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# Advances in Domestication and Culture Techniques for Crayfish *Procambarus acanthophorus*

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## 1. Introduction

### 1.1 Ecological importance

The crayfish are a group of crustaceans that habit in different environments in the world, both in lotic systems as lentic, in addition to caverns, which makes them cosmopolitan organisms with a wide range of tolerance to environmental conditions. Over 600 crayfish species are known to exist in the worldwide, with at least 100 species in Australia and about 300 in the Americas (Holdich, 1993), mostly (85%) in North and Central America (Rojas, 1998). Species in Mexico include one in the *Orconectes* genus, 10 in the *Cambarellus* genus and 44 in the *Procambarus* genus, the latter also distributed in Belize, Honduras and the United States (Villalobos, 1948; Hobbs, 1984; Rojas, 1998; López, 2006). The genus *Procambarus* habits in temporary water bodies, during the dry season can be seen in small holes in the soil, similar to the anteaters, which conduct to tunnels and chambers with sufficient moisture for the crayfish to survive to the drying (López, 2008). The crayfish have been adapted in various ways, according to environmental conditions that occur in the places they want to colonize. The first adaptation is their ability to spend a lot of time, even months, faced with the lack of water and breathe atmospheric oxygen (Huner, 1995) in some cave environments it has been recorded that these organisms exhibit a diminution in their effective breathing rate as a response to the decrease in the concentration of oxygen, and undersupply of food (Mejía, 2010). Despite their abundance, less than a dozen crayfish species are cultivated worldwide and only two species constitute sizable commercial fisheries (Huner, 1994). Crayfish have a high potential for use in aquaculture systems because they are at the bottom of the trophic chain, feeding largely on carrion and detritus, for that they are therefore considered fundamental for maintaining ecological balance in

natural ecosystems (Rojas, 1998), and is possible their maintained in control conditions. The global diversity of crayfish allows to establish a productive activity associated with diverse environments, even in reduced environments with eutrophication, sulfate-reducing bacteria and that there is a proliferation of algae (Sánchez et al., 2009).

## 1.2 Aquaculture importance

Crayfish like other decapods crustaceans, have some biological characteristics that make them potentially important species for aquaculture, among which include: adaptation to conditions of captivity and handling; accept artificial feeds of different origins (shrimp, fish aquatic plants, vegetables), can even be fed diets with vegetable protein (75%); have a relatively short life cycle (two years or less). First studies indicates that these organisms can breed in captivity at early age (about four months), and first spawning had high survival rates (> 75%), with reproduction all year, and some females had more than one spawning per year.

The physiological characteristics of crayfish allow them to adapt to extreme climatic variations, diversifying their potential habitats, ensuring reproduction and contributing to progeny survival under adverse conditions. This occurs under natural or artificial conditions, making them promising organisms for use in aquaculture systems (Gutiérrez-Yurrita, 1994; Rodríguez-Almaraz & Mendoza-Alfaro, 1999). Of the crayfish species which can be cultivated in subtropical environments, *Procambarus acanthophorus* stands out for its biological attributes, such as high number of progeny per spawn, resistance to a wide range of environmental and water quality conditions, and successful performance in captivity (Arrignon, 1985; Cervantes, 2008; Cervantes-Santiago et al., 2010a).

## 1.3 Advances in laboratory research for aquaculture facilities

### 1.3.1 Environmental requirements of the species

Advances in laboratory research indicate that crayfish *P. acanthophorus* has a high potential for being used in aquaculture, for that reason different trials were done to determinate the best biotechnology for semi intensive and intensive culture conditions in monoculture and polyculture facilities.

Studies under laboratory conditions had showed that crayfish *P. acanthophorus* (Villalobos, 1948; 1993), can be quickly adapted to conditions of captivity, despite coming from natural environments. In the study the organisms were maintained at an average temperature of  $26 \pm 2^{\circ}\text{C}$  and photoperiods between 12 and 14 h light: dark, oxygen concentrations from 0.5 to 5  $\text{mgL}^{-1}$ , indicating that they may like other crustaceans endure low contents  $\text{O}_2$ . The pH tolerance of the species ranges from 6 to 9, the ammonium concentration is about 0.5  $\text{mL}^{-1}$ , and hardness greater than 200  $\text{mL}^{-1}$  as calcium carbonates.

### 1.3.2 Feeding

Even though it is known that crayfish accept balanced food for aquaculture species as shrimp. Laboratory experiment with 20 formulated diets containing different protein (200, 250, 300, 350 and 400  $\text{gkg}^{-1}$ ) and lipid (60, 80, 100 and 120  $\text{gkg}^{-1}$ ) levels (Table 1) on growth and survival in juvenile crayfish (*P. acanthophorus*) during 12-week nutritional trial, indicate

Ingredients (g kg <sup>-1</sup> )	Diets																			
	200/60	200/80	200/100	200/120	250/60	250/80	250/100	200/120	300/60	300/80	300/100	300/120	350/60	350/80	350/100	350/120	400/60	400/80	400/100	400/120
Fish meal <sup>1</sup>	77.1	77.1	77.1	77.1	96.3	96.3	96.3	96.3	115.6	115.6	115.6	115.6	134.8	134.8	134.8	134.8	154.1	154.1	154.1	154.1
Soya meal <sup>2</sup>	327.9	327.9	327.9	327.9	409.8	409.8	409.8	409.8	491.8	491.8	491.8	491.8	573.8	573.8	573.8	573.8	667	681	695.1	702.6
Wheat bran <sup>3</sup>	58.1	58.1	58.1	58.1	72.6	72.6	72.6	72.6	87.2	87.2	87.2	87.2	101.7	101.7	101.7	101.7	88.3	53.5	18.6	0.0
Fish oil <sup>4</sup>	22.3	32.3	42.3	52.3	20.4	30.4	40.4	50.4	18.4	28.4	38.4	48.4	16.5	26.5	36.5	46.5	14.6	24.6	34.6	44.6
Corn oil <sup>5</sup>	20.8	30.8	40.8	50.8	18.6	28.6	38.6	48.6	16.3	26.3	36.3	46.3	14	24	34	44	12.4	23.3	34.2	44.7
Corn starch	431.3	411.3	391.3	371.3	319.8	299.8	279.8	259.8	208.3	188.3	168.3	148.3	96.7	76.7	56.7	36.7	1.1	1.0	0.9	0.0
Vitamin and mineral premix <sup>6</sup>	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Carboxy-methylcellulose	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Soya lecithin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Pigment	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin C <sup>7</sup>	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Proximate composition (g·kg <sup>-1</sup> dry base)																				
Moisture	63.1	54.9	63.6	59.4	66.2	61.3	59.4	52.3	92.5	82.4	82.9	79.1	62.7	57.4	75.5	53.7	66.5	75.2	67.4	56.1
Crude protein	217.6	219.6	220.0	227.7	276.9	271.9	271.2	268.2	317.9	316.9	315.4	319.4	377.2	373.0	376.5	381.5	434.4	424.5	425.9	420.9
Ether extract	68.3	95	116.9	134.8	67.5	91.1	114.7	136	62.6	81.4	98.4	112.8	63.4	84.1	110.7	135.1	62.3	86.4	104.9	126.1
Ash	42.3	46.0	44.8	45.9	56.4	55.3	54.8	54.6	65.5	63.9	64.5	64.6	74.1	77.3	74.5	77.3	83.5	83.5	82.1	99.4
Crude fiber	10.7	11.9	11.0	12.3	10.8	16.1	20.4	20.9	25.8	22.9	21.0	20.1	29.3	31.6	34.1	40.8	34.8	36.5	30.4	28.2
NFE	598.0	572.6	543.7	519.9	522.2	504.3	479.5	468.0	435.7	432.5	417.8	404.0	393.3	376.6	328.7	311.6	318.5	293.9	289.3	269.3
Energy (MJ/100g <sup>-1</sup> )	18.92	18.83	20.01	17.24	18.42	18.67	18.75	17.24	18.29	18.25	18.46	19.00	18.54	18.92	19.17	18.83	18.08	18.04	17.58	18.46

