Acclimatization of the *Metapenaeus affinis* and the half-lethal concentration at different salinities and temperatures in the laboratory.

Wafaa Abdulhameed Abdulrazzaq[*,1] Raghad Z. Khalaf [2] , Tariq H. Y. Al-MAliky[3]

[1] Department of Biology, College of Science, University of Basrah,Iraq.

[2]Department of Ecology, College of Science, University of Basrah, Iraq.

[3]Department of Marine Biology, Marine Science Centre, University of Basrah, Iraq.

Abstract:

The study examined how six salinity levels (1, 5, 10, 15, 20, and 25) mg/L and three temperatures (20, 25, and 30)°C affected the survival of *Metapenaeus affinis*. We examined survival rates and median lethal concentrations (LC_{50}) under these conditions. And at Temperature 20 °Cthe LC_{50} (values=66.66±11.54) at salinities of (1, 5, 10, 15, 20, and 25) mg/L were (10, 11, 8, 6, 4, and 3) days, respectively. The numbers for 25°C were (13, 15, 11, 9, 7, and 5) days, in that order. At 30°C, the numbers were (12, 13, 9, 7, 5, and 4) days. The best acclimation happened at salinities of (1 and 5)mg/L at all tested temperature levels, as shown by survival rates and LC_{50} values. Statistical analysis indicated that the daily survival rates were completely unique ($P < 0.05$) between the six salinity levels at all three temperatures (20, 25, and 30)°C.

Keywords: Crustacean biology, Environmental conditions, Salinity, Temperature.

Introduction

Acclimatization is a physiological process that refers to the shrimp's ability and capacity to adapt to surrounding environmental fluctuations and changes, represented by temperature, salinity, pH, and dissolved oxygen, to improve and strengthen its ability to grow and survive in cultured environments. "Acclimatization" refers to a set of biological responses exhibited by an organism when exposed to fluctuating and unstable environmental conditions, whether in natural ecosystems or unnatural (cultivated) environments. These responses manifest as physiological, biochemical, and behavioral changes that enable shrimp to continue growing and surviving under unfavorable or harsh conditions, such as changes in temperature, salinity, pH, and dissolved oxygen. Moreover, these biological (adaptive) changes play a crucial role in stabilizing growth rates, enhancing survival rates, increasing disease resistance, and improving and boosting productivity in aquaculture systems (Abaas *et al.*, 2024; Boyd, 2017). Undoubtedly, adopting the acclimatization process before introducing shrimp into aquaculture environments reduces mortality rates, increases survival rates, enhances energy consumption efficiency, and improves shrimp resistance to changing environmental conditions (Boyd & Tucker, 2012; Fotedar & Evans, 2011).The process of acclimating shrimp is a fundamental procedure in their cultivation, as it gradually adjusts and regulates their response to new environmental factors to reduce the gap between the natural environment and the cultivation environment. Undoubtedly, sudden changes in environmental conditions weaken the shrimp's immunity and increase their susceptibility to viral, bacterial, and parasitic infections. Therefore, a slow acclimatization to environmental conditions provides the shrimp with sufficient time to adapt to the new variables, which in turn reduces stress and enhances the shrimp's response to the new environment, leading to increased immunity, reduced disease incidence, and lower mortality rates (Xiong *et al*., 2024; Lightner *et al.*, 2020). It is worth noting that there are three essential steps for acclimatization (Jory, 2016), which are transfer to the acclimatization environment, monitoring and adjusting environmental conditions, and evaluation and follow-up. It is necessary to mention the existence of fundamental environmental challenges and factors associated with and affecting the acclimatization process, such as temperature, salinity, pH, and dissolved oxygen. (Topuz & Kir, 2022; Suárez *et al*., 2022). There are many local studies on this type of shrimp that have addressed some of the life factors affecting its presence and growth, including (Al-Maliky*et al*., 2009; Al-Maliky, 2022a; Al-Maliky, 2022b; Al-Maliky*et al et al*., 2023).

The study aimed to determine the optimal salinity and temperature for the life of the white leg shrimp and to identify the median lethal concentration of this important crustacean in our aquatic environment**.**

Materials and Methods

The shrimp samples were transferred to 18 experimental tanks, which were plastic tanks with dimensions of (35×25×19) cm in length, width, and height, respectively, and a capacity of (22) liters. They were equipped with dechlorinated pure water and provided with continuous aeration systems. Additionally, nets were used to cover the tanks.Each tank was filled with 12 liters of dechlorinated pure water. Additionally, the tank water was replaced at a rate of 50-70%, and the bottom of the tank was cleaned regularly every two or three days. Cooling systems were used on hot days and heating devices on cold days to regulate the water temperature, as it is considered an essential factor for the continuity of theexperiment.

Six saline concentrations (1, 5, 10, 15, 20, 25) mg/L were prepared for the shrimp larvae tanks, with adherence to the gradual addition of salinity concentration at (10, 15, 20, and 25) mg/L. Each salt concentration was subjected to three different thermal treatments (20, 25, 30) °C.

After finishing the preparation of the tanks, the samples were distributed in the experimental tanks according to their lengths and sizes, with a total of 90 individuals whose lengths ranged between (60 and 70) mm. (5) shrimp individuals were allocated per replicate, with (3) replicates for each salinity concentration. The feed was provided once daily until saturation (2008, New). (Figure 1). The lethal salinity concentration LC50 for half of the individuals (50%) was also determined.

