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Influence of Different Temperature-Salinity Combinations on the Oxygen Consumption in the Juveniles Jinga Shrimp *Metapenaeus affinis*

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ARTICLE INFO ABSTRACT

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The Shatt al-Arab River in Iraq has seen a significant spread and subsequent disappearance of certain shrimp species, potentially due to historical fluctuations in temperature and salinity. This study examines the effects of combined temperature and salinity on the oxygen consumption, metabolic rate, and thermal coefficient of juvenile *Metapenaeus affinis*. Salinity tolerance tests revealed that shrimp survived transfers up to 15ppt but experienced over 50% mortality at 20 ppt, with the highest survival (100%) at 5ppt and the lowest (17%) at 20ppt. Oxygen consumption increased with salinity at 20-21 \degree C, with peak rates at 15 ppt (0.036mg O2/h) and lowest at 1ppt $(0.006mg O₂/h)$. At 25-26°C, oxygen consumption was higher at 10 and 15 ppt $(0.040 \text{mg } O_2/h)$ and lower at 5 and 10 ppt $(0.010 \text{mg } O_2/h)$ O₂/h). At 29-30 $^{\circ}$ C, the highest consumption rate was 0.046mg O₂/h at 1ppt, while the lowest was 0.016 mg O₂/h at the same salinity in the second and third hours. Temperature significantly affected oxygen consumption, with the highest rate (0.018mg O_2/h) at 29-30°C and 15ppt, and the lowest rate $(0.006mg O₂/h)$ at 20-21^oC and 1 ppt. Metabolic rates peaked at 0.072 mg O_2/g at 29-30°C and 15 ppt and were lowest at 0.024mg O_2/g at 20-21°C and 1 ppt. These findings provide valuable insights for aquaculturists in developing practices to enhance growth and survival in varying environmental conditions.

INTRODUCTION

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 In the 1980s and 1990s a number of shrimp species spread in the Iraqi local environment, especially in the Shatt al- Arab River, such as *Metapenaeus affinis* **(Miquel, 1983)**, *Atyphaera desmarestii mosopotamica* **(Al-Adhub, 1987)**, *Caridina Babulti basrensis* **(Al-Adhub & Hamzah, 1987)** and *Exopalaemon styliferus* **(Salman & Bishop, 1990)**. After the 2000s, these species disappeared and other species were recorded as invasive species to the local environment like *Macrobrachium nipponense* **(Salman** *et al.,* **2006)**, *Macrobrachium lar* **(Ghazi & Hassan, 2021)**, *Macrobrachium equidens* **(Hassan** *et al.,* **2023)**. There are various reasons for this situation, including climate changes especially temperature, pollution, and different levels of salinity in the

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Shatt al-Arab due to the lack of drainage that negatively affect the marine environment in the region **(Habashy & Hassan, 2011; Al-Mahmood** *et al.,* **2015)**. One of the threatened species is the jinga shrimp, *Metapenaeus affinis* (H. Milne Edwards, 1837), it is dispersal from the Arabian Sea to South India, and live at depths between 55 to 90m, mainly on mud bottom **(Fischer & Bianchi, 1984)**. The jinga shrimp is one of the dominant and highly valued penaeid shrimps along the coastal waters of Iraqi, and juveniles can travel for long distances from the marine toward the estuary of the Shatt al-Arab (inland waters), moreover the jinga shrimp is considered as a commercial shrimp in this area **(Salman** *et al.,* **1986; Salman** *et al***., 1990; Abbas & Ghazi, 2021)**.