Table 1. Formulation and proximate composition of experimental diets containing different protein/lipid ratios fed *P. acanthophorus*.

that the protein requirement for young *P. acanthophorus* is in a range between 210 and 280 gkg<sup>-1</sup>, without observing a specific requirement of lipids, the results also suggest that in culture, it is possible to use foods with a maximum of 279 gkg<sup>-1</sup> of protein and 60 gkg<sup>-1</sup> lipid for better growth in crayfish, which can use up to 75% protein of vegetal source and only 25% from animal source, with growth performance and uptake efficient (Cervantes, 2006 ) (Table 1).

The study results indicate that nutritional diets can be used with protein content between 211 and 232 gkg<sup>-1</sup> to feed growing crayfish in order to minimize feed costs, indicating that these organisms consume protein from vegetal source and assimilated efficiently (1.09:1 FCR) regardless of sex. This was verified by the assessment of carcass composition of crayfish fed with 20 experimental diets and by sex, where females were found to store more lipids (%) and body caloric energy (MJ/100 g) without significant differences in relation with the values reported for males. Other important information obtained during the nutritional study was the detection of ovigerous females in treatments 200/120, 250/60 and 400/120, but could not be attributed to the generation of egg protein or lipids tested, which suggests that in general all the diets allowed the bodies to cover their energy requirement for basic functions, but also could reproduce and promote sexual maturation.

The crayfish survival ranged from 66% to 86%, without differences between treatments. Because of the lowest survival recorded in the juvenile fed with the 250/12 gkg<sup>-1</sup> diet, although this treatment yielded the most efficient parameters (WG, SGR and DWG), a correlation analysis was performed between weight gain and survival to determine a possible influence of mortality on growth. The weight gain survival ratio was not significant ( $r^2 > 0.037$ ;  $P > 0.1365$ ), indicating that crayfish growth was only affected for the experimental diets.

### 1.3.3 Life cycle and reproduction

A vital aspect to consider when determining an organism culture potential, is its reproductive capacity under controlled conditions, which in turn depends on its ability to adapt to the culture system, feed and water quality. Factors reported to significantly affect crayfish reproductive capacity include water temperature, photoperiod, and sex ratio (Yeh & Rouse, 1995; Carmona-Osalde et al., 2002; 2004a, 2004b). The results in laboratory conditions demonstrate that the crayfish *P. acanthophorus* is a candidate for aquaculture production in a closed cycle since it effectively reproduces in captivity. During the time that organisms captured from the wild remained in captivity, it was observed mating and reproduction, from which ovigerous females were obtained, indicating that breeding in captivity could be obtained. Under the study conditions, *P. acanthophorus* exhibited the peak of the reproductive activity during November and December, when average water temperature was 25 °C. The lowest reproductive activity occurred in February and March, when wide variations in water temperature (25 ± 5°C) may have affected organism metabolism and consequently their reproductive cycle (Figure 1).

In a similar researches, Rodríguez-Serna et al. (2000) reported that reproduction in *P. llamas* occurs year around, although, in contrast to *P. acanthophorus*, this species has three spawning peaks between November and June. Its maximum activity is in May and June and its minimum in August and October, when temperatures above 26.7 °C negatively affect its reproductive efficiency. Temperature is clearly a limiting factor for reproduction in

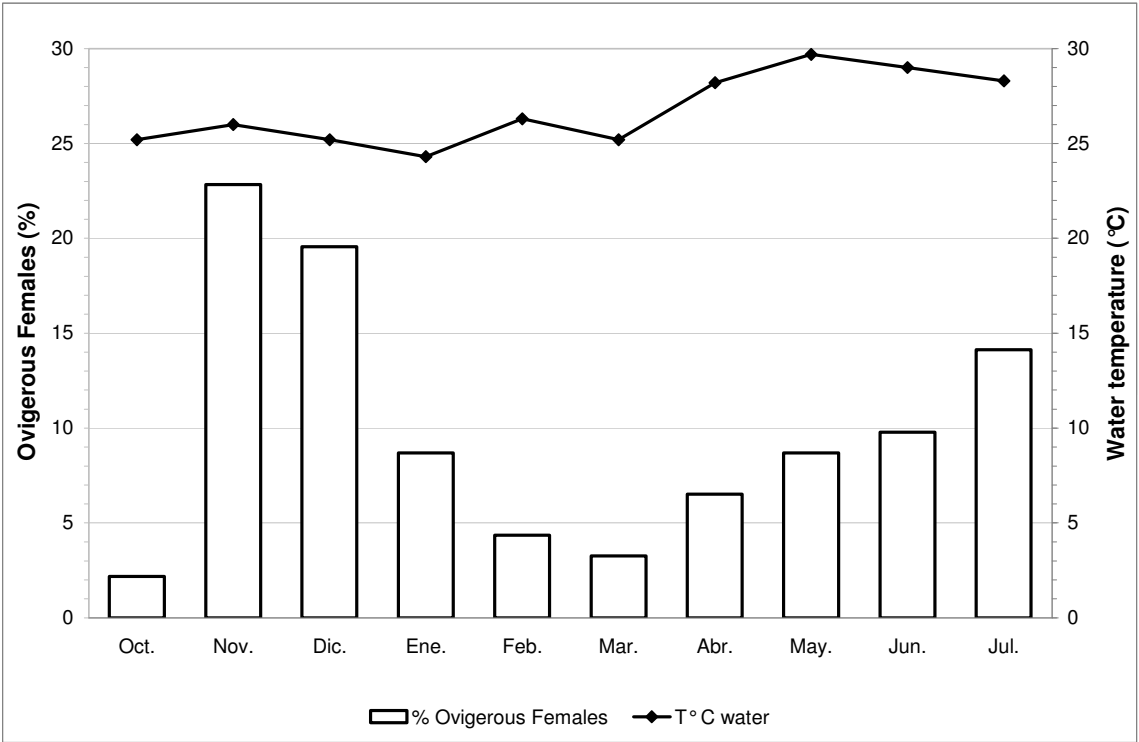


Fig. 1. Proportion of ovigerous females versus water temperature (°C) during a ten-month period.

