



# A Response-Surface Analysis of Temperature-Salinity on Growth and Survival of the Caridean Shrimp, *Macrobrachium americanum* (Bate, 1868)

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## ABSTRACT

The effect of different levels of temperature (23, 26, 29, and 32°C) and salinity (0, 5, and 10‰) on growth performance and survival of *Macrobrachium americanum* was studied under controlled laboratory conditions. Sub-adult's prawns (10.29 ± 0.27 g) were reared in these conditions for 72 days. Each experimental group contained 25 prawns in an approximately 100-L water capacity tank. The experimental design was factorial with three repetitions per treatment. Prawns were fed (commercial shrimp feed with 35% protein, 12% lipid) daily at a ratio of 10% body weight, twice a day (10:00 and 18:00 h). The optimal growth intervals were from 26 to 29°C and from 3 to 11‰ of salinity, and survival was at 23 to 29°C and 0 to 5‰. The greatest effect of the temperature-salinity interaction was on the upper extremes of response. The analysis of response surfaces showed that the final weight and weight gain increased as temperature increased and, in the upper end, salinity had a linear synergistic effect, with a greater effect on growth at high temperatures. *M. americanum* attains its best growth in freshwater and low salinities (< 11‰) in subtropical zones.

## Article Information

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## Authors' Contribution

JTTP, FLR and MRC conceived and designed the experiments. FLU and SCV performed the experiments. IGV and ARL analyzed the data. JTTP wrote the manuscript.

## Key words

Caridean shrimp, *Macrobrachium americanum*, Specific growth rate, Optimal growth

## INTRODUCTION

Salinity and temperature affect the physiology and, partially, the distribution and survival of aquatic organisms (Re *et al.*, 2005). Temperature and salinity are factors that directly and indirectly influence aquatic organisms. The first factor directly controls aspects of the organism's activity and the second indirectly influences the physiological response, such as nutrition, metabolism, growth, life cycle, and intra- and inter-specific relationships (Kinne, 1971). In brackish waters, studies of water temperature and salinity on prawns have shown that salinities above their osmotic point have a greater effect on survival than temperature (Ch and Shailender, 2013), although high mortality is registered in extreme temperatures (Aktas and Cavdar, 2012; Crisp *et al.*, 2017). Studies of the effect of salinity and temperature on prawns

of commercial interest in the world have focused mainly on *M. rosenbergii* in larval-postlarval production (Mohanty *et al.*, 2016) and in juvenile stages (Habashy and Hassan, 2011; Chand *et al.*, 2015). The separate or combined effect of these two environmental factors on growth and survival of the *Macrobrachium* species in Latin America has been studied in *M. amazonicum* (Guest and Durocher, 1979), *M. holthuisi* (Moreira *et al.*, 1979), *M. carcinus* (Choudhury, 1971) *M. acanthurus* (Signoret *et al.*, 1997) and *M. tenellum* (Signoret *et al.*, 1997; Vega-Villasante *et al.*, 2011; Rodriguez-Flores *et al.*, 2012).

In *M. americanum*, studies have been performed on the effect of embryological development and survival of eggs and larvae, finding that the best temperature for the development of eggs and larvae was 29°C (Sainz-Hernández *et al.*, 2016) and a salinity of around 20‰ is required at the beginning of larval development, decreasing to around 15‰ upon reaching the postlarval stage (Holtzman and Pfeiler, 1984). Within the models to study the individual and synergistic response of the salinity-temperature effect, the analysis of response surfaces has been used in *M.*

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*rosenbergii* to determine the best parameters for growth and survival with good results and a better understanding of the interaction (Agard, 1999). The aim of this study was to determine the influence of salinity and temperature on growth and survival of *M. americanum* sub-adults by using response-surface techniques to understand better the single and combined effect of the two factors and their influence on growth performance.

## MATERIALS AND METHODS

### Animal collection and experimental site

Caridean shrimp *M. americanum* ( $10.29 \pm 0.27$  g) were captured in the Ameca river, in the State of Nayarit, Mexico ( $20^{\circ}53'27.00''$  N,  $105^{\circ}07'30.20''$  W). The study was conducted in the Coastal Bioengineering Laboratory of the National School of Fishing Engineering, in the University of the State of Nayarit, Mexico.

