Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 28 (5): 1071 – 1083 (2024) www.ejabf.journals.ekb.eg

Proximal Composition Profile of the Jing Shrimp *Metapenaeus affinis* **in the Iraqi Waters**

Ashwaq T. Abbas¹ , Abdul-Hussein H. Ghazi1* , Anteesar N. Sultan²

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¹Department of Natural Marine Science, Marine Science College, University of Basrah, Iraq ²Department of fishes and marine resources, University of Basrah, Agriculture College ***Corresponding Author: gabdulhussein@gmail.com**

ARTICLE INFO ABSTRACT

Article History: Received: Sept. 5, 2024 Accepted: Sept. 22, 2024 Online: Sep. 27, 2024

Keywords: Body composition, Profile, *Metapenaeus affinis*, Iraqi water

An increasing global demand on shrimp has been obviously recorded in recent years due to their richness in protein, lipid, vitamins and minerals. Therefore, the present study aimed to analyze the body composition, mineral content, amino acids, and fatty acids in both marine and freshwater Jing shrimp (*M. affinis*). Body composition, lymph ions, body ions, fatty acids and amino acids of shrimp under study were analyzed, and statistical analysis was conducted. The study found that marine shrimp had higher protein $(27.15 \pm 2.41\%)$ and lower fat content $(1.32 \pm 0.54\%)$ compared to freshwater shrimp $(25.16 \pm 2.51\%)$ protein and $2.31 \pm 0.91\%$ fat), while carbohydrates were higher in freshwater shrimp $(4.30 \pm 1.40\%)$ than in marine shrimp $(3.13 \pm 1.45\%)$. Environmental conditions revealed marine shrimp lived in temperatures of 22-26°C, salinities of 30-32g/ L, and dissolved oxygen levels of 6.8-7.7mg/ L, whereas freshwater shrimp thrived in temperatures of 20-25°C, salinities of 3-4g/ L, and higher dissolved oxygen levels of 8.0-9.3mg/ L. Additionally, marine shrimp had elevated concentrations of sodium, magnesium, and chloride ions, while potassium and calcium ions were lower, with a higher proportion of these ions present in their lymph. Sixteen amino acids were identified, with valine and phenylalanine being the most concentrated in both environments, alongside six fatty acids categorized as two saturated, two monounsaturated, and two polyunsaturated. In conclusion, it is crucial to comprehend the differences in the body composition of the shrimp, in marine and freshwater environments, to enhance their quality, initiate promising breeding techniques, maintain biodiversity as a marine resource, and sustain the global food economy.

INTRODUCTION

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 Seafood is rich in protein, carbohydrates and fats with enzymes **(Li** *et al***., 2011)**. The group of decapoda belonging to crustaceans includes shrimps, lobsters and crabs, and has received attention because it is a food rich in lipids, proteins, and other nutrients such as minerals and vitamins **(Bono** *et al***., 2012)**. Shrimp is a marine product that enjoys an increasing global demand and its farming provides opportunities for achieving self-

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sufficiency through export to global markets **(FAO, 2022)**. Therefore, shrimp farming is an important part of the blue economy and contributes to enhancing food security and can relieve pressure on natural lifts and protect biodiversity in the seas and oceans **(Chethurajupalli & Tambireddy, 2022)**. Studying the body components of shrimp is of great importance, as shrimp represent a valuable marine resource and play a significant role in the global food economy. They are a major source of protein, and understanding their body composition helps farmers enhance breeding techniques, improve production, and increase overall quality **(Palafox** *et al.,* **1997)**.