 Dissolved oxygen (DO) is the major factor for studying water quality since all aquatic organisms need oxygen to survive and grow **(Anongponyoskun** *et al.,* **2012)**. The life cycle of *M. affinis* is completed between inland and marine waters. Therefore, this species is considered migratory between brackish water (1- 2ppt) and marine water (30ppt) **(Gerami** *et al.,* **2012)**. Moreover, salinity and temperature are important factors and have influence on oxygen consumption **(Duan** *et al.,* **2022)**. Knowledge of the effects of different salinity levels on dissolved oxygen will benefit aquaculture by promoting optimal growth and survival **(Schuler, 2008; Cobo** *et al.,* **2014)**. Many papers have been written on the combined effects of salinity and temperature on oxygen consumption, metabolic rate, and thermal coefficient (Q10) **(Stern** *et al.,* **1984; Rosas** *et al.,* **2001; Manush** *et al.,* **2004; Spanopoulos** *et al.,* **2005; Allan** *et al.,* **2006; González** *et al.,* **2010; Garcia-Guerrero** *et al.,* **2013; Vinagre** *et al.,* **2015; Rahi** *et al.,* **2021; García-Guerrero** *et al.,* **2022)**. The purpose of this research was to determine the effects of different salinity concentrations and temperatures on the oxygen consumption rate in the juveniles of *Metapenaeus affinis* shrimp, as well as assessing their metabolic rate and thermal coefficient (Q10).

MATERIALS AND METHODS

A total of 60 juvenile shrimps, *M. affinis,* were collected from the al-Mas'hab area, southern sector of Iraqi inland water near Al- Hammar marshes; this is located within an area that lies between 30° 39^{\prime} 34.27" N; 47° 39 \prime 13.81" E. (Map 1). The shrimp were captured by trawl net (Fig. 1), and they were kept in laboratory aquaria containing water adjustment to the salinity level of resource water, and acclimated for 12 hours at 4ppt prior to the experiment. Additionally, the salinity of the resource water varied between 3 to 4ppt. and specimens weighed from 1.0 to 1.3g. The effect of a rapid change of salinity on the oxygen consumption was estimated every one hour and for four consecutive hours, this is a time when juveniles reach the point of stress due to lack of oxygen to less than 4mg/ l (initial DO 8.5mg/ l). The oxygen consumption was measured with a YSI oximeter, salinity by refractometer and controlled on temperature by a thermostat heater, during this time shrimp were not fed. We used four plastic tanks,

tightly closed to prevent the exchange of external air, the size of one tank is two liters, each tank contains three individuals of shrimp, and tanks were kept in the dark (Fig. 2). The juveniles were transferred from the acclimatization tanks directly to the experimental tanks (rapid change) containing different salinities by adding sea salts (1, 5, 10 and 15ppt). The temperatures were controlled within the specified ranges using an electric heater, and it was noted that the temperature ranges were set at $\pm 1^{\circ}$ C. Therefore, the chosen ranges were 20- 21, 25- 26, and 29- 30°C. Total oxygen consumption (OCT) (mg O_2/h /per shrimp) was calculated as: $OCT = (Ct_0 - Ct_1) \times (Cv)$.

Where, Ct_0 is the initial oxygen concentration (mg/L); Ct_1 is the final oxygen concentration, and Cv is water volume in liters.

Oxygen consumption per gram of weight (OCg) was calculated as: OCg (mg/g/h) = OCT/WP, where WP is the shrimp's weight (g).

The thermal cofinance (Q10) value of the shrimp was determined by using the equation $Q10=(R2/R1)^{10/T2}$ –T1

Where, R_2 and R_1 ¹ are the oxygen consumption at the respective temperatures T_2 and T_1 .

Map 1. A map representing the samples collection area in the Al-Mas'hab region in southern Iraq

Fig. 1. Collection process in trawling nets (right), transportation process using plastic bags (left), and individuals selected to conduct oxygen consumption experiments (bottom)

Fig. 2. Designing an oxygen consumption experiment in plastic tanks with measuring devices and thermometers installed to adjust the temperature

RESULTS

 Before studying oxygen consumption, the half lethal concentration (LC50) of salinities was determined to establish the ranges in which oxygen consumption experiments are conducted. It was noted that at a salinity of 1ppt, the survival rate was 95%. At a salinity of 5ppt, there were no deaths (100% survival), and at a salinity of 10ppt, the survival rate was 80%. When the salinity increased to 15ppt, the survival rate dropped to 58%, and at 20ppt, mortality occurred in more than half of the juveniles, resulting in a survival rate of only 17%. Consequently, the highest salinity concentration was excluded from the oxygen consumption experiments (Fig. 3).

