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Research Article

Growth and survival of juvenile cauque river prawn *Macrobrachium americanum* fed with diets containing different protein levels

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ABSTRACT. The effect of five diets with different crude protein levels (27, 33, 38, 43, and 48%) on growth and survival of *Macrobrachium americanum* prawns was determined. Optimum dietary protein level was also calculated. Specimens were collected in the Coyuca River, Guerrero, Mexico, and classified into seven weight groups with 0.67 g intervals amplitude. Five diets with different levels of crude protein were formulated and supplied to individuals of the seven weight groups as an experimental treatment. Weight gain percentage (WG%), specific growth rate (SGR), food intake (FI), protein intake (PI), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) were calculated weekly along the 70 days experiment. A quadratic model was fitted to weekly mean weight gain and to WG% rates data to estimate protein requirement for every week of the experiment and weight group. A mortality model was also fitted to mortality data to compare mortality trend among the different experimental diets. 33% protein level resulted in the best treatment because of the parameters calculated, cost to produce and survival trend. Weekly optimum level of protein calculus varied on a range from 39.4 to 43.3% and optimal crude protein percentage for the seven weight ranges was between 49% for smaller prawns (0.248-0.918 g) and 35.7% for the larger prawns (4.271-4.940 g). Thus, it is recommended juvenile feeding prawns with different crude protein levels while the culture time elapses to achieve the maximum weight gain.

Keywords: *Macrobrachium americanum*, prawn, protein-optimum, survival rate, nutrition, juvenile, aquaculture.

INTRODUCTION

Macrobrachium prawns have the potential for freshwater aquaculture and large areas are suitable for its production in Mexico (García-Guerrero *et al.*, 2013). It can be executed in simple facilities at low-cost (Valenti & New, 2000; Moraes-Valenti & Valenti, 2007). Some species are already appreciated as high cuisine products and source of high-quality protein (Gupta *et al.*, 2007). One good example of this is *Macrobrachium americanum* (Bate, 1868), which is an endemic species distributed along the Pacific coast rivers from Mexico to Peru (Wicksten & Hendrickx, 2003). This species has aquaculture potential and has already demanded as cuisine product in local markets. It is likely to attain higher prices in the market if a proper stable quota of biomass is offered to national and international markets (Ponce-Palafox *et al.*, 2002; García-Guerrero *et al.*, 2013). However, technology for its culture is not available yet.

Supply the optimal protein in food is one of the main requirements for a good profit on cultured animals regarding survival, growth rates, and feed conversion factors (Cortés-Jacinto *et al*., 2003). A basic nutrient in any balanced diet is protein, one of the major nutrients required for growth and energy production as well as for all physiological processes. Dietary proteins provide the amino acids for the synthesis of muscle, connective tissue and hemolymph respiratory proteins (Harrison, 1990). However, it has been observed the reduction of growth when at low or exceeded protein in the food. If protein level in the diet is low, prawns may not have enough available protein for tissue formation, resulting in growth reduction, whereas an excess could increase energy expenditure and undesired toxicity on water due ammonia excretion (Goda, 2008; Méndez-Martínez *et al*., 2017).

A protein-efficient diet will reduce the production cost that is a very important aspect, considering that feeding may represent up to 60% of total production costs (Du & Niu, 2003). The optimum dietary protein levels on formulated food for prawns depend on many factors such as age, feeding strategy, protein quality, dietary energy level and protein sources (Méndez-Martínez *et al*., 2018). Optimum dietary protein level for crustaceans ranges between 23 and 60% depending on those factors (Teshima *et al*., 2006). For *Macrobrachium rosenbergii*, an herbivorous-omnivorous and the best-studied freshwater prawn, New (2002) suggested that a 35% of protein on food must be given for the first two months after postlarvae stocking and 30% from month three to harvest. However species-specific differences in protein requirements need to be considered in establishing recommended levels. Considering the above mentioned the study was designed to determine the optimum dietary protein of *M. americanum* juveniles of seven groups of size subjected to different protein content diets during a seven-week experiment.