 Metapenaeus affinis popularly known as Jing shrimp, is one of the dominant and highly valued penaeid along the Shatt al- Arab River and Iraqi waters, individuals can migrate from marine toward Shatt al-Arab Estuary **(Salman** *et al.,* **1986; Salman** *et al.,* **1990)**. This species is regarded as one of the important and commercially seafood product that has an increasing exportation rate in the sources of food in the Arabian Gulf countries, Iraq and Kuwait **(Mathews, 1989; Safaie & Kamrani, 2003; Kamrani** *et al.***, 2005; Abbas & Ghazi, 2021)**. Several studies have investigated the differences in body composition between marine and freshwater shrimp **(King** *et al.,* **1990; Dincer & Aydin, 2014; Dupetti** *et al.,* **2017; Zou** *et al.,* **2019)**. On the other hand, studying minerals and ions such as calcium, magnesium and sodium is important for determining the safety for human consumption and nutritional value of shrimp **(Koosej** *et al***., 2017)**. These minerals play a fundamental role in the health and growth of shrimp as well as in the health benefits for the consumer, and can also help in understanding the effect of environmental conditions such as salinity, soil type and temperature on the physiological and adaptations of shrimp **(Tantuloand & Fotedar, 2007)**. Additionally, these minerals and ions are also essential in various biological processes such as respiration and reproduction and can be used to improve shrimp farming methods in fresh and salt water farms, which contributes to the development of aquaculture **(Molina** *et al***., 2019)**.

 Studying amino acids helps understand the quality of protein and its nutritional importance, and helps improve general health, and some amino acids play a vital role in strengthening the immune system and improving body functions such as digestion and growth. Additionally, studying amino acids contributes to improving shrimp feeding methods to ensure the best growth rates and achieve the best survival rates **(Achuthan & Parulekar, 1984; Dupetti** *et al.***, 2017**). On the other hand, studying saturated and unsaturated fatty acids are of great importance in determining the nutritional value of shrimp, especially omega-3 fatty acids, which are essential for human health, addittionally shrimp is considered as an important source of these fatty acids **(Bhavan, 2009; Zou** *et al***., 2019)**. Fatty acids in shrimp have significant health and pharmaceutical applications, particularly in developing nutritional supplements that leverage the beneficial properties of omega-3 fatty acids. Moreover, these fatty acids can provide insights into environmental pollution, as changes in their proportions and concentrations may reflect ecological impacts **(Ramasamy** *et al.,* **2017; Fitriana** *et al.,* **2021)**. This

study aimed to analyze the body composition, mineral content, amino acids, and fatty acids in both marine and freshwater Jing shrimp (*M. affinis*).

MATERIALS AND METHODS

Samples collection

 Shrimp samples in this study were collected from the marine and freshwater environments during the period from 12/13/2022 to 12/13/2023 by dragging a net using a fishing boat for 15 minutes. Active and healthy shrimp were selected according to certain characteristics such as transparent body color, swimming behavior, and complete moulting (Pic. 1a). Shrimps were placed in 25-liter plastic bags containing water from the collection area, a quarter filled with water and the rest with air, and then transferred to the Invertebrates Laboratory at the Marine Sciences College.

Environmental factors

 Environmental factors were measured in the field, including temperature, salinity, oxygen concentration, and pH. A multi-purpose device of the type was used to measure temperature, salinity and pH. An oxygen meter was used to measure the dissolved oxygen level, which is the same device used in the oxygen measurement experiments in this study. The devices were calibrated before starting to take field measurements.

Body composition of shrimp *M. affinis*

 A total of 250g of shrimps were taken in 4 replicates (Pic. 1 b), and dried using an oven at 60° C for 24 hours until the weight stabilized. From knowing the difference between the dry weight and the wet weight, the moisture content was estimated %, and the dry samples were kept in the desiccator for the purpose of analyzing the body components, which included protein, fats, carbohydrates, mineral, amino acids and fatty acids.

