Research Article

A Comparative Study of Bacterial Contamination from Shrimp Ponds in Two Different Environments

Tariq H.Y. Al-Maliky*, Mahmood S. Hashim and Amal S. Al-Sheraa

Marine Biology Department, Marine Science Centre, University of Basrah, Basrah, Iraq.

Abstract | The bacterial content of the water of *Metapenaeus affinis* and *Macrobrachium nipponense* juvenile shrimp cultured in two salinities (3 and 15 g.L^{-1}) were observed during 45 days. The initial and final weights of *M. affinis* were (0.939 ± 0.069 and 1.581 ± 0.183 g) in 3 g.L^{-1}, and (0.939 ± 0.065 and 2.274 ± 0.294 g) in 15 g.L^{-1}. While for *M. nipponense*, it was (0.914 ± 0.086 and 1.308 ± 0.070 g) in 3 g.L^{-1}, and (0.908 ± 0.070 and 1.086 ± 0.107 g) in 15 g.L^{-1}. The environmental factors were temperature 24-29 °C, acidity 7-8, and dissolved oxygen 5-10 g.L^{-1}. Bacterial contamination (CFU m.L^{-1} 10^{6}) was evident in treatment D and included four types of bacteria: *Bacillus* spp., *Escherchia coli, Staphylococcus* spp., *Streptococcus* spp., and *Enterococcus* spp. The survival rates of *M. affinis* during different breeding periods in the two salinities remained as follows: (93.33 ± 5.77, 9.00 ± 10.00, 83.33 ± 5.77 %) and (93.33 ± 5.77, 90.00 ± 10.00, and 86.67 ±5.77 %), respectively. The survival rates of *M. nipponense* during different breeding periods in the two salinities are as follows: (100 ± 0, 93.33 ± 5.77, 93.33 ± 5.77 %), (60.00 ± 10.00, 50.00 ± 10.00, and 43.33 ± 5.77 %), respectively. The results of the statistical analysis showed that the shrimp *M. affinis* (A) in salinity 3 was significant (P>0.05) between the average weights of all the average weights during all periods, while there were no significant differences (P<0.05) between the average weights of the 15 and 30-day periods. In treatment (B) 15, significant differences (P>0.05) were found between all periods of the experiment. Shrimp *M. nipponense* in salinity 3 (C) treatment, there are significant differences (P>0.05) between the initial weight and all periods, but there were no significant differences (P<0.05) between it and the first period of 15 days, also there were no differences (P<0.05) between the periods 30 and 45 days. While in the treatment of salinity 15 (D), significant differences (P>0.05) were found between the last period (45 days) and the average weight of the initial and 15 days, but there were no significant differences (P<0.05) between the other periods. And the total bacterial content found significant differences (P>0.05) between treatment D and the combination of treatments A, B, and C, and there were no significant differences (P<0.05) between them.

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***Correspondence** | Tariq H.Y. Al-Maliky, Marine Biology Department, Marine Science Centre, University of Basrah, Basrah, Iraq; **Email:** tariq.yasien@uobasrah.edu.iq
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Introduction

Aquaculture is one of the fastest-growing and developing sectors, as it is estimated that it will provide about 60% of the consumer's need for aquatic organisms, of which shrimp produced from fresh and saltwater constitute approximately 15%, and crustaceans are the most important in global fish products as they constitute 23.0% of it and 8.3% of it is aquaculture (FAO, 2018). The amount of shrimp marketing to global markets is estimated at more than 3.4 million tons annually, at an average price between 3800 to 8800 US dollars.ton^{-1}. Shrimp offered in global markets in 2018, confirms the ability of this sector to provide the need for shrimp throughout the year, and it has the possibility of increasing production at the lowest cost, and its rapid growth compared to its presence in the natural environment, and since farmed shrimp possesses many specifications and resistances to its success, some of which are borne by ranges. A wide range of salinity and temperature and the importance of the location of the farm from the water source, and thus the ease of supplying water and disposing of waste, and the chosen culture method, especially the high-density method. On the other hand, the farm has Disadvantages, including the impact of waste on the environment and thus the accompanying possibility of infection with pathogens, especially from the family Enterobacteriaceae (Landsman *et al*., 2019).

