

*Short Communication*

## Growth and survival of males of *Cryphiops caementarius* (Palaemonidae) with diets supplemented with common salt

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**ABSTRACT.** The aim was to determine the growth and survival of males of the *Cryphiops caementarius* shrimp fed with diets supplemented with common salt. Three experimental diets supplemented with 1, 2 and 3% of common salt and a control diet without common salt were used, with three replicates, respectively. Twelve aquariums (55 L) were used and six individual culture containers (284 cm<sup>2</sup>) installed in two groups of three levels were introduced into each one. In each culture, the container stocked a shrimp, and in total six shrimp per aquarium was randomly stocked. Seventy-two male shrimps (3.87 g in weight) were used. The experiment lasted 90 days. Results indicated that the diet supplemented with 2% of common salt favored the growth in weight of shrimp males, and it was only different ( $P < 0.05$ ) with the diet with 3% common salt that caused slow growth in weight. Diets with common salt did not affect shrimp survival ( $P > 0.05$ ).

**Keywords:** *Cryphiops caementarius*, shrimp, growth, salt in the diet, nutrition, survival.

In the Peruvian coast rivers, there are eight shrimp species of the genus *Macrobrachium*, three of *Palaemon* and one of *Cryphiops* (Méndez, 1981). *Cryphiops caementarius* Molina, 1782, is distributed from the Taymi River in northern Peru to the Maipo River in Chile (Viacava *et al.*, 1978; Zúñiga, 2002), but are commercially extracted from the Ocoña, Majes - Camaná and Tambo rivers in Arequipa, Peru (Yépez & Bandín, 1996; Wasiw & Yépez, 2015), which in 2015 it reached 1042 ton (PRODUCE, 2016).

Crustaceans require mineral supplements in the diet to compensate losses during the shedding (Kanasawa, 1985) and for the basal metabolism and growth (Roy & Davis, 2010). Sodium and chlorine are osmotically active in the hemolymph (Castille & Lawrence, 1981). Sodium is involved in the extra-cellular ion balance, the osmoregulation, the enzymatic regulation and the absorption of carbohydrates, and chlorine in gastric digestion (Guillaume *et al.*, 2004).

In *Penaeus vannamei*, the diet with 0.5% sodium chloride improves osmoregulatory capacity and the growth (Gong *et al.*, 2004) and with 1% of sodium and 1% of potassium growth is promoted (Davis *et al.*, 2005; Liu *et al.*, 2014). On the other hand, with 4% of

sodium chloride, the food conversion factor decreases, the weight increases, the protein content increases, there is more quantity of inosinic acid and sodium in the muscle that improves the quality of the shrimp meat (Zhou *et al.*, 2012). In *Macrobrachium rosenbergii*, the diet with 1.5 to 2.0% of sodium chloride increases the activity of the enzymes in the intestine and hepatopancreas, which together with the digestibility of the nutrients favor growth, decrease the feed conversion factor, raise the rate of protein efficiency and maintains survival (Keshavanath *et al.*, 2011).

In *C. caementarius* the requirement of sodium chloride in the diet has not been investigated. In nutritional studies with the species, food supplies are used to establish the level of protein in the diet for juvenile growth (Ayvar, 1982), the energy balance is determined (Zúñiga & Ramos, 1987), the silage digestibility (Rubio, 2010), the soy lecithin supplementation for growth and survival (Reyes, 2012) and the yeast for hemocyte proliferation (Cornejo *et al.*, 2015). Therefore, the objective was to determine the growth and survival of *C. caementarius* males with diets supplemented with common salt.

*C. caementarius* shrimps were harvested from the Pativilca River (10°09'S, 77°00'W) and were transported individually in 200 mL plastic buckets (the buckets were drilled to allow water flow) which were conditioned into containers (45 L) with water from the same river and continuous aeration. The density was 50 shrimps per container. Land transport lasted 4.5 h and there was no mortality. The shrimps were acclimated for a week in the same transport system and were fed with the control diet. The species of shrimp was verified according to the Méndez' key (1981) and the male sex was verified by the presence of gonopores in the coxopodites of the fifth pair of pereopods.

The cultivation system (Reyes, 2012) consisted of circular containers (19 cm in diameter and 284 cm<sup>2</sup> total area), each one with openings on the sides to allow water flow; in addition, a 0.5" diameter PVC pipe was placed that protruded the water level and through which food pellets were introduced. Each culture container was placed in two groups of three levels that were conditioned inside each one of the 12 aquariums (0.186 m<sup>2</sup> and 55 L). Each aquarium had an air-water-lift type water recirculation system (0.43 L min<sup>-1</sup>) with percolating biological filter (2.5 L), which filter bed was of crushed shell and gravel, in the same proportions (Fig. 1). The biological filters were activated with Nutrafin Cycle.

