Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*

Efecto de la temperatura sobre el incremento en peso, sobrevivencia y preferencia térmica de juveniles del acocil *Cherax quadricarinatus*

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ABSTRACT

The objective was to determine the effect of temperature on the development of Australian crayfish *Cherax quadricarinatus* juveniles and its thermal preference under laboratory conditions. The determination of the effect of temperature was made based on the results of growth and survival of juvenile crayfish *C. quadricarinatus*. The crayfish were cultivated and fed with commercial shrimp pellets for 90 days at four different temperatures (20, 25, 28, and 31 °C). The greatest weight increase and total biomass were obtained at 28 °C and the highest survival (83%) at 25 °C. The thermal preference of crayfish after the assay was also examined in crayfish of each treatment. The thermal preference of crayfish was always between 23 °C and 26 °C.

Key words: *Cherax quadricarinatus*, growth rate, survival, temperature, thermal preference.

RESUMEN

Con la finalidad de conocer el efecto de la temperatura en el desarrollo del acocil australiano *Cherax quadricarinatus*, y determinar su preferencia térmica bajo condiciones de laboratorio, se realizó un estudio para evaluar el efecto de la temperatura sobre el incremento en peso y la sobrevivencia en juveniles del acocil *C.quadricarinatus*. Los organismos se cultivaron y alimentaron con una dieta comercial para camarón durante 90 días a cuatro diferentes temperaturas experimentales (20, 25, 28, y 31 °C). Los juveniles que se cultivaron a 28 °C produjeron los mejores resultados en términos de crecimiento y los cultivados a 25 °C presentaron los mejores resultados en términos de sobrevivencia (83%). Asimismo, se evaluó la preferencia térmica posterior al ensayo en estos acociles en cada uno de los tratamientos, cuyos valores estuvieron entre 23 °C y 26 °C.

Palabras clave: *Cherax quadricarinatus,* preferencia térmica, supervivencia, temperatura, tasa de crecimiento.

INTRODUCTION

The redclaw crayfish *Cherax quadricarinatus* (von Martens, 1898), a native of northern Australia, has been a subject of research at least since 1985 (Love & Langenkamp, 2003; Vazquez & López-Greco, 2007). It is adaptable to a wide range of weather conditions, can be fed commercial pellet diets, and can be raised inexpensively (Jones & Ruscoe, 2001; Campaña-Torres *et al.*, 2003; Cortés-Jacinto *et al.*, 2005; Vazquez & López-Greco, 2007). Crayfish can grow to 40–60 g after six months of cultivation (Masser & Rouse, 1997; Jones & Ruscoe, 2001). After more than 25 years of research, production techniques are well developed; however, there are gaps in the understanding of its response to different temperatures that is important to understand when a species is produced outside its native region. In crayfish, as in all Decapoda, the selection of a particular cultivation temperature provides the possibility of extending or shortening growth rate (*Cherax quadricarinatus*; Jones, 1994; Allan *et al.*, 2006). Temperature is the most important environmental factor in crayfish growth because it directly affects metabolic rate (Schmidt-Nielsen, 1997; *Cherax quadricarinatus;* Díaz *et al.*, 2004, Díaz-Herrera *et al.*, 2006). As a consequence, growth (*Penaeus semisulcatus* De Haan, 1844; Kir & Kumlu, 2008), behavior (Forward, 1990), nutrient requirements, and food digestion (*Perca fivescens* (Mitchill, 1814); Tidwell *et al.*, 1999) could be altered. Variations in these factors affect weight increase and survival of *Cherax quadricarinatus* (García-Guerrero *et al.*, 2003a). Extreme temperatures might also cause deformities or metabolic failure (Payette & McGaw, 2003); hence, survival is also affected (Jones, 1994; García Guerrero *et al.*, 2003a; Hamasaki, 2003).