Shrimp is considered as the most important and economical in the world's fisheries and the second non-oil product and the most prevalent in Asia within aquaculture, which accounts for an annual growth rate of 8.6%. This development resulted in shrimp farming methods, including intensive farming, to counterproductive results with non-positive effects on the environment from pollution and the spread of diseases, therefore, the development of sustainable shrimp farming requires responsible practices to improve their operational efficiency and help reduce environmental degradation of coastal ecosystems (Zhang and Liu, 2015; Portley, 2016; Rivera *et al*., 2018). In addition, feed constitutes 50% of the production cost of a shrimp farm (Tacon and Nunes, 2013). The production of freshwater aquaculture exceeded 591 thousand tons in 2017, but this important sector is affected by bacteria and the resulting dangers (Zhou *et al*., 2019). Some factors affecting the production of intensive shrimp farms and the material returns resulting from them were studied and the reasons of failure of continue their success due to the losses resulting from the lack of control over the water quality of their ponds upon sudden changes in the surrounding conditions and thus the spread of deadly epidemics (Nguyen *et al*., 2020).

Prakash and Karmagam (2013) studied the effect of the presence of bacteria in the water of freshwater shrimp ponds, *Macrobrachium rosenbergii*, and the most important bacterial genera were *E. coli*, *Enterobacter* sp., as well as other types of bacteria found in fish farms, including *Bacillus*, *Staphylococcus* and *Streptococcus* (Reyes *et al*., 2021). There are many studies on bacteria in aquaculture farms (Abraham and Sasmal, 2009; Suantika *et al*., 2017; Islam *et al*., 2018; Reza *et al*., 2020).

The local commercial shrimp is concentrated in the waters of southern Iraq in Basra through the territorial waters of the northwest of the Arabian Gulf and in the internal waters of the Shatt al-Basra Canal, Shatt al-Arab River, Hammar Marsh, Tigris, and Euphrates rivers (Al-Maliky, 2013). There are many local studies on the species of shrimp *M. affinis* and *M. nipponense*, including environmental studies and the growth and effect of probiotics (Al-Maliky, 2009, 2015, 2022; Al-Maliky *et al*., 2021).

The current study aimed to identify the most important bacterial species (bacterial contamination) in shrimp ponds, *M. affinis* and *M. nipponense* in low saline 3 g.L^{-1} and medium 15 g.L^{-1} environments, thus providing all the necessary preparations for each environment while comparing the effect of salinity on tiger shrimp in both salinities.

Materials and Methods

The experiment was designed by preparing two environments, one of which was Oligohaline water of low salinity 3 g.L^{-1} and the other of (Mesohaline) water of medium salinity 15 g.L^{-1}, with three replicates for each environment (treatment) and lasted for 45 days and was fed on a commercial diet, twice daily (9 am and 2 pm) and measurements were made weekly for each of the juveniles' weights and some other environmental conditions.

Juvenile shrimp

Juvenile shrimp were obtained from inland river waters in Basra, southern Iraq, distributed with 10 juveniles for each refiner, and in both ponds calibrated their water in two salinities (3 and 15 g.L^{-1}) with four treatments (A, B, C, and D) for both types of shrimp *M. affinis* (A and B) with weights (0.939 ± 0.069 g and 0.939 ± 0.065 g), respectively, and *M. nipponense* (C and D) shrimp with weights (0.914 ± 0.086 and 0.908 ± 0.070 g), respectively. Use a sensitive scale to measure weights every 15 days until the end of the experiment. Survival rates based on (Maica *et al.*, 2014) are as follows: Survival (%) = 100 (final number of shrimp/initial number of shrimp).

Measurement of bacterial content

The method of serial dilution of the bacterial isolate was followed using nutrient agar medium and water samples under the sterilization. Water sampling was performed to determine the total bacteria, once every 15 days and at the end of the experiment; Samples were determined by the dilution method in which the sample (i.e., concentration 100) was diluted in 0.86% physiological solution to a concentration of 10^{-2}, and then a sample of 100 µl was distributed over sterile TCBS (thiosulfate-citrate-bile salts-sucrose agar) (Huys, 2002). The total number of bacteria was calculated as follows (Huys, 2002; Tao *et al.*, 2021):

$$\textit{Bacterial count } (CFU\ mL^{-1}\ 10^{6}) = \textit{number of colony } c \times \textit{dilution factor} \times 10$$

Statistical analysis

The results were examined using (ANOVA) analysis to determine the effect of bacterial content on shrimp growth at the $p < 0.05$ level, from treatments (A, B, C, and D) for two types of local shrimp in salinities (3 and 15 g.L^{-1}), and shrimp survival rates, when they were to find significant differences, the LSD test was applied with $\alpha = 0.05$. The results were analyzed based on Excel 2010 and according to the SPSS statistical analysis system.