Seventy-two male shrimp (3.87 ± 0.12 g total weight) were used with complete cephalothorax appendages and no signs of lacerations in the body and appendages, randomly selected from a batch of 100 specimens. In each culture container, a shrimp was randomly stocked and six shrimp per aquarium (32 ind m<sup>-2</sup>) were stocked in total.

The basal diet was prepared according to the formulation of Reyes (2012) and consisted of 30% fish meal, 21% soy meal, 16.7% corn meal, 2% fish oil, 0.5% soybean oil, 0.5 % corn oil, 22% rice powder, 3% sugar cane molasses, 2% zeolite, 1% soy lecithin, 1% common salt and 0.3% vitamins and minerals. The proximal analysis of the basal diet was performed in the Colecibi S.A.C. Laboratory, using the method UNE-EN ISO 5983-2 Part 2 Dec. 2006, where the factor 6.25 was used for the protein content; besides, moisture, lipids, ash, and fiber were also determined (AOAC, 1990). Carbohydrates were determined by the formula: 100 - (%protein + % fat + % fiber + % ash). For the analysis of the basal diet, common salt was excluded and 30.26% crude protein was obtained, 14.44% total fat, 2.92% crude fiber, 13.08% moisture and 10.75% ashes. Three experimental diets supplemented with 1, 2 and 3% of common salt (99.1% sodium chloride) and a control diet without common salt, each one with three replicates, were used. The daily ration (09:00 and 18:00

h) was 5% of the wet biomass that was readjusted monthly.

Monthly samplings of the stocking population were performed for 90 days. The survival was determined by direct observation. The total weight was determined on the ADAM AQT600 digital scale (±0.1 g). With the data, the growth parameters were determined (El-Sherif & Ali, 2009) and the production was estimated (Reyes, 2012).

$$\text{Survival (\%)} = \frac{(N_i \times 100)}{N_o}$$

$$\text{Absolute Growth} = X_2 - X_1$$

$$\text{Percentage Gain (\%)} = \frac{[(X_2 - X_1) \times 100]}{X_1}$$

$$\text{Absolute Growth Rate} = \frac{(X_2 - X_1)}{t_2 - t_1}$$

$$\text{SGR (\% day}^{-1}\text{)} = \left[ \frac{(\ln X_2 - \ln X_1)}{t_2 - t_1} \right] \times 100$$

$$\text{Production (kg m}^{-2}\text{)} = \frac{(X_2 \times D_2)}{1000}$$

where X1 and X2 were the wet weight (g), initial and final; t1 and t2 the duration in days; ln X1 and ln X2 the natural logarithm of the initial and final weight; N<sub>o</sub> and N<sub>i</sub> is the initial and final number of the sown shrimps, respectively; SGR: Specific Growth Rate D2 is the final density.

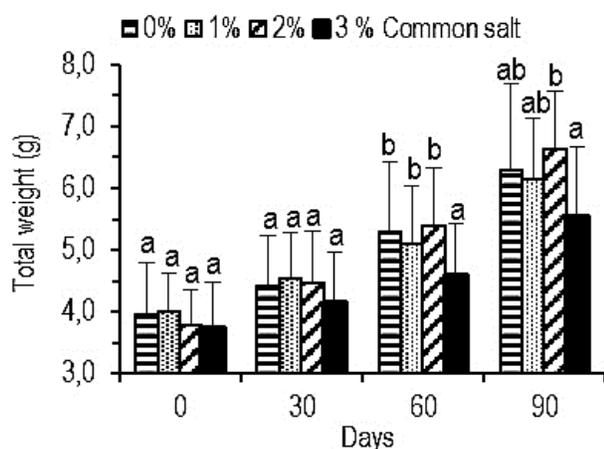
The water quality was monitored every week and dissolved oxygen and the water temperature was determined with Hatch LDO digital oximeter (±0.01 mg L<sup>-1</sup>; ±0.01°C), pH with digital pH meter Oakton 110 (±0.01 units), total ammonia and Nitrites with the Nutrafin Colorimetric Test (±0.1 mg L<sup>-1</sup>).

A completely randomized statistical design was used. The normality of the data was determined by the Kolmogorov-Smirnov test. The differences among the averages of the treatments were determined by one-way analysis of variance and with Duncan's multiple range test, in all cases with one significance level of 5%. The statistical processing was performed with SPSS software version 23 for Windows.

The *C. caementarius* shrimp accepted all diets supplemented with common salt and these did not alter their dietary behavior. The weight growth of shrimps from all treatments was similar ( $P > 0.05$ ) during the first 30 days of culture. At 60 days, the weight of control shrimps and those fed with 1 and 2% of salt in the diet were higher ( $P < 0.05$ ) than those consuming 3% salt diet (Fig. 2). However, at 90 days of culture,



**Figure 1.** *C. caementarius* culture system in individual containers installed in aquaria with water recirculation and percolator biological filter.



