



Effect of photoperiod and temperature on growth and activity of digestive enzymes in juveniles of the long-arm river shrimp *Macrobrachium tenellum* (Smith, 1871) (Caridea: Palaemonidae)

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ABSTRACT

We studied the influence of temperature and photoperiod on the growth, survival, and digestive enzyme activity of the juveniles of *Macrobrachium tenellum* (Smith, 1871) kept in tanks and fed with a diet containing 20% chitin. The regime of the 60-day experiment included two temperatures (26 °C and 30 °C) and two photoperiods (14L/10D and 10L/14D). In addition to survival, the evaluation included total growth, growth rate, specific growth rate, and feeding efficiency. Trypsin, chymotrypsin, lipases, amylases, and chitinases were also measured. The optimal final weight and specific growth rate were the result of a combination of photoperiod 14L/10D at both temperatures. There was no significant effect on survival. Among the enzymes tested for digestive activity, only chitinase in the 14L/10D treatment at a temperature of 30 °C proved to be significant.

Key Words: aquaculture, chitinase, nutrition, physiology, water parameters

INTRODUCTION

The caridean freshwater shrimps are very diverse, with a worldwide distribution throughout tropical and temperate climates (Hernández *et al.*, 2007; Valencia & Campos, 2007). Members of the caridean family Palaemonidae are distributed in marine, brackish, and freshwater habitats (De Grave *et al.*, 2008). *Macrobrachium* (Spence Bate, 1868) is the most diverse, consisting of species that live mostly in coastal tropical regions worldwide (Espinosa-Chaurand *et al.*, 2011). Some species are very common on the Pacific continental side of Mexico, as is the case of *M. tenellum* (Smith, 1871), which adapts well to captivity (Espinosa-Chaurand *et al.*, 2011; García-Guerrero *et al.*, 2013). There is nevertheless scarce knowledge on its culture requirements of species of *Macrobrachium*, such as its proper temperature and photoperiod regimes, which are the major factors regulating molt cycle in all crustaceans but particularly in *Macrobrachium* (see Hartnoll, 2001). Investigations on this topic may help to improve small-scale aquaculture practices of species of *Macrobrachium* (Vega-Villasante

et al., 2011; García-Guerrero *et al.*, 2015). Temperature is perhaps the most important limiting factor for metabolic functions in all crustacean but particularly in shrimps, directly determining metabolic rate and growth (Kumlu *et al.*, 2000; Hoang *et al.*, 2003; Lagerspetz & Vainio, 2006). Temperature can highly affect food intake and digestion in farmed crustaceans (Croll & Watts, 2004). Individuals of *Macrobrachium tenellum*, for example, stop feeding when temperature decreases to less than 20 °C (Hernández *et al.*, 1995). Previous investigations (F. Diaz-Herrera, pers. comm.) indicate that the thermal preference of *M. tenellum* is from 27 °C to 30 °C so reproduction, metabolism, digestion, and growth are optimal at this temperature.

Because species of *Macrobrachium* are omnivores and pass through different life stages, living first in the brackish water of estuaries and then in rivers, it is assumed that these changes in habitat and diet are accompanied with changes in feeding requirements. Those changes could have also an effect on the activity of digestive enzymes because digestibility depends on the type of food ingested. The habitat in which organisms live can

have a major influence in their development, with photoperiod and light intensity among the most important factors in aquatic species (Hoang *et al.*, 2003). Most crustacean depends on photoperiod variations as an external stimulus, which start many biochemical and physiological processes related to digestion and growth, such as tissue growth and circadian rhythms of enzymatic activity (Casillas-Hernández *et al.*, 2006; Moreno-Reyes *et al.*, 2015). Photoperiodicity can control growth during early life stages of most aquatic animals because of its effect on feeding rhythms and influence on feeding behavior. Changes in the photoperiod might affect appetite, food intake, and feed conversion ratio in freshwater shrimps and thus have an effect on their growth (Espinosa-Chaurand *et al.* (2012, 2013). Light intensity and photoperiod influence most physiological functions (Vega-Villasante *et al.*, 2015) and endocrine control (Brito *et al.*, 2001), and may even increase cannibalism (Hecht & Pienaar, 1993). Studies on decapods, particularly those involving early stages, have shown that changes of feeding habits or digestive enzyme activity are associated with light intensity and day length (Deering *et al.*, 1995; Bermudes & Ritar, 2008; Matsuda *et al.*, 2012). As a result, physiological activity, feeding behavior, and even mobility are affected by both light intensity and day length, which in turn will affect growth and survival (Gardner & Maguire, 1998). Espinosa Chaurand (2013) suggested that enzymatic activity and enzyme levels in *M. tenellum* could be affected by changes in the photoperiod.