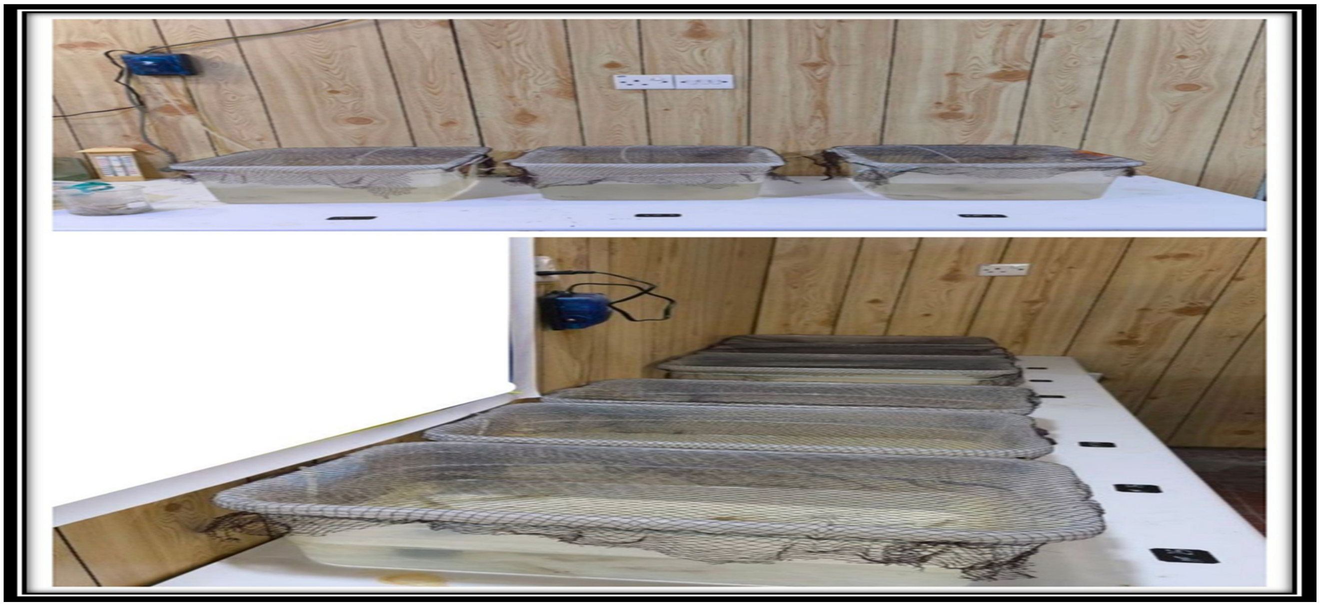

Figure1. The tanks used in the acclimatization experiment of shrimp*M. affinis***.**

Statical analysis

The data were statistically analyzed using the IBM SPSS Statistics (Version 20) software, where statistical significance was determined using a Two-Way ANOVA to identify differences at significance levels equal to or less than 0.05.

Results and Discussion

Table 1. Survival rates deviation and half-lethal concentration of the shrimp *M. affinis* at six salinity concentrations at a temperature of 20 °Cover the experimental period (in days) (± standard).

Salinity Concentrations (mg/L)
Time Duration (Days)

Time (Days)	1 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
1	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100
2	00 ±100	00 ±100	00 ±100	00 ±100	11.54 ±86.66	11.54 ±86.66
3	00 ±100	00 ±100	00 ±100	11.54 ±93.33	11.54 ±73.33	**11.54 ±66.66**
4	00 ±100	00 ±100	00 ±100	11.54 ±86.66	**11.54 ±66.66**	11.54 ±53.33
5	00 ±100	00 ±100	11.54 ±93.33	11.54 ±73.33	11.54 ±53.33	23.09 ±26.66
6	00 ±100	00 ±100	11.54 ±86.66	**11.54 ±66.66**	23.09 ±26.66	00 ±00
7	11.54 ±93.33	00 ±100	11.54 ±73.33	11.54 ±53.33	00 ±00	00 ±00
8	11.54 ±86.66	11.54 ±93.33	**11.54 ±66.66**	23.09 ±26.66	00 ±00	00 ±00
9	11.54 ±73.33	11.54 ±86.66	11.54 ±53.33	00 ±00	00 ±00	00 ±00
10	**11.54 ±66.66**	11.54 ±73.33	23.09 ±26.66	00 ±00	00 ±00	00 ±00
11	11.54 ±53.33	**11.54 ±66.66**	00 ±00	00 ±00	00 ±00	00 ±00
12	23.09 ±26.66	11.54 ±53.33	00 ±00	00 ±00	00 ±00	00 ±00
13	00 ±00	23.09 ±26.66	00 ±00	00 ±00	00 ±00	00 ±00

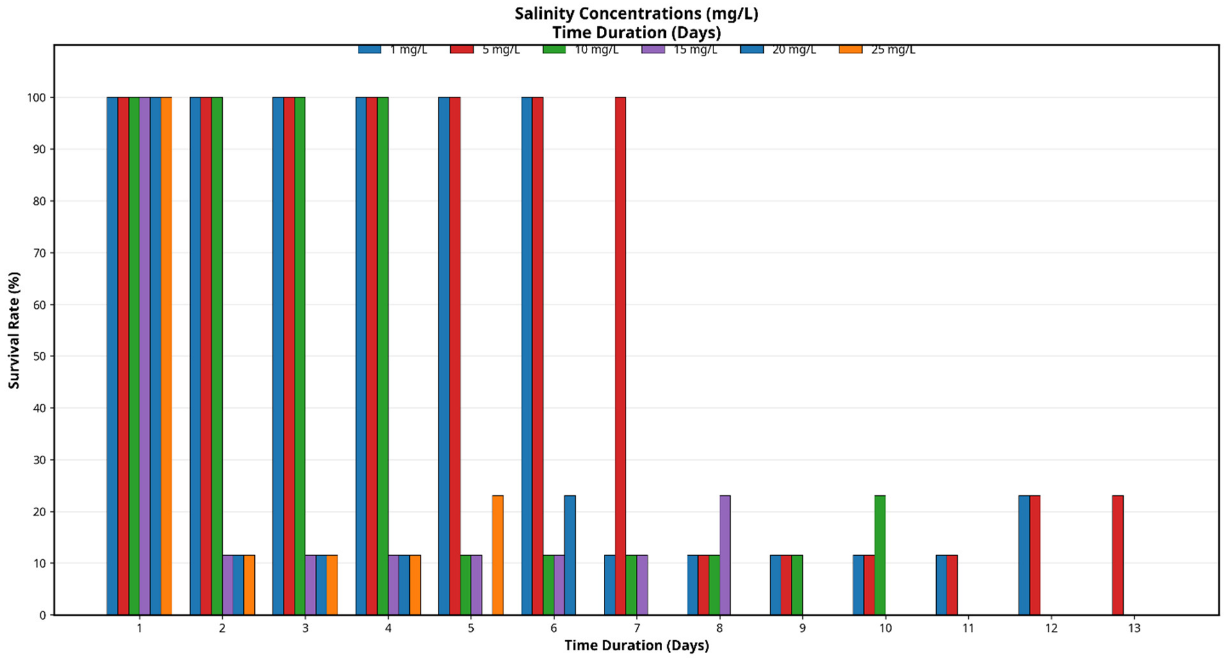

Figure 2. Survival rates (%) of *M. affinis* at different salinity concentrations in temperature of 20°C.