Pic. 1. Sample preparation of shrimp *Metapenaeus affinis* for analysis of body components, **(a):** caught shrimp, **(b):** dried shrimp

Body ions analysis of shrimp *M. affinis*

 To examine the ionic components, a quantity of shrimp was dried, 5g of dry weight was taken and placed in a 250ml glass flask, 15ml of concentrated nitric acid HNO3 was added to it and left for 24 hours at laboratory temperature with occasional stirring, then placed in a centrifuge at 3500 rpm/15min, after which 1ml of the filtrate was taken, and the volume was completed to 10ml with deionized water and stored until use (Pic. 2).

Pic. 2. Sample preparation of shrimp *Metapenaeus affinis* for ions body analysis, **(a):** dry shrimp weight, **(b)** sample digested for analysis

Lymph ions analysis of shrimp *M. affinis*

 Immediately after catching the shrimp, 0.5ml of lymph was withdrawn using a microneedle from the abdominal pouch located at the base of the first abdominal segment near the fifth pair of walking legs after drying it with cotton to avoid drawing any moisture, then immediately placed in special tubes containing sodium esters as an anticoagulant (Pic. 3). The samples were digested by taking 0.1ml of lymph and 1ml of concentrated nitric acid and left for 2 hours, and the volume was completed to 10ml by adding deionized water and stored in a freezer. Ions were determined using a flame photometer (PFP7) after calibration with standard solutions of sodium chloride at concentrations of 0.5, 1, 1.5, 2 and 2.5mol/ l and potassium chloride at concentrations of 0.06, 0.12, 0.25 and 0.5mmol/ l.

Amino acid analysis of shrimp *M. affinis*

We used a German-made HPLC type AAA device from Sykam, model 2012, for amino acid analysis. 0.2g of dried samples was weighed and 12ml of 6M HCl acid was added to it. The mixture was placed in an oven at 110°C for 24h. The mixture was filtered with 0.8 micrometer filter paper, washed twice with distilled water and then placed in a rotary device at 50°C. After drying, 10ml of distilled water was added, and then the mixture was returned to the rotary device until dry. 0.02 M HCl acids were

added to it at a volume of 3.5ml. The acidity was neutralized by adding a base, and the sample was injected into an amino acid measuring device after adding the Orthophthalaldehyde (OPA) reagent to the sample (Pic. 4a).

Pic. 3. Lymph ions analysis of shrimp *Metapenaeus affinis*, **(a):** lymph aspiration area and method; **(b):** 0.1 ml of lymph aspiration and mixing with nitric acid for digestion

Fatty acid analysis of shrimp *M. affinis*

 A Japanese Shimadzu GC device, model 2010, was used to analyze the fatty acids of shrimp under study (Pic. 4 b). 2g of the sample was taken after grinding it, mixed with 6ml of hexane and placed in a shaking water bath for an hour at a temperature of 60° C. The sample was dried to a certain extent for the purpose of focusing on the rotating device. A volume of 200ml of the sample was taken, then 2ml of KOH was added to it, and the mixture was placed in a water bath again at a temperature of 50°C for half an hour, after which it was left to reach the laboratory temperature. 1ml of distilled water and 1ml of hexane were added, and the mixture was placed in a centrifuge at 10,000 rpm for 10 minutes. Then the upper layer (organic) was withdrawn and filtered using 0.45 micrometer filter paper and injected into a GC device. According to the analytical program, the studied fatty acids were recorded.

Pic. 4. (a) HPLC device for amino acid analysis; **(b)** GC device for analysis of fatty acids in shrimp *Metapenaeus affinis*

Statistical analysis

 The ready-made statistical program called the Statistical Package for Social Sciences (SPSS v.20) was used in the statistical analysis to test the significant differences between the averages and by the L.S.D. test at a significance level of 0.05.

RESULTS

Environmental factors

 Environmental factors were recorded in the field during the collection of marine and river samples. In the marine environment, the water temperature ranged between 22- 26°C, salinity between 30-32g/ l, pH between 7.8-8.5, and dissolved oxygen level between 6.8-7.7mg/ l. In the river environment, the water temperature ranged between 20-25°C, salinity between 3-4g/ l, pH between 7.2-7.5, and dissolved oxygen level between 8.0-9.3mg/ l.