The aim of this study was to analyze the effect of different temperature and photoperiod regimes on growth, digestive enzyme activity, and survival in the juveniles of *M. tenellum*. Acknowledgement of the effect of variation in light and temperature on enzyme activity could provide useful information for the culture and management of *M. tenellum* and other freshwater species of shrimps.

MATERIAL AND METHODS

Juveniles of *Macrobrachium tenellum* were collected in the Chachahua Lagoon (15°58'2"N, 97°32'28"W), Oaxaca, Mexico. They were transported in a 250 l tank with permanent aeration to the laboratory and acclimated for two weeks at 25 °C. During acclimation, shrimps were fed with commercial shrimp pellet (40% crude protein; Camaronina 40® Agribands Purina, Ciudad Obregón, Mexico).

An experiment of four randomized treatments was designed to evaluate the effects of photoperiodicity and temperature. Treatments consisted on the combination of two different temperatures (26 °C and 30 °C) and two different photoperiods, 14 h light (L)/10 h dark (D), and 10 h L/14 h D. A control treatment (environmental temperature and photoperiod of 12L/12D) was included. Every treatment had six replicates and every replicate consisted on non-translucent plastic containers (50 l). Water temperature was maintained with submersible 300 Watt heaters (RENA Aquatic Supply, Charlotte, NC, USA) installed in the recirculating tray of every treatment. Oxygen concentration was measured daily with a Hanna® HI98186 oximeter (Hanna Instrumenta, Padova, Italy) and maintained above 5 mg O₂ l⁻¹. The photoperiod was controlled by placing white light lamps or black plastic curtains depending on the desired regime. In each container, a 0.5 m² plastic mesh was placed in the water to provide shade and shelter. Ten specimens (0.2 ± 0.04 g) were placed in every container.

Shrimp were fed with pelletized food prepared following De los Santos-Romero *et al.* (2017) (Table 1). Pellets were used because they had given optimal results in previous research (De los Santos-Romero *et al.*, 2017). The conversion factors suggested by Anh *et al.* (2009) (4.2 for carbohydrates, 5.56 for proteins, and 9.54 for lipids) were applied to estimate energy values.

A pelletized food ration consisting of 7% of the average weight of individuals was provided during acclimation. The ration was adjusted to 10% of their average weight for all treatments every two weeks, and this was considered as *ad libitum* (Cortés-Jacinto *et al.*, 2003). The shrimp were fed every day at 18.00 h. Survival was registered daily, and the trial lasted 60 days. At days 0, 15, 30, 45, and 60, all shrimp were individually weighed with a digital scale (SD ± 0.001 g).

The following parameters were calculated: average individual weight gain (g) (AIWG = final weight – initial weight), gain weight per day (g day⁻¹) (GWPD = (final weight – initial weight) t⁻¹), % gain weight (GW = 100 × (final weight – initial weight)/initial weight), and specific growth rate (%) SGR = ((LogN final weight – LogN initial weight) t⁻¹) × 100. Feed conversion ratio was calculated as FCR = supplied food (g)/weight gain (g), and feed efficiency ratio as FER = weight gain (g)/supplied food (g). SGR and FCR were calculated after Cortés-Jacinto *et al.* (2003); GW and GWP after Gitte & Indulkar (2005); AIWG and SR after Vega-Villasante *et al.* (2011); and FER after Hasan *et al.* (2012).

Analysis of enzyme activity

Two shrimp per replica (eight per treatment) were randomly taken on days 0, 30, and 60. Molt stage was not evaluated or considered for this sampling. After sampling, individual weight was registered and individuals were stored at –20°C until analysis. Only the cephalothorax was used for the analysis of enzyme activity, the rest was discarded. The hepatopancreas and intestine were dissected and separated and the dissected parts were individually weighed and homogenized with distilled water (4 °C) at a v/w ratio of 4 ml of water per gram of tissue. Raw extracts were separated by centrifugation at 14,000 rpm for 10 min at 4 °C and the crude extract was kept at –40 °C until analyzed for soluble proteins and enzyme activity.