The results of the current study showed a variation in survival rates among the six different salinity concentrations during the experimental period of 13 days at a temperature of 20°C. In the 1 mg/l concentration, the survival rate was recorded at 76.92%, while in the 5 mg/lconcentration, the survival rate was 84.61%. The 10 mg/l concentration recorded a survival rate of 61.53%, and the 15 mg/L concentration recorded a survival rate of 46.15%. The 20 mg/lconcentration recorded a survival rate of 31.28%, and the 25 mg/l concentration recorded a survival rate of 25.64%. Thus, the highest survival rate was for the 5 mg/L concentration, followed by the 1 mg/L concentration, then the 10 mg/L concentration, followed by the 15 mg/L concentration, then the 20 mg/l concentration, and finally the 25 mg/l concentration, which represented the lowest survival rate recorded during the experiment. The results of the statistical analysis indicated a significant difference ($P < 0.05$) in survival rates among the six different salinity concentrations during the experimental period, as shown in Table 1 and Figure 2. In addition, the results of the current study showed daily variations in survival rates across the six salinity concentrations over the 13-day experiment at a temperature of 20°C. On day (1), the recorded survival rate was (100%), and on day two, it was (95.55%). On day three, the rate was (88.88%), while on day four, it was (84.44%). On day five, the rate was (74.44%), and on day six, it was (63.33%). On day seven, the rate was (53.33%), and on day eight, it was (45.55%). On day nine, the rate was (35.55%), and on day ten, it was (27.77%). On day eleven, the rate was (20%), and on day twelve, it was (13.33%). On day thirteen, the survival rate was 4.44%. The results of the statistical analysis showed a significant difference ($P < 0.05$) in daily survival rates across the six salinity concentrations at a temperature of °C.

Table 2. Survival rates and median lethal concentration of the shrimp *M. affinis* at six salinity concentrations at a temperature of 25°C during the experimental period (in days) (± standard deviation).

Salinity Concentrations (mg/L)
Time Duration (Days)

Time (Days)	1 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
1	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100
2	00 ±100	00 ±100	00 ±100	00 ±100	11.54 ±86.66	11.54 ±86.66
3	00 ±100	00 ±100	00 ±100	11.54 ±93.33	11.54 ±73.33	**11.54 ±66.66**
4	00 ±100	00 ±100	00 ±100	11.54 ±86.66	**11.54 ±66.66**	11.54 ±53.33
5	00 ±100	00 ±100	11.54 ±93.33	11.54 ±73.33	11.54 ±53.33	23.09 ±26.66
6	00 ±100	00 ±100	11.54 ±86.66	**11.54 ±66.66**	23.09 ±26.66	00 ±00
7	11.54 ±93.33	00 ±100	11.54 ±73.33	11.54 ±53.33	00 ±00	00 ±00
8	11.54 ±86.66	11.54 ±93.33	**11.54 ±66.66**	23.09 ±26.66	00 ±00	00 ±00
9	11.54 ±73.33	11.54 ±86.66	11.54 ±53.33	00 ±00	00 ±00	00 ±00
10	**11.54 ±66.66**	11.54 ±73.33	23.09 ±26.66	00 ±00	00 ±00	00 ±00
11	11.54 ±53.33	**11.54 ±66.66**	00 ±00	00 ±00	00 ±00	00 ±00
12	23.09 ±26.66	11.54 ±53.33	00 ±00	00 ±00	00 ±00	00 ±00
13	00 ±00	23.09 ±26.66	00 ±00	00 ±00	00 ±00	00 ±00

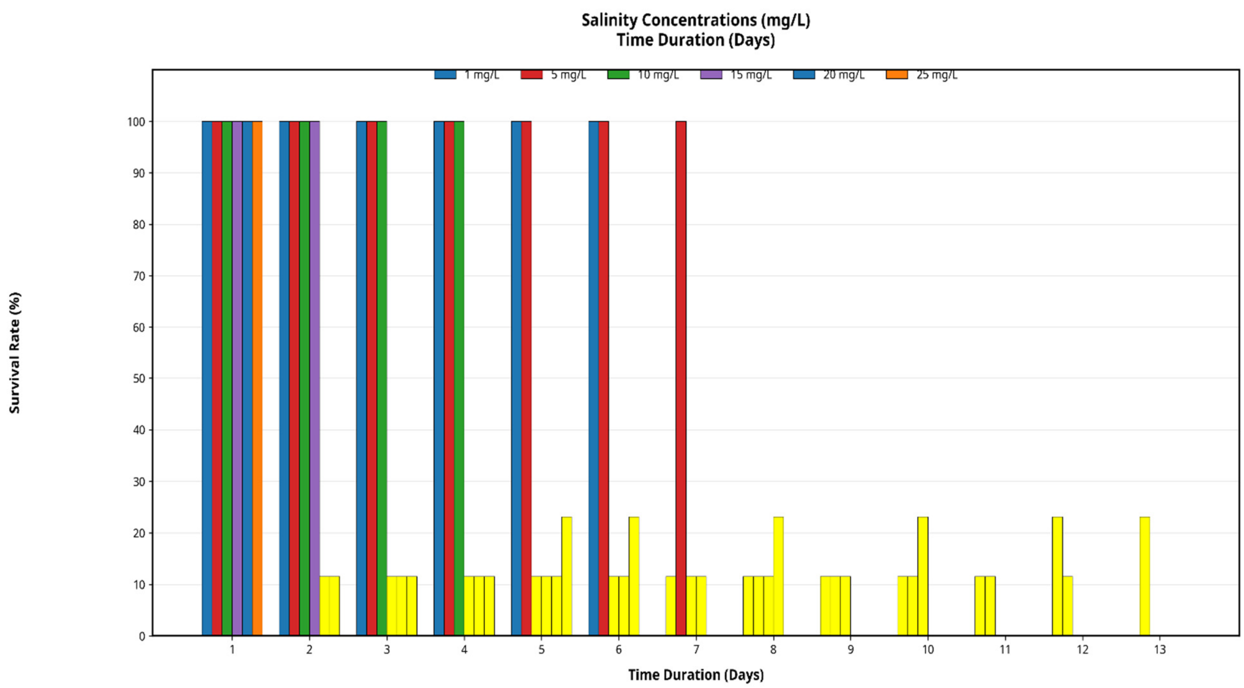

Figure 3. Survival rates (%) of*M. affinis* at different salinity concentrations in temperature of 25°C.