### Experimental design

Prawns were acclimated during 2 weeks at different temperatures (23, 26, 29, and 32°C) and salinities (0, 5, and 10‰). The experiment was carried out in a cooled room, the water was maintained at 23°C and under a 12 h L:12 h D photoperiod. Temperatures above 23°C were maintained with a 300-W thermostat-controlled immersion heater, and low aeration assured mixing of the water. Prawns were stocked at 29°C and 0‰, which were increased or decreased at a rate of 3°C/h and 2 g/L/h, respectively. Seawater was obtained from an unpolluted coastal site at the beachfront and freshwater from drinking water sources (dechlorinated tap water). Temperature and salinity were controlled daily to maintain the variations within  $\pm 0.5^{\circ}\text{C}$  and  $\pm 0.5\text{‰}$ , respectively, and were measured with an YSI Model 85 salinity-conductivity-temperature meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Each experimental group contained 25 prawns in approximately 80-L water in a 100-L capacity tank. The experimental design was factorial with three repetitions per treatment. Prawns were fed (commercial shrimp feed with 35% protein, 12% lipid) daily at a ratio of 10% body weight, twice a day (10:00 and 18:00 h). Feed and fecal matters were removed daily. The prawns were cultured for 72 days under these conditions. The initial weight ( $W_i$ ), final weight ( $W_f$ ), food conversion ratio (FCR), weight gain (WG), specific growth rate (SGR), condition factor (K), and survival were calculated according to Ponce-Palafox *et al.* (2014).

### Response-surface analysis

The influences of salinity and temperature and their combinations on growth and survival were examined

using response-surface regression analysis (JMP PRO 13, 2016). To predict the optimal point (zone where SGR is maximum without having a negative effect on the prawn), a second-order polynomial function was fitted. The function correlated the relationship between independent variables and response. To correlate the two variables (temperature and salinity), the following equation was used:

$$SGR = b_0 + b_1T_1 + b_2S + b_{12}TS + b_{11}T^2 + b_{22}S^2 \dots (1)$$

Where  $b_0$  is the model constant;  $T$  and  $S$  are independent variables,  $T$ = temperature ( $^{\circ}\text{C}$ ) and  $S$ = salinity ( $\text{g L}^{-1}$ );  $b_1$  and  $b_2$  are linear coefficients ( $b_1$ = linear effects of temperature and  $b_2$ = linear effects of salinity);  $b_{12}$  is crossproduct coefficient ( $b_{12}$ = interaction effects between temperature and salinity); and  $b_{11}$  and  $b_{22}$  are the quadratic coefficients ( $b_{11}$ = quadratic effects of temperature and  $b_{22}$ = quadratic effects of salinity) (Box and Youle, 1955). The software JMP Pro 13 (Version 13.2.1, created by SAS for statistical discovery, 2016, USA) was used for experimental design, data analysis, and quadratic model building. The standard response of shrimp to SGR as a result of temperature and salinity was obtained by solving the regression equation and by analyzing the response-surface contour plots using the Statistica software (Version 7, Stat Soft, Inc 2004, USA). The complete response-surface model for each response parameter was a second-order polynomial with orthogonal central composite designs.