All enzymatic analyses were made four times, including a blank, in which the enzyme reagent was added after the reaction had ceased. Proteins were quantified by the method of Bradford (1976) and absorbance was measured at 595 nm with bovine serum albumin (05470, Sigma-Aldrich, St. Louis, MO, USA) as standard. Lipase activity was determined following Versaw *et al.* (1989) using β-naphthyl caprylate (Sigma-Aldrich) as substrate. Lipase activity was expressed as lipase units mg protein⁻¹ (one lipase unit was the amount of enzyme required for an increase of 0.01 absorbance units at 540 nm/min). Amylase activity was measured as described by Vega-Villasante *et al.* (1993), using 1% starch in 50 mmol Tris-HCl at pH 7.5 as substrate. This activity was expressed as amylase units mg protein⁻¹ (one amylase unit was defined as the amount of enzyme needed to increase absorbance units by 0.01 at 550 nm/min). Trypsin activity was determined

Table 1. Approximate analysis of the diet of *Macrobrachium tenellum* used in the study.

	g 100 g ⁻¹ of dry food	
	Average value	SD
Humidity (%) ¹	4.783	± 0.37
Crude protein ²	44.137	± 0.19
Total lipids ³	6.517	± 0.20
Crude fiber ⁴	1.557	± 0.09
Ash ⁵	9.700	± 0.38
Nitrogen-free extract	38.090	± 0.77
Gross energy (kcal g ⁻¹)	4.741	

¹Differences in moist and dry weight; ²AOAC (micro-Kjendahl method);

³AOAC (Soxhlet method); ⁴AOAC (Weende method), ⁵AOAC (oven at 550 °C).

using BAPNA (B4875; Sigma-Aldrich) as substrate (García-Carreño & Haard 1993), adapted to a 96-well microplate by adding 10 μ l crude extract, 160 μ l 60 mmol Tris-HCl at pH 8.0, 10 μ l 192 mmol CaCl₂ at pH 8.0, and 10 μ l 9.6 mmol BAPNA dissolved in DMSO in each unit. Chymotrypsin activity was determined, using 9.6 mmol SAAPNA (S7388; Sigma Aldrich) dissolved in DMSO. Absorbance at 414 nm for both enzymes was recorded every 15 s for 30 min. At the end of the assay, a linear coefficient was calculated to determine the increase of absorbance per second. Enzyme activity was calculated using the molar extinction coefficient of *p*-nitroaniline (8800). Chitinase activity was determined according to De los Santos-Romero *et al.* (2017) by mixing 20 μ l of crude extract, 50 μ l 60 mmol Tris-HCl at pH 8, and 530 μ l with chitin azure as substrate (C3020; Sigma-Aldrich). The mixture was shaken with a vortex at 120 rpm, inclined at 45°, and incubated during 2 h. The reaction was stopped by centrifugation at 14,000 rpm for 10 min at 4 °C. The supernatant was immediately separated and absorbance was measured at 570 nm. One chitinase unit was defined as the amount of enzymes required for an increase of 0.001 absorbance units at 570 nm/min.

Statistical analysis

After determining survival rate (%), individual weight gain (g), gain weight per day (g/day), specific growth rate (%), feed conversion ratio, feed efficiency ratio, and enzyme activity (U/mg soluble protein) were compared with a test that allowed simultaneous assessment of the effect of two variables (two-way ANOVA; Zar, 2010). Normality tests were performed (Kolmogorov-Smirnov test, $\alpha = 0.05$). Significant differences among treatments were determined by the Duncan multiple range test ($P > 0.05$). Data were analyzed with Minitab 17 statistical package (Minitab, College Park, PA, USA).

RESULTS

Water temperature during the trial was of 22 ± 1.4 °C, pH was maintained at 8.1 ± 0.15 , and dissolved oxygen measured at 6.7 ± 0.49 mg O₂l⁻¹. Table 2 shows the results on all growth parameters obtained for all treatments during the study. The 30 °C treatment combined with a photoperiod of 14L/10D yielded the best results in terms of growth: IWG (1.147 ± 0.2 g) and GWPD (0.019 ± 0.0033 g day⁻¹). The minimum and maximum growth intervals for the other parameters were as follows: GWP (64.81 to 370.87%), SRG (0.83 to 2.55%), and FCR (16.7:1 to 2.2:1).

The final weight (IGW) of all treatments is given in Figure 1. Weight increased with time, with statistically significant differences after day 45 ($P < 0.05$; Fig. 2). The highest growth rate was observed at day 45, with a decrease until the last days in all treatments (Fig. 3). The lowest survival was 52.7% (14L/10D at 30 °C), and the optimal survival (83.2%) showed an inverse relationship with the maximum growth. FCR showed no statistically significant differences among treatments ($P \leq 0.05$).

