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Research Article

EVALUATION OF THE LARVAL DEVELOPMENT OF THE PRAWN MACROBRACHIUM ROSENBERGII (DE MAN, 1879) AT DIFFERENT TEMPERATURES UNDER CONTROLLED CONDITIONS

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Abstract

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a commercially important species with great potential for farming in Mexico. Its larvae require adequate conditions for optimal development, making the farming of this species a complex process and, thus, one not widely undertaken in the country. Finding the ideal temperature for the larval development of *M. rosenbergii* is an important factor as it assists both larvae growth and the speed of metamorphosis into postlarvae. Three temperature gradients were studied (ambient, 28±0.5 °C, and 30±0.5 °C) in nine 70 L tanks, with an initial density of 1100 larvae in each and thermostats used to control the temperature. The larvae developed slowly and had a low survival rate, with 18 larvae surviving at an ambient temperature (27.07 °C on average) by the end of the experiment. In contrast, although larval development improved at 28±0.5 °C, the survival rate remained low with a final larva count of 20, while, at 30±0.5 °C, both survival and growth showed better culture efficiency, with a final larva count of 105. The ANOVA test applied showed significant differences, revealing a value of p=<0.001 with a 95% confidence interval, while the Tukey test applied identified the 30±0.5 °C treatment as both different and the optimal temperature for a commercial culture for this species.

Keywords: Prawn, Larvae, Development, Temperature, Survival.

INTRODUCTION

All of the prawn species that have been subject to commercial farming pertain to the genus *Macrobrachium*, which is distributed in both the tropical and subtropical regions of the world, in rivers, lagoons, swamps, irrigation channels, and estuarine waters (New, 2002). Some of these species are of high commercial value, such as *Macrobrachium tenellum*, *Macrobrachium americanum, Macrobrachium carcinus*, and *Macrobrachium rosenbergii* (Kent, 1995). Native to the Indo-Pacific, *M. rosenbergii* is the most farmed of the aforementioned prawn species and has, therefore, been introduced in a large number of countries (New, 2002). The species lives in freshwater regions that have access to estuarine waters with low salinity (10–14‰), where its larval development takes place (Brown *et al.,* 2010). The production of larvae and postlarvae is influenced by various physical and chemical factors, among which temperature, salinity, pH, and dissolved oxygen are notable. Temperature is closely related to larval growth and development, wherein exposure to inadequate temperatures arrests the development of various crustacean species and even leads to high mortality rates (Anger, 2001; Brown *et al*., 2010). Temperature plays a crucial role in the development of *M. rosenbergii*, which requires warm waters with an optimal temperature range of between 28 and 31 °C for its larva, with temperatures lower than this arresting its growth, while temperatures above 33 °C begin to have a fatal impact, which can also happen with small changes of 1 °C (New, 2002). In Veracruz, postlarvae production takes place in locations where the temperature often varies drastically from season to season, with minimum and maximum temperatures of 26 °C and 32 °C, respectively, in the summer (Jauregui, 2004), causing fluctuations in surface water temperature. Given the foregoing, the present study aimed to determine whether or not the use of temperature regulating instruments, such as thermostats, is necessary in culture systems. Moreover, it sought to determine, via a review of the literature on various temperature gradients, the most useful gradient for application in the region of interest and those with similar climates. Ling and Merica (1961) conducted the first studies on larvae and described some of the larval stages of *M. rosenbergii*. They achieved the first successes in the farming of the species due to a serendipitous incident involving the accidental ingress of soy sauce into the tanks, which led them to conclude that larvae require salinity throughout their development, knowledge which has been key to the farming of this species. New's manual (2002) for *M. rosenbergii* cultures narrowed to three factors (telson, rostrum, and pleon) the list of characteristics on which to focus during the stages of larval development, all of which factors undergo significant changes during the transitions between larval stages. In 1971, Dobkin studied 655 *M. acanthurus* larvae, applying a temperature range of 26 °C to 30 °C throughout their development in previously filtered seawater, which was subject to three different salinity concentrations (35‰, 23.5‰, and 12‰) for the 43 to 56 days that constituted the organisms' metamorphosis. Subsequently, Gómez-Díaz (1987) applied 30 salinity and temperature combinations on *M. rosenbergii* breeding larvae collected from different geographical locations, with the purpose of establishing whether survival was influenced by the adults' place of origin, and observed that the larvae developed faster at 28 °C and 13.6‰ salinity. Later that year, Gómez-Díaz and Kasahara (1987) conducted five daily observations on individually raised larvae and described 18 larval stages for *M. rosenbergii*, some of which lasted less