The results of the current study showed a variation in survival rates among the six different salinity concentrations over the duration of the experiment, which lasted 17 days at a temperature of 25°C. At a concentration of (1) mg/L, the survival rate recorded was (80.0%), while at a concentration of (5) mg/L, the survival rate recorded was (86.66%). At a concentration of 10 mg/L, the survival rate recorded was (63.13%), and at a concentration of 15 mg/l, the survival rate recorded was (51.37%). At a concentration of 20 mg/L, the survival rate recorded was (41.17%), and at a concentration of 25 mg/L, the survival rate recorded was (36.86%). Therefore, the highest recorded survival rate was for the concentration of 5 mg/L, followed by 1 mg/L, then 10 mg/L, followed by 15 mg/L, then 20 mg/l, and finally 25 mg/l, which recorded the lowest survival rate during the experiment period. Accordingly, the results of the statistical analysis showed a significant difference ($P < 0.05$) in survival rates among the six different salinity concentrations over the duration of the experiment at a temperature of °C (Table 2), and Figure 3.

Additionally, the results of the current study showed daily variation in survival rates during the 17-day experimental period at 25°C. On day 1, the recorded survival rate was 100%, and it remained 100% on day 2 and day 3. However, the survival rate began to decrease on day 4, recording 97.77%. On day 5, it reached 94.44%, and on day 6, it was 86.66%. On day 7, it recorded 82.22%, and on day 8, 73.33%. On day 9, it reached 61.11%, while on day 10, it recorded 53.33%. On day 11, it reached 44.44%, and on day 12, it was 35.55%. On day 13, it reached 27.77%, and on day 14, it was 23.33%. On day 15, it reached 20%; on day 16, 13.33%; and on day 17, 4.44%. Based on this, statistical analysis revealed a significant difference ($P < 0.05$) in daily survival rates for six different salinity concentrations at 25°C. This aligns with Al-Maliky (2017) study during his cultivation of *M. affinis* shrimp at a salinity of 1 mg/L and a temperature ranging between (24 and 30)°C, where survival rates were high, between (75 and 90)%. The study also aligns with Al-Maliky*et al.* (2019) study on the same shrimp, where the optimal environmental conditions for its viability were found to be at 25°C. However, it differed from Al-Maliky *et al*. (2021) study on *M. affinis* shrimp cultivation using a probiotic system, where high survival rates reached 93% at a salinity ranging between (12 and 17) mg/L and a temperature between (24 and 27)°C. This discrepancy may be attributed to differences in the cultivation method and the size of the shrimp used.

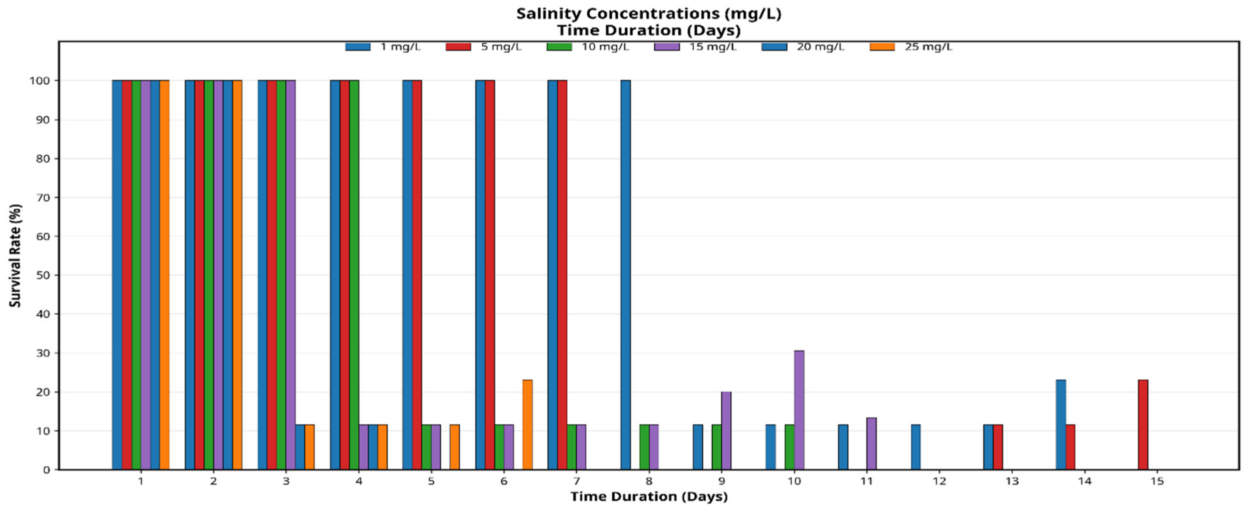

Figure 4. Survival rates (%) of *M. affinis* at different salinity concentrations in temperature of 30°C.

Table 3. Survival rates and half-lethal concentration of the shrimp *M. affinis* at six salinity concentrations at a temperature of 30°C (± standard deviation).