Chitinase was the only enzyme showing statistically significant differences in its activity ($P \leq 0.05$; Fig. 4). The other enzymes (lipase, amylase, trypsin, and chymotrypsin) did not show this trend (Fig. 5), with no statistically significant differences among treatments ($P \leq 0.05$).

DISCUSSION

Chitin was included in the shrimp diet to follow the results of De los Santos-Romero *et al.* (2017), who evaluated the addition of chitin as an ingredient of the pelletized food. Results show that this type of food directly affects development of individuals when temperature and photoperiod are manipulated. This effect can be reflected in enzymatic activity during digestion, which in turn affects survival and growth rate. The interaction between the highest temperature and the longest photoperiod resulted in optimal growth, which agrees with previous research with other species of *Macrobrachium*. Most of the previous research nevertheless analyzed these effects separately, so that the effect of the interaction remained unclear. Hernández-Sandoval (2008) observed that in *M. occidentale* (Holthuis, 1950) growth increased with a temperature increase of 25 °C to 28 °C, but beyond 31 °C both growth and survival decreased. Madlen (2010) showed that growth in *M. rosenbergii* (De Man, 1879) declined above 34 °C, perhaps because of failure of enzymatic function. New (1995), Madlen (2010), and Anger (2013) reported that the growth rate of *M. rosenbergii* increased from 24 °C to 29 °C. Trinidad *et al.* (2010) indicated that higher average sizes (1.72 g and 5.55 cm) in *Macrobrachium jelskii* (Miers, 1877) were obtained at 29.97 ± 0.81 °C. The same trend is observed even considering differences in species and size of individuals. Other non-tropical species of *Macrobrachium* might respond similarly. Yue *et al.* (2009) reported that growth and survival of the crayfish *Procambarus clarkii* (Girard, 1852) decrease beyond 34 °C, mostly because of a combination of high oxygen and energy demand. Our research suggests that growth could be improved by manipulating temperature and photoperiod together by having an effect on the molt cycle and, therefore, on growth, an observation previously made by Aiken & Waddy (1992) and Pervaiz (2015a). Nevertheless, the general opinion is that temperature exerts a crucial effect on the metabolism of shrimps, and that variations in photoperiod could only cause significant differences under extreme temperature intervals, and its direct effect seems to be mostly on the efficiency of digestion. It is suggested that this effect on the efficiency of digestion is due to the similarities in the digestive system of crustaceans, having the same general enzymatic activity and with the same origin, but with subtle differences due to adaptations of species to particular conditions (McGaw & Curtis, 2013). Because photoperiod could have an effect on enzymatic activity, photoperiodicity might enhance growth depending on circumstances, as previously stated for most tropical decapods (Mazlum *et al.*, 2011). Kartamulia & Rouse (1992) suggested that higher temperatures may cause extreme energy and oxygen exhaustion, inducing stress and even mortality as a consequence. Although the fastest growth in our research was observed at 30 °C,

Table 2. Growth parameters in juveniles of *Macrobrachium tenellum* maintained at different photoperiod and temperature regimes, all in grams. S, survivor (%); IWG, individual weight gain (g); GWPD, gain weight per day (g day⁻¹); GWP, gain weight (%); SRG, specific growth rate (%); FCR, feed-efficiency ratio; L, light period; D, dark period.

TREATMENT	S \pm SE	IGW \pm SE	GWPD \pm SE	GWP \pm SE	SRG \pm SE	FCR \pm SE
10 L/14 D 26 °C	75.0 \pm 3.6	0.758 \pm 0.19	0.0126 \pm 0.0032	248.50 \pm 65.8	1.97 \pm 0.30	4.71 \pm 1.06
10 L/14 D 30 °C	66.8 \pm 4.3	0.950 \pm 0.11	0.0158 \pm 0.0019	310.38 \pm 38.7	2.33 \pm 0.15	2.49 \pm 0.27
14 L/10 D 26 °C	66.8 \pm 6.0	1.130 \pm 0.11	0.0188 \pm 0.0018	368.56 \pm 36.6	2.55 \pm 0.14	2.22 \pm 0.39
14 L/10 D 30 °C	52.7 \pm 6.7	1.147 \pm 0.20	0.0191 \pm 0.0033	370.67 \pm 63.9	2.51 \pm 0.24	2.42 \pm 0.34
CONTROL	83.2 \pm 4.3	0.205 \pm 0.02	0.0034 \pm 0.0002	64.81 \pm 5.9	0.83 \pm 0.06	16.75 \pm 5.69