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than 24 hours. Valenti (1996) indicates that the temperature for optimal larval development should be within the range of 28 to 31 °C and recommends the avoidance of abrupt changes. New's (2002) manual stipulates an ideal temperature range for larvae growth of 28 to 31 °C, indicating that larvae grow slowly and their metamorphosis is delayed at temperatures below 24 to 26 °C, while temperatures above 33°C cause high levels of mortality. The manual states that a temperature change as small as 1 °C can cause a shock to larvae, thus causing fatalities, and indicates that temperature is of the utmost importance to the timeframe of the stages of larval development. Lal *et al*. (2012) carried out an experiment on *Macrobrachium lar* larvae in 800 ml testing jars, to which 5 mm aeration hoses were attached, and subjected the subjects to three temperature treatments: 26±0.5 °C; 28±0.5 °C; and, 30±0.5 °C. Their results revealed that the ideal temperature for these organisms is 30±0.5 °C, which, of the three treatments, generated the best survival and growth rates. Makombu *et al*. (2014) observed the complete larval development of *Macrobrachium vollenhovenii* in four 65 L tanks connected to a biological filter, an experimental design which was based on that described by Uno and Kwon (1969) for *M. rosenbergii*. They reported that metamorphosis into postlarvae ranged from 41 to 74 days at a temperature of 26°C. Kumar-Mohanty *et al*. (2016) used combinations of three temperatures with four salinity levels in order to ascertain the best survival rates in each of the stages up to the postlarval stage, finding that a salinity of 12% and a temperature of 31 °C are optimal for larval development. Furthermore, after applying various temperature and salinity combinations, John *et al*. (2017) found that an ambient temperature of 29 °C and 12‰ salinity result in a high survival rate for larvae of the same species.

MATERIALS AND METHODS

The present study was carried out in the aquaculture laboratory of the Postgraduate College, Campus Veracruz, in the state of Veracruz, Mexico. The experiment used nine 65 L tanks, measuring 60 cm x 46.7 cm x 37.8 cm, to which 4" PVC filters were attached, while oyster shells were placed in the interior of the tank in order to aid the proliferation of nitrogen-consuming bacteria and an *air lift* recirculation system based on Domínguez-Mora's (2019) model was then installed. A pump with two air outlets was attached to each tank, one to facilitate the *air lift* system and the other equipped with an aerator stone, while both were fitted with aquarium hoses. Thermostats were used for temperature control, while 12-watt lightbulbs were used to maintain the photoperiod. Hoods were constructed from screen-printing mesh (size 24), attached with silicone, in the form of a bag to the outlet tube, and changed every third day to avoid a diminished flow of water due to the build-up of excess organic material resulting from the death of the larvae. The seawater was obtained at Boca del Río Beach in Boca del Río, in the state of Veracruz, transported in 10 L barrels and 20 L water jugs, and filtered using screen-printing mesh (200 threads per cm). Sodium hypochlorite was then added at a proportion of 0.5 ml per litre of saltwater, with a power head used to aid circulation and homogenization and, also, aerate the water. Five drops of a sodium thiosulphate pentahydrate solution was added per litre of 100 g/L distilled water to dechlorinate the water. A ratio of 40% saltwater, at 35‰, to 60% freshwater was used to achieve a salinity of 12‰, with the temperature, pH, salinity, nitrate, nitrite, and ammonia parameters then measured. The *M. rosenbergii* larvae were fed *Artemia salina* cysts, seven grams of which were placed in six litres of water at 30‰ salinity, while a balanced feed comprising 200 g tilapia fillets, one egg, 60 g of rice flour, and a capsule containing omega-3, 6, and 9 was also prepared. Three treatments with varying temperatures were establi Table 1).

Table 1. Experimental treatments for *M. rosenbergii*.