*P. llamas*i, with optimum spawning at 21 °C, although breeder length and degree of female sexual maturity can also affect spawning (Carmona-Osalde et al., 2004a).

In the study, a total of 92 ovigerous females were recorded from the 192 placed in the reproduction tanks during the study. ANOVA showed no significant variation for the number of ovigerous females at the three sex ratios used in the treatments, with 30 ovigerous females at 1:1, 40 at 1:3 and 22 at 1:5 male: female ratios, respectively (Figure 2).

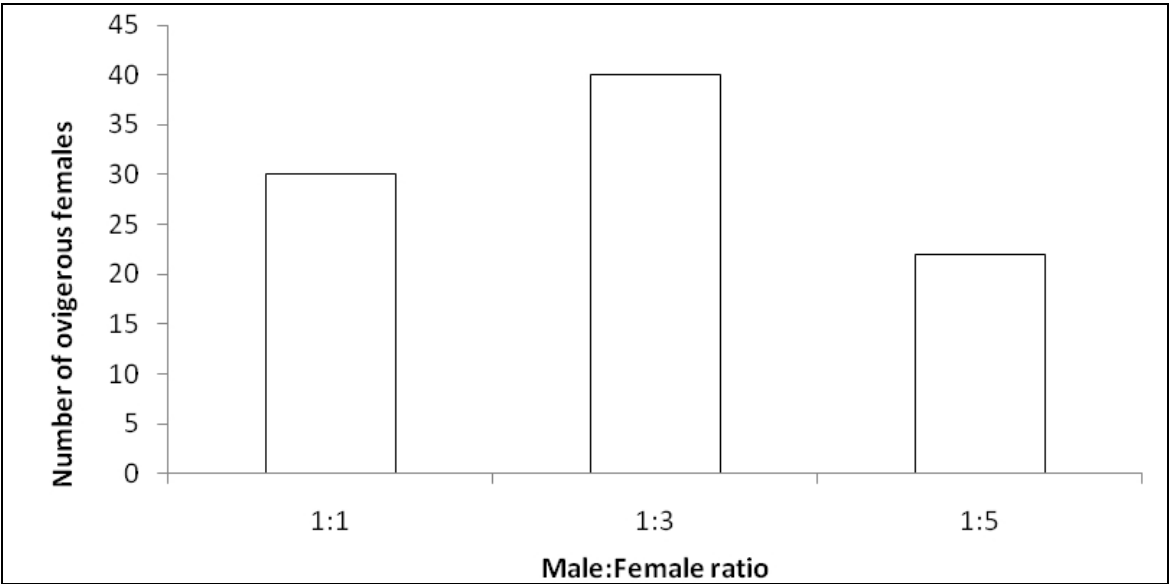


Fig. 2. Number of ovigerous females per sex ratio treatment



The results indicate that a higher quantity of gravid females can be obtained if sex ratio of 1:3 (male: female) is using during reproduction, which indicates that the ratio male: female is a variable that can affect the mass production juveniles. This is higher than the 1M: 1F ratio reported for optimum reproductive efficiency in penaeid shrimp (Martínez, 1999), but lower than the 1M: 5F ratio reported for *Cherax quadricarinatus* (Yeh & Rouse, 1995). In the crayfish *Astacus astacus*, a 1M: 3F ratio is inefficient since all females do not reproduce at this ratio, for that reason a 1M: 2F ratio is recommended for best results (Taugbol & Skurdal, 1990). In *P. llamas*, higher densities resulted in higher mating and ovigerous female rates during reproduction, meaning density significantly influenced female sexual maturation and possibly also male maturation (Carmona-Osalde, et al., 2004a).

Other interesting result is that mating was first observed when females reached an average length of 30 mm, although the first viable spawns did not occur until females reached 37 mm total length. This is therefore considered the female length at first sexual maturity for *P. acanthophorus*. This is similar to *P. llamas* females, which exhibit initial reproductive behavior at 30 mm but have viable spawns only between 40 and 60 mm length. This indicates that crayfish mature and reproduce at an early age, an advantage for production under controlled conditions.

Aspects of reproductive biology such as fertility vary by species, ranging from as little as five eggs in *Astacus pachypus* up to 960 in *Cherax destructor* (Lee & Wickins, 1992). Egg counts for *P. acanthophorus* females in the present study ranged from 77 to 467 per female, with an average of 240.9. The ranges are similar with reported average fertility ranges for other crayfish species: 100-700 eggs in *P. clarkii* (Lee & Wickins, 1992; McClain & Romaine, 2007); 200-700 eggs in *P. llamas* (Rodríguez-Serna et al., 2000); 300-400 eggs in *P. zonangulus* (Reynolds, 2002); and 323 eggs in *P. leniusculus* (Celada et al., 2005).

Egg count per female in crayfish depends on organism age and length. For instance, egg production in *A. leptodactylus* varies according to female length, with organisms measuring 47 to 76 mm producing between 200 and 400 eggs (average = 305.9), and 72 mm long females producing a average maximum of 588 eggs (Köksal, 1988; Mustafa et al., 2004). In the present study, total female length in *P. acanthophorus* had a linear, positive and significant ( $p \leq 0.01$ ) relationship ( $r^2 = 0.654$ ) with egg count. This is similar to the positive relationship between cephalothorax length and egg count reported for *Austropotamobius pallipes* where females with a 25 mm minimum carapace length produced a maximum of 80 eggs (Brewis & Bowler, 1985). The number of eggs per spawn can also depend on egg size. The crab *Cambaroides japonicus* produces only 22 to 75 eggs per spawn but these are 2.13 to 2.50 mm in diameter; this is significantly larger than mean egg size in other crustaceans and may contribute to this species high survival rate (Nakata & Goshima, 2004).

Independent of egg count, not all eggs hatch. This can be caused by biotic and/or abiotic factors that lead to losses during embryo growth. Temperature was the main factor causing egg loss in the present study since at higher temperatures fungi began to grow on the egg masses, affecting water quality in the hatchery units. Rodríguez-Serna et al. (2000) reported a similar incident in *P. llamas* which seriously affected egg survival. Water quality and stability are clearly key elements during crayfish incubation since this is apparently a phase when pathogenic microorganisms attack eggs. Maximum egg viability in the present study was 97.4% in 56 mm long females. Embryo survival varied according to female length but did not exhibit a clear pattern; it was highest (42%) in 56-60 mm females and almost absent in 66-70 mm females. This decrease may be the result of female age since egg quality, and

therefore viability, generally decreases as age increases, although this cannot be emphatically stated in the present case because the breeders were collected from the wild and their ages were therefore not exactly known. Two suggestions arise from the above results for commercial production of *P. acanthophorus*. First, breeders should be between 41 and 60 mm in length to ensure the highest possible egg and viable progeny counts. Second, adequate female nutritional condition and genetic quality need to be ensured since these are expressed in progeny quality and survival, perhaps by maintaining well-fed breeder stocks and employing constant selection to improve genetic quality.