However, most previous studies of this species assess temperature effects together with other variables, such as age, reproduction condition or diet (e.g. Hernández *et al.*, 1995; Ponce Palafox *et al.*, 1997; Hewitt & Duncan, 2001; Meade *et al.*, 2002; Jackson & Burford, 2003; Allan *et al.*, 2006; Díaz- Herrera *et al.*, 2006; Rodríguez-Flores *et al.*, 2012). Other studies show that there are different approaches to recognize the effects of temperature, such as nutrient or oxygen consumption (e.g., Forward, 1990; García-Guerrero *et al.*, 2003a; Hamasaki, 2003; Spanopoulos *et al.*, 2005). Perhaps, the most evident effect of water temperature is on weight gain and survival, since both are highly temperature-dependent (Jones, 1994; Rodríguez-Flores *et al.*, 2012), as well as an indicator of metabolic efficiency. Cultivation temperature may influence survival and growth at all stages, since the physiological response of aquatic organisms is strictly related to biochemical reactions that are temperature dependent (González *et al.*, 2010). Since there are always several factors involved, such as management, density, and diet, this phenomenon must be studied under different schemes to understand weight gain and thermal preferences before reaching conclusions. Most analyses of this topic come from cultivation ponds, and it is often restricted to data related to stocking or harvest. Since other factors could mask this effect, it is necessary to perform a specific experiment designed to determine the effect of temperature on weight gain. According to King (1994), thermal tolerance range for *C. quadricarinatus* is approximately 16-32 °C; however, within its temperature range, crayfish may have greater weight gain and survival (Power *et al.*, 1998; Love & Langenkamp, 2003). For this reason, 20 to 30 °C was selected as temperature range for this study. Additionally, crustaceans have a thermoregulatory response that enables them to actively move to a habitat with a more comfortable temperature (Lagerspetz & Vainio, 2006; González *et al.*, 2010). Since mobility may produce consequences on the condition of redclaw crayfish and few tests have been carried out (Diaz *et al.*, 2004), a thermal preference assay was included in this research.

This study assessed the effect of four temperature treatments on weight gain and survival of juvenile redclaw crayfish within its normal temperature range in experimental tanks and temperature preferences within this range.

MATERIALS AND METHODS

Survival and growth experiment. A batch of 300 juvenile redclaw crayfish were obtained from the stock maintained in the CIIDIR-IPN Laboratory (Guasave, Sinaloa, Mexico, 25°54′42.24″N, 108°4′ 30.31″W). This stock included 9 adult females (18.5 ± 2.1cm; 44.4 ± 7.8 g) and 4 males (21.3 ± 2.1cm; 56.7 ± 9.44 g) that were fed once a day with commercial shrimp pellets containing 35% crude protein (Purina, Cd. Obregon, Sonora, Mexico) (1.0 g per crayfish) and fresh squid meat (2 g per crayfish). From the batch, three females were berried after three days and each was placed in a 60 L fiberglass tank with fresh tap water at 25 °C and maintained, as described in García-Guerrero *et al.* (2003a,b). Once the hatchlings turn into free juveniles, they were harvested and placed in a 1000 L nursery tank. The tank was aerated to oxygen saturation and maintained at 25 °C. Folded pieces of plastic net served as shelter. Juveniles were fed, as described in Cortés-Jacinto *et al.* (2003).

After 21 days, juveniles reached 0.75 g ± 0.23 g; at random, 160 crayfish were divided in four groups, 40 for each experimental temperature. The effect of four temperatures on survival and growth were recorded, with four replications at each temperature. Each group of 40 specimens were divided in sets of 10 (a replicate) and every set was placed in a 60 L rectangular plastic tank. The tanks were kept at the experimental temperatures (20, 25, 28, and 31 °C) with fresh tap water (initially from the nursery tank), with permanent aeration and kept at oxygen saturation. Within 24 h, water in all 12 tanks was increased to the experimental temperature, using submersible, calibrated 100 W heaters (Mars Fishcare North America, Chalfont, PA). Before trials, temperature in the experimental room was lowered to 20 °C. For the lowest temperature, no heaters were needed. Each tank was covered with a piece of fine net to provide shade. Temperature was monitored three times each day (08:00, 14:00, and 21:00 h) with a precision mercury thermometer (±0.5 °C), and if necessary, heaters were recalibrated. During trials, juveniles in each tank were fed a diet of pelleted shrimp (Purina®, 35% crude protein) at dawn and dusk at the rate of 5% of total weight. About 50% of the water was changed every other day with water at the same temperature. Uneaten food and feces were siphoned each day from each tank at 10:00 h. Every two weeks, all juveniles were counted and weighed on a digital balance (CL501, Ohaus, Pine Brook, NJ; 500 g ± 0.1 g) and then returned to their tanks. For each treatment, the feed ration for the next 15 days was recalculated from the new average weight. The trials lasted 16 weeks; final weight and survival were recorded for all crayfish.