### Data analysis

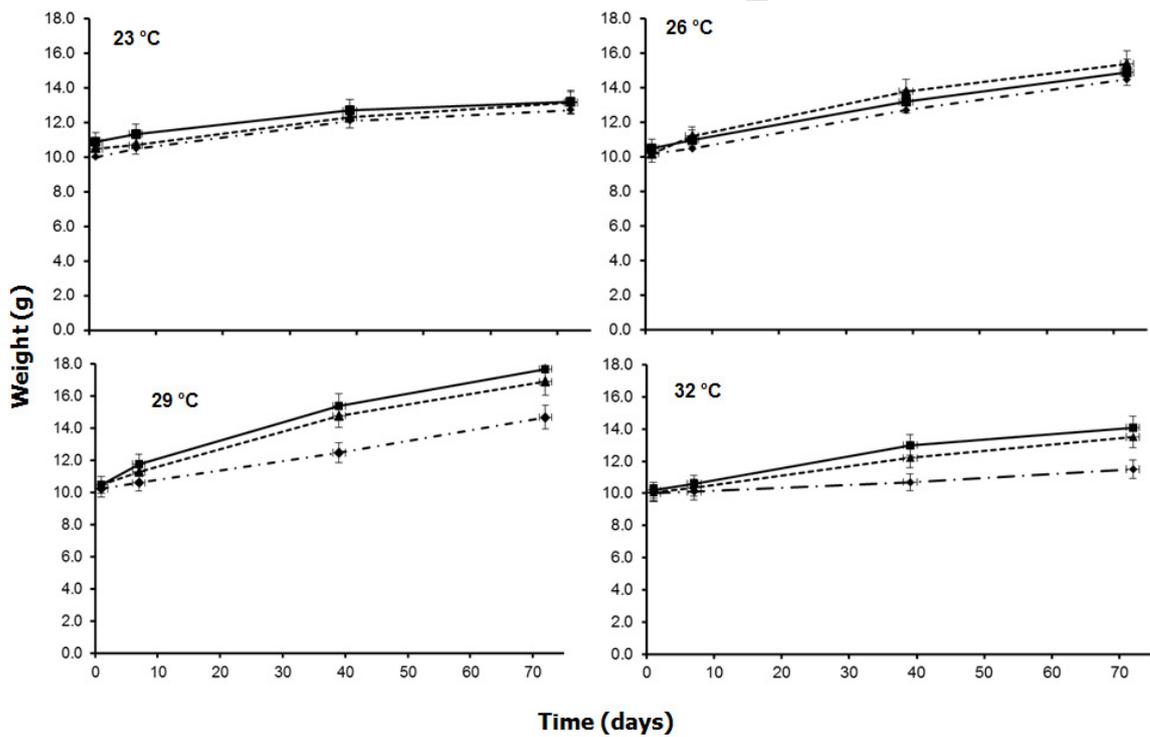
Data were assessed for homogeneity of variance using Levene's test. Analysis of variance ( $p < 0.05$ ) was used to test the individual and combined effects of temperature (23, 26, 29, and 32°C) and salinity (0, 5, and 10‰) on the growth and survival parameters of *M. americanum*. Tukey's multiple comparisons test ( $P < 0.05$ ) was used to compare treatments (Steel and Torrie, 1980). Before analysis, regressor variables were transformed to deviations from the mean to reduce correlation between the linear and quadratic regressors.

## RESULTS

The results for weight of *M. americanum* at different salinities and temperatures after a period of 72 days are shown in Figure 1. Up to 10 days, there are no differences ( $P > 0.05$ ) in growth in the three salinities at all temperatures tested. At 40 days, significantly ( $P < 0.05$ ) lower growth was observed for prawns grown at 0‰ of salinity at 29 and 32°C. At the end of the experiment (72 days), the organisms at salinities of 5 and 10‰ grew significantly more than those at 0‰ and temperatures of 29 and 32°C. The increase in prawn weight at 29°C was the largest of all treatments.

**Table I. Growth, production, and survival parameters of *M. americanum* at different temperatures and salinities in a recirculating system.**