Fig. (4) shows the effect of different salinities on oxygen consumption $\frac{mg}{O2/h/per}$ shrimp) in juvenile shrimp at a temperature of 20- 21°C. We observed an increase in oxygen consumption with increasing salinity. At 1ppt, we noticed a gradual increase in consumption until the third hour: 0.06, 0.016, and 0.018, respectively, after which there is a decrease in oxygen consumption rate to 0.012. While at a salinity of 5ppt, we observed an increase in oxygen consumption in the first hour (0.018), dropping to 0.016 and 0.012 in the second and third hours, respectively, then rising to 0.019 in the fourth hour of the experiment. Oxygen consumption levels converged at 10ppt salinity, with a gradual increase until the third hour of the experiment: 0.020, 0.022, and 0.024, respectively, then a decrease occurred in the fourth hour to 0.019. At high salinity of 15ppt, oxygen consumption in the first hour recorded 0.024 and in the second hour 0.020, rising in the third and fourth hours to 0.036 and 0.034, respectively.

 By raising the temperature to 25- 26°C, we noticed differences in the rate of oxygen consumption depending on the difference in salinity. At a salinity of 1ppt, we noticed stability in oxygen consumption in similar ranges, ranging between 0.018, 0.016, 0.016 and 0.014 in the four hours of the experiment, respectively. At a salinity of 5ppt, there was an increase in the consumption rate in the first and second hours, reaching 0.024 and 0.038, respectively, then the consumption decreased in the third hour to 0.014, and at the end of the experiment (fourth hour) it reached 0.01. When the salinity increased to 10ppt, there was an increase in the consumption values for the first three hours, reaching 0.022, 0.034, and 0.040, respectively, and a decrease in the fourth hour of the experiment to 0.010. At high salinity of 15ppt, we noticed that oxygen consumption rose in the first hour to 0.040 then decreased in the second hour to 0.026, followed by another rise in the third hour to 0.032, and a slight decrease slight in the fourth hour reaching 0.030 (Fig. 5).

When the temperature was risen to an extreme temperature, we generally noticed an increase in oxygen consumption. At a salinity of 1ppt, we noticed an increase in consumption in the first and second hours: 0.024 and 0.046, respectively, and it decreased in the third and fourth hours at the same rate, reaching 0.016. At 5ppt salinity, consumption converged in the first and second hours, reaching 0.023 and 0.024,

respectively, and decreased to 0.030 and 0.026 in the third and fourth hours of the experiment, respectively. At the salinities of 10 and 15ppt, the juvenile shrimp exhibited similar physiological patterns. Oxygen consumption in the first hour was 0.038mg/ O2 at 10ppt and 0.040mg/ O2 at 15ppt. In the second hour, the consumption decreased to 0.022mg/ O2 at 10ppt and 0.030mg/ O2 at 15ppt. However, in the third and fourth hours, there was an increase in consumption. At 10ppt, the rates were 0.024mg/ O2 in the third hour and 0.028mg/ O2 in the fourth hour. At 15ppt, the rates were 0.032mg/ O2 in the third hour and 0.038mg/ O2 in the fourth hour (Fig. 6).

When testing three temperature ranges (20-21, 25-26, and 29- 30°C) with different salinities, a difference was recorded in the rate of total oxygen consumption (mg/O2/h/per shrimp). At a salinity of 1ppt, the consumption according to the difference in temperature ranges reached between 0.024, 0.032, and 0.028, respectively. At 5ppt salinity, the total consumption was 0.032, 0.040 and 0.052, respectively. At 10ppt salinity, the total consumption was 0.044, 0.052 and 0.056, respectively. Total oxygen consumption increases with salinity up to 15ppt, reaching 0.056mg/ O2 in the 20- 21°C temperature range, 0.064mg/ O2 in the 25- 26°C range, and 0.072mg/ O2 in the 29- 30°C range (Fig. 7).