MATERIALS AND METHODS

Prawn collection

A total of 105 *M. americanum* juveniles from the Coyuca River, Coyuca de Benítez, Guerrero, Mexico (17°03'36.14"N, 100°01'42.80"W; 69-132 m above sea level) were collected using a cast-net. The weight of the individuals ranged between 0.25 and 4.94 g. All specimens were acclimated to laboratory conditions and fed with a commercial shrimp pellet (Camaronina® 35% protein) for one week before the experiment (García-Guerrero & Apun-Molina, 2008). Individuals were grouped in seven weight ranges (WR) of 0.67 g length, and 15 individuals included per group (Table 2).

Experimental diets

Five diets with different levels of crude protein (CP) were formulated with Mixit-win® (Agricultural Software Consultants Inc., San Diego, CA, USA) (Table 1). Each different protein amount in the diet (27, 33, 38, 43, 48%) was considered as a treatment, and every treatment had three replicates. So, a total of 15 experimental units were used to test the effect of the five diets.

Proximate analyses of ingredients and diets were determined according to AOAC (2016) to corroborate the real protein level in diets. Each diet was prepared as reported by Cortés-Jacinto *et al.* (2003).

Experimental design

The 15 experimental units used for this experiment were 30 L containers cover with Styrofoam trays and a bottom area of 0.12 m². In each experimental seven *M. americanum* juveniles were placed (average density of 58.3 juveniles per m²), one of each the seven weight ranges previously defined. Each unit was provided with PVC tubes (15 cm long×1.27 cm diameter) and three pieces of the plastic net (25×30 cm) for shelter and to minimize cannibalism over soft prawns by increasing the area (Mariappan *et al*., 2004; Méndez-Martínez *et al*., 2018).

Daily water exchange was 30% by siphoning while uneaten feed and feces were discarded. All experiments were maintained with tap freshwater under this procedure: temperature 28.6 ± 0.65°C (Correia *et al*., 2000), pH 8.0-8.2, dissolved oxygen concentration was always maintained in saturation using air diffusers (8.7 ± 0.3 mg L^{-1}) (Yamasaki-Granados *et al*., 2013). Juveniles were fed three times per day (5:30, 12:30 and 19:30 h). The total daily ration was set at 5% of total prawn biomass and later diminished to 4% depending on consumption.

Estimation of yield parameters

The experiment lasted 70 days and all parameters were registered every week starting from the first day. Growth was measured as weight increase: weight gain percentage (WG%), specific growth rate (SGR), food intake (FI), protein intake (PI), protein efficiency ratio (PER) and food conversion ratio (FCR) (Table 3). Survival rate (SR) was estimated also. Parameters were calculated as follows:

$$WG\% = (FBW - IBN) \times 100 \quad (1)$$

$$SR = (\text{final number/initial number of prawns}) \times 100 \quad (2)$$

$$SGR = (\ln FBW - \ln IBW)/t \times 100 \quad (3)$$

$$FI = (SF / \text{number of juveniles}) / t \quad (4)$$

Table 1. Formulation and proximate composition of five diets (g kg^{-1} in dry matter) for *M. americanum* juveniles.

Ingredient	T (CP %)				
	27	33	38	43	48
Fish meal[1]	111	209	308	406	505
Whole wheat flour[1]	625	536	447	357	268
Soybean paste[1]	100	100	100	100	100
Fish oil[1]	44	35	26	17	8
Squid meal[1]	50	50	50	50	50
Alginic acid[2]	20	20	20	20	20
Soy lecithin[3]	10	10	10	10	10
Mineral premix in diet[4]	25	25	25	25	25
Vitamin premix [5]	3	3	3	3	3
Ascorbic acid[6]	1	1	1	1	1
Calcium carbonate[7]	10	10	10	10	10
Choline chloride[8]	60	60	60	60	60
Proximate composition (g kg^{-1} as fed-basis)					
Crude protein[9]	268 ± 2.8	327 ± 1.0	378 ± 3.6	428 ± 0.7	477 ± 1.6
Ether extract[9]	48 ± 1.3	59 ± 7.1	66 ± 0.8	80 ± 0.5	75 ± 0.4
Ash[9]	62 ± 0.6	79 ± 0.8	97 ± 0.4	114 ± 0.2	129 ± 0.9
Fiber[9]	5 ± 0.5	5 ± 0.9	5 ± 1.6	5 ± 0.9	3 ± 0.4
Nitrogen free extract [9]	617 ± 3.5	531 ± 4.9	455 ± 3.9	373 ± 1.5	317 ± 2.0
Humidity[9]	69 ± 4.5	74 ± 2.4	59 ± 1.1	60 ± 2.0	57 ± 3.3