Salinity Concentrations (mg/L)
Time Duration (Days)

Time (Days)	1 mg/L	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
1	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100
2	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100	00 ±100
3	00 ±100	00 ±100	00 ±100	00 ±100	11.54±86.66	11.54±86.66
4	00 ±100	00 ±100	00 ±100	11.54±93.33	11.54±73.33	**11.54±66.66**
5	00 ±100	00 ±100	11.54±93.33	11.54±93.33	**11.54±66.66**	11.54±53.33
6	00 ±100	00 ±100	11.54±86.66	11.54±86.66	11.54±53.33	23.09±26.66
7	00 ±100	00 ±100	11.54±86.66	**11.54±66.66**	23.09±26.66	00 ± 00
8	11.54±93.33	00 ±100	11.54±73.33	20.0 ±60.00	00 ± 00	00 ± 00
9	11.54±86.66	11.54±93.33	**11.54±66.66**	30.55±33.33	00 ± 00	00 ± 00
10	11.54±73.33	11.54±86.66	11.54±53.33	13.33±13.33	00 ± 00	00 ± 00
11	11.54±73.33	11.54±86.66	23.09±26.66	00 ± 00	00 ± 00	00 ± 00
12	**11.54±66.66**	11.54±73.33	00 ± 00	00 ± 00	00 ± 00	00 ± 00
13	11.54±53.33	**11.54±66.66**	00 ± 00	00 ± 00	00 ± 00	00 ± 00
14	23.09±26.66	11.54±53.33	00 ± 00	00 ± 00	00 ± 00	00 ± 00
15	00 ± 00	23.09±26.66	00 ± 00	00 ± 00	00 ± 00	00 ± 00

The results of the current study showed differences in the duration of stay (in days) according to the varying salt concentrations (1, 5, 10, 15, 20, 25) mg/L at a temperature of 30 °C. At a concentration of 1 ppt, the recorded duration of stay was 14 days, while at a concentration of 5 mg/l, the recorded duration was 15 days. At a concentration of (10) ppt, the duration of stay was recorded as 11 days, and at a concentration of (15) mg/L, the duration of stay was (10) days. At a concentration of 20mg/L, the duration of stay was (7) days, and at a concentration of (25) mg/L, the duration of stay was (6) days. Accordingly, the longest duration of stay (15 and 14) days was recorded for the low salinity concentrations (5 and 1)mg/L, respectively). Then, a decrease in the duration of stay to (11 and 10) days was recorded for the medium salinity concentrations of (10 and 15)mg/L, respectively. The shortest duration of stay (7 and 6) days was recorded for the high salinity concentrations (20 and 25)mg/L, respectively). Therefore, the results of the statistical analysis indicated significant differences ($P < 0.05$) in the duration of stay (in days) among the six different salinity concentrations at a temperature of 30°C during the experimental period, as shown in Table 3 and Figure 4.

The results of the current study showed a clear variation in survival rates among the six different salinity concentrations over the duration of the experiment, which lasted 15 days at °C. At a concentration of (1) mg/L, the survival rate recorded was (78.22%), while at a concentration of (5) mg/l, the survival rate was (84.44%). At a concentration of 10 mg/L, the survival rate was (59.55%), and at a concentration of 15 mg/L, the survival rate was (49.77%). At a concentration of (20) mg/L, the survival rate was (33.77%), and at a concentration of 25 mg/L, the survival rate was (28.88%). Thus, the highest recorded survival rate was for the concentration of (5) mg/l, followed by (1) mg/L, then (10) mg/L, followed by (15) mg/L, then (20) mg/l, and finally (25) mg/L, which recorded the lowest survival rate during the experiment period. The results of the statistical analysis indicated a significant difference ($P < 0.05$) in survival rates among the six different salinity concentrations over the duration of the experiment at °C.

Furthermore, the results of the current study revealed a clear daily variation in survival rates throughout the experimental period, which lasted for 15 days at a constant temperature of 30 °C. On the first day, the recorded survival rate was (100%), and on the second day, it was (100%). Subsequently, the survival rate began to decline on the third day, recording 95.55%. On the fourth day, it was (88.88%), and on the fifth day, it was (84.44%). On the sixth day, it reached 75.55%, while on the seventh day, it was 63.33%. On the eighth day, the recorded rate was (54.44%), and on the ninth day, it was (47.44%). On the tenth day, it was (37.77%). On the eleventh day, it was (31.11%), while on the twelfth day, it was (23.33%). On the thirteenth day, it was (16.66%), and on the fourteenth day, it was (13.33%). Finally, on the fifteenth day, it was (4.44%). Consequently, statistical analysis indicated a significant difference ($P < 0.05$) in daily survival rates for six different salinity concentrations at 30°C, as shown in Table 14 and Figure 18, for the variation in daily survival rates (mean survival rate + standard deviation) over the experimental period.

The results of the current study, as shown in Table 4, indicate that the lethal concentration 50 (LC50) for the shrimp *M. affinis* across six different salinity concentrations and three different temperature ranges showed the best

Table 4. The median lethal concentration of *M. affinis* across six salinities at different temperatures over a period of days.

Temperature ℃	Salinity mg/L	LC50 (day)	LC50
20	1	10	66.66 ±11.54
	5	11	66.66 ±11.54
	10	8	66.66 ±11.54
	15	6	66.66 ±11.54
	20	4	66.66 ±11.54
	25	3	66.66 ±11.54
25	1	13	66.66 ±11.54
	5	15	66.66 ±11.54
	10	11	66.66 ±11.54
	15	9	66.66 ±11.54
	20	7	66.66 ±11.54
	25	6	66.66 ±11.54
30	1	12	66.66 ±11.54
	5	13	66.66 ±11.54
	10	9	66.66 ±11.54
	15	7	66.66 ±11.54
	20	5	66.66 ±11.54
	25	4	66.66 ±11.54

