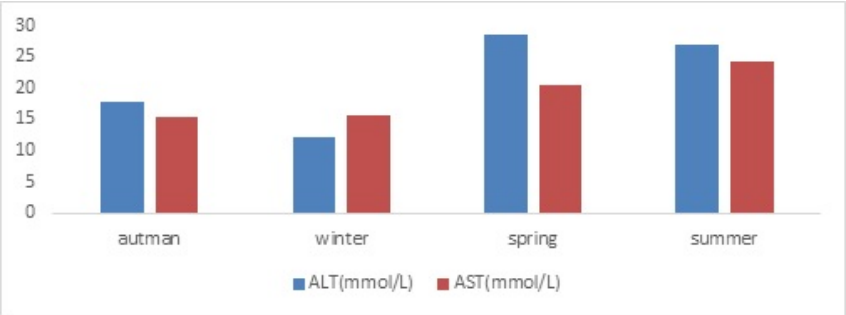


Figure 1. Seasonal changes in ALT and AST enzyme concentrations in male blue crab *P. segnis*

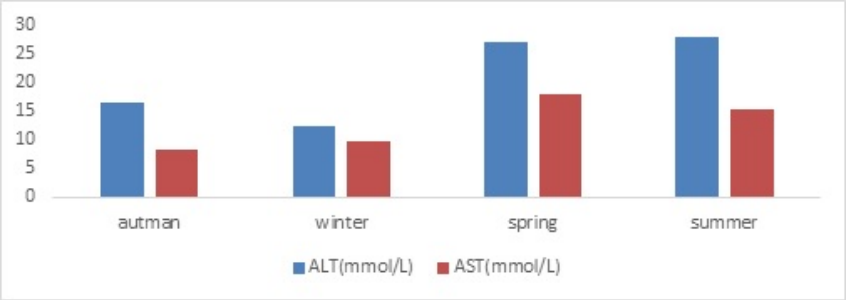


Figure 2. Seasonal changes in ALT and AST enzyme concentrations in female blue crab *P. segnis*

**Total protein, glucose, and cholesterol concentrations in the lymph**

Figure (7) depicts seasonal variations in total protein, glucose, and cholesterol concentrations in the lymph of the male of E. hepuensis. During the fall and spring, the highest and lowest concentrations of total protein were 1.4 and 2.9, respectively, while the highest and lowest concentrations of glucose were 9.7 and 5.9 in the spring and autumn, respectively. During the winter and spring, the highest and lowest concentrations of cholesterol were 1.8 and 1.0, respectively, while the highest and lowest concentrations of total protein in females were 2.7 and 1.4, respectively. During the summer and autumn, the highest and lowest concentrations of glucose were 7.0 and 3.0, respectively, and the highest and lowest concentrations of cholesterol were 1.9 and 1.3. The statistical analysis of data collected during the fall and spring seasons (Fig. 8) revealed that there were no significant differences between male and female concentrations at the P 0.5 level between the seasons.

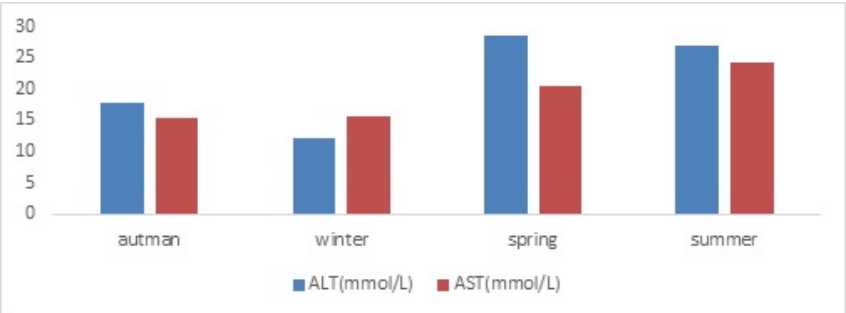


Figure 3. Seasonal changes in the concentrations of total protein, glucose and cholesterol in male blue crab lymphocytes

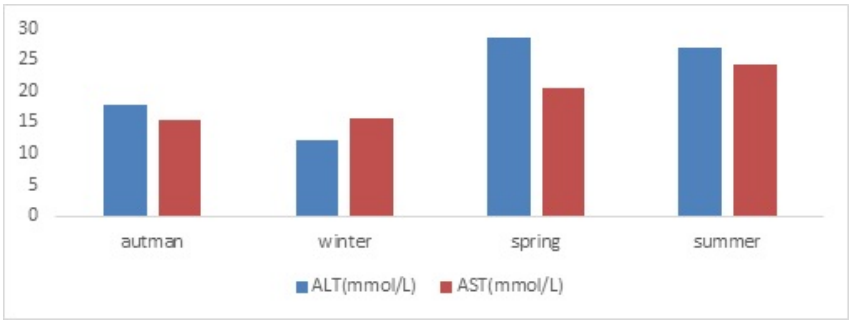


Figure 4. Seasonal changes in the concentrations of total protein, glucose and cholesterol in female blue crab lymphocytes