1.3.4 Physicochemical parameters

Water chemical and quality parameters during the crayfish reproduction trial were within tolerance ranges for organisms of the same sex (Malone & Burden, 1988; McClain & Romaine, 2007; Cervantes-Santiago et al., 2010b): temperature, 23.8±2.2 °C; dissolved oxygen, 5.7±0.18 mg L<sup>-1</sup>; total hardness, 110 mg L<sup>-1</sup>CaCO<sub>3</sub>; pH, 8.67±0.13; ammonium, 0.18±0.10 mg L<sup>-1</sup>; N-nitrite, 0.25±0.20 mg L<sup>-1</sup>; and N-nitrate, 32.5±20.6 mg L<sup>-1</sup>.

1.3.5 Fertility

The ratio between total female length and egg counts ( $r^2=0.6541$ ) was positive, linear and significant ( $p\leq0.01$ ), defined by the equation  $y= 8.4126X - 216.4313$ . Average ovigerous female length was 54.4 mm (max = 71 mm; min = 37 mm) and average egg count per female was 240.9 (±S.D. 93.08) (max = 467; min = 77) (Figure 3).

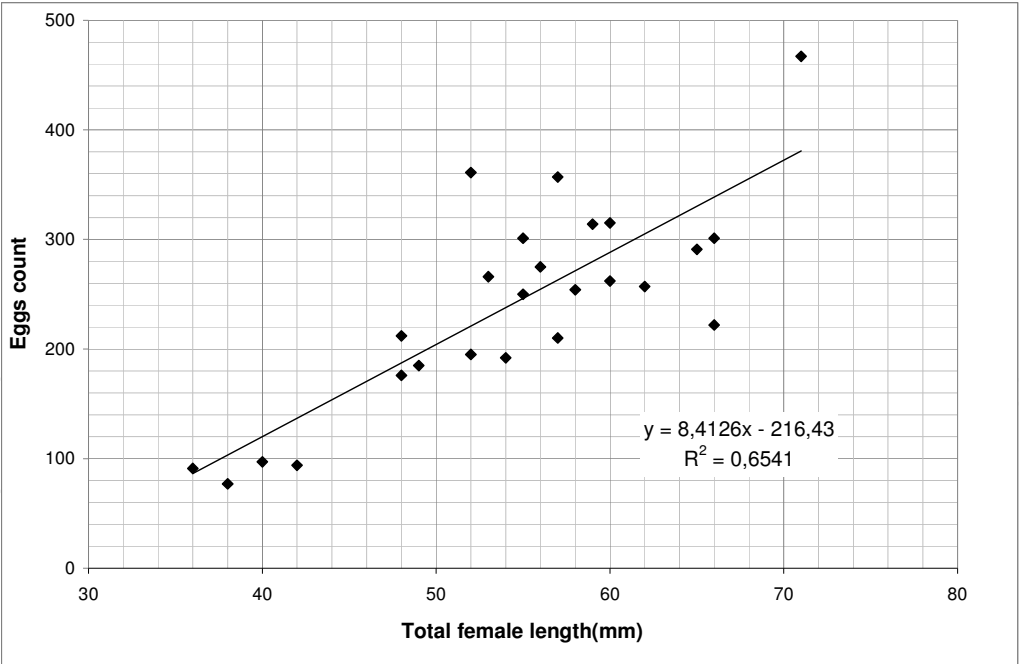


Fig. 3. Ratio of egg count to total female length in *Procambarus acanthophorus* during study.

1.3.6 Egg viability

Average egg viability was 29.1% (± S.D. 31.7; n=66), with a maximum of 97.4% and a minimum of zero. Overall, females between 41 and 60 mm had the highest egg viability



(97.4%). Those within the 46-50 mm size had an average viability greater than 40%, while those in the 66-70 mm had the lowest (2.9%) (Figure 4).

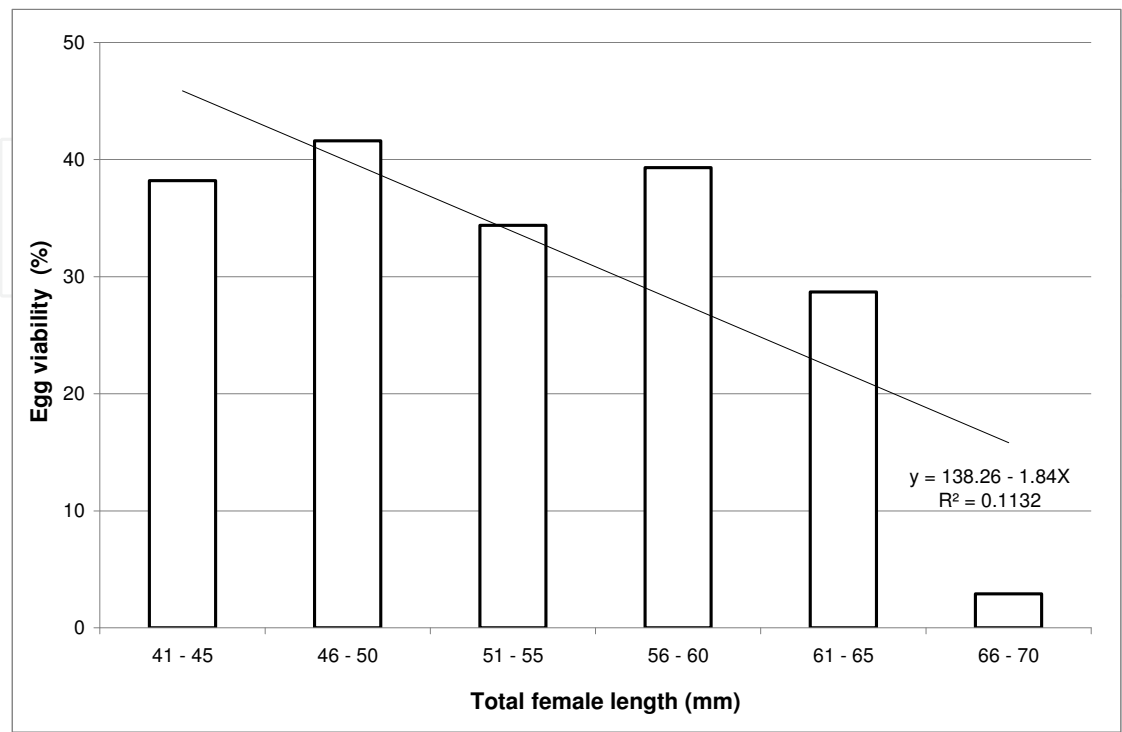


Fig. 4. Ratio of egg viability to total female length in *Procambarus acanthophorus* during study.

Water temperature probably affected egg viability since when it surpassed 30 °C, the eggs detached from the female’s abdomen and were soon infected by fungi.