Results and Discussion

Shrimp culture is the process of stabilizing water salinity, pH, dissolved oxide, and other quality elements, so the temperature has a direct impact on the success of the culture from its failure to its importance in increasing food consumption and thus increasing the metabolism of shrimp food and growth (Abraham and Sasmal, 2009). Table 1, shows some of the environmental factors measured during the experiment period and were within the appropriate ranges for shrimp rearing and growth, noting that the dissolved oxygen values decreased slightly in the last periods of the experiment, but remained at a valuable level within the optimal levels of culture, and this may be due to the increase in the weight of the farmed *M. affinis* shrimp. Thus, the increased consumption of oxygen, while the shrimp *M. nipponense* farmed in medium salinity, maybe due to its unsuitability for growth, and thus the occurrence of microbial contamination and the accompanying problems that lead to mortality. The average temperature in the current study was higher than what was recorded in the study by Maica *et al.* (2014) for rearing Shrimp *L. vannamei* in my salinity (4, 16) ‰ within the 36-day rearing period, which may be due to the different rearing area and rearing season, as well as the dissolved oxygen values, were comparatively less while the pH values are close for the current study. And the study of Anh *et al.* (2019) showed that The temperature and pH rates were comparable to the current study. The study found water pH values in all ponds Table 1, within the ideal range (7.5-8.5) for the ideal for shrimp farming (Chiu, 1988; Abraham and Sasmal, 2009). Das and Saksena (2001) observed a positive correlation between pH and salinity with shrimp growth.

In other studies, there were high levels of dissolved oxygen and pH for shrimp ponds that have a large area and a high density of shrimp and bacteria to create beneficial bacteria in maintaining water quality. The large area of the tanks provides a high percentage of oxygen and is sufficient for the needs of shrimp during the period of their rearing (Wang *et al.*, 2020; Riandi *et al.*, 2021).

Table 1: *Some environmental conditions measured in shrimp pond water in environments (3 and 15 g.L^{-1}) for 45 days (mean ± standard deviation).*

Parameter	Treatments			
	A	B	C	D
Temperature (°C)	27.33±3.05	26.9 ±1.66	26.67±2.51	26.9 ±1.66
Dissolved Oxygen (mg·L-1)	7.00±1.00	7.10 ±1.49	7.67±1.52	6.63±1.41
pH	7.90±0.37	7.82 ±0.56	8.00±0.30	8.09 ±0.20

This study considers the breeding of shrimp in Brackish water because it is located within a water salinity ranging between 0.5-18.0 g.L^{-1}. Figure 1, shows that treatment of *M. affinis* during the experiment period in two different environments, we note an increase in the weights of shrimp juveniles with each period, and this is an indication of the appropriate environmental conditions in the rearing ponds and the effectiveness of the diet used for feeding, and consequently the weight gains with the length of the experiment. The weights of the juveniles raised in medium saline water were better than those raised in low salinity ponds. This proves that these shrimp are marine living and enter the river water for incubation to supply food and protection and then return to the marine waters to complete their life. And the statistical analysis of the growth rates of *Penaeus monodon* were also different during different feeding within the culture in marine waters with very short growth periods that did not exceed four days for each period (Putra *et al*., 2018).

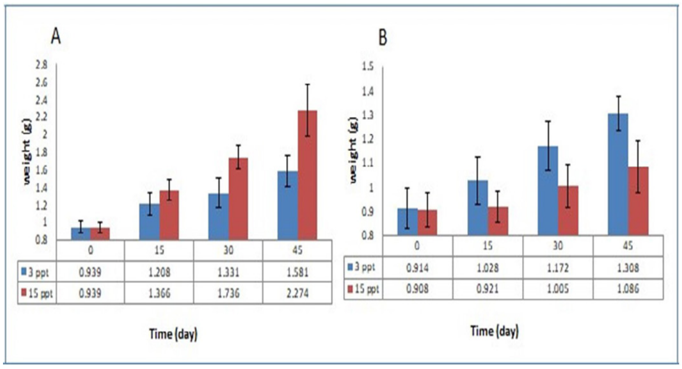

Figure 1: *Comparison of growth of shrimp Metapenaeus affinis (A), Macrobrachium nipponense (B) in ponds with two salinity (3 and 15 g.L^{-1}).*