Discussion

These clear variations in the experimental duration and survival rates among different salinity concentrations are attributed to a reduction in the effort dedicated to ion exchange and osmoregulation, which in turn leads to a decrease in energy consumption for metabolic processes. This suggests that increasing salinity concentrations negatively and directly impacts the survival duration of shrimp (Kültz, 2015). The current study recorded the longest experimental duration and highest survival rates at low salinity concentrations (1 and 5) mg/L, due to the reduced effort expended. Therefore, the results recorded in the current study do not differ from those recorded in the study by (Abbas et al., 2024). Subsequently, the experimental duration decreased, and survival rates declined at medium salinity concentrations (10, 15) mg/L, due to the onset of salinity stress on the shrimp to maintain osmotic balance and ion exchange, which leads to an increased energy consumption for metabolic processes.(Suresh & Lin, 1992). Therefore, the results recorded in the current study are consistent with those recorded in the study by Castille & Lawrence (1981), which indicated that moderate salinity concentrations are not directly lethal but reduce the duration of the experiment while decreasing survival rates due to salt stress. The current study recorded the shortest duration of the experiment and a sharp decline in survival rates at high salinity concentrations (25 and 20) mg/L, as these concentrations exceed the physiological tolerance (Kültz, 2015). Therefore, the results of the current study are consistent with the study by Abbas et al. (2024). Therefore, it must be noted that the duration of the experiment and the survival rates were relatively high at low salinity concentrations (1 and 5) mg/L and then gradually decreased as salinity increased until reaching their lowest levels at a concentration of 25 mg/L. The results of the current study are consistent with those recorded in the study by Silva *et al.* (2010), which indicated that increased salinity concentrations reduce the survival rates of the shrimp Farfantepenaeus subtilis due to increased osmotic pressure. Additionally, the study by Davis *et al.* (2002) on species more tolerant to high salinity concentrations, such as the shrimp *Litopenaeus vannamei*, showed that this species has a wide salinity tolerance range. This indicates that salinity tolerance depends on the species and the environment in which the shrimp live (freshwater or marine). Accordingly, the results of the current study differ from the results recorded in the study by Davis *et al.* (2002).

In the context of the current study on the acclimatization of *M. affinis* shrimp at 25°C across six salinity concentration ranges (1, 5, 10, 15, 20, and 25) mg/L, it appears that salinity plays a definitive role in the survival duration, continuity of the experiment, and survival rate, with the highest survival duration and rate recorded at a salinity concentration of 5 mg/L, followed by 1 mg/L. More survival duration and survival rate were apparent with 5 and 1 mg/L, while with the increasing salinity concentration of (10, 15, and 20) mg/L, the shortest survival duration and the lowest survival rate were recorded at 25 mg/L. Therefore, it can be said that this shrimp species' optimal salinity range lies within a low to a moderate salinity concentration, as the organism's requirement for an adequate osmotic balance is less energy cost for ion switches in the exchange process of the organism while the energy lost is shifted to the vital biological processes that are necessary for the organism to continue to live (Péqueux, 1995). Moreover, in a study carried out by Salman *et al.* (1990), it was also noted that M. affinis juveniles recorded the highest survival rate at a 5 mg/L salinity concentration, while at salinities of 15 and 20 mg/L, the survival rate dropped drastically due to the osmotic pressure increase and the energy cost of maintaining the metabolic rate. In a similar study, an increase in the osmotic pressure leads to an increase in energy required for metabolic processes, and, therefore, the juvenile *M. affinis* at 25 mg/L recorded a high oxygen consumption for the metabolic processes, while at 5 mg/L the oxygen consumption was low. (Abbas *et al*., 2024).

The current study rewrites the study done by Freire *et al.*(2008). Freire studied the basic physiological processes of the crustaceans and mentioned in his study that deviation from the optimal salinity range leads to more effort to control the process of osmosis and thus reduces the chances of survival. Freire's study agrees with the current study. Moreover, the current study also contradicts the study done by Davis *et al.*(2002). Davis believed and showed through his study that the shrimp *Litopenaeus vannamei* demonstrates a wide range of salinity, i.e., tolerance, and that the survival rate for the shrimp remains high at salinities of 40 parts per thousand. According to Saoud & Davis (2003), some age groups of the shrimp *Farfantepenaeus aztecus*were also studied for the survival of that species in a range of salinity, from medium to high concentrations of 30 mg/L, and the survival rate did not decline sharply. Therefore, *M. affinis* shrimp is confirmed as a species that has adapted to the low salinity coastal and estuarine environments. Further increases in salinity beyond optimal levels will lead to increased physiological stress and reduce survival chances.

Conclusions

The results of the current study on the acclimatization of shrimp with six different salinity concentrations (1, 5, 10, 15, 20, and 25) mg/L at a temperature of 30°C. The longest duration and highest survival rate were observed at low concentrations (1 and 5) mg/L, respectively). The concentration (5) mg/L recorded the longest duration and highest survival rate, followed by the concentration (1) mg/L. Then, the duration and survival rates began to gradually decrease with increasing salinity concentrations to (10 and 15) mg/L, respectively. After that, a significant decline in duration and survival rates was recorded at high concentrations (20 and 25) mg/L, respectively, due to the noticeable effect of different salinity concentrations on the shrimp's efficiency, adaptability, growth, and survival at a temperature of 30°C. Low salinity concentrations, particularly the concentration of 5 mg/L, showed longer durations and higher survival rates because they represent the optimal salinity range where osmotic pressure on the shrimp decreases due to the minimal differences between the salinity concentrations inside and outside the shrimp's body. This reduces the energy consumed for osmotic regulation and ion exchange processes through the gills and other regulatory mechanisms, which in turn provides more energy for physiological processes such as feeding, growth, and stress reduction.

As for high salinity concentrations (20 and 25) mg/L, particularly the concentration of 25 mg/L, the shortest duration and lowest survival rate were recorded. This is due to the inverse osmotic pressure experienced by the shrimp, which causes water loss from the shrimp's cells and tissues as salts enter the body tissues. This necessitates an increased energy expenditure for physiological processes. When the temperature is increased to 30 °C, metabolic rates rise, requiring increased oxygen consumption, which exacerbates the effect of salinity stress on shrimp. This negatively impacts survival rates, leading to a significant decrease. It can be concluded that high temperatures limit the appropriate salinity range for continued survival. Accordingly, the results of the current study are consistent with the findings of Abbas *et al*. (2024), which recorded a low survival rate (17%) at a concentration of 20 mg/L and a temperature ranging from (29 to 30) °C, indicating that this species is highly sensitive and has low tolerance to high salinity at elevated temperatures. However, the results of the current study are not consistent with the findings of Lebata & Primavera (1997) on *Metapenaeus anchistus* shrimp, which recorded a high and complete survival rate

(100%) at a concentration of 35 mg/L. Therefore, temperature and salinity have different effects on survival rates depending on the species and environment. The results of the current study indicated a clear variation in cumulative daily survival rates and differences in survival duration (in days) for *M. affinis* shrimp due to varying temperatures (20, 25, 30) °C. At 20°C, the rate was recorded as 54.36% for 13 days. At 25°C, the rate was recorded as 59.87% for 17 days. While at 30 °C, the rate was recorded as 55.77% for 15 days. Based on this, statistical analysis showed significant differences ($P < 0.05$) in cumulative daily survival rates among the three different temperature ranges (20, 25, 30) °C.