Treatment name	Temperature	Observations
Treatment A	Ambient temperature	Temperature fluctuating freely in accordance with environmental conditions
Treatment B	28 ± 0.5	Regulation via thermostat
Treatment C	30 ± 0.5	Regulation via thermostat

A totally random design was chosen, with three numbered tanks used for each treatment, thus giving: A1, A2, and A3; B1, B2, and B3; and C1, C2, and C3. The data were entered into an Excel 2016 spreadsheet, from which the following descriptive statistics were obtained for each parameter: average; median; maximum and minimum number; and, standard deviation. These were later graphed using the same software to organize and depict the level of progress of the larval stages. A variance analysis (ANOVA) was carried out, using the statistical software packages Past3 and Statistica 7 on the results obtained from the survival counts. Said packages were used to process the data obtained from the Excel spreadsheet and determine whether there were any significant differences among the treatments, the assumptions of normality, and variance homogeneity. New's (2002) manual, which presents 12 larval phases, the first 11 of which corresponded to larvae and the twelfth to a postlarval stage, along with their characteristics, was used as a guide for the identification of the larval stages. In order to evaluate the time elapsed in the change from one larval stage to another, a sample of ten organisms was collected on each of the 34 days for which study was conducted. Said time periods were registered in an Excel spreadsheet, which was used to calculate the time period between phases for each of the treatments. Once hatching had occurred, all larvae were captured and placed in a container with 2 L of water and mixed uniformly with a circular motion, with a 50 ml beaker then used to take a total of ten samples, from which an approximate number of the larvae that had hatched per volume was then calculated. The total number of hatched larvae were divided among the nine tanks, giving 1100 larvae per tank.

RESULTS

The 28 °C and 30 °C treatments presented minimal temperature fluctuations due to the control provided by the thermostats, although some minor variations were observed.

A fluctuation of 3.1 °C was observed for Treatment A due to changes in the ambient temperature occurring from the outset of the experiment to the second day of the period in which parameters were measured. By the last day of the experiment, the temperature for this treatment had reached 28.6 °C, giving minimal and maximal fluctuations of 0.56 and 3.17, respectively. At 28 °C, Treatment B presented minimal and maximal daily fluctuations of 0 °C and 0.9 °C, respectively, while Treatment C, at 30 °C, presented minimal and maximal daily fluctuations of 0 °C and 0.73 °C, respectively, making it the most stable of the experimental treatments applied. The tendency followed by the temperature in the different experimental treatments reveals a notable fluctuation in temperature for Treatment A, namely that a wide temperature range was observed for the ambient temperature. Treatments B

and C presented minimal fluctuations due to the temperature control provided by the thermostats installed (Figure 1).

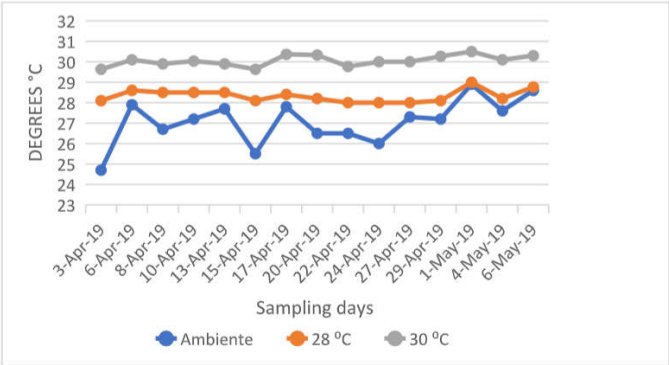

Figure 1. Temperature fluctuations observed for the different experimental treatments