Thermal preference assay. This assay was performed (Hall *et al.*, 1978) in a compartmentalized water trough (Fig. 1) set with a temperature range from 15 °C to 37 °C, which corresponds to the temperatures found in the native environment of the crayfish (Masser & Rouse, 1997). The trough was 300 cm long × 20 cm diameter made of a plastic pipe cut lengthwise to obtain a channel-shaped configuration divided into 15 equal aerated compartments filled with tap water. Each compartment had a precision mercury thermometer and was maintained at oxygen saturation, which depends on temperature (Fig. 1). Compartments were separated by plastic walls open at the bottom, so the crayfish can pass to the adjacent chambers. On the previous day, air conditioning in the room was lowered to the minimum and in, due time, this controlled the lower water temperature in the trough. Two 300 W submersible heaters, set to 37 °C were placed at one end of the trough while the temperature of the room was lowered to 15 °C. A temperature gradient was generated in the trough after two or three hours. Temperature in the different compartments, were 15, 16, 17, 18, 19, 22, 23, 24, 25, 26, 27, 29, 33, 35, and 37 ± 0.5 °C. Before the test, the crayfish were acclimated for 7 days, with each set maintained at its previous experimental temperature and, after the end of the weight-increase experiment, at different temperatures. After the formal observations, an assay was carried out to observe how much time the animals needed to move to a particular compartment and stay there for more than two hours. Then, the experiment started at 09:00. Animals were individually marked on their carapace for identification. Specimens of each treatment set were tested separately, placed one-by-one in the compartments that corresponded to its acclimation experimental temperature. Each trial lasted 8 h, which was previously determined as sufficient time for each crayfish to move into the compartment of preference and stay there, as indicated by our previous test and the work of Hall *et al.* (1978). This was done three times for each treatment with all specimens in each treatment on successive days, starting always at 11:00 h. The chamber which each crayfish preferred was recorded. Systematic recording of oxygen concentration in each chamber was done to maintain oxygen saturation, which decreases in each chamber with the temperature from 10.07 at 15 °C to 6.86 at 37 °C.

Statistical analysis. Analysis of variance (ANOVA) between weights was run among the medians of the same day, but of different temperatures. When statistical differences were found, Student's Newman-Keuls multiple comparison test was applied (Zar, 1999). Survival were calculated as a Kaplan and Meier (1958) product limit estimator, based on a series of horizontal steps of declining magnitude in which, when a large enough sample is taken, approaches the true survival function for that population. The value of the survival function between successive, distinct sampled observations is assumed to be constant (Kaplan & Meier,