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$$PI = (FI)\ (PF) / SF \quad (5)$$

$$FCR = FI\ (g) / \text{weight gain}\ (g) \quad (6)$$

$$PER = \text{weight gain}\ (g) / \text{protein intake}\ (g) \quad (7)$$

In these equations, FBW is the final body weight (g), IBW is the initial body weight (g), t is the time in days, SF is the amount food (g) supplied, PF is the grams of protein in the food.

Table 2. The seven different prawn groups of *Macrobrachium americanum* with a weight range of 0.67 g and the number of juveniles per group.

Group	Weight range (g)	n
WR1	0.248 - 0.918	26
WR2	0.919 - 1.589	9
WR3	1.59 - 2.259	14
WR4	2.26 - 2.929	17
WR5	2.93 - 3.599	20
WR6	3.6 - 4.27	11
WR7	4.271 - 4.94	8

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to determine the effects of crude protein (CP) level and the initial prawn weight on SGR. The nonparametric Friedman's test tested all others variables (WG%, FI, PI, FCR, and PER) measured-nonparametric Friedman's test. Significant levels were considered at $P < 0.05$.

A quadratic model was fitted to weekly mean weight gain to estimate protein requirement (Shearer, 2000). The quadratic model was defined as

$$Y = \alpha + ßx + cx^2 \quad (8)$$

where x is the protein level, Y is the response variable, α is the constant, $ß$ and c are the linear coefficient and the quadratic coefficient respectively, and $x = -ß/2c$ is the inflection point that represents the optimum protein level for the best growth (Shearer, 2000). The quadratic model was also fitted to WG% rates data calculated for each WR as well as the optimum requirement of CP.

Table 3. Crude protein (CP %) productivity parameters (mean ± SE) and their significant differences in *M. americanum* juveniles after 70 days of culture. Values within columns with the same superscript are not significantly different ($P > 0.05$). SR: survival rate, WG: Weight gain, SGR: specific growth rate.

CP (%)	SR (%)	WG (%)	SGR (% day^{-1})	Food intake (g day^{-1} ind^{-1})	Protein intake (mg day^{-1} ind^{-1})	FCR	PER
27	85.71	9.58 ± 0.99	0.07 ± 0.08[a]	0.12 ± 0.008[a]	25 ± 2.08	0.71 ± 0.20[a]	0.82 ± 0.05[a]
33	90.47	15.87 ± 1.06[a]	0.27 ± 0.09[a]	0.11 ± 0.008[a]	26 ± 2.42[ab]	1.26 ± 0.21[a]	0.79 ± 0.06[a]
38	90.47	16.09 ± 1.02[a]	0.27 ± 0.09[a]	0.12 ± 0.008[a]	33 ± 3.23[abc]	1.27 ± 0.20[a]	0.86 ± 0.06[a]
43	76.19	14.83 ± 0.99[a]	0.12 ± 0.08[a]	0.11 ± 0.008[a]	35 ± 2.78[abc]	1.20 ± 0.20[a]	0.78 ± 0.05[a]
48	90.47	14.41 ± 0.95[a]	0.08 ± 0.08[a]	0.12 ± 0.007[a]	44 ± 3.45[ac]	0.96 ± 0.19[a]	0.84 ± 0.05[a]

A mortality model (Pauly, 1983) was fitted to mortality data to describe the decrease in many individuals and to compare the mortality trend among the different experimental diets and not only the final value. The model is defined as:

$$y = a \times e^{(-b\times t)} \quad (9)$$

where a is the y-intercept, e is the Euler number (2.71828), b is the slope (mortality rate), and t is the number of prawn remaining at the end of time "t".