**Figure 2.** Growth in weight of *C. caementarius* males fed with diets supplemented with common salt for 90 days. Equal letters on the bars in the same growing period indicate that there is no significant difference ( $P > 0.05$ ).

the growth in weight of the shrimps fed with 2% of salt in the diet was higher ( $P < 0.05$ ) than those ones with 3% of salt but not with the 1% fed with salt and not with the Control group (Table 1). A shrimp fed 3% salt in the diet died at 55 days of culture and showed no signs of a dying cause. The estimated production of shrimp fed with 2% salt in the diet ( $0.214 \text{ kg m}^{-2}$ ) was only higher ( $P < 0.05$ ) than those with 3% salt ( $0.169 \text{ kg m}^{-2}$ ) (Table 1).

The water quality parameters were similar ( $P > 0.05$ ), except for the total ammonia of the control that was higher ( $0.10 \text{ mg L}^{-1}$ ) and significant ( $P < 0.05$ ) only with those where the diet was used with 1% of salt ( $0.06 \text{ mg L}^{-1}$ ) (Table 2).

The results show for the first time, in adult males of *C. caementarius*, that the 90 day ingestion of the diet with 2% of salt favored ( $P < 0.05$ ) the growth in weight, in relation to that of 3% of salt. This suggests that supplementation with 2% salt in the diet was sufficient

to contribute to the weight growth, which is in agreement with Keshavanath *et al.* (2011) who determined in *M. rosenbergii* that with 1.5 to 2.0% of sodium chloride in the diet improves the growth and stimulates the enzymatic activity in the intestine and hepatopancreas.

Although the enzymatic activity varies according to the species, sex, shrimp size, and environmental and nutritional conditions, as it is suggested for other freshwater crustaceans (López-López *et al.*, 2005; Coccia *et al.*, 2011), the adults of *C. caementarius* would have less enzymatic activity. In *C. quadricarinatus* the proteases decrease with the animal age (Figueiredo & Anderson, 2003). However, the growth results of *C. caementarius* suggest that sodium chloride ions contributed to the digestibility and assimilation of nutrients. This is because the chlorine intervenes in the gastric digestion and sodium in the carbohydrate absorption (Guillaume *et al.*, 2004), although the weight was not different ( $P > 0.05$ ) with the control and with 1% of salt, but could be evidenced with longer culture time. In *M. rosenbergii*, the growth in weight is greater with the diet with 2% of sodium chloride and during 120 days of culture (Keshavanath *et al.*, 2011).

In crustaceans, the salt intake from the gut to the hemolymph is explained by various mechanisms such as the simultaneous co-transport of ions and the co-transport of sodium with amino acids and glucose in *M. rosenbergii* (Ahear & Tornquist, 1977; Ahear & Maginniss, 1977) and the exchange of sodium for hydrogen in *Procambarus clarkii* (Zetino *et al.*, 2001), among others. According to these studies, it is likely that sodium chloride ions from the ingested diet entered the hemolymph of *C. caementarius* and contributed to the osmotic balance, which probably decreased energy expenditure, whose surplus would have been channeled for the animal growth, as suggested in other crustaceans (Keshavanath *et al.*, 2011).

**Table 1.** Growth parameters by weight (mean average  $\pm$  standard deviation) of *C. caementarius* males fed with diets supplemented with common salt for 90 days. TW: total weight, AG: absolute growth, PG: percentual gain, AGR: absolute growth rate, SGR: specific growth rate. Values in the same row with different superscripts are significantly different ( $P < 0.05$ ).