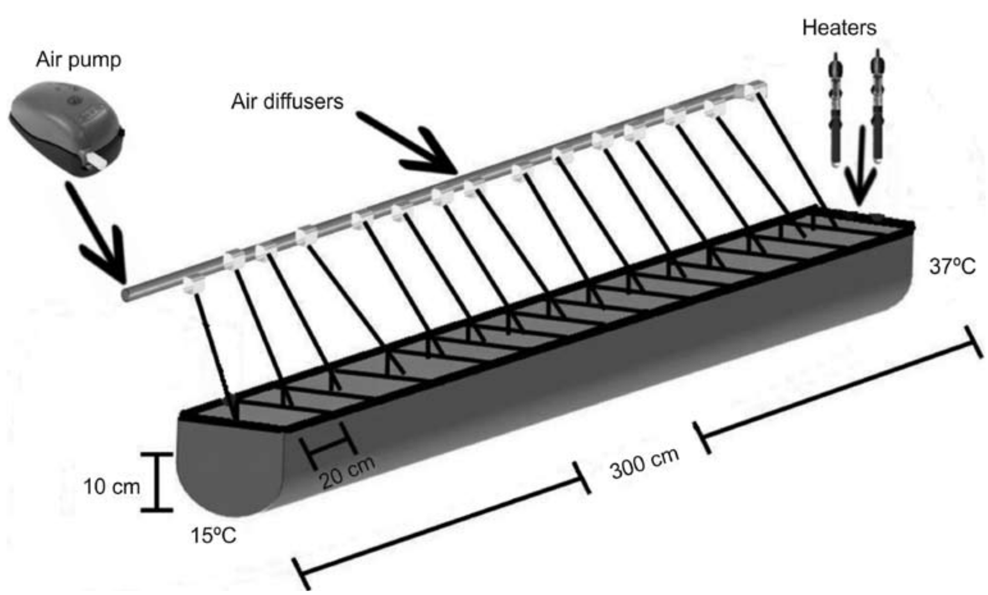

Figure 1. Experimental trough used for *thermal* preference tests.

1958). Final average biomass (weight) per treatment was the sum of weights of all crayfish under one particular treatment divided by the total number of crayfish. Thermal preference was determined with a table of results of the frequencies of selected temperatures, which implies the number of crayfish in each chamber after 8 h (Hall *et al.*, 1978; Reynolds & Casterlin, 1979a, b). Since each test was performed three times, one-way ANOVA was run to find differences between treatments; the average results were used in Table 2. Significance was set at $p < 0.05$.

RESULTS

Final average weight, final total biomass, increase in weight for each treatment, and survival are presented in Table 1. Average weight increase (±SD) per treatment is shown in Fig. 2. In most treatments, differences in weight over time were significantly different after day 45, since at this time, there were considerable differences in growth between crayfish. After day 45 and until the end of the trials, crayfish maintained at 28 °C were significantly heavier than those raised at other temperatures (Fig. 2). Survival was significantly affected by water temperature over time (Fig. 3). Crayfish raised at 25 °C had the highest survival (83%) and those at 20 °C had the lowest (38%).In the preference assay, crayfish in all treatments tended to prefer the range from 23 °C to 26 °C (Table 2). Crayfish raised at 20 °C chose higher temperatures and those raised at 28 °C and 31 °C chose lower temperatures compared with their cultivation temperature (Table 2).