RESULTS

Growth

The values calculated for all yield parameters and the significant differences among the five different protein treatments are shown (Tables 3-4). There was only a significant difference in diet 27% CP for WG%. It had the smaller value for SGR, and WG% was significantly ($P < 0.05$) smaller than obtained for all other diets, it was the worst treatment. 38% CP diet had the highest and best values for SR (90.48), WG% (16.09 ± 1.02), SGR (0.27 ± 0.09); and the highest and worst values for FI (0.12 ± 0.008), FCR (1.27 ± 0.20) and PER (0.86 ± 0.06). 33 CP (%) had the same values of 38% CP diet for SR, SGR, and all other variables values were very close (Table 3). Considering only these yield parameters, 33% CP diet seems to offer the best results. It has the lowest protein content, and there were no significant differences in WG% or SGR between the remaining treatments (Table 3).

Table 4 presents values of weight range groups. WR3 was the only one significantly different from the others in WG%, food, and protein intake, FCR and PER values. Even though there was a difference in WG%, it was not observed for SGR as we could expect. The groups formed on FCR, PER, food and protein intake seem to cause by size differences since larger individuals consumed more food than small ones and had FCR and PER values are also bigger.

The quadratic model fitted to WG% weekly-data for each experimental diet group, and its inflection point calculus showed that optimum CP is not constant and varied along the experiment on a range from 39.4% to 43.3% (Fig. 1), mean value was 42.2%.

WR curves fitted to WG% rates data (Fig. 2) show how protein level on a diet is related to weight gain for the different WR. Optimum CP percentage on a diet calculated had a range from 49 to 36 (Table 5). The smallest WR seems to have higher optimum CP on diet and vice versa, as expected.

Survival

By diet groups, 33%, 38% and 48% CP diets had the same highest survival rates (90%), followed by 27% CP (86%) and 43% CP (76%) (Table 3). Mortality started at different moments for each treatment. It was first observed at the third week on 48% CP group, last observed at the ninth week on 27% CP group and at sixth week for 33% CP group (Fig. 3). 27% CP group had the highest rate (-0.05) and 43% CP the lowest (-0.007). 33% CP, 38% CP, and 43% CP had similar slope values. Meanwhile, WR groups it seems small individuals had higher survival rates than the big ones. In fact, first two WR had very high values (92% and 100% respectively), and last two had the lower survival rates (81.81% and 75%) (Table 4).

DISCUSSION

Water quality was maintained as recommended for prawns (García-Guerrero *et al.*, 2011; Langer *et al.*, 2011; Ding *et al.*, 2015) for all experimental units of all treatments. Diets were formulated using highly digestible ingredients (approximately 87%) similar to those used in experimental diets formulated for prawns and other decapod crustaceans (Campaña-Torres, 2001; Campaña-Torres *et al.*, 2005; Cortés-Jacinto *et al.*, 2009, Méndez-Martínez *et al.*, 2017, 2018). It was observed that *M. americanum* juveniles were able to

Table 4. Weight range productivity parameters (mean ± SE) and their significant differences in *M. americanum* juveniles after 70 days of culture. Values within columns with the same superscript are not significantly different ($P > 0.05$).