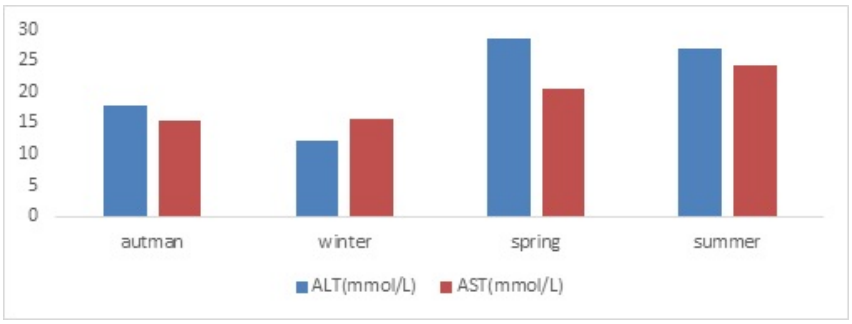


Figure 5. Seasonal changes in the concentration of ALT and AST in male of *E. hepuensis*

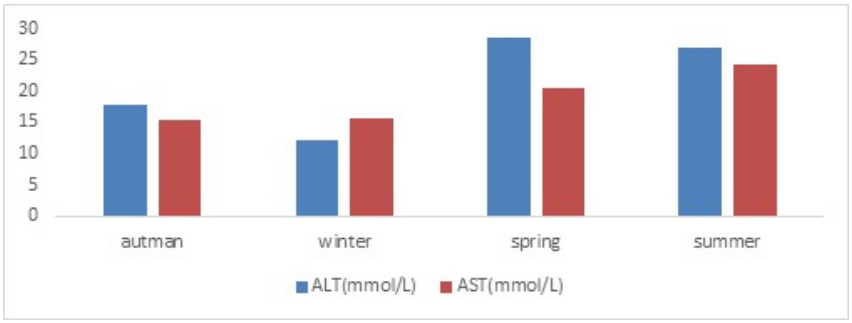


Figure 6. Seasonal changes in the concentration of ALT and AST in female of *E. hepuensis*

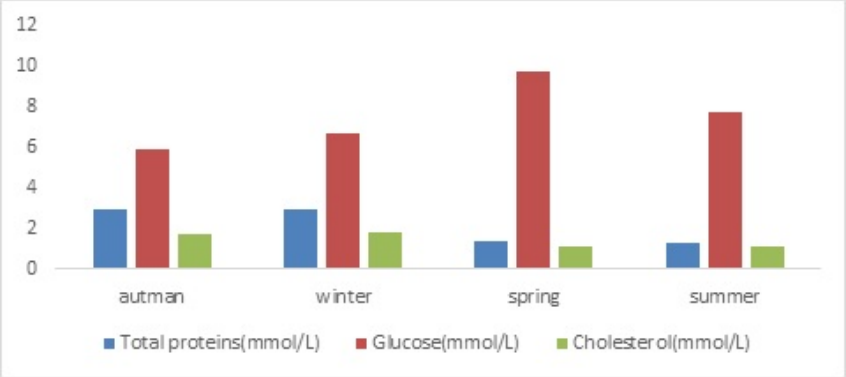


Figure 7. Seasonal changes in the concentrations of total protein, glucose and cholesterol in the lymph of male of *E. hepuensis*

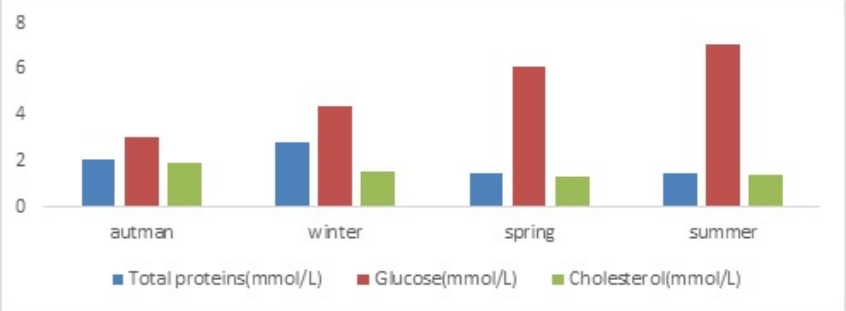


Figure 8. Seasonal changes in the concentrations of total protein, glucose and cholesterol in the lymph of female of *E. hepuensis*

## Discussion

The total hemolymph content of crustaceans is a valuable indicator since it is used to determine the stress levels in the crab animal's environment. Crustaceans have an open circulatory system (Bauchau, 1981), which allows hemolymph to flow freely throughout the body (2000).The current investigation found the maximum concentration of ALT enzyme in the *E. hepuensis* lymph during the spring season. Energy and basic substances are involved in the exchange of many hormones and enzymes involved in the processes of sexual maturation and puberty to the reproductive process, so the demand for energy rises, and the level of amino transfer enzymes, including ALT enzyme, rises to produce pyruvate from the Chris cycle and release the required energy. The results recorded the lowest level of ALT enzyme during the Autumn season in the two study stations, and this is due to the change of environmental factors during this season, and requirement for energy, such as proteins and other nutrients, is lower at the start of the spring season than it is throughout the fall season, therefore it lowers. The requirement for energy causes the enzyme ALT to decrease in activity, which is consistent with a study (Moss et al., 1986) that found that aminotransferases like ALT and AST keep a balanced assembly of free amino acids during protein synthesis.