Fig. (8) shows that the metabolic rate $(mg/O2/g)$ was clearly associated with an increase in temperature and salinity. At a temperature of 20- 21°C, the metabolism increased exponentially with an increase in salinity, and was 0.024, 0.032, 0.040, and 0.058 at salinity (ppt) 1, 5, 10, and 15, respectively. At a temperature of $25 - 26^{\circ}\text{C}$, metabolic rates were recorded at 0.032, 0.040, 0.052, and 0.064, respectively. At 29- 30°C metabolic rates increased from 0.028 at a salinity of 1ppt, and increased to 0.052 at a salinity of 5ppt, also increased to 0.056 at a salinity of 10ppt, as well as to 0.072 at a salinity of 15ppt.

Fig. 3. The lethal concentration (LC 50) for shrimp *Metapnaeus affinis* within two hours of a sudden increase in salinity to concentrations 1, 5, 10, 15, and 20ppt

Fig. 4. Oxygen consumption rate (mg/h/ind./ww) of Juveniles *Metapenaeus affinis* at different salinity at constant temperature 20- 21°C

Fig. 5. Oxygen consumption rate (mg/h/ind./ww) of Juveniles *Metapenaeus affinis* at different salinity at constant temperature 25- 26°C

Fig. 6. Oxygen consumption rate (mg/h/ind./ww) of juveniles *Metapenaeus Affinis* at different salinity at constant temperature of 29- 30°C

Fig. 7. Combined between different temperatures and salinities on total oxygen consumption (mg/h/ind./ww) after four hours for juveniles *Metapenaeus affinis*

Fig. 8. Metabolic rate (mg O2 /g) for *M. affinis* juveniles at four experimental salinities and three temperature degree combination

Table (1) shows the thermal efficiency coefficient at temperature ranging from 20- 25, 25- 30, and 20- 30°C, and at different salt concentrations of 1, 5, 10, and 15ppt. At salinity of 1ppt, the highest Q10 rate was achieved in the range of 20- 25°C, and reached 1.77, and the lowest rate was achieved at a temperature of 25- 30°C and reached 0.76, while in the thermal abrasion of 20- 30°C, it reached 1.36. On the other hand, at 5ppt salinity, the Q10 average ranged between 1.56, 1.69, and 2.64, respectively. Additionally, at 10ppt salinity, the Q10 ranged between the highest rate in the

temperature ranging 20- 30°C and reached 1.61, and the lowest rate in the temperature ranged between 25- 30°C, and reached 1.51, while in the temperature range of 20- 25°C the Q10 reached 1.40. A similar pattern was observed at a salinity of 15ppt, with the highest Q10 value of 1.28 recorded in the 20- 30°C temperature range. The lowest Q10 value of 1.12 was observed in the 25- 30°C range, while in the 20- 25°C range, the Q10 value reached 1.14.

Temperature range °C	Salinities (ppt)			
20-25	177	1.56	1.40	l 14
25-30	0.76	1.69	1.15	112
20-30	.36	2.64	$.6^{\circ}$	

Table 1. Estimation of the thermal coefficient (Q10) for *M affinis* juveniles at different salinities

DISCUSSION

 M. affinis have an migrate phase in their life cycle and thus get exposed to wide fluctuations in salinity, since salinity is an important environmental variable that has a significant influence on dissolve oxygen of estuarine and marine animals, knowledge of the oxygen consumption under different salinity regimes will be useful to aquaculturists in formulating improved culture practices for maximizing growth and survival **(Gerami,** *et al***., 2012)**. Aquatic organisms in high salinity environments often expend more energy to osmotic balance and regulate the flow of water and salts across their membranes **(Anongponyoskun** *et al.,* **2012)**. In this study, the salinites of 1 and 5ppt achieved a higher survival rate, this is because the salinity of the source is close to the salinity of the experiment. On the other hand, the survival rate decreased to 80% in the salinity of 10ppt and decreased to 58% in the salinity of 15ppt due to the extent of the salinity being far from the salinity of the source water. What confirms this is the decrease in the survival rate to 17%, meaning the percentage of deaths reached less than half the number when the salinity increased to 20ppt. Therefore, the experiment was stopped. Generally, salinity decreases the solubility of oxygen in water, this is because the presence of salts reduces the amount of space available for oxygen molecules. Consequently, high salinity environments tend to have lower dissolved oxygen levels compared to freshwater environments. Salinity seems to be a crucial factor for the survival due to its impact on shrimp respiration **(Duan** *et al.,* **2022).**