Comparison of body components

 Table (1) shows the body components of shrimp *M. affinis* in the marine and freshwater environments expressed as a percentage (%). It was noted that the protein rate in marine shrimp was 27.15 ± 2.41 compared to 25.16 ± 2.51 in freshwater, and carbohydrates were $3.13 + 1.45$ and $4.30 + 1.40$ respectively, while fat rate in marine shrimp was 1.32 ± 0.54 compared to 2.31 ± 0.91 in freshwater. As for moisture and ash, they were $67.33 + 2.51\%$ and $1.07 + 0.41$ in marine individuals, and $67.02 + 2.32$ and 1.21 + 0.61 in river individuals, respectively. No significant differences were recorded (*P* < 0.05) between body compositions of shrimps in the two environments.

Comparison of ions and minerals in the body and lymph

 The concentration of the main ions in the body and lymph of shrimp living in the marine and freshwater environment were compared, expressed in values (mg/L), as shown in Table (2). In the body of marine shrimp, the concentrations of sodium, magnesium and chloride ions were higher than their concentrations in the body of freshwater shrimp. In marine shrimps, they were 191.60, 8.20 and 255.70, respectively, and in freshwater shrimp, they were 52.91, 2.90 and 184.21, respectively. While potassium and calcium ions recorded lower concentrations in marine shrimp, reaching 4.10 and 10.71, respectively, and in freshwater shrimp, they were 4.40 and 21.71, respectively. The results of the statistical analysis of the shrimp body ions showed significant differences (*P*>0.05) in sodium and chloride ions, while there were no significant differences (*P*<0.05) in the rest of the ions.

When comparing the concentration of these ions (mg/l) in the internal fluid of shrimp (lymph) inhabiting different environments, fluctuations in the concentrations of some ions were observed depending on the environment. In general, sodium, magnesium, calcium and chloride ions formed a higher percentage in the lymph of marine shrimp, reaching 168.90, 4.70, 34.64 and 193.31 respectively, compared to a lower concentration in the lymph of freshwater shrimp, which was 163.50, 2.81, 27.12 and 116.90 respectively, while a similarity was recorded in the percentage of potassium ions in the lymph of marine and freshwater shrimp, reaching 1.46 and 1.41 respectively. The results of the statistical analysis showed that there were significant differences (*P*>0.05) for shrimp living in the marine and freshwater environments in the lymph ions represented by sodium, magnesium and potassium, and there were no significant differences (*P*<0.05) in calcium and chloride ions.

Minerals concentration

The concentration of minerals (mg/l) was measured in shrimp from both marine and freshwater environments, revealing differences in mineral content. Phosphate levels were 0.16mg/ l in marine shrimp compared to 0.28mg/ l in freshwater shrimp. Iron concentrations were 0.26 and 1.34mg/ l, respectively, while zinc measured 0.05mg/ l in marine and 0.09mg/ l in freshwater shrimp. Manganese levels were 0.01 and 0.07mg/ l, respectively, and copper concentrations were 0.28mg/ l in marine and 0.25mg/ l in freshwater shrimp. Sulfur content was higher in marine shrimp (9.87mg/ l) compared to freshwater shrimp (7.46mg/ l), while silica was higher in freshwater shrimp (12.14mg/ l) than in marine shrimp (7.34mg/ l). Elements such as lead, cadmium, selenium, and nickel were present at concentrations of less than 0.01mg/ l in both environments (Table 3).