Temperature (°C)	Salinity (‰)	W <sub>i</sub> (g)	W <sub>f</sub> (g)	FCR	SGR (%/day)	K condition	Survival (%)
23	0	9.8	12.4 <sup>c</sup>	1.8 <sup>b</sup>	0.33 <sup>d</sup>	0.64 <sup>d</sup>	84.0 <sup>a</sup>
23	5	10.8	13.2 <sup>c</sup>	1.7 <sup>b</sup>	0.28 <sup>d</sup>	0.72 <sup>c</sup>	84.0 <sup>a</sup>
23	10	10.5	13.1 <sup>c</sup>	1.7 <sup>b</sup>	0.31 <sup>d</sup>	1.25 <sup>a</sup>	84.0 <sup>a</sup>
26	0	10.2	14.5 <sup>b</sup>	1.5 <sup>a</sup>	0.49 <sup>b</sup>	0.97 <sup>a</sup>	80.0 <sup>a</sup>
26	5	10.5	14.9 <sup>b</sup>	1.5 <sup>a</sup>	0.49 <sup>b</sup>	0.67 <sup>d</sup>	80.0 <sup>b</sup>
26	10	10.2	15.4 <sup>b</sup>	1.4 <sup>a</sup>	0.57 <sup>b</sup>	0.60 <sup>d</sup>	80.0 <sup>b</sup>
29	0	10.2	14.7 <sup>b</sup>	1.3 <sup>a</sup>	0.50 <sup>b</sup>	0.97 <sup>a</sup>	76.0 <sup>b</sup>
29	5	10.5	17.7 <sup>a</sup>	1.3 <sup>a</sup>	0.73 <sup>a</sup>	0.80 <sup>b</sup>	76.0 <sup>b</sup>
29	10	10.5	16.9 <sup>a</sup>	1.4 <sup>a</sup>	0.66 <sup>a</sup>	0.99 <sup>a</sup>	76.0 <sup>b</sup>
32	0	10	11.5 <sup>d</sup>	1.9 <sup>b</sup>	0.19 <sup>c</sup>	0.74 <sup>c</sup>	72.0 <sup>b</sup>
32	5	10.2	14.1 <sup>b,c</sup>	1.5 <sup>a</sup>	0.45 <sup>c</sup>	0.87 <sup>b</sup>	72.0 <sup>b</sup>
32	10	10.1	13.5 <sup>c</sup>	1.6 <sup>a,b</sup>	0.40 <sup>c</sup>	0.68 <sup>d</sup>	60.0 <sup>c</sup>

Fig. 1. Weight (g) of *M. americanum* reared at different temperatures and salinities in recirculation systems for 72 days.

The maximum SGR was found at the temperature of 29°C and at the salinity of 5‰, whereas the lowest SGR was at the temperature of 32°C and salinity of 0‰ (Fig. 2). Growth parameters showed significantly ( $P < 0.05$ ) that the highest final weight and SGR were determined at the temperature of 29°C and salinities of 5 and 10‰, with a

low FCR (Table I). The highest K condition factor was found at 29°C and 10‰ salinity and the lowest final weight, SGR, and K condition factor were measured at 32°C and 0‰ salinity. In general, the significantly lowest survival ( $P < 0.05$ ) was found with the 32°C treatment at all the salinities studied. The prawns that revealed an isometric

growth ( $b = 3.010$ ) were those maintained at 26°C and 10‰ of salinity (Table II). Prawns maintained in salinities of 10‰ at all temperatures tended to isometry in general terms. The weight gain declined above 32°C and was strongly reduced below 23°C and above 33°C. The growth rate also decreased when salinity was below 5‰ (Fig. 3). There was a tendency to decrease the survival as salinity increased above 10‰ and temperature above 30°C (Fig. 4). Constant estimates, including significance and 95% confidence intervals of the model equation, generated for final weight are presented on Table III. The linear effect of salinity and quadratic effects of temperature, significantly ( $P < 0.05$ ) contributed to the variation in final weight. The linear effect of temperature and interactive effects between temperature and salinity were not significant ( $P > 0.05$ ). The coefficients model was calculated on the experimental responses. The fitted model was made according to the following equation:

$$Wf = 14.275 + 0.06 \times T + 0.1475 \times S + (T - 27.5) \times ((S - 5) \times 0.016333333) + (T - 27.5) \times ((T - 27.5) \times -0.15) + (S - 5) \times ((S - 5) \times -0.0385) \dots (2)$$

where  $T$ = temperature (°C);  $S$ = salinity (‰), and SGR= specific growth rate (%/day).