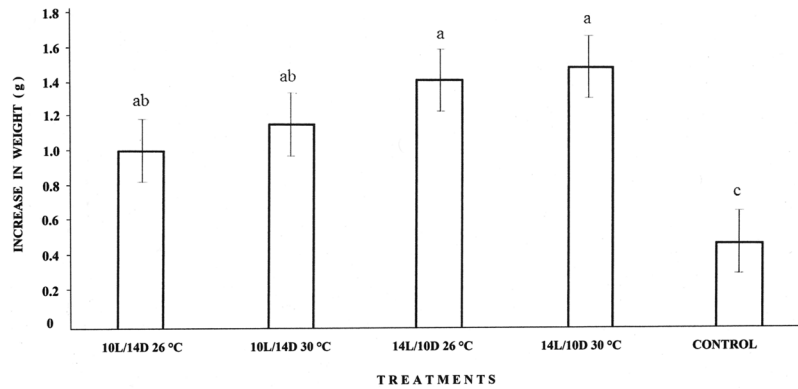


Figure 1. Increase in weight for juveniles *Macrobrachium tenellum* after 60 days under different photoperiod and temperature regimes. Different letters indicate statistical differences at $P \leq 0.05$.

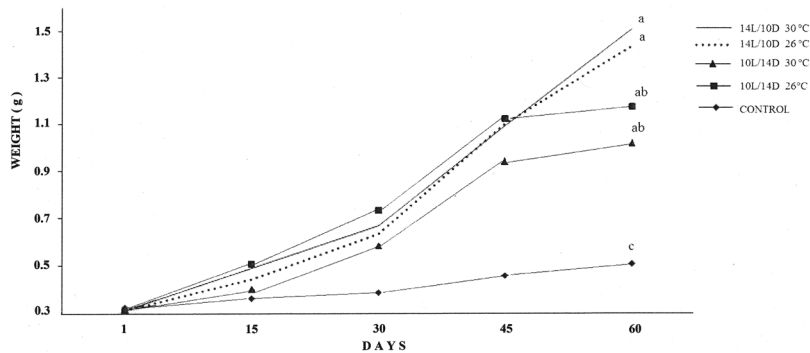


Figure 2. Increase in weight in relation to time in juveniles of *Macrobrachium tenellum* kept under different photoperiod and temperature regimes. Different letters indicate statistical differences at $P \leq 0.05$.

this treatment had significant differences in terms of growth rate only when compared to the control (22.8 °C), where there was very slow growth. None of the extreme temperatures are therefore recommended in practice.

Growth rate was also affected by water temperature, and high growth rate produced high FCR as previously observed in *M. rosenbergii* (Chavez *et al.*, 1991). Feeding in this species can also increase when temperatures increased from 23 °C to 33 °C, increasing growth (Niu *et al.*, 2003). *Macrobrachium tenellum* was reported to grow well from 25 °C to 32 °C (Espinosa-Chaurand *et al.*, 2011), but our research shows that optimum growths seems to be narrower. A change in the growth rate when temperature is far from optimal could be due to a low efficiency in energy use or to a metabolic rate unsuitable for good growth. Hernández *et al.* (1995) suggested that juveniles of *M. tenellum* can survive in a wide temperature interval, but optimal growth occurs only close to 29 °C. García-Ulloa *et al.* (2008) and Vega-Villasante *et al.* (2011), however, reported average weight increase of 0.015 g/day at 27 °C for *M. tenellum*, which is similar to our results. These results are expected in tropical freshwater shrimps such as *M. tenellum*, and is consistent with results of Ponce-Palafox *et al.* (2006) and Vega-Villasante *et al.* (2011), who found that the optimal growth of this species is between 29 °C and 32 °C. Digestion, feeding, and food absorption is therefore optimal within this temperature range.

In terms of survival, significant differences were observed between the control and all treatments, which suggests that even if the growth rate is affected by the treatments, none of the treatments were outside the range for the species. De los Santos-Romero *et al.* (2017) reported that 22.8 °C could be optimal in terms of survival, hence, both temperatures used here are close to a range in which an inverse relationship of survival with growth is observed, as described by García-Ulloa *et al.* (2008),

Vega-Villasante *et al.* (2011), and Espinosa-Chaurand *et al.* (2012). These results suggest that survival is temperature dependent, but other factors may affect the results depending on experimental conditions, among which feeding and physiological conditions are important (Yue *et al.*, 2009).