Figure 1, shows the treatment of B for *M. nipponense* during the experiment period in two different environments (3 and 15 g.L^{-1}) and the values of the weights during the first period after 15 days. We notice an increase in the weights of shrimp juveniles with each period. This indicates an appropriate environmental conditions in breeding ponds and the effectiveness of the diet used in feeding and consequently weight gain with the length of the experiment. The weights of the juveniles raised in brackish water were better than those raised in medium salinity ponds. This proves that this shrimp is adapted to the environment of fresh living water and is one of the shrimp that entered our environment in 2002 due to the activity of the aquaculture sector in the neighboring country Iran. And between the statistical analysis and the study of Anh *et al*. (2019) about the growth of shrimp *L. vannamei*, its initial weights were similar to those of the current study, while the final weights were different, and it may be due to the type of shrimp farmed, the difference in the culture period and the rearing system.

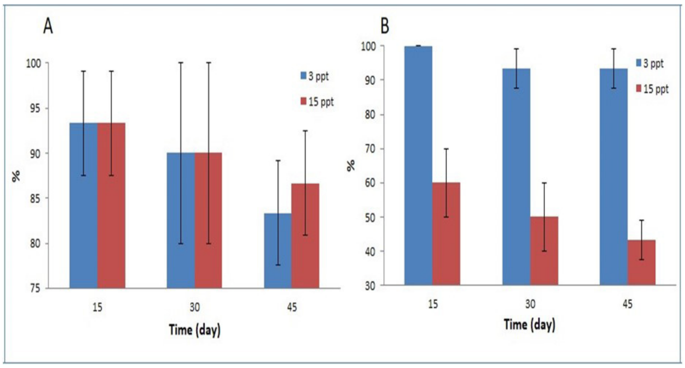

Figure 2: *Comparison of survival rate (%) of shrimp Metapenaeus affinis (A), Macrobrachium nipponense (B), in ponds with two salinity (3 and 15 g.L^{-1}).*

Figure 2, We note the survival rates in brackish water treatments with high and close values. This is evidence that the local shrimp (inland water shrimp) has high survival rates, which results in the possibility of benefiting by establishing local shrimp farms and thus expecting economic feasibility for them. While we note the medium salinity water treatments, the survival advantage of shrimp *M. affinis* compared to the shrimp *M. nipponense* may be caused by the osmotic effect of saltwater on the second type of shrimp that is adapted to living in brackish water, unlike the first type of shrimp that is adapted to live in saltwater. It is not affected by medium salinity water and is preferred by it. Survival rates by the study of Maica *et al*. (2014) are contrast with the values of the current study, especially in coefficients A and B, and this is an indication that the type of shrimp has a wide saline tolerance range for survival, but its survival rates and higher growth are within the higher saline range, and its survival within the lower saline ranges is only a characteristic for growth to be more desirable and widespread. The survival rates of shrimp *P. monodon* were also high during different feeding within the culture in marine waters, and this may be the reason for the short period of rearing. The study by Anh *et al*. (2019) on shrimp *L. vannamei* during four treatments with different survival rates close to the current study if we take into account that the culture period which is 75 days.

We note from Table 2, that five species of bacteria were recorded in the water of shrimp ponds, and the

Table 2: *Recording of the most important types and total number (CFU mL^{-1} 10^{6}) of bacteria in the four treatments in ponds for M. affinis and M. nipponense with salinities (3 and 15 g.L^{-1}). (Mean ± Std. Deviation).*