The AST enzyme, also known as aspartae aminotransferase, is an essential enzyme in amino acid metabolism (Almo et al., 1994). The current study's findings revealed that higher average AST concentrations were recorded in the spring, and that this increase was due to a variety of factors, including an increase in vital and physiological activities as a result of

the high level of nutrition and the start of the reproductive cycle. Because of the decrease in physiological activity, the study found the lowest rate of AST concentration during the fall season. AST is one of the most important enzymes in stimulating the metabolism of amino acids, which is responsible for the formation of important protein compounds in the body, whether they are carriers, receptors, enzymes, or hormones, and is involved in a variety of physiological activities including reproduction, shedding, and sexual maturation.

Total protein is one of the energy sources in crustaceans, so it is directly affected by seasonal temperature variations, which may reduce its content in the crustacean as a result of the increased demand for energy to meet heat stress, and this is consistent with his study (Matozzo et al., 2011), which found that the concentration of total protein decreases when temperatures rise above 30°C. Proteins are the principal source of energy in crustaceans and one of the core components of crustacean hemolymph; they are involved in many critical physiological processes such as growth and development and motion generation (Berg et al., 2002). Furthermore, hemolymph proteins in invertebrates lack immunoglobulin and albumin (Terwilliger et al., 1998), and the total protein level in crustacea is used as an important tool in understanding the physiological processes of these animals when they are exposed to different environmental conditions (Lorenzon et al., 2007).

According to the findings of the current study, the highest concentration of total protein in the hemolymph of *P. segnis* and *E. hepuensis* was found in the fall season. An average of the concentration of total protein during the summer and spring seasons due to changes in environmental factors, particularly temperature, which rises during the summer and, in order to maintain body temperature, decreases movement and nutrition, and reproductive activity ceases, reducing the need for a high concentration of total protein. Its concentration falls, and the current study's findings correspond with (Akbar, 1988; Soundarapandia and Singh, 2008), which confirmed that the biochemical components of the animal vary depending on the season as well as the animal's size. Temperature, food availability, maturity stage, and so on are all influences. According to the findings, the concentration of total protein in *E. hepuensis* hemolymph is higher than in *P. segnis*, which may be attributed to differences in food and environment, as corroborated by Sakhar and Kamble (2014). They suggested that the variation in total protein concentrations in *E. hepuensis* hemolymph could be due to diversity. Size, as well as the surroundings, can play a vital effect in modifying total protein concentration in food or due to sex.

In most species, glucose is the primary source of energy for aerobic and anaerobic respiration (Boerio et al., 1991). Because of the physiological importance of glucose in the life of living organisms, it has been used by many scientists as one of the biochemical criteria approved as a vital evidence of the organism's influence on toxic and polluting substances in the environment, specifically in the aquatic environment ( Rao and Jayshree, 1990). Aquatic creatures are one of the most important sources of energy communicated through hemolymph, which is one of the primary components of hemolymph in invertebrate species, and their effect is usually reflected in the variation in the overall amount of hemolymph. Its content was determined in the hemolymph of *P. segnis* and *E. hepuensis*. The results revealed a seasonal change in Its concentration over the course of the year. The current study found that the maximum rate of glucose concentration occurred throughout the summer and spring, and that it decreased during the winter. This is owing to an increase in cancer physiological activity during the summer, when the animal's reproductive cycle begins. According to Schein et al. (2005), seasonal fluctuation plays a significant role in the glucose production process, and the study's findings support this (Valle et al., 2009).

Cholesterol is a primary energy reserve as well as a limiting factor in crustacean degeneration, and it is required for the physiological and structural relationship of cellular and intercellular membranes to be maintained (Luvizotto

Santos et al., 2003). The decline in metabolic and reproductive activity of both species is related to the decrease in the rate of development, nourishment, and mobility, as well as the level of energy consumed for such processes. The current results show that the rate of its concentration is lowest during the spring and summer, and this is due to an increase in energy demand, as higher temperatures stimulate the processes of growth, reproduction, and egg production in crustaceans, and these vital activities increase the demand for energy, resulting in a decrease in the level of cholesterol, which is one of the important sources of energy. This is consistent with (Vingre et al., 2007), who confirmed a significant decrease in cholesterol level in *Ocypods quadrata* female and male crabs during the spring season, and (Kucharski and Da silva, 1991; Rosa and Nunes, 2003) who noted that the level of Cholesterol decreased in *Chasmagnathus granulate* Dana (1851) in hepatopancrease during the summer and spring, and increased significantly in the gonads. He attributed its high level to its relationship to the degree of development of these crustaceans' ovaries. This explanation is compatible with the current study's findings, which show that the lowest rate of cholesterol concentration occurs during the summer, when temperatures are at their maximum. The rise in temperature, as well as the increase in growth rates, imply that the demand for cholesterol is increasing.

## Acknolwdgments

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## Conflicts of Interest

The authors declare that there is no conflicts of interest.

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