The effect of the photoperiod on growth is not easy to measure because it is not as influential as temperature. It is often masked by other factors or the organism may respond to it only under certain conditions. The effect of the photoperiod seems to be very particular, mostly on certain processes such as digestion. It has a direct effect on enzyme activity because circadian rhythms are affected by it (Shan *et al.*, 2008). Enzymatic activity was significantly higher in *M. tenellum* in treatments with longer daylight. Hoang *et al.* (2003) found that an altered 7L:5D regime induced better growth in *Penaeus merguensis* (De Man, 1888) than the regular regime (12L:12D), and reported that the photoperiod significantly improved weight gain this species, although its effect on molting was not significant. The growth rate of juveniles of *M. rosenbergii* at a photoperiod of 24L/0D was the highest among those considered (Lin, 1997), which suggests that permanent light may improve growth at least in this species. Wang *et al.* (2006) did not find any effect of photoperiod on the growth of juvenile *Fenneropenaeus chinensis* (Osbeck, 1765) when the light period was lower than 12h day⁻¹, and Withyachumnarnkul *et al.* (1990) found that juveniles of *M. rosenbergii* at a 24-hour light regime were heavier than those maintained at other regimes. Yue *et al.* (2009) observed that the crayfish *Procambarus digueti* (Bouvier, 1897) reduced their locomotory activity at regimes of 20L:4D, suggesting that there is less energetic expenditure, allowing individuals to channel more energy to growth. Several investigators have reported that the light period and intensity can affect growth indirectly since it has an effect on both feeding behavior and circadian rhythm, as in the case of the shrimp *Litopenaeus vannamei* (Boone,

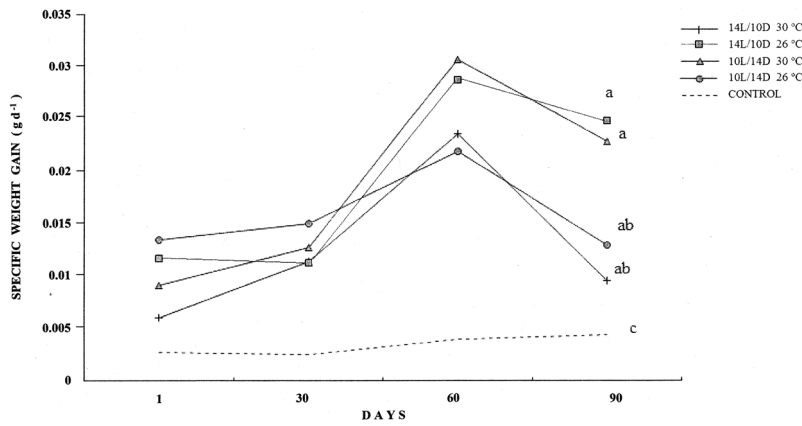


Figure 3. Specific growth gain in relation to time in juveniles of *Macrobrachium tenellum* kept under different photoperiod and temperature regimes. Different letters indicate statistical differences at $P \leq 0.05$.

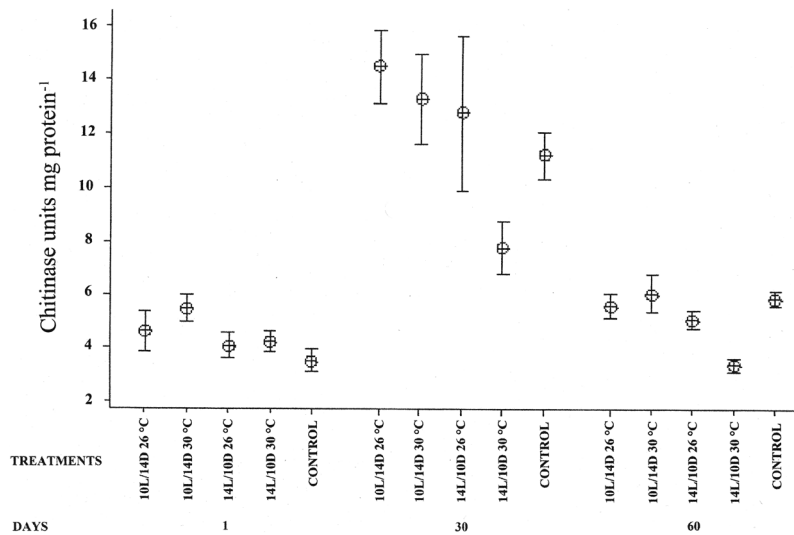


Figure 4. Chitinase activity in juveniles of *Macrobrachium tenellum* kept under different photoperiod and temperature regimes. One chitinase unit is defined as the amount of enzymes required for an increase of 0.001 absorbance units at 570 nm/min.