1.3.7 Female length at first sexual maturity

The present reproductive biology results for the crayfish *Procambarus acanthophorus* show it to be apt for use in aquaculture systems. Based on specimens grown in captivity, it is known that it reproduces year around and females are sexually mature at 37 mm length (13 weeks) (Cervantes, 2008). This allows for constant reproduction and implementation of a continuous breeder replacement program to ensure high quality and genetically variable spawns. Under the study conditions, spawn viability was variable (29.1- 97.4%), although this could probably be kept above 44% by controlling environmental parameters (e.g. keeping water temperature below 30 °C) and thus providing a regular supply of juveniles for culture. In addition, the species did not exhibit aggressiveness or territoriality during the study. These results, in conjunction with previous studies indicating these species preference for feeds containing vegetable protein sources (Cervantes et al., 2007), confirm that the crayfish *P. acanthophorus* is a candidate for culture under controlled conditions, be it as a preservation strategy for commercial purposes.

1.3.8 Embryology development

Incubation of crayfish *P. acanthophorus* eggs has a duration period of three to four weeks, depending on temperature and embryonic development inside the egg. At the time of

hatching, the larvae present physical characteristics and eating behavior similar to an adult (Cervantes, 2006; 2008). A detailed description of embryonic development until juvenile stage, suggests that the development lasts 21 to 27 days on average, where it can identify nine embryonic stages, four post-embryonic and one juvenile. Also was observed that embryonic development, presents 11 color changes of the eggs, which are however asynchronous the same ovigerous mass, so it is considered that the coloring of eggs during embryonic development is not a clear indicator the stage of development.

Under the laboratory conditions, fourteen embryonic development stages were identified for *P. acanthophorus* using the structure descriptions and nomenclature of Anderson (1982). The females produced fertile eggs which exhibited nine embryonic stages, with an average total elapsed time of  $15 \pm 3$  days. After embryonic stages, crayfish had four post-embryonic stages and a final juvenile stage, which lasted an average of 10 days. Total elapsed time of development from fertilization through juvenile stage was on average  $25 \pm 3$  days. Egg diameter and later embryo and juvenile length increased constantly until reaching a final length 600 times larger than initial diameter of a recently fertilized egg) (Figures 5 to 18).

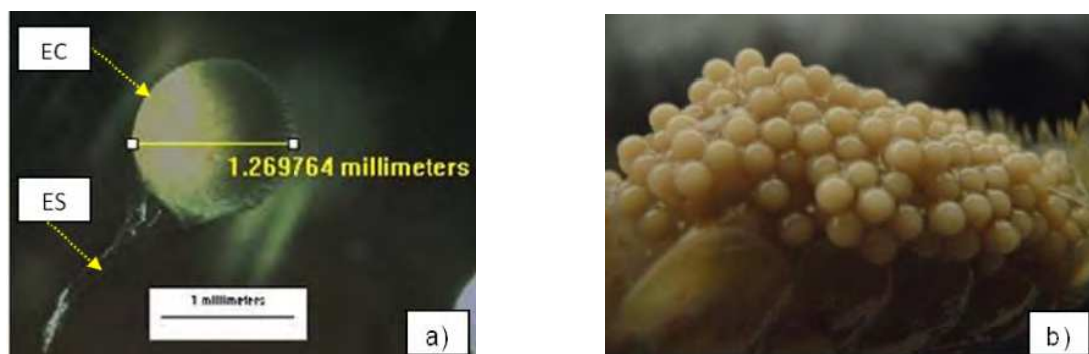


Fig. 5. Stage 1 (days 1-2; 0-11% development). - a) The recently fertilized eggs have a spherical shape and large quantities of mucus are present evidence of recent spawning. b) Egg mass has uniform light beige color which corresponds to the vitellus. By day 2 the vitellus has divided and small drops appear, probably the beginning of scission. It is observed the egg capsule (EC) and egg stolon (ES).

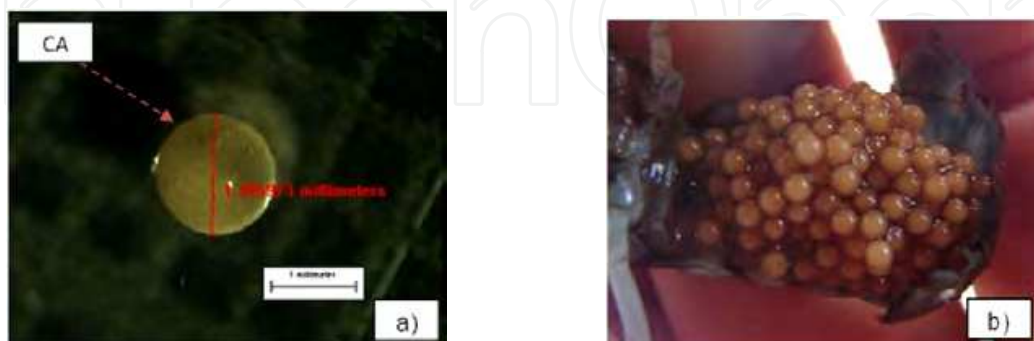


Fig. 6. Stage 2 (days 3-4; 11-22%). - a) Cellular division continues and the vitellus is completely divided into small drops. A region of greater cell accumulation (CA) is observed which corresponds to the zone where the blastopore will form. b) The egg mass begins to change color (yellow-olive green).

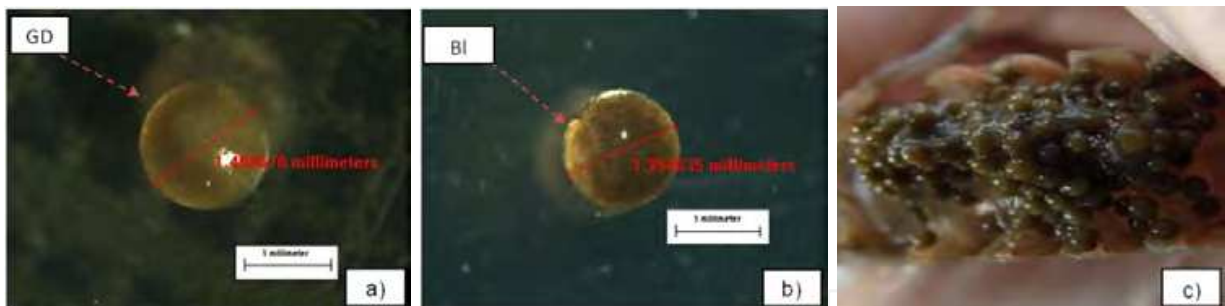


Fig. 7. Stage 3 (days 5-6; 22-33%). - a) Cell division continues and a group of cells begins to form on the egg ventral surface, corresponding to the germinal disk (GD). Starting on day 6, the cell layer begins to expand and form a depression, corresponding to the gastrula. b) The gastrula's ventral plate sinks in to form a groove, which is how the blastopore (Bl) appears; the forward portion of the caudal papilla starts to develop. The blastopore then closes and the rear portion of the caudal papilla appears. d) The egg mass changes in color from dark beige to a translucent olive green.