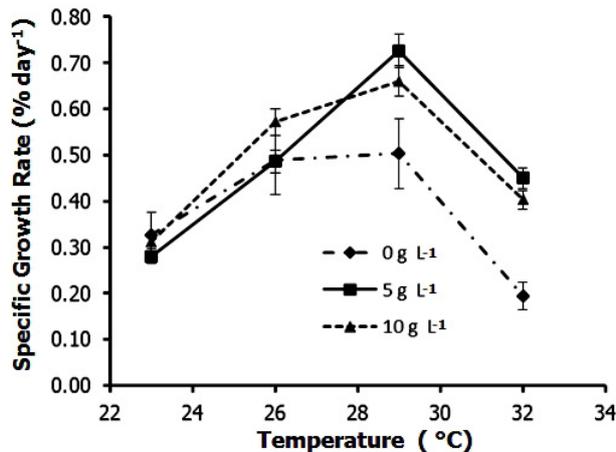


Fig. 2. Specific growth rate (%/day) of *M. americanum* reared at different temperatures and salinities in a recirculating system.

The effect of the temperature-salinity interaction on the final weight was shown in Figure 5; the plot was circular, which indicates the synergistic effect of the two factors. When temperature was in the range of 26 to 29°C and the salinity was 3 to 10.5‰, the best final weight was obtained. When salinity was maintained >10.5‰, and temperature was gradually increased from 32 to 34°C and decreased from 23 to 22°C, the final weight decreased rapidly indicating that the salinity-temperature effect is

greater in the tested lower and upper ends.

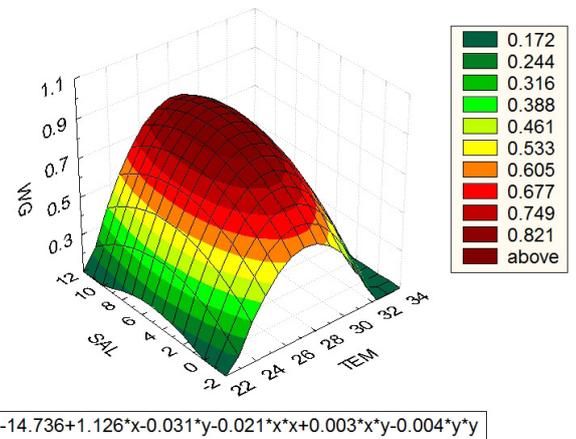


Fig. 3. Surface plot showing weight gain (g/week) of *M. americanum* reared at different temperatures and salinities in a recirculating system.

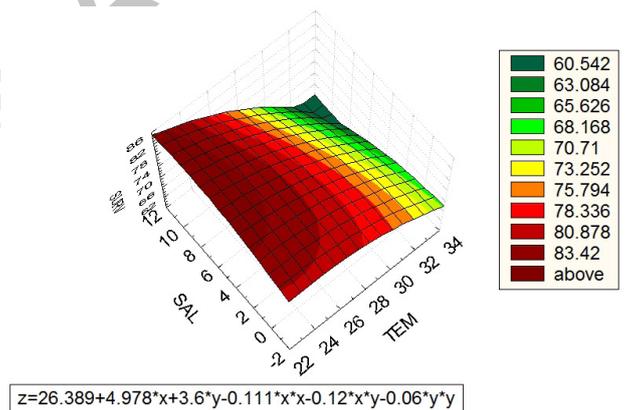


Fig. 4. Surface plot showing survival (%) of *M. americanum* reared at different temperatures and salinities in a recirculating system for 72 days.

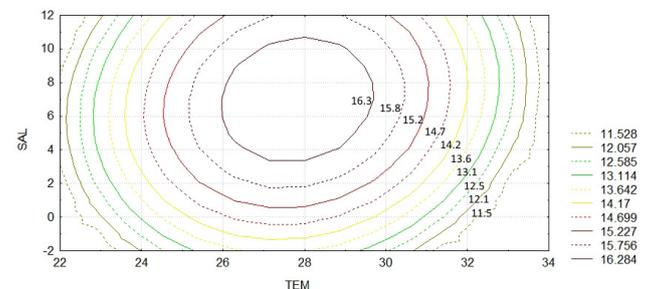


Fig. 5. Response-surface contour plot showing  $W_f$  (g) of *M. americanum* reared at different temperatures and salinities in a recirculating system.