1831), in which a low-intensity (600 lux) light induces less growth because individuals eat less (Guo *et al.*, 2013). As observed here with the longest photoperiod (14L/10D) additional light induced growth at 26°C. No differences in the growth rate of *Penaeus indicus* (Milne Edwards, 1837) were found between photoperiods of 0L/24D and 14L/10D (Patikawa & Wemmo, 2014) and 24L/0D and 12L/12D (Vijayan & Diwan, 1995).

Feeding habits differ among species based on feeding habits and circadian rhythms, which influence the activity of digestive enzymes. For example, Hoang *et al.* (2003) reported that the survival of the juveniles of *Portunus pelagicus* (Linnaeus, 1758) and *P. merguensis* (De Man, 1988) showed no significant differences in growth if maintained under photoperiods of 12L/12D and 14L/10D ($P > 0.01$). Hoang *et al.* (2002) mentioned that the effect of photoperiod on the growth of *P. merguensis* is gradual and may decrease over time, suggesting that there is a relationship between the influence of photoperiod and age. Growth of *P. merguensis* was not affected by the photoperiod if water temperature is below 24 °C (Hoang *et al.* (2002). Despite studies that show how photoperiod affect crustacean growth, there is no strong evidence for the American species of *Macrobrachium* and whether the photoperiod might interfere with molting. Studies with *M. rosenbergii* suggest that daylight length may not have a significant effect on growth (Chávez *et al.*, 1991). Similar trends were observed for the

brachyuran crab *Scylla serrata* Forskål, 1775 (Quinitio *et al.*, 2001), the caridean shrimp *Palaemonetes argentinus* Nobili, 1901 (Díaz *et al.*, 2003), and the shrimp *Penaeus monodon* Fabricius, 1798 (Hasan *et al.*, 2012).

Survival in *M. tenellum* showed significant differences ($P < 0.05$) between the control (22.8 °C; 12L/12D) and the rest of the treatments, and there was no direct effect of the photoperiod, as previously observed by Vega-Villasante *et al.* (2015). Such effect does not necessarily apply to all species of *Macrobrachium*, however, because Chavez *et al.* (1991) found that an increase in photoperiod (15L/9D) combined with low temperature (24 °C) could lower survival (21%) in *M. rosenbergii*, and Tidwell *et al.* (2001) reported that survival in the same species was significantly higher at 24L/0D (72%), but 59% and 58%, respectively, at 12L/12D or 0L/24D. Hecht & Pienaar (1993) and Gardner & Maguire (1998) observed that the photoperiod is related to cannibalism in the brachyuran crab *Pseudocarcinus gigas* (Lamarck, 1818). Both investigators found that this crab finds food visually, thus, more hours of darkness diminish its chances to find food. More hours of darkness can promote cannibalism or death if food is unavailable. Wang *et al.* (2006) and Guo *et al.* (2013) found that the shrimp *Fenneropenaeus chinensis* had stronger enzymatic activity when light was more intense. The photoperiod is therefore not a direct and determinant factor on growth and survival itself and its effect is