Weight range	SR(%)	WG(%)	SGR (% day^{-1})	Food intake (g day^{-1} ind^{-1})	Protein intake (mg day^{-1} ind^{-1})	FCR	PER
1	92.30	14.04 ± 0.77^{a}	0.04 ± 0.06^{a}	0.11 ± 0.12^{ac}	3.16 ± 3.45^{abcd}	0.28 ± 0.15^{b}	0.79 ± 0.04^{abc}
2	100.00	13.30 ± 1.52^{a}	0.12 ± 0.12^{a}	0.08 ± 0.07^{a}	2.00 ± 1.71^{abc}	1.03 ± 0.30^{ab}	0.52 ± 0.08^{ab}
3	85.70	18.97 ± 1.08	0.27 ± 0.09^{a}	0.10 ± 0.12^{ac}	2.84 ± 3.67^{abcd}	1.26 ± 0.22^{a}	0.75 ± 0.06^{abc}
4	76.47	14.63 ± 0.99^{a}	0.16 ± 0.08^{a}	0.11 ± 0.07^{ac}	2.93 ± 1.95^{abcd}	1.13 ± 0.20^{a}	0.74 ± 0.05^{abc}
5	90.00	11.89 ± 0.92^{a}	0.21 ± 0.08^{a}	0.12 ± 0.09^{abc}	3.47 ± 2.59^{acde}	1.11 ± 0.19^{a}	0.86 ± 0.05^{acd}
6	81.81	12.74 ± 1.37^{a}	0.19 ± 0.11^{a}	0.15 ± 0.11^{bc}	4.27 ± 3.08^{de}	1.46 ± 0.27^{a}	1.09 ± 0.07^{cd}
7	75.00	13.53 ± 1.46^{a}	0.13 ± 0.12^{a}	0.14 ± 0.06^{abc}	3.89 ± 1.91^{acde}	1.31 ± 0.20^{a}	1.00 ± 0.08^{acd}

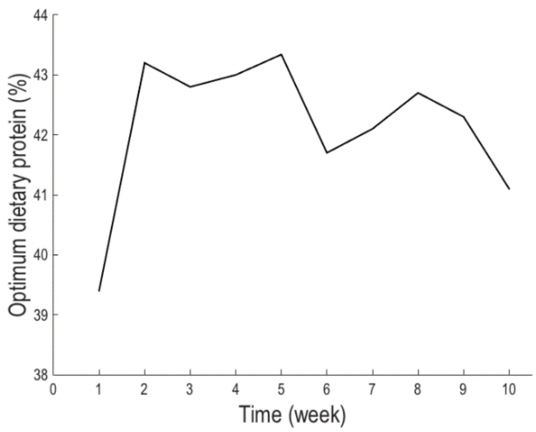

Figure 1. *M. americanum* juveniles optimum CP variation in diet along with the experiment (70 days).

digest the presented diets efficiently. Therefore, differences in growth rates could be attributed to different treatments.

The highest WG (16.1%) obtained on the 70 experimental days is lower if compared to that reported for *M. rosenbergii* by New (2002), which may have a requirement from 35 to 40% of dietary protein depending on stage (Teshima *et al*., 2006). This author reported that 0.33 g juveniles stocked at a density of 6 ind m^{-2} in a temperate zone reached an average of 30 g in 106 days in ponds without shelter and nearly 37 g in ponds provided with shelter. These weights increase several times its initial size on a similar time, producing larger prawns than those of present experiment. However, it has to be considered that *M. rosenbergii* may vary in its food requirements and has well-developed culture techniques and very adapted to pelletized food. It is possible, in agreement with present results, that *M. americanum* has a slower growth in comparison with other *Macrobrachium* such as *M. rosenbergii* even considering this, is possible to observe that 27% CP was the only diet that results in a significant lower WG%. Because of this, 33% CP is

Table 5. Optimum dietary protein (%) calculated from models fitted to WG% rates for every weight range.

Weight range (g)	Optimum dietary protein (%)
WR1 (0.248 - 0.918)	29
WR2 (0.919 - 1.589)	49
WR3 (1.59 - 2.259)	37
WR4 (2.26 - 2.929)	38
WR5 (2.93 - 3.599)	38
WR6 (3.6 - 4.27)	40
WR7 (4.271 - 4.94)	36

suggested as best option since it had very similar results to 38% CP but at a lower cost. Is possible that the lack of proper culture techniques limits its growth potential at commercial scale. So, it is necessary to improve the knowledge on its growth potential by the formulation of a specific diet meeting its requirements to know if the slow growth rate is reasonable for this species or if it is due to improper diet.