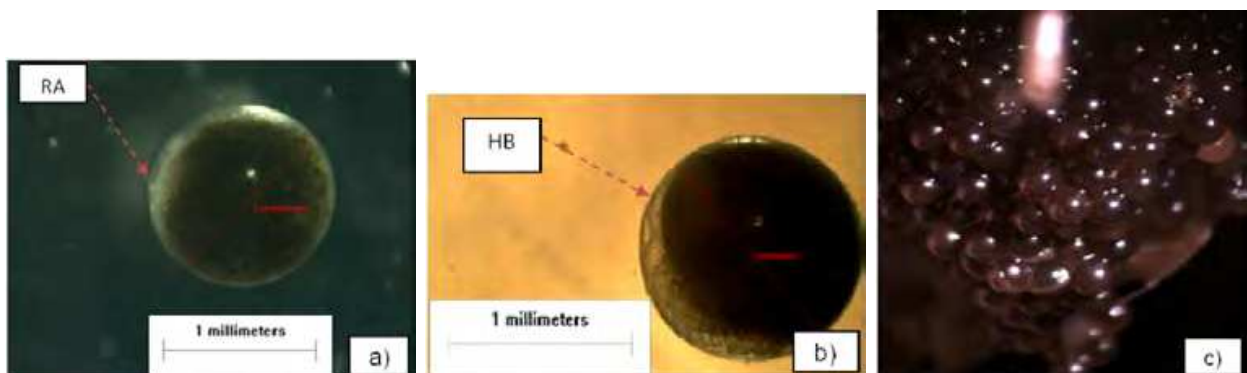


Fig. 8. Stage 4 (days 7-8; 33-44%). - a) An embryo with rudimentary anterior appendages (RA) is evident. Outlines of the frontal lobules and antennules, antennae and mandible can be distinguished. b) On day 8 the heart beat (HB) and embryo contractions can be. c) The egg mass slowly changes in color from olive green to brown.



Fig. 9. Stage 5 (days 9-10; 44-55%).- a) Primordial eyes or ocular lobules (OL) appear as two elevations in front of the body, a transversal groove appears in the vitellus, crossing the middle of the egg, and a long, thin, anterior-curved caudal papilla is present. The embryo contracts more frequently and the vitellus is clearly visible. b) The egg mass has a translucent, light yellow color and the ocular spots appear as black dots.

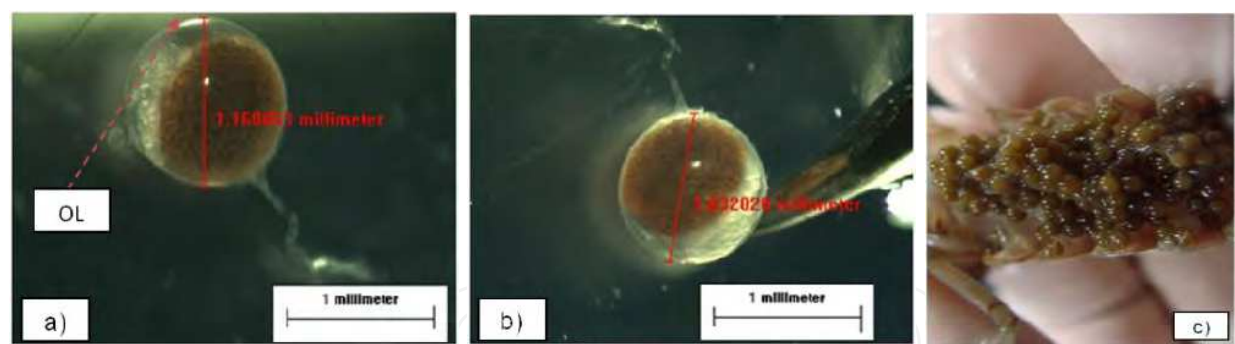


Fig. 10. Stage 6 (days 11-12; 55-66%). - a) OL are well-defined on the anterior body, while the abdominal somites and periopods remain rudimentary, although the chelae are visible. b) The caudal papilla is folded after and covered by the periopods, almost reaching the head. c) The egg mass is heterogeneously colored, with tones varying from light brown, to olive green and khaki.

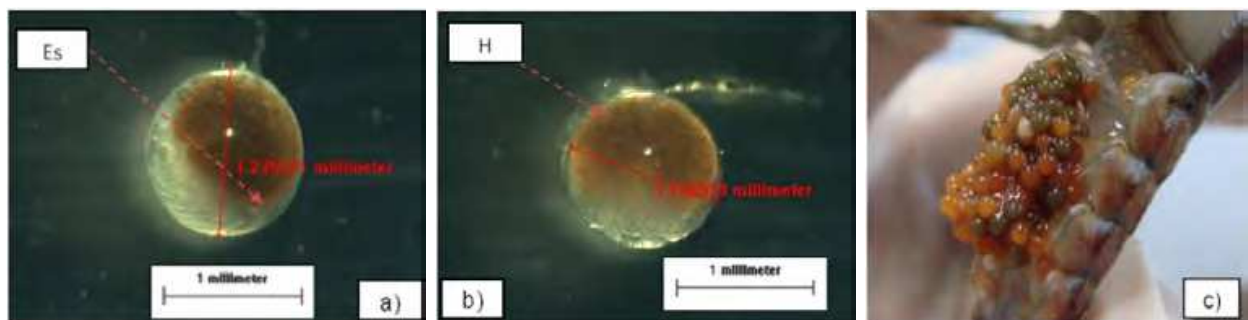


Fig. 11. Stage 7 (days 13-14; 66-77%). - a) Eyespots (Es) are visible, and deep grooves cross through the vitellus along the dorsal medial line. b) The heart (H) can be seen to beat strongly and regularly, Embryo interior is clear and more complex, the periopods are elongated and thin, and a small rostrum appears between the eyes. c) Egg mass coloring is heterogeneous, varying from olive green to bright orange.

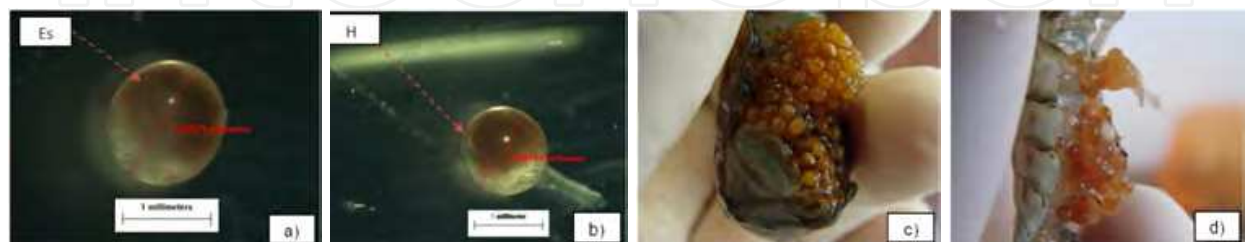


Fig. 12. Stage 8 (days 15-17; 77-88%). - a) The embryo occupies approximately three quarters of the egg ventral surface. b) The thoracic appendages are more developed and the chelae are totally formed. The eyes are sessile and elongate. c) The egg mass has taken on a translucent bright orange color. d) The embryo and eyes are clearly visible.