Parameter	Common salt in the diet			
	0%	1%	2%	3%
TW initial (g)	3.95 $\pm$ 0.84 <sup>a</sup>	3.99 $\pm$ 0.63 <sup>a</sup>	3.78 $\pm$ 0.59 <sup>a</sup>	3.74 $\pm$ 0.73 <sup>a</sup>
TW final (g)	6.30 $\pm$ 1.40 <sup>ab</sup>	6.15 $\pm$ 0.98 <sup>ab</sup>	6.63 $\pm$ 0.95 <sup>b</sup>	5.54 $\pm$ 1.13 <sup>a</sup>
AG (g)	2.35 $\pm$ 0.27 <sup>ab</sup>	2.16 $\pm$ 0.28 <sup>ab</sup>	2.85 $\pm$ 0.27 <sup>b</sup>	1.80 $\pm$ 0.30 <sup>a</sup>
PG (%)	37.30 $\pm$ 4.17 <sup>ab</sup>	35.05 $\pm$ 4.20 <sup>ab</sup>	43.00 $\pm$ 2.39 <sup>b</sup>	32.43 $\pm$ 5.56 <sup>a</sup>
AGR (g day <sup>-1</sup> )	0.026 $\pm$ 0.009 <sup>ab</sup>	0.024 $\pm$ 0.009 <sup>ab</sup>	0.032 $\pm$ 0.009 <sup>b</sup>	0.020 $\pm$ 0.010 <sup>a</sup>
SGR (% TW day <sup>-1</sup> )	0.519 $\pm$ 0.120 <sup>ab</sup>	0.479 $\pm$ 0.120 <sup>ab</sup>	0.625 $\pm$ 0.066 <sup>b</sup>	0.435 $\pm$ 0.160 <sup>a</sup>
Production (kg m <sup>-2</sup> )	0.203 $\pm$ 0.022 <sup>ab</sup>	0.198 $\pm$ 0.005 <sup>ab</sup>	0.214 $\pm$ 0.012 <sup>b</sup>	0.169 $\pm$ 0.025 <sup>a</sup>

**Table 2.** Physical and chemical parameters of water (mean average  $\pm$  standard deviation) of *C. caementarius* males culture fed with diets supplemented with common salt for 90 days. Values in the same column with different superscripts are significantly different ( $P < 0.05$ ).

Common salt in diet	Temperature (°C)	O <sub>2</sub> (mg L <sup>-1</sup> )	pH	Nitrite (mg L <sup>-1</sup> )	Total ammonia (mg L <sup>-1</sup> )
0%	19.05 $\pm$ 0.46 <sup>a</sup>	7.17 $\pm$ 0.40 <sup>a</sup>	7.49 $\pm$ 0.08 <sup>a</sup>	0.19 $\pm$ 0.21 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>
1%	19.08 $\pm$ 0.39 <sup>a</sup>	7.26 $\pm$ 0.62 <sup>a</sup>	7.51 $\pm$ 0.11 <sup>a</sup>	0.13 $\pm$ 0.10 <sup>a</sup>	0.06 $\pm$ 0.03 <sup>b</sup>
2%	19.14 $\pm$ 0.47 <sup>a</sup>	7.30 $\pm$ 0.44 <sup>a</sup>	7.48 $\pm$ 0.13 <sup>a</sup>	0.18 $\pm$ 0.21 <sup>a</sup>	0.08 $\pm$ 0.02 <sup>ab</sup>
3%	19.05 $\pm$ 0.44 <sup>a</sup>	7.22 $\pm$ 0.58 <sup>a</sup>	7.49 $\pm$ 0.22 <sup>a</sup>	0.14 $\pm$ 0.06 <sup>a</sup>	0.07 $\pm$ 0.03 <sup>ab</sup>

In the case of the diet supplemented with 3% salt that caused slow growth in weight ( $P < 0.05$ ) of *C. caementarius*, from 60 days of cultivation, suggests that this concentration of salt would interfere with the digestive process or with the assimilation of nutrients. In fish, high concentrations of sodium chloride (12%) in the diet interfere with the balance of essential components that make up the diet and slow down the growth (Arockiaraj & Appelbaum, 2010).

On the other hand, the death of a shrimp fed 3% of salt in the diet, which occurred at 55 days, was not related to salt concentration, since the dead shrimp showed no signs of a cause of death in a cultivation system where interaction and cannibalism were impossible. In addition, the temperature, pH, and oxygen of the culture water were similar to those recorded for the natural environment where they are considered favorable for *C. caementarius* (Yépez & Bandín, 1996; Wasiw & Yépez, 2015). Likewise, the nitrites and the total ammonia were similar to normal laboratory conditions where experiments were performed with the same culture system (Reyes, 2012; Graciano & Vásquez, 2014; Cornejo *et al.*, 2015). Consequently, it is suggested that shrimp death may be due to poor animal handling during sampling, as it has been reported in the same species (Reyes, 2012).

Therefore, it is preferable to use a diet without salt (0%) than with excess salt (3%) because of this excess of salt-affected ( $P < 0.05$ ) the growth in weight of the shrimp. However, according to the results and as *C. caementarius* is marketed whole and sold by weight, it is advisable to supplement the diet with 2% of salt. Also due to a greater gain in weight (43%), although it was not significant with the diets that had low salt concentrations but was able to increase production to 0.214 kg m<sup>-2</sup>, which was close to that reported for the species in similar growing conditions (0.313 kg m<sup>-2</sup>, Reyes, 2012). This difference in production would probably be due to the small size of the shrimp used in the research. It is necessary to continue nutritional studies where salt combinations are included to optimize the growth of the species in the different stages of ontogenetic development.

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