The quadratic model used to estimate optimum protein requirement (Shearer, 2000) showed that if only results presented in Table 3 are considered we would underestimate the *M. americanum* need of CP on a diet. The calculated weekly CP optimum had an average value of 42.2 (range = 39.4-43.3), and the maximum was registered during the fifth week (Fig. 1). 33% CP diet had less protein than the average value calculated by the inflection points, and even below the minimum optimum CP(%) estimated. The optimum CP value in the last week was 41.1%. Therefore if predictions are only based on the final inflection points (week 10) to determine the optimum CP in the diet as has been done in previous studies (Cortés-Jacinto *et al.*, 2003; Goda, 2008), there may be an underestimated optimum for early stages too. That is why size should be considered while formulating and using diets. So, it is suggested to feed prawns with CP from 39.4% to 43.3% depending on size.

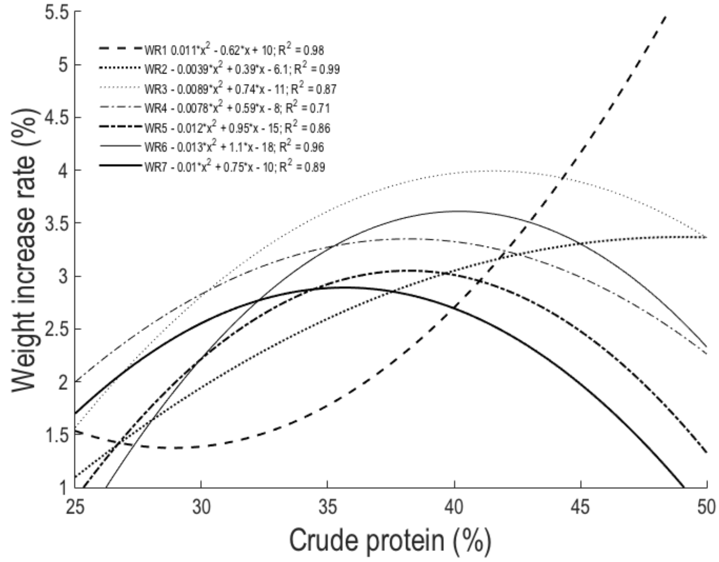

Figure 2. Rate curves of WG% for each weight range (WR) of *Macrobrachium americanum* fed with different amounts of crude protein in their diet. The *x* and *y* values presented in the legend represent the inflection points calculated for every fitted curve.

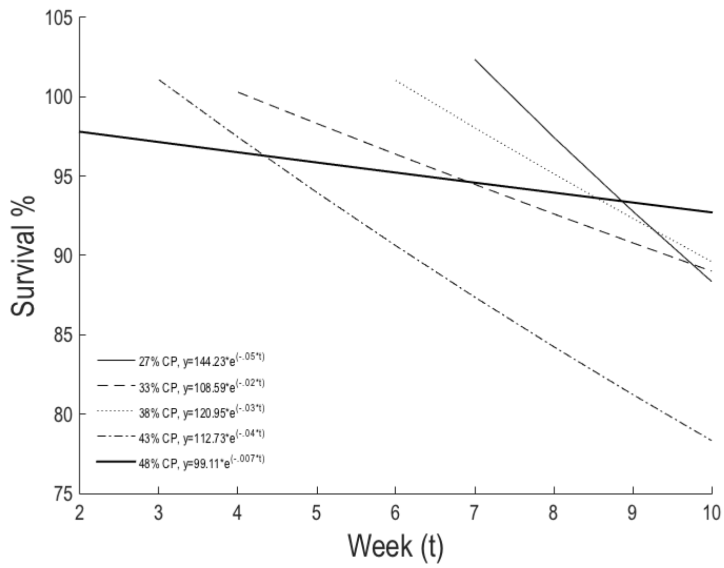

Figure 3. Curves of mortality fitted to survival data for each experimental treatment of *Macrobrachium americanum* fed with different amounts of protein in their diet.