Bacteria species	Rearing shrimp in brackish water			
	A	B	C	D
Bacillus spp	5.67±1.53^{a}	4.67±1.53^{a}	5.00±1.00^{ab}	6.00±1.00^{b}
Escherchia coli	5.00±1.00^{a}	3.75±0.96^{a}	7.33±4.93^{ab}	11.00±1.41^{b}
Staphylococcus spp.	1.67±0.58^{a}	1.33±0.58^{a}	1.33±1.15^{a}	6.00±1.00^{b}
Streptococcus spp.	1.33±1.15^{a}	1.33±0.58^{a}	1.33±1.15^{a}	6.00±2.00^{b}
Enterococcus spp.	1.33±0.58^{a}	1.00±0.00^{a}	1.00±1.00^{a}	4.00±1.00^{b}
Total	15.00±3.00^{a}	12.33±3.06^{a}	12.67±0.58^{a}	33.67±0.58^{b}

total bacterial content was close in ponds of treatments A, B, and C, but it was somewhat high in treatment D. The presence of bacteria of each of *Bacillus* spp. and *E. coli* showed significant differences (P >0.05) between treatment D and between treatments A and B, which did not show a significant difference between treatment C, while it showed *Staphylococcus* spp. and *Streptococcus* spp. and *Enterococcus* spp. had significant differences (P>0.05) between treatment D and the combination of treatments A, B, and C, which had no significant differences (P<0.05). And the total bacterial content found significant differences (P>0.05) between treatment D and the combination of treatments A, B, and C, and there were no significant differences between them (P<0.05). And the bacterial content in the current study is less than what was mentioned in the study of Riandi *et al*. (2021) in shrimp ponds of *L. vannamei* during different grower experiment treatments.

Low salinity water negatively affects marine shrimp and thus negatively affects survival rates, which gave indications of its impact with other environmental factors on shrimp farms, coinciding with the incidence of diseases (Miaca *et al*., 2014).

From Table 3, we notice that the bacteria in all periods were low in numbers, but they were medium only when rearing in the medium saline water of the eastern shrimp, and this is an indication of a problem in the water quality and therefore the lack of shrimp growth with a low survival rate, meaning there is a death of the shrimp and the resulting disintegration of the dead shrimp and an increase Bacteria numbers because of it. It was noted that bacteria were rare in the first period of the experiment, but began to exist significantly during the second period of the experiment and were more numerous and diverse during the last period at the end of the experiment, especially in the eastern breeding ponds with salinity 15 g.L^{-1}. Bacterial infection was much less compared to other culture ponds which have increased weights and high survival rates for juveniles due to consumption of all food. While Riandi *et al*. (2021) mentioned the presence of high bacteria due to the high amounts of both the faces and the accumulated feed deposited at the bottom of the shrimp pond, which can then be decomposed by beneficial bacteria to transform it into beneficial nutrients for shrimp growth. The reason for the large numbers of bacteria in those The ponds are due to sedimentary organic matter, fodder residues, fecal matter, and changes in the levels of some environmental conditions and water quality from the source supplied to the pond, and that bacterial contamination has been associated with shrimp farms since the past twenty years, which resulted in great material damage to the owners of those farms with low survival rates and few production (Mastan and Begum, 2016).

Table 3: *Bacterial content amount "++, +, −" medium, low, and no density in treatments of M. affinis and M. nipponense shrimp ponds with two salinities (3 and 15 g.L^{-1}).*

Treat-ments	No. of bacteria in ponds of culture (day)			
	0	15	30	45
A	-	+	+	+
B	-	+	+	+
C	-	+	+	+
D	-	+	+	++

The shrimp ponds under study have a suitable environment because they contain some bacterial genera in a small number and are free of bacteria belonging to the genus Vibrio, whose presence in large numbers is dangerous in shrimp ponds in fresh and marine waters, and consequently, large losses occur in shrimp farms (Flores-Miranda *et al*., 2012).

Conclusions and Recommendations

The appearance of microbial contamination significantly during the last period of treatment D, in which the survival rates of shrimp *M. nipponense* decreased in the medium brackish water environment compared to the increase in the survival rates of C in brackish water. While the growth of juvenile shrimp *M. affinis* B increased in brackish water A compared to brackish water A, and higher survival rates in the two culture environments.

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Novelty Statement

Salinity levels suitable for the growth of two species of shrimp and their effect on their survival rates were determined.

Auther's Contribution

Tariq H.Y. Al-Maliky: Suggest a topic and shrimp farming, write and discuss the results
Mahmood S. Hashim and Amal S. Al-Sheraa: Both contributed to the review and discussion of the results and classification of bacteria.

Conflict of interest
The authors have declared no conflict of interest.

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