In the first larval stage for Treatment A, the eyes of the larva were found to be sessile and completely fixed to the head, while the telson was without division and trapezium-shaped. An excitation of the eyes was observed in the second phase, while, by the third, the telson had divided into three parts, which developed into uropods. This treatment also saw the early development of the fourth stage, with the uropods beginning to bifurcate without the appearance of setae, which, once they had fully appeared, became more elongated and thinner as the individual progressed into the fifth stage. From the sixth stage onwards, protrusions could be seen in the abdominal segments, which would later be replaced by the pleopods to then become double-branched in the seventh stage. The eighth stage saw the emergence of setae in the pleopods, while fully developed uropods could be observed by the ninth phase. Eleven larval stages were observed in Treatment B, stages which were similar to those recorded for the foregoing treatment, although the observations yielded varying details due to the difference in the duration of the metamorphosis, in that the changes observed in Treatment B occurred faster. While the first four stages are shown in Figure 11, due to the early observation time and the speed of the metamorphosis, only an intermediate point between the second and third phase was observed, in which the uropods began to appear. The transition to phase four occurred rapidly for Treatment B, with the corresponding characteristics presenting in less than a day. The fourth stage presented when the uropods began to bifurcate and present setae. Stages five, six, seven, and eight were all observed, as indicated by the complete bifurcation of the uropods and the appearance of setae on them. The protrusions on the abdomen, their subsequent bifurcation, and the appearance of setae on the new pleopods are characteristics that present in the order corresponding to their respective larval stages. Every phase occurred without any irregularity in terms of either advanced or delayed development. The ninth stage presents the appearance of internal appendages on the endopods, while the rostrum presents, in the tenth stage, with three to four dorsal teeth, largely unmarked, and the eleventh stage presents many dorsal teeth and a serrated rostrum. The stages identified for Treatment C, at 30°C, were similar to those observed for the treatments undertaken at 28°C, with a rapid metamorphosis observed in the early stages of the third stage, as were more advanced characteristics than those observed for Treatment B. The fourth phase presented normally, with the bifurcation of the uropods. The fifth stage was notable for the early presentation of characteristics such as bifurcated uropods and the quick onset of the sixth stage. Both the seventh and eighth stages presented their respective characteristics. Phases nine, ten, and eleven presented complete development similar to that observed in the foregoing treatments; however, more advanced development was observed in the eleventh phase, presenting a more pronounced serration of the rostrum than that observed in the previous treatment. A difference was found in the number of days between the different larval stages, with one day each observed for phases one, three, and four, while the sixth stage presented the longest transition phase, with eleven days at ambient temperature and nine days at 28°C and 30°C. The phase F12 corresponds to the metamorphosis into postlarvae, which, as can be seen in the graph, only presented in the treatments conducted at 30 °C (Figure 2).

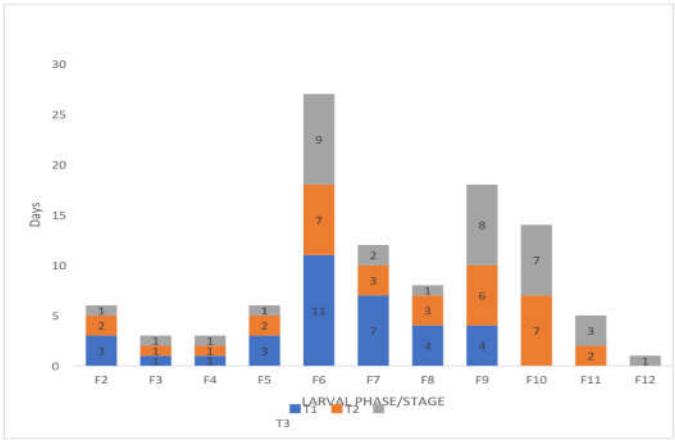

Figure 2. Number of days for each larval stage by treatment, where T1 is Treatment A, at ambient temperature, T2 is Treatment B, at 28 °C, and T3 is Treatment C, at 30°C

The metamorphosis into the twelfth stage was observed after 35 days in the treatment conducted at 30°C, while, in that same time period, the larvae produced at ambient temperature developed up to the ninth phase and those produced by the treatment at 28°C did reach the eleventh phase, but did not transform into postlarvae (Figure 3).

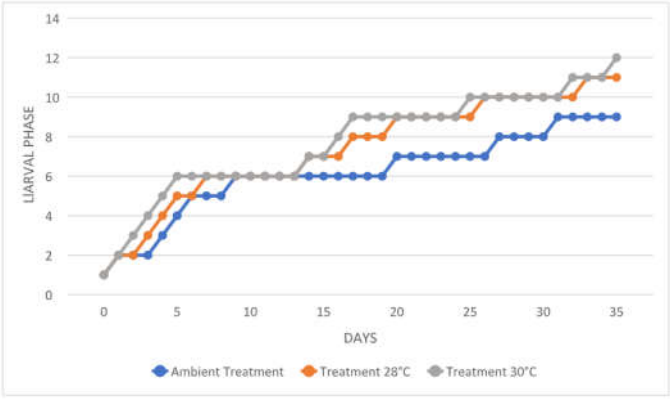

Figure 3. Progress of each larval stage, in days, by treatment

In order to avoid wide genetic variations among the larvae, all of the approximately 10,000 larvae used in the present research were taken from one female and divided between the nine experimental units, giving 1100 larvae per tank. One further larva count was conducted after 12 days, from which it was observed that the larvae population had decreased to 243 for Treatment A, at ambient temperature, to 227 for Treatment B, at 28°C, and to 300 for Treatment C, at 30°C. By the end of the experiment, the final count revealed 18 larvae for Treatment A, at ambient temperature, 20 larvae for Treatment B, at 28°C,

and 105 for Treatment C, 30°C, with all of said values corresponding to the average count obtained per treatment and their three respective replicas. The count undertaken halfway through the experiment was statistically normal, although the final count was not, presenting a value lower than the significance value (P>0.05) (Table 2).