The results of acclimating the shrimp *M. affinis* at three thermal levels (20, 25, 30) °C with six different salinity concentrations (1, 5, 10, 15, 20, 25) mg/L indicated the interaction between temperature and salinity on the duration and survival rates and the ability of this species to acclimate. The results of the current study indicated that the temperature (25°C) is the most suitable and optimal, as it recorded the longest survival duration, lasting 17 days, and the highest survival rates. This is followed by the temperature (30°C) and then the temperature (20°C). The temperature (25 °C) represents the thermal range suitable for physiological processes, as osmotic regulation, ionic exchange, and metabolism are more efficient and active compared to higher and lower temperatures. At a temperature of 30°C, there is a relative decline in the duration and rates of survival due to the exacerbation of thermal-salinity stress, increased oxygen demands, and elevated metabolic rates, which exceed the shrimp's ability to adapt. However, at a temperature of 20 °C, a decrease in duration and survival rates was observed due to the slowing of metabolic activity and reduced efficiency of ion exchange and osmotic regulation, which negatively affects growth rates and survival rates. It can be concluded that this species prefers to inhabit waters with warm temperatures, which are compatible with its natural environment represented by the waters of Shatt al-Arab and the marshes in southern Iraq through its daily migration (Ali *et al*., 2015).

The results also show that a salt concentration of 5 mg/L is optimal and best, as it recorded the longest durations and the highest survival rates, followed by a concentration of 1 mg/L, then (10, 15, and 20) mg/L, and lastly a concentration of 25 mg/L that recorded the shortest durations and lowest survival rates. This shows that this type can osmoregulation at low salinities because low osmotic pressure is created between the external environment and the body fluids, thus requiring less energy for the transport of water and ions across the cell membranes, thus increasing survival rates (Zhu *et al*., 2020). For high salinities (20 and 25) mg/L, particularly at 25 mg/L, survival rates were low because of high osmotic pressure for the shrimp, and thus high energy was expended to maintain the internal equilibrium of the body. This means that chronic high salinity exposure for a long time leads to high energy expenditure and thus low survival rates. Several authors have documented the salinity ranges of the Shatt al-Arab waters, where the salinity is as high as 37 mg/L as a result of the inflow of saline water from the Arabian Gulf, but it is less than 10 mg/L in freshwater zones such as the southern Iraqi marshes (Al-Edany, 2020).

ACKNOWLEDGEMENTS

I extend my gratitude to the staff of the Aquaculture Laboratory and the other members of the Marine Biology Department at the Marine Science Center for their assistance in completing our article.

References

Abbas, A. T., Ghazi, A. H. H.& Sultan, A. N. (2024). Influence of different temperature-salinity combinations on the oxygen consumption in the juveniles *Metapenaeus affinis*. Egyptian Journal of Aquatic Biology & Fisheries, 28(4), 1479–1494. https://doi.org/10.21608/ejabf.2024.368142 (https://www.google.com/search?q=https://doi.org/10.21608/ejabf.2024.368142)

Ali, M. H., Salman, D. S.& Al-Adhub, A. H. (2015). Socioeconomic study for the wealth of marine shrimp in the territorial sea of Iraq. Iraqi Journal of Aquaculture, 12(2), 59–70. https://www.iasj.net/iasj/article/111303 (https://www.google.com/search?q=https://www.iasj.net/iasj/article/111303

Al-Edany, A. H. (2020). Assessment of water quality in Shatt Al-Arab River, Southern Iraq. Iraqi Journal of Science, 61(5), 1120–1130. https://doi.org/10.24996/ijs.2020.61.5.15

Al-Maliky, T. H. Y.; Ali, M.H. & Al-Nor, S.S. (2009). Comparison of the growth rates of Jinga Shrimp Metapenaeus affinis (H. Milne Edwards, 1837) in different types of Aquaculture Systems. A thesis submitted to the College of Agriculture – University of Basrah, In Partial Fulfillment of the Requirements for the degree of Master of Science in Fisheries Resources. 108 pp.

Al-Maliky, T. H. Y. (2017). Breeding of shrimps *Metapenaeus affinis* in cages. International Journal of Marine Science, 7(41), 394–398. https://doi.org/10.5376/ijms.2017.07.0041 (https://www.google.com/search?q=https://doi.org/10.5376/ijms.2017.07.0041)

Al-Maliky, T. H. Y., Al-Noor, S. S.& Ali, M. H. (2019). Effect of different temperatures on food consumption of juvenile shrimp *Metapenaeus affinis*. Arthropods, 8(1), 32–37. http://www.iaees.org/publications/journals/arthropods/articles/2019-8(1)/32-37.pdf (https://www.google.com/search?q=http://www.iaees.org/publications/journals/arthropods/articles/2019-8(1)/32-37.pdf)

Al-Maliky, T. H. Y., Al-Maliky, A. M. J., Al-Maliki, G. M. J.& Boyd, C. A. (2021). Effects of prebiotic and molasses on water quality, growth and survival of *Metapenaeus affinis* and Macrobrachium nipponense. Egyptian Journal of Aquatic Biology and Fisheries, 25(4), 767–783. https://doi.org/10.21608/ejabf.2021.191632 (https://www.google.com/search?q=https://doi.org/10.21608/ejabf.2021.191632)

Al-Maliky, T. H. Y. (2022a). The study of annual shrimp stocks in Masshab (Al-Hammar Marsh), Basrah, Southern Iraq. International Journal of Applied Science, 5(4), 9–16. https://doi.org/10.5281/zenodo.7115160 (https://www.google.com/search?q=https://doi.org/10.5281/zenodo.7115160)