WR3 had the best growth response in general for all diet treatments; it had an optimum calculated rate of 4.6 that would be reached at 37.2% of CP in diet, whereas the WR7 had the lowest estimated rate (2.9%) with 35.7% CP. WG% rates tend to be higher at small body sizes (Ra'anan *et al.*, 1991). WR1 seems to have an optimum CP in the diet of 28.9% but, in fact, the optimum level would be >49%. Protein levels required, in the earliest stages, are supposed to be higher. Because the general trend, for most animals, is that the weight gain rate becomes slower as size increases (Lim & Persyn, 1989), so it is possible to assume that if WR2 had an optimum of 49%, then WR1 would have a CP optimum higher. Maybe the curve fitted is underestimating the optimum CP level due to the nature of the data.

Dietary trends observed in this experiment for *M. americanum* are better in comparison to those reported for *M. rosenbergii* by New (2002) (35% protein for postlarvae and 30% after three months of culture and until harvest). Du & Niu (2002) also reported the best growth rates were obtained between 20% and 30% of protein level in the diet for *M. rosenbergii* juveniles (0.075 ± 0.022 g). They also reported ammonia excretion rate was similar between prawns fed 20 and 30% protein level diets but increased beyond 30%, suggesting that 30% protein is near to optimum CP in the diet for the growth of *M. rosenbergii*. Beyond 30%, some of the ingested protein is wasted as ammonia lowering water quality, whereas prawns fed with 30% protein in their diet may require less energy for basic physiological maintenance. So it seems *M. americanum* is a more like carnivorous-omnivorous species compared with *M. rosenbergii*.

The use of a mortality model in experiments such as this has been justified before as useful for populations with discontinuous or irregular survival along a trial since survival is higher or lower depending on culture stage and age (Miller, 2003). When a species requirement is not determined, stress during culture is another factor that may turn irregular the survival, making difficult to describe it by only defining survival as a percentage. Because of this, mortality slopes varied considerably among treatments (Fig. 3). The highest occurred in 27% CP (-0.05) and is mainly associated with the lowest protein content, which may not comply with the minimum required amount, pushing the prawns into cannibalism. Cannibalism is enhanced when such prawns have low protein amounts in the diet, particularly over soft specimens (García-Guerrero & Apún-Molina, 2008; Méndez-Martínez *et al.*, 2018). In fact, food consumption, growth, food-storing behavior are all altered under a lack of an adequate food scheme (Karplus, 2005). In fact, 48% CP diet had the smallest mortality slope, possibly due to the high amount of protein on a diet. Behavioral issues are also involved. Aggressive or dominant prawns may obtain more amount of food, so they can grow faster, while subordinate prawns may have limited food and, hence, show lower growth rates. Because *M. americanum* is naturally aggressive and territorial, this behavior could be one of the leading causes of cannibalism and dominance, causing low growth rates in captivity and may help to explain the relatively low survival of 27% CP group. If also, food has low quality, this lack of nutrients may cause even slower growth (Kulesh, 2009). We consider that 33% CP is a proper treatment because it showed mortality at the sixth week of the experiment and has a medium slope (Fig. 3). However, although other treatments had similar SR values, overall productivity parameters led to suggest that 33% CP was the best treatment regarding growth and survival. But more studies are required for better understanding of protein digestibility and use by prawns in addition to the proper amount in the diet. For example, it is known circadian, and molt cycles are related, particularly post-molt period generates a need of a gradual accumulation of nutrients for growth and energy production (Amer-Hamsa, 1982). Circadian rhythm in crustaceans varies along the day having an effect on digestive enzymatic activity and therefore in protein digestion (Nolasco-Soria & Vega-Villasante, 1998; Casillas-Hernández *et al.*, 2006).

In conclusion, the optimum protein requirement for growth of *M. americanum* juveniles may vary ranging from 36% to 49% depending mostly on size. If the final inflection points are considered as criteria in an experiment to determine the optimum CP in the diet, this may help to determine when food with lower protein must be given, but still maintaining best results.

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