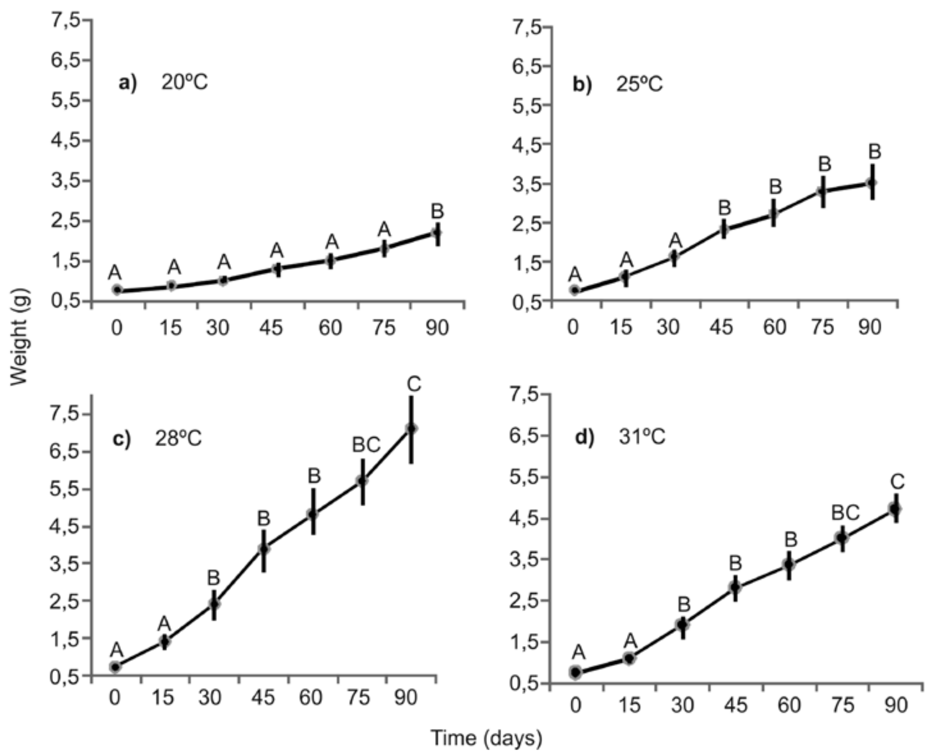

Figure 2 a-d. Average weight increase (± SD) for temperature trials of juvenile redclaw *Cherax quadricarinatus* raised at different temperatures. Different letters indicates statistical differences when compared on the same day at different temperatures ($p < 0.05$). a) 20°C, b) 25°C, c) 28°C, d) 31°C.

Table 1. Average initial weight (IW), final survival (S), average final weight (FW), final biomass (FBio), and linear equation of weight increase for *Cherax quadricarinatus* maintained at four temperature treatments.

(°C)	IW (g)	S (%)	FW (g)*	FBio (g)	Linear equation	r^2
20	0.57± 0.06	38	2.08 ± 0.12 (a)	18.70	y = 0.2411x + 0.3786	0.9674
25	0.69 ± 0.07	83	3.25 ± 0.17 (a)	68.20	y = 0.4911x + 0.2143	0.9887
28	0.84 ± 0.09	63	6.68 ± 0.13 (b)	100.20	y = 1.0411x – 0.4857	0.9937
31	0.66 ± 0.07	54	4.12 ± 0.16 (a)	53.60	y = 0.6875x – 0.0714	0.9921

Cells with different letters (a, b) are statistically significant ($p < 0.05$).

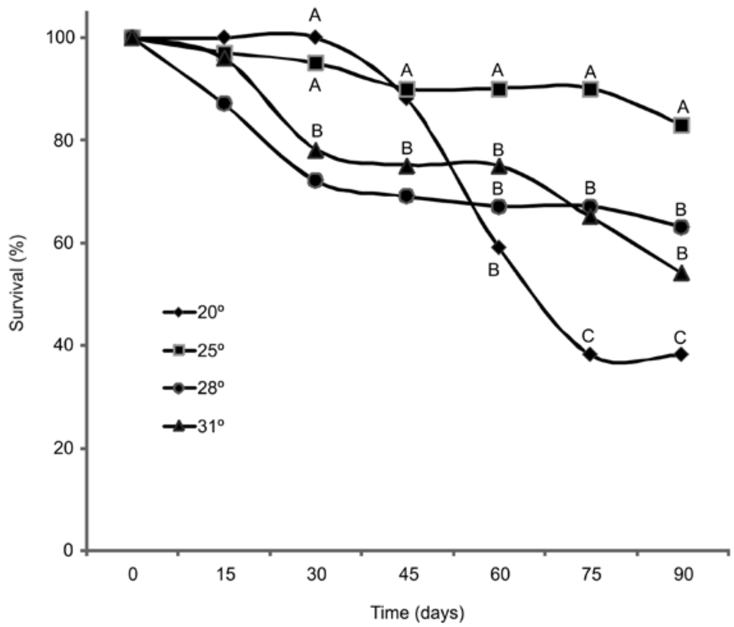

Figure 3. Survival (%) during the trials of juvenile redclaw *Cherax quadricarinatus* raised at different temperatures. Dots with different letters had statistical differences, when compared on the same day at different temperatures ($p < 0.05$).

Table 2. Temperature selected by juvenile redclaw crayfish *Cherax quadricarinatus* raised at four experimental temperatures. N = numbers of organisms.

T (°C)	N	21-22	23-24	25-26	27-28
20	19	—	4 (a)	9 (a)	6
25	39	3 (a)	33 (b)	3 (b)	—
28	30	2 (a)	27 (b)	1 (b)	—
31	20	9 (b)	9 (c)	2 (b)	—

Cells with different letters (a, b) are statistically significant ($p < 0.05$).