**Table II. Estimated parameters of the length (LT)–weight (WT) relationships for *M. americanum* cultured at different salinities and temperatures in a recirculating system for 72 days. Values for the slope (b) are shown.**

Temperature (°C)	Salinity (‰)	a	b	r <sup>2</sup>
23	0	0.0048	3.3392	0.9585
23	5	0.0088	3.2619	0.9559
23	10	0.0041	3.6507	0.9609
26	0	0.0805	2.3127	0.8727
26	5	0.035	2.5467	0.9974
26	10	0.013	3.0108	0.9673
29	0	0.0083	3.2977	0.9955
29	5	0.0027	3.8082	0.9685
29	10	0.0156	2.9890	0.9964
32	0	0.0176	2.9564	0.9483
32	5	0.0045	3.5600	0.8299
32	10	0.0142	2.9811	0.9858

## DISCUSSION

The results of this study show that juvenile and adults of *M. americanum* can tolerate a wide range of temperature and low salinity, although these factors will have independent and synergistic effects on growth, survival, and production, which is a similar pattern to that of several crustacean species (Ruscoe *et al.*, 2004) found in areas adjacent to the coastal lagoon-estuarine systems of the tropical regions of the American Pacific. In general, the specific growth rate and the weight gain increased from 23 to 29°C and at 32°C began to decline in all salinities until reaching the lowest at 34°C (Fig. 3). This behavior is similar to several *Macrobrachium* species from tropical

regions (Habsby *et al.*, 2011; Lopez-Uriostegui *et al.*, 2017). The decline in the growth of crustaceans at high temperatures has been explained because the high demand of metabolism is close to the caloric intake in these conditions, leaving minimal energy for growth (Firkins and Holdich, 1993); likewise molting frequency increases. If the osmotic gradient is also high, the prawns will use less water in the ecdysis producing a decrease in the size of the organisms, as opposed to low salinities (0 to 10‰) where there is greater availability of water with the consequent increase in size (Yen and Bart, 2008), as has been determined in the prawn *M. rosenbergii* (Goodwin and Hanson, 1975; Chand *et al.*, 2015). In this latter species the osmotic point has been determined at 17 ‰ (Steel, 1980), but its best growth occurs in slightly brackish waters, around 5‰ (Nair and Salin, 2012), similar to that found in this work for *M. americanum*, where salinity had an effect on its culture and showed adequate results in the range of salinities from 4 to 11‰. The analysis of response surfaces showed that the final weight and weight gain increased as the temperature increased and, in the upper end, the salinity had a linear synergistic effect, having a greater effect on growth at high temperatures. The response surface approach to study the response of the physiological performance of *M. americanum* showed the optimum conditions of salinity-temperature for its growth and survival, and provided fundamental information for the study of the environmental physiology of this species.

In conclusion, the present study clarifies the effects of temperature and salinity and their interaction on growth and survival of *M. americanum*. The results confirm a relatively wider tolerance to temperature than to salinity. The greatest effect of the temperature-salinity interaction was on the upper extremes of response. The optimal growth intervals were from 26 to 29°C and from 3 to 11‰ of salinity and for survival, they were 23 to 29°C and 0 to 5‰. The information obtained provides basic data to continue

**Table III. Parameter estimates from polynomial surface responses of temperature and salinity on final weight of the caridean shrimp *M. americanum*.**

Parameter	Estimate W <sub>f</sub> (g)	Std error	Wald Chi-square	Prob > Chi-Square	95 % CI	
					Low	High
Constant model	14.275	2.2215463	41.289678	<0.0001*	9.9208492	18.629151
Temperature	0.06	0.07737	0.6013921	0.438	-0.091642	0.2116423
Salinity	0.1475	0.0635659	5.3843774	0.0203*	0.0229131	0.2720869
(T-27.5)*(S - 5)	0.0163333	0.0189517	0.7427688	0.3888	-0.020811	0.053478
(T-27.5)*(T-27.5)	-0.15	0.0288341	27.062645	<0.0001*	-0.206514	-0.093486
(S - 5)*(S - 5)	-0.0385	0.0220199	3.0569734	0.0804	-0.081658	0.0046582
Scale	0.8989577	0.366998	6	0.0143*	0.179655	1.6182605

with the study of the physiological response of the species under the two factors used, and to improve the implementation of management strategies in intensive and semi-intensive farming systems.

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#### Statement of conflict of interest

No potential conflict of interest was reported by the authors.

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