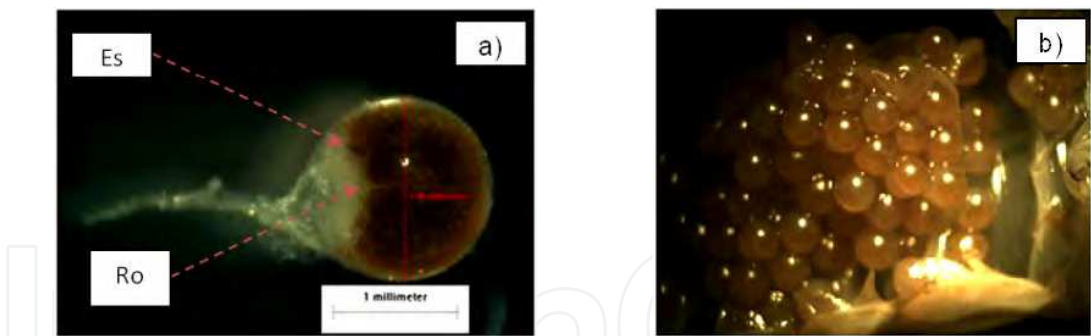


Fig. 13. Stage 9 (day18; 88-99%). - a) Shortly before hatching, the embryo appears compressed inside the egg such that the appendages seem flat and overlapped; there is no space remaining in the chorion. The chelae have grown in front of the eye base, the rostrum (Ro) is visible between the eyes and a groove sagittally crosses half the embryo. b) Egg mass color is bright yellow, rudimentary appendages are visible on the translucent embryos and the eyes are clearly identifiable.

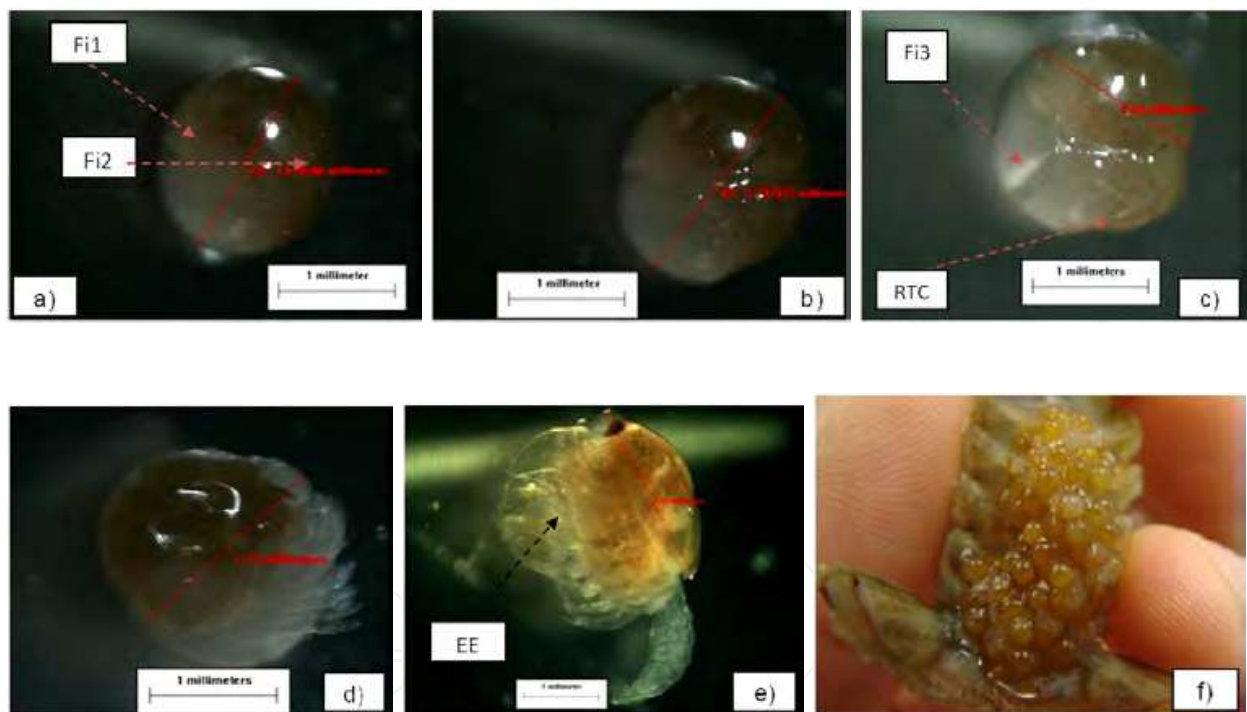


Fig. 14. Stage 10. Hatching (day 18; Development 100%). - The hatching process lasts an average of 10 to 15 min, from when the chorion breaks to when the embryo is completely free. a, b) It begins with the first fissure (Fi1) in the surface of the chorion barely visible, fissure (Fi2) of the chorion covers the folds of the eyes and maxillae; c) notable fissure (Fi3) accompanied by total rupture of the chorion (TRC). d) Breaking the chorion, the periopods are the first to get out. e) Expulsion of the embryo (EE) to the outside of the chorion. f) External appearance of hatchlings, births shown asynchronously. This is considered a critical phase in embryo survival since exiting the chorion requires considerable energy expenditure.

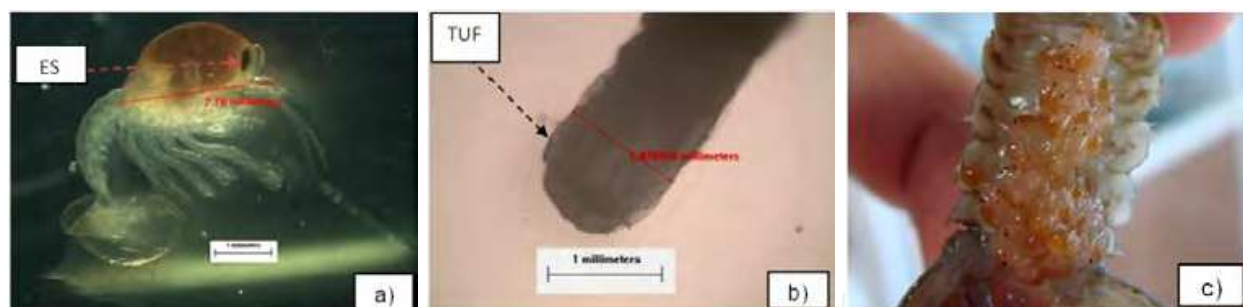


Fig. 15. Post-embryonic stage I (days 18-19). - a) The cephalothorax is formed by a yellow, elongated dorsal hump containing the remaining vitellus, which supplies nutrients to the organism during the following post-embryonic stages; the rest of the body is translucent. The eyes are round and sessile (ES) and contain dark pigment on about one quarter of the overall surface. The antennae and antennules are caudally curved and have sensory villi. b) The partially developed telsons and uropods are fused together in the membranous ligaments that attach the organisms to the chorion interior. c) The hatched organisms remain attached to the mother's pleura.



Fig. 16. Post-embryonic stage II (days 20-21). - a) The eyes are pedunculate (PE) with dark pigment, the dorsal hump which corresponds to the yolk remaining (YR) that nourishes the organism is smaller than in the previous stage (almost half its original size) and the cephalothorax has almost reached its final anatomy. Red dots begin to cover the entire body, the beginning of chromatophore pigmentation. b) The telson and uropods (TUS) appear to be separate with bristles at the ends. c) The organisms remain attached to the mother's pleura.