Body composition (%)	Shrimp		
	Marine	Freshwater	
Protein	$2.41 + 27.15a$	$2.51 + 25.16a$	
Carbohydrate	$1.45 + 3.13$ a	$1.40 + 4.30a$	
Lipid	$0.54 + 1.32$ a	$0.91 + 2.31a$	
Moisture	$2.51 + 67.33a$	$2.32 + 67.02a$	
Ash	$0.41 + 2.07a$	$0.61 + 1.21a$	

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Ions	Ions in body (mg/l)		Ions in lymph (mg/l)	
	Marine	Freshwater	Marine	Fresh water
Na	$191.60 + 8.41$ b	$52.91 + 5.22$ a	$9.24 + 168.90$ a	$163.50 + 15.40$ a
Mg	$8.20 + 1.11 a$	$2.90 + 1.02$ a	$4.70 + 1.27$ a	$2.81 + 0.94$ a
$\mathbf K$	$4.10 + 1.15$ a	$4.40 + 1.16$ a	$1.46 + 0.71$ a	$1.41 + 0.41$ a
Ca	$10.71 + 2.28$ a	$21.71 + 5.71$ a	$34.64 + 2.51$ b	$27.12 + 3.71$ a
Cl	$255.70 + 16.10$ b	$184.21 + 12.51$ a	$193.31 + 12.31 b$	116.90 $+8.41$ a

Table 3. Concentration of minerals (mg/L) in the body and lymph of shrimp *Metapenaeus affinis* in the Iraqi waters

Amino acids concentration

 Table (4) shows the percentage and concentration of amino acids in shrimp in marine and freshwater environments, as 16 amino acids were recorded with differences in percentages and concentration (values expressed in mg/100 g dry weight). The two amino

acids Valine and Phenylalnine recorded the highest concentration and percentage in shrimp of both environments, as their concentration in the marine environment reached 549.96 and a percentage of 58.40%, while in the shrimp of the freshwater environment, it reached 661.34 and a percentage of 71.20%, respectively. While some amino acids were observed to differ between marine and freshwater shrimp, the amino acid Serine achieved a high concentration of 92.77 and a percentage of 9.85%, while its concentration was lower in river juveniles with a concentration of 21.34 and a percentage of 2.30%, respectively. Glutamine achieved a lower concentration in marine shrimp compared to freshwater shrimp and reached 42.12 with a percentage of 4.48% compared to a concentration of 49.04 and 5.30%, respectively. The amino acid Histamine was also observed to be higher in marine shrimp and reached 39.96 with a percentage of 4.24% compared to a concentration of 35.36 with a percentage of 3.79% in freshwater shrimp. The concentration of aspartic acid decreased in marine shrimp to 24.96 with a percentage of 2.66% compared to a higher concentration of 52.33 and a percentage of 5.33% in freshwater shrimp. On the other hand, the amino acid Methionin achieved a significantly higher concentration in marine shrimp reaching 22.01 with a percentage of 2.34% compared to a very low percentage in freshwater shrimp reaching 0.01mg/ 100g dry weight. The concentration of the amino acid Alanine was higher in marine shrimp, reaching 46.38, or 4.92%, and its concentration decreased in freshwater shrimp to 4.03mg/ 100g dry weight. The concentrations of the remaining amino acids were low and were similar in marine and freshwaters shrimp.

Fatty acids concentration

 Six fatty acids were identified during the study, two saturated fatty acids, two monounsaturated fatty acids, and two polyunsaturated fatty acids (expressed as mg/100 g/dry weight). The concentration of saturated fatty acid Palmitic acid (C16:0) in marine shrimp was 7.82 and 8.27% compared to 7.42 and 9.58% in freshwater shrimp, while the concentration of stearic acid (C18:0) was 15.21 and 16.08% and 12.35 and 15.94% in marine and freshwater shrimp, respectively. As for the concentration of monounsaturated fatty acids, the concentration of palmitolleic acid (C16:1) was 13.76 and 14.55% in marine shrimp and 12.82 and 16.55% in freshwater shrimp, and the concentration of monounsaturated fatty acid Oleic acid (C18:1) in marine and freshwater shrimp was 16.05 and 16.91% and 14.15 and 18.28% respectively. As for the two polyunsaturated fatty acids, the concentration of Linoleic in marine reached 20.20 with a percentage of 21.34% and in freshwater shrimp 16.20 with a percentage of 20.91%, while the concentration of polyunsaturated fatty acid Linolenic reached 21.62 with a percentage of 22.85% in marine, while in freshwater shrimp, its concentration reached 14.52 with a percentage of 18.74% (Table 5).