DISCUSSION

From many studies, it is known that crustaceans, as in most poikilothermics, show weight gain rates and survival that are temperature-dependent processes; however, when a batch of specimens, coming from a similar environment, but subjected to different temperature, the effect may require time before being noticed (Garcia-Guerrero *et al.*, 2010). In our study, differences took several weeks. Previous works state that physiological functions and metabolic reactions, such as those involved with oxygen consumption (Massanbau & Forgue, 1996; Spanopoulos *et al.*, 2005), use of nutrients (Tidwell *et al.*, 1999; García-Guerrero *et al.*, 2003a, b), or enzyme activity (Wang *et al.*, 2006) are efficient only within certain temperature ranges. The effect of temperature on metabolism was not measured here, but it is known that metabolism rate is determined mostly by water temperature (García-Guerrero *et al.*, 2003a; García-Guerrero, 2010) and has a direct effect on growth (Meade *et al.*, 2002; Hammond *et al.*, 2006) and survival (Thomas *et al.*, 2001; García-Guerrero *et al.*, 2003a; Carmona *et al.*, 2004) For crayfish, this is well documented (Chen *et al.*, 1995; Payette & Mc Gaw, 2003; Carmona *et al.*, 2004; Yue *et al.*, 2009). Hence, differences in growth and survival observed should result from differences in water temperature since that may cause diverse responses (Lagerspetz & Vainio, 2006). Studies of the effect of cultivation temperature on redclaw crayfish are few, but some are comparable; most results suggest a temperature range, rather than a particular temperature. For example, Meade *et al.* (2002) found reduced weight gain at the limits of the range between 16 °C and 32 °C, with the largest crayfish at 28 °C and the smallest at <21 °C. Masser and Rouse (1997) found that redclaw crayfish grow best at 23-29 °C, but usually die at temperatures over 33 °C. Jones (1994) found that juvenile redclaw grow at 20 °C to 34 °C, but this study used native crayfish in its native area raised in ponds. Austin (1995) and Díaz-Herrera *et al.* (2006) state that juvenile redclaw crayfish may survive even more extreme conditions (10-30 °C), but have weight gain only from 25 °C to 30 °C. Diminished growth and survival at the extreme temperatures in our study support these findings.

Temperature usually has similar effects on related species. Morrissy (1990) finds good weight gain and survival of *Cherax tenuimanus* Smith, 1912 at 13-30 °C. Mills (1986) obtained similar results with blueclaw crayfish *Cherax destructor* Clark 1936 at 15-36 °C, which has more tolerance for warm water than *C. quadricarinatus* (Huner & Brown, 1985). Tradeoff of faster weight gain at higher temperatures is explainable by its adaptation in its area of origin (Díaz-Herrera *et al.*, 2006). At the temperature extremes in our study, higher weight gain and survival occurred in juveniles raised at 31 °C, compared to crayfish raised at 20 °C, which suggests that, particularly in *C. quadricarinatus*, specimens are adapted to higher temperatures, even though very high temperature are lethal (García-Guerrero, 2010). Temperate and tropical crayfish are not resistant to low temperatures (Karplus *et al.*, 1998). Additionally, although our results demonstrate the convenience of cultivation at 28 °C for maximizing weight gain, their physiological processes may be more efficient at 25 °C, as suggested by the highest survival rate at this temperature. As others confirm, at certain temperatures, animals may not grow rapidly, but they make better use of nutrients (Whiteley *et al.*, 2001; García-Guerrero *et al.*, 2003b). Higher temperatures shorten the molt cycle and lead to faster growth (Hartnoll, 2001), but only within a suitable range (García-Guerrero *et al.*, 2003a). We found the best combination of growth and survival between 25 °C and 28 °C. Chen *et al.* (1995) report that high temperatures induce crayfish to remain buried in pond mud without activity or eating, which reduces growth. In contrast, tropical crustaceans cultivated in cold water dramatically reduce or even stop feeding (Wyban *et al.*, 1995). Huner and Barr (1991) and Wyban *et al.*, (1995) report that the red swamp crayfish *Procambarus clarkii* Girard, 1852 feed and molt only when the temperature is above 12.8 °C; growth is inhibited above 32 °C. When the green tiger prawn *Penaeus semisulcatus* is maintained through the winter, it consumes only one-third of the regular ration and has molting difficulties (Kumlu & Kir, 2005; Kir & Kumlu, 2008). Since the green tiger prawn is marine and native to the Indo Pacific Ocean (Holthuis, 1980), it is not well adapted to cold or widely fluctuating temperatures. A similar response seems to occur to redclaw crayfish, since in this study, crayfish grew slower at temperatures below 25 °C. Even if juvenile redclaw survive over a wide range of temperatures, a narrower interval (25-28 °C) is recommended for weight gain and higher survival rates.