 Oxygen needs to be increased when shrimp move to marine waters due to the increase in energy expended in metabolic processes accompanied by osmoregulation

(Havird *et al***., 2014; Rahi** *et al.,* **2020)**. The current study discussed the oxygen consumption and metabolic rate in shrimp when they suddenly moved to salt concentrations different from the salinity of the source water. A relative increase in the rate of oxygen consumption was observed when they suddenly moved to a salinity of 15ppt and a relative decrease when the salinity concentration decreased to 1ppt. These changes in the level of oxygen consumed with different salinity are associated with changes in the effective transport of ions **(Rhai** *et al***., 2018)**. There are a number of studies that addressed changes in metabolic rates when moving to environments with different salinity concentrations **(Tantulo & Fotedar, 2006; Joseph & Philie, 2007; Ye** *et al.,* **2009; Rahi** *et al***., 2018; Rahi** *et al.,* **2020; Jaffer** *et al***., 2020; Rhai** *et al.,* **2021)**, these sources indicated that there are physiological changes associated with a change in salinity concentration that make the organism tend toward a short-term loss of energy. In many organisms, high salinity resulted in decreased oxygen consumption, the results in current study, agree with previous studies of **Stern** *et al.* **(1984)**, **Rosas** *et al***. (2001)**, **Spanopoulos** *et al***. (2005)**, **Garcia- Guerrero** *et al.* **(2013)** and **García-Guerrero** *et al.* **(2022)**. The interactions between salinity and temperature are complex and can significantly influence oxygen consumption in aquatic ecosystems. Changes in temperature and salinity can alter community structures and ecosystem functioning, leading to shifts in species within local habitats, particularly among aquatic organisms. In the current investigation, we observed differences in the rate of oxygen consumption depending on variations in salinity and temperature.

 Higher temperatures and salinities together can significantly raise metabolic rates, leading to greater oxygen consumption **(Villarrea** *et al.,* **1994)**. Organisms in such environments may experience increased metabolic stress as they struggle to balance oxygen intake with elevated energy demands for maintaining homeostasis **(Bett & Vinatea, 2009; Kieffer & Wakefilld, 2009; Abdul Wafi** *et al.,* **2021)**. If oxygen becomes critically low, it can lead to reduced growth, impaired physiological functions and some species may migrate to areas with more favorable conditions **(Allan** *et al.,* **2006)**. Hence, we believe that high temperatures and salinities in past periods may have caused some species to shift from or become depleted in the local environment.

 Thermal coefficient (Q10) is a measure of how temperature affects the rates of biochemical reactions, expressing the percentage increase in reaction rate when the temperature is raised by 10°C, changes in temperature and salinity affect the osmotic pressure inside the cells, and high or low osmotic pressure can change the activity of enzymes and other chemicals and thus affect Q10. During the current study, shrimp showed a similarity in Q10 values with differences in temperature and salinity, and this explains the ability of this species to tolerate wide ranges of temperatures between 20- 28°C, in addition to its wide tolerance to differences in salinity, as it is a migratory species between the marine and freshwater **(Salman** *et al.,* **1990)**. The current study agrees with a study of **Retes** *et al.* **(2008)** that concluded that the value of Q10 ranges between 2- 3. Sudden changes in temperature can cause environmental stress to the shrimp, which leads to changes in biochemical processes and increases the shrimp's energy consumption **(Haas, 2007; Huang** *et al.,* **2023)**.

CONCLUSION

 The research clearly shows that salinity and temperature levels have a direct effect on the rate of oxygen consumption in juvenile shrimp. As salinity and temperature increase, the rate of oxygen consumption also rises. Juvenile shrimp demonstrate a noticeable adaptation to decreasing oxygen levels by reducing their consumption rate in proportion to the oxygen concentration in their environment.

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