SFA (Saturated fatty acid); MUFA (Mono saturated fatty acid) and PUFA (Poly saturated fatty acid)

DISCUSSION

 Environmental factors play an influential role in the growth and survival activities of living organisms, including shrimp, at low temperatures, metabolic activities decrease, these factorsreduce growth and survival rates in individuals, while high temperatures also negatively affect increased activity, which in turn leads to increased nutrition and waste excretion, which leads to a reduction in the amount of dissolved oxygen available to the

living organism **(FAO, 2022)**. On the other hand, salinity clearly affects the increase in the activities of ionic regulation and ion exchange to achieve internal stability through the balance of body and external fluids in shrimp **(Castille & Lawrence, 1981)**. In addition, appropriate salinity reduces stress posed on the organism, which leads to converting food energy into increased growth **(Diaz** *et al***., 2001)**. The results showed that shrimp living in the marine environment have a higher content of fatty acids compared to shrimp living in the river environment. The main reason is due to the nature of nutrition, as marine shrimp feed on a wide range of algae, crustaceans and marine plankton with high levels of saturated and unsaturated fatty acids, while shrimp living in freshwater feed on food components that are lower in their content of fatty acids, represented by aquatic plants and inorganic materials **(Ghazi, 2020)**. Marine individuals also live in a somewhat stable environment such as temperature compared to river water, which allows for the accumulation of types of fatty acids that are essential in building flexible membranes to maintain cells, while temperature changes in the river environment are greater, hence they are expected to affect the composition of fatty acids in the body **(Bhavan** *et al***., 2008; Pirest** *et al.,* **2018)**. The concentration of amino acids varies between shrimp living in marine and freshwater environments, as observed in the current study. One reason for this difference is that marine shrimp live in water with a high salt content, unlike shrimp in freshwater environments **(Bell & Sargent, 2003)**. These differences influence nutrient availability and absorption, which indirectly impacts the type and concentration of amino acids in the body **(Palafox** *et al***., 1997)**. The food available in marine environments tends to be richer in amino acid components, which is reflected in their higher concentration in marine shrimp **(Ghazi, 2020)**. Additionally, physiological and genetic factors, such as the need for physiological adaptations in response to environmental conditions, can also contribute to these differences **(Bhavan, 2009)**.

Marine shrimp live in a high-salinity environment, requiring larger amounts of amino acids to support growth, reproduction, and physiological processes for maintaining internal balance **(Dupetti** *et al.,* **2017; Rahi** *et al.,* **2018)**. The study's results highlight differences in ion and mineral concentrations between shrimp in marine and freshwater environments, showcasing their biochemical and physiological adaptations. Marine shrimp, due to their salty environment, have higher concentrations of ions like sodium and chloride, as they need to excrete excess salts via their gills and bodies **(Li** *et al.,* **2011)**. In contrast, freshwater shrimp have efficient mechanisms to retain salts by absorbing them from their environment **(Abdul-Sahib & Ajeel, 2005)**. This study confirms that differences in ions and minerals, such as calcium, potassium, and magnesium, align with the respective environments where the shrimp live.

CONCLUSION

According to the results, the Jing shrimp (*M. affinis*) has demonstrated good nutritional value, comparable to shrimp species of significant economic importance. A notable difference was observed in the chemical composition, including protein and lipid content. Additionally, variations were found in the concentrations of certain ions and minerals between shrimp living in different environments, as well as differences in the fatty and amino acid content, which were influenced by the living environment.

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