Table 2. P values for the Shapiro-Wilk normality test

Count	Statical (W)	P value	Value of n
Halfway	0.8357	0.05175	9
Final	0.7712	0.003619	9

The Levene's test applied shows a homogeneity in the data obtained from the halfway and final counts, from which a contrast can be observed in the equality of variances, due to the fact that these values are higher than the significance value (P>0.05). These values are presented in Table 3.

Table 3. Values obtained from the Levene's test applied for variance homogeneity

Count	Value of F	P (>F)
Halfway	0.4526	0.6566
Final	0.7379	0.517

The ANOVA test applied for the halfway count indicated no significant difference in the survival of the organisms up to that point for the three different treatments applied in the present study. The P value was 0.216 with a confidence interval of 95% (Figure 4).

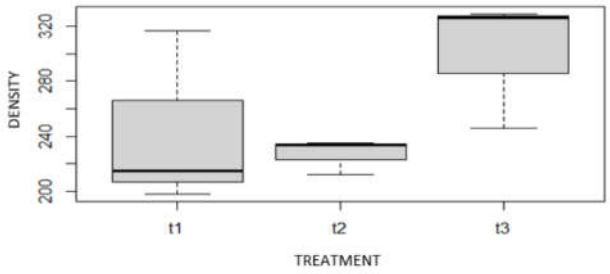

Figure 4. Boxplots comparing medians, wherein t1, t2, and t3 correspond to treatments A, B, and C, respectively

The ANOVA test conducted on the final count shows a significant difference, having obtained a value of p=<0.001 with a confidence interval of 95%. The Tukey test applied in order to identify differences among the treatments revealed no differences between treatments A and B, which were both found to be different to Treatment C. These values are presented in Table 7, which highlights, in color, Treatment C as different (Table 4).

Table 4. Comparison of the treatments via the Tukey test

Count	Treatment A	Treatment B	Treatment C
Treatment A	-	0.9297	0.004751
Treatment B	0.9297	-	0.00214
Treatment C	0.004751	0.00214	-

A non-parametric Kruskal-Wallis test was conducted due to the fact that the normality test found a non-normal distribution in the final count, confirming a difference among the treatments on obtaining a value of P=0.0649 in terms of/compared to the significance value (p>0.05). The boxplots presented in Figure 22 show a marked difference between Treatment C and the other two treatments (Figure 5).

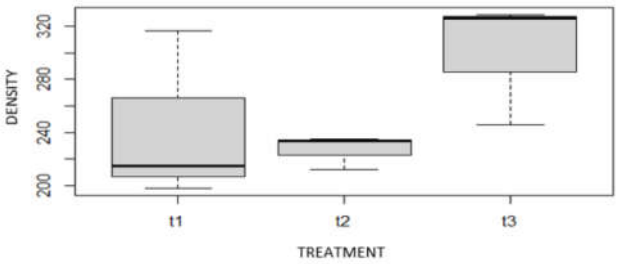

Figure 5. Boxplots for the final count, wherein t1, t2, and t3 correspond to treatments A, B, and C, respectively