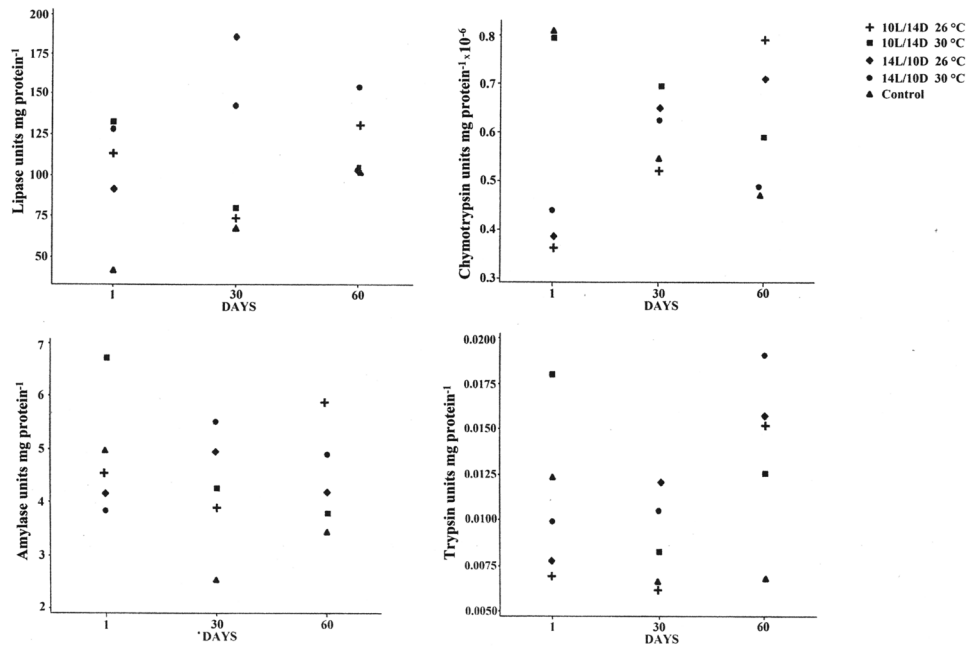


Figure 5. Enzymatic activity of amylase, lipase, trypsin, and chymotrypsin in juveniles of *Macrobrachium tenellum* kept under different photoperiod and temperature regimes. One unit of enzyme is defined as the amount required for an increase of 0.001 absorbance units at 550 nm/min for amylase, 540 nm/min for lipase, and 414 nm/min for trypsin and chymotrypsin.

not as clear as that of temperature. It can act in combination with other factors that can mask its effect.

Explaining the combined effect of temperature and photoperiod on enzymatic activity could lead to a better understanding of this interaction. Enzymatic activity influences digestion because of its effect on digestibility, which also depends on food type and feeding habits. Ceccaldi (1989) and Zhang *et al.* (2014) mentioned the importance of the effect of temperature and photoperiod on chitinase activity because the intensity of this activity can determine the efficiency of digestion as well as molting. Wang *et al.* (2004) found that the growth of *Fenneropenaeus chinensis* and *Palaemon serratus* (Pennant, 1777) is significantly different at different photoperiods, suggesting that there is a relationship between duration of daylight and enzyme activity, affecting growth because of its direct effect on digestion. But enzyme activity also depends on dietary regimes and physiological condition (Brito *et al.*, 2001). An elevated enzyme activity may occur when a particular component is scarce in the diet (García-Carreño & Haard, 1993). An increase in amylase activity might be due to low levels of carbohydrates in the diet and the rise in trypsin activity could be due to low protein ingestion or to amino acids deficiency in the food (Rivas-Vega *et al.*, 2006). Chitin in the diet may also alter enzymatic activity because it has low digestibility (Brito *et al.*, 2001) and several investigators have found that enzyme activity could change as an adjustment mechanism to a low amount of nutrients in the digestive tract, particularly protein, or due to poor digestibility. We nevertheless found no evidence to support an effect of the combined interaction of temperature and photoperiod on enzymatic activity because all enzyme activity measurements revealed no significant differences. Previous work on this phenomenon in *Macrobrachium dayanum* Henderson, 1893 (Pervaiz *et al.*, 2015b) revealed that activity rate was slower at low temperatures than at higher ones, independently from the photoperiod. Pervaiz *et al.* (2015b) also stated that specimens under permanent light or darkness, will also induce lower food consumption as a result of the circadian rhythm, thus altering enzyme activity. Long daylight may cause an increase in daily

enzymatic or hormonal activity at certain intervals depending on the species (Vega-Villasante *et al.*, 1993).

Some statistical differences were observed when light exposure was lower (10 h) combined with low temperatures (26 °C). Light variations are also involved with most physiological functions, which are affected by enzyme activity, but the magnitude of this effect will always depend on the combination of various intrinsic and extrinsic factors. An additional problem associated with the determination of enzyme activity could be the stage of molt cycle, season, and sexual condition, hence, no results can be conclusive because enzyme activity may influence other processes as a result of its effect on digestion.

Temperatures of 26 °C and 30 °C in combination with 14 hours of light seem to be the optimal for growth in *M. tenellum*, which is consistent with the environmental conditions in the natural environment of the species. Such combination of temperature and light allows proper enzyme activity, particularly of chitinase. The combined effect of temperature and light on growth and survival could be better understood if other processes involved in this interaction are included, such as reproductive status and molt cycle.

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