Al-Maliky, T. H. Y. (2022b). A study on shrimp fishing quantities offered in some markets of Basrah, Iraq. Egyptian Journal of Aquatic Biology and Fisheries, 26(5), 307–317. https://doi.org/10.21608/ejabf.2022.261234 (https://www.google.com/search?q=https://doi.org/10.21608/ejabf.2022.261234)

Al-Maliky, T. H. Y., Hashim, M. S.& Al-Sheraa, A. S. (2023). A comparative study of bacterial contamination from shrimp ponds in two different environments. Sarhad Journal of Agriculture, 39(1), 126–133. https://dx.doi.org/10.17582/journal.sja/2023/39.1.126.133

Boyd, C. E.& Tucker, C. S. (2012). Pond aquaculture water quality management. Springer. https://doi.org/10.1007/978-1-4615-5407-3

Boyd, C. E. (2017). Water quality: An introduction. In Aquaculture Production Systems. Wiley-Blackwell. (https://www.google.com/search?q=https://www.wiley.com/en-us/Aquaculture%2BProduction%2BSystems-p-9781118250884)

Castille, F. L.& Lawrence, A. L. (1981). Osmoregulatory capabilities of five species of shrimp. Contributions in Marine Science, 24, 181–194. https://repositories.lib.utexas.edu/handle/2152/20421 (https://www.google.com/search?q=https://repositories.lib.utexas.edu/handle/2152/20421)

Davis, D. A., Arnold, C. R.& Blake, B. (2002). Response of *Litopenaeus vannamei* to salinity stress. Journal of the World Aquaculture Society, 33(4), 395–402. https://doi.org/10.1111/j.1749-7345.2002.tb00118.x

Fotedar, R.& Evans, L. (2011). Health management during handling and transport of crustaceans. Journal of Invertebrate Pathology, 106(1), 143–152. https://doi.org/10.1016/j.jip.2010.09.013

Freire, C. A., Onken, H.& McNamara, J. C. (2008). Ion transport in crustacean gills: Structure-function analysis. Comparative Biochemistry and Physiology Part A, 151(3), 213–229. https://doi.org/10.1016/j.cbpa.2008.06.003

Jory, D. E. (2016). Acclimating shrimp postlarvae before pond stocking. Global Seafood Alliance. https://www.globalseafood.org/advocate/acclimating-shrimp-postlarvae-before-pond-stocking/

Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. Journal of Experimental Biology, 218(12), 1907–1914. https://doi.org/10.1242/jeb.118695

Lebata, M. J. H. L.& Primavera, J. H. (1997). Effect of salinity on survival of *Metapenaeus anchistus* juveniles and subadults. UPV Journal of Natural Sciences, 2(2), 83–90.

Lightner, D. V. Tang, K. F. J., Wang, J., & White, B. L. (2020). Viral diseases of farmed shrimp in Asia and the Americas: Review and management strategies. Journal of Invertebrate Pathology, 177, 107528. https://doi.org/10.1016/j.jip.2020.107528

New, M. B.& Singholka, S. (1982). Freshwater prawn farming: A manual for the culture of *Macrobrachium rosenbergii* (FAO Fish. Tech. Paper 225). FAO. https://www.fao.org/3/ac135e/ac135e00.htm (https://www.google.com/search?q=https://www.fao.org/3/ac135e/ac135e00.htm)

Péqueux, A. (1995). Osmotic regulation in crustaceans. Journal of Crustacean Biology, 15(1), 1–60. https://doi.org/10.2307/1548595

Salman, S. D., Ali, M. H.& Al-Adhub, A. H. Y. (1990). Abundance and seasonal migrations of the penaeid shrimp *Metapenaeus affinis* within Iraqi waters. Hydrobiologia, 196(1), 79–90. https://doi.org/10.1007/BF00008893 (https://www.google.com/search?q=https://doi.org/10.1007/BF00008893

Saoud, I. P.& Davis, D. A. (2003). Salinity tolerance of brown shrimp *Farfantepenaeus aztecus* as it relates to postlarval and juvenile survival, growth, and osmoregulation. Estuaries, 26(5), 1213–1219. https://doi.org/10.1007/BF02803515

Silva, E. F., Calazans, N., Soares, M., Soares, R.& Peixoto, S. (2010). Effect of salinity on survival, growth, food consumption and haemolymph osmolality of the pink shrimp Farfantepenaeus subtilis. Aquaculture, 306(1-4), 352-356. https://doi.org/10.1016/j.aquaculture.2010.04.025

Suárez, J. A., Pineda, A. S.& Gálvez, J. A. (2022). Environmental stressors and their impact on shrimp aquaculture: A review. Aquaculture Reports, 23, 101076. https://doi.org/10.1016/j.aqrep.2021.101076

Suresh, A. V.& Lin, C. K. (1992). Tilapia culture in saline waters: A review. Aquaculture, 106(3-4), 201–226. https://doi.org/10.1016/0044-8486(92)90253-H

Tang, S. K., Akatsu, S., El-Zahr, C., Al-Abdul-Ellah, K. M., & Abdallah, M. (1985). Preliminary observations on the relative growth and production of sobaity cultured in open circulation tanks at three stocking densities. Kuwait Bulletin of Marine Science, 6, 95–107.

Topuz, B.& Kir, M. (2022). Effects of thermal stress on crustaceans: Implications for aquaculture under climate change. Aquaculture Research, 53(12), 4321–4333. https://doi.org/10.1111/are.16024

Xiong, J., Zhu, J.& Zhang, J. (2024). Microbes and pathogens associated with shrimps: Environmental drivers and implications for aquaculture health management. Frontiers in Marine Science, 11, 1397708. https://doi.org/10.3389/fmars.2024.1397708

Zhu, F., Huang, Y., Du, T., Li, Q., Pan, H., Li, Y. & Ding, Z. (2020). Multi-omic approach provides insights into the osmoregulation of the euryhaline crab Scylla paramamosain. Scientific Reports, 10(1), 21734. https://doi.org/10.1038/s41598-020-78792-1