DISCUSSION

The temperature fluctuation was evident in the treatment conducted at an ambient temperature and was an important factor in the slow development of the organisms. New (2002) states that temperatures below 26°C cause this type of development, a finding also described by Dobkin (1971). The stages observed in the present study concur with that reported by New (2002), wherein changes to the telson, pleon, and rostrum are identified, which contrasts with that found by Ling and Merica (1961), who reported eight stages. Uno and Kwon (1969) reported eleven stages, which is closer to the results described by New (2002) and is corroborated by the findings of the present study. While Gómez-Díaz and Kasahara (1987) obtained 18 larval stages, their observations are not comparable with the those obtained by the present study. This is due to the fact that the former involved five observations per day, while the observation schedule in the latter was organised around the maintenance and cleaning of the maternity and feeding systems. In the present study, Replica 1 of Treatment A confirms the classification proposed by Jalihal *et al*. (1993), which comprised more than ten stages. Sandifer and Smith (1985) concur with the authors cited above, indicating that *M. rosenbergii* larvae undergo 11 larval stages over a period ranging from 20 to 50 days, a timeframe also observed in the present study. The timeframe observed for treatments conducted at an ambient temperature was longer, to the extent that they could be discarded, in terms of their utility, for use in a system intended for commercial production. This finding is similar to that reported by Dobkin (1971), who subjected *M. acanthurus* larvae to similar temperature ranges. However, Dobkin's study differed by 2 °C to the temperature range used in the present study, wherein the ambient temperature fluctuated from 24.7 to 28.6 °C throughout the experiment, within a range of 26 to 30 °C, with a complete metamorphosis observed over 43 to 56 days, a result inconclusive for *M. rosenbergii* larvae. The present study aimed to find, among other objectives, the ideal temperature for reducing the metamorphosis timeframe for use in commercial production. Gomez-Diaz (1987) reported a timeframe of approximately 21 days for the transformation into the post-larval stage under conditions of 28 °C and 13.6‰ salinity. This finding contrasts with that observed in the present study, wherein the metamorphosis had not been completed even after 35 days of treatment at the same temperature and a salinity of 12‰. Morales-Valenti and Valenti (2010) confirm that a constant temperature of 30 °C is ideal for the larval development of the genus *Macrobrachium*, while Lal *et al*. (2012), in a study conducted on *M. lar* larvae, found a temperature of 30°C, with changes no greater than ±0.5 °C, to be ideal for ensuring higher levels of survival and growth. This indicates that the results obtained by the present research is applicable for other *Macrobrachium* species, with Makombu *et al*. (2014)

obtaining a metamorphosis time of 41 to 76 days with a temperature of 26 °C. From this, it can be inferred that a temperature below 28 °C considerably increases the timeframe in which the organisms complete their development up to the post-larval stage. Kumar-Mohanty (2016) defines the best combination for *M. rosenbergii* larvae culture as 12‰ salinity and a temperature of 30 °C, with John and collaborators (2017) later showing that 12‰ salinity and a temperature of 29 °C result in a high survival rate. The results of the present study also concur with those reported by Morales-Valenti and Valenti (2010) and Kumar-Mohanty (2016), in that a higher survival rate and faster development is observed at 30 °C. This reinforces that established by the above-discussed authors, namely the great commercial potential of prawn cultures conducted at this temperature. The foregoing is further supported by the statistical data, which show a marked difference between the treatments, in that the treatment conducted at 30 °C obtained a higher survival rate than the rates obtained at both an ambient temperature and 28 °C, which were statistically equal. A lower timeframe is observed for treatments conducted at an ambient temperature than for those conducted at 28 °C, as are differences in terms of survival rate. Given the foregoing, it can be inferred that, with some of the above discussed authors reporting total population losses for some treatment replicas conducted at temperatures below 28 °C, a 10% survival rate represents a clear advantage compared to treatments conducted at 30 °C.

Conclusion

Temperature fluctuation is an important variable for *M. rosenbergii* larvae culture, directly influencing the speed of larval development and survival rate. Therefore, a significant difference is observed for metamorphosis, on exposure to these temperatures, among the results obtained for the various treatments. Similar results for the larval stages were observed for both treatments B and C, although the latter obtained complete metamorphosis before the former as well as a more advanced morphology in the last two stages of larval development. The timeframe required by the treatments to develop the organisms is noteworthy, given that 30°C is found to be the ideal temperature at which the culture timeframe can be reduced and, thus, be applied commercially. Moreover, the final survival rate of the larvae at said temperature should also be noted in terms of obtaining a higher density, which could be of great commercial benefit. Furthermore, the treatment conducted at 30°C is shown to be the best option, taking into account the fact that the survival rates for treatments A and B are statistically equal and far lower than those obtained for Treatment C. For this reason, the application of a temperature of 30 °C for *M. rosenbergii* larvae culture is viable, considering both the 35-day metamorphosis timeframe and the higher survival rate observed at that temperature. However, further studies on the influence of other more specific conditions in terms of food and/or water quality may help to improve the survival rates for *M. rosenbergii* larvae. Finally, the use of thermostats is recommended to regulate the temperature of the culture during larval development, as is constant monitoring in order to achieve high survival rates, rapid development, and, thus, higher yields.

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