Using thermal preference, the species-dependent responses can be modified with acclimation (Reynolds & Casterlin, 1979a, b; Hernández *et al.*, 1995; Diaz Herrera *et al.*, 2004). The preferred temperature occurs when energy is used most efficiently (González *et al.*, 2010). Specific adaptations and previous individual experience guides this behavior (Lagerspetz & Vainio, 2006).

Most crustaceans avoid harsh conditions by moving to an area that is more suitable for the physiological functions that ensures survival, growth, and reproduction (González *et al.*, 2010). Juveniles choose temperatures that are more suitable for growth; adults prefer temperatures that are more suitable for reproduction (González *et al.*, 2010; Tropea *et al.*, 2010). In our study, we suggest that juveniles prefer conditions related to growth despite the preferred range, which was narrow, compared to other studies (Lea, 1984; Punzo, 1985; Lagerspetz & Vainio, 2006). It seems that the temperature for routine metabolism, swimming, cardiac contraction, and efficient food conversion are chosen for optimal growth, but influenced by acclimation temperature (Reynolds & Casterlin, 1979a, b;). Perhaps the only previous documented report similar to this issue with redclaw crayfish is Diaz *et al.* (2004), where the juveniles, previously acclimated to several temperatures, preferred 28.7 °C. Further, they state that the responses to thermal stress of red claw juvenile crayfish showed that acclimation temperatures influenced the thermal resistance of the crayfish. In our study, when the acclimation temperature was at the critical thermal maximum, juveniles preferred lower temperatures, which is a response to differences in acclimation, diet, animal management, adaptation to different environments, or the specific differences between populations. From Table 2, we suggest that, if a crayfish is exposed at certain times to higher than optimal physiological temperature, they will choose a compensatory temperature when they have the possibility to do so. Only crayfish kept at 25 °C before the trials, which is close to the optimal temperature, had a preference for the same or nearly the same experimental temperature, hence, not engaging in compensating behavior. This behavior is observed in other species, in *Procambarus clarkii* (Espina *et al.*, 1993), the giant tiger prawn *Penaeus monodon* Fabricius, 1798 (Chen & Chen, 1999), and the whiteleg shrimp *Litopenaeus vannamei* Boone, 1931 (González *et al.*, 2010). For juvenile northern pink shrimp *Farfantepenaeus duorarum* Burkenroad, 1939, the preferred temperature is 30.3 °C (Reynolds & Casterlin, 1979b); for the blue shrimp *L. stylirostris* Stimpson, 1874, 27.8 °C (Re *et al.*, 2006), and for *L. vannamei* 27-30 °C (Hernández *et al.*, 2006). This same range for juvenile *L. vannamei* was reported by Ponce-Palafox *et al.* (1997), as best for survival and growth. All of these authors agree that thermal preferences are highly connected to the conditions of its life history. While the best temperatures for a species depend on its life history, preferences are influenced by acclimation. Hence, the preferred temperature usually is a good indicator of the thermal requirements to achieve homeostasis. Knowledge of preferred temperature help to select conditions to successfully cultivate a shrimp or crayfish, depending on specific conditions and purposes (Reynolds & Casterlin, 1979a, b). Since commercial producers regularly prefer high yield, redclaw crayfish raised at 28 °C will lead to heavier crayfish and higher total biomass, compared to crayfish raised at 25 °C. However, raising crayfish at 25 °C will lead to higher survival. Manipulation of temperature to achieve a desired biomass over a shorter time still needs intensive study. Protocols for cultivation should consider the effects of water temperature on weight gain and survival in areas with broad temperature changes, as well as nutritional requirements at different temperatures in the subtropical areas of Northwestern Mexico.

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