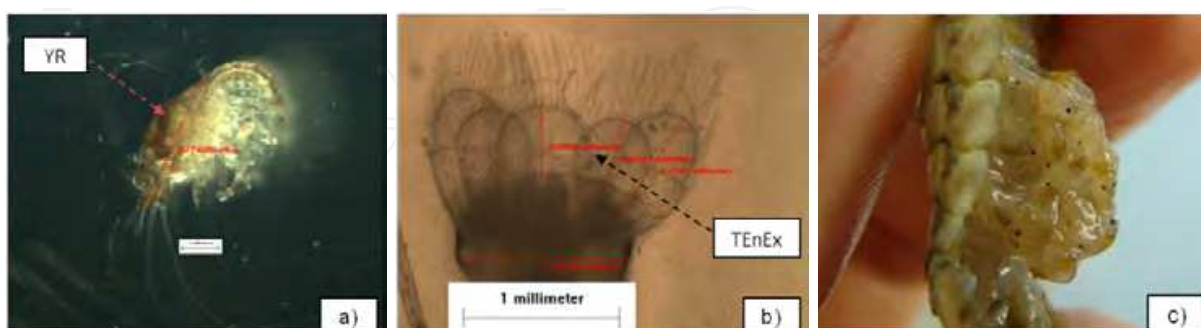


Fig. 17. Post-embryonic stage III (days 22-23). - a) The number and size of chromatophores increases over the entire body, but peduncles eyes not have dark pigment. Yolk Reserves (YR) are still present in the vitellus but almost exhausted, no exogenous feeding activity is observed. b) Telson and uropods are larger-well defined, this last are divided in endopodites and exopodites (TEnEx) which are still immobile and short but well-defined. c) Independent locomotion does not yet occur and the organisms remain attached to the mother's periopods.



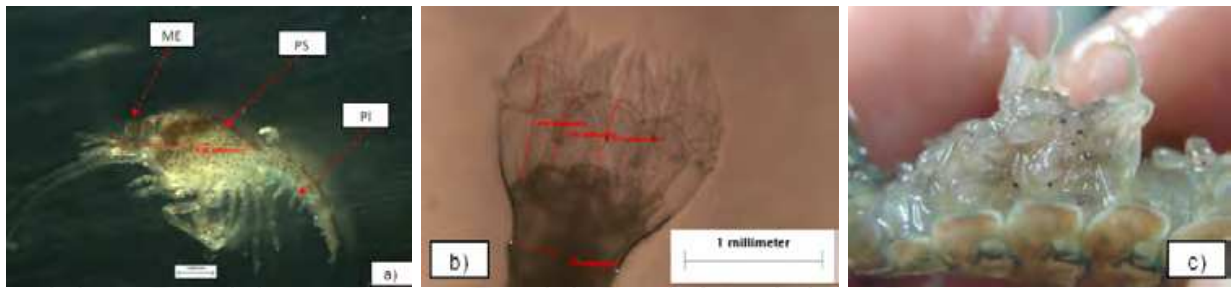


Fig. 18. Post-embryonic stage IV (days 24-25).- a) Fully development eyes or mature eyes (ME), pigmentation spots (PS) dispersed throughout the body, pleopods (PI) are visible; the vitellus is exhausted but exogenous feeding has not begun. b) Telson elongated and uropods compound (protop, endopod, exopod) with bristles and sensorial filaments at the ends; but independent movement does not yet occur. Three pairs of pleopods (PI) are visible on the abdominal somites, and c) the organisms remain attached to the mother's pleopods.

Descriptions were based on the external morphological changes observed in the embryos during ontogeny because the most important ontogenic events during development in *P. acanthophorus* occur in the embryos while still in the chorion. These define the different stages and morphological changes in ways similar to those reported by Montemayor et al. (2010) in *P. regiomontanus* (Villalobos, 1954) (Cambarideae family); Sandeman & Sandeman (1991) in *C. destructor*, and García-Guerrero et al. (2003) in *C. quadricarinatus* (both of the Parastacidae family).

Embryonic development in some crustaceans is highly dependent on water temperature (Bottrell, 1975; Herzig, 1983). In the present study, the crayfish *P. acanthophorus* embryos developed at an average temperature of  $23.8 \pm 2.2$  °C during January-March. This coincides with Cervantes (2008), who reported that this species can reproduce year round under laboratory conditions as long as average temperature is kept at 25 °C. Auvergne (1982) stated that optimum temperature for each life stage in crustaceans is species dependent; for instance, *Astacus astacus* (Linnaeus, 1758) has a range of between 18 to 20 °C whereas *Procambarus clarkii* (Girard, 1852) requires a range of 22 to 26 °C. In further examples, Sandeman & Sandeman (1991) reported satisfactory development in *C. destructor* eggs incubated at 19 °C; García-Guerrero et al. (2003) described successful embryo development in *C. quadricarinatus* at 26°C; and García-Guerrero & Hendrickx (2009) reported proper development in fertilized *Macrobrachium americanum* eggs at 24 °C.

As embryo development progressed, egg length increased; initial egg diameter was 1.3 mm and total length of juvenile organisms was  $6 \pm 1$  mm. This development trajectory differs from the 16 stages (22 days) reported for *P. regiomontanus*, with eight embryonic stages, eight post-embryonic stages and an average juvenile size of 2 cm (Montemayor et al., 2010). However, both these *Procambarus* species have embryonic development periods near the 30-day average for cambarid crustaceans. *Procambarus clarkii* completes its embryonic development in an average of three to four weeks (McClain & Romaine, 2007), and *P. llamasii* completes it in 27 to 30 days (Rodríguez-Serna et al., 2000). These contrast with the longer development periods of other species. *C. quadricarinatus* has a 42-day development period with ten embryonic stages, two post-embryonic stages and a juvenile stage (García-Guerrero et al., 2003), while *C. destructor* has a 40-day development period with an unknown number of embryonic stages, at least two post-embryonic stages and a juvenile stage (Sandeman &

Sandeman, 1991). In stark contrast to all the above species, *Austropotamobius pallipes* (Lereboullet, 1858), a cold water species, requires over seven months to complete embryo development (Holdich & Lowery, 1988).

During the embryonic development trial, selected live eggs were kept in Petri dishes to evaluate survival. Artificially incubated fertile eggs were found to remain viable after development stage eight. This means that egg lots could be artificially incubated to reduce development period and synchronize times in mass production settings and/or if only small lots of reproductive-age females are available. The present study is the first report of embryonic development and artificial incubation of eggs in *P. acanthophorus*. It constitutes a significant contribution to the biology of this and other decapods crustaceans with potential for use in alternative, sustainable aquaculture systems.

1.3.9 Culture

1.3.9.1 Optimum density for growth

Advances in knowledge for the commercial culture of crayfish have shown that in this specie territorial habits are not present, allowing stocking densities as high as 100 orgm<sup>-2</sup> without affecting the growth density and survival, which is considered an advantage in production. In addition, laboratory studies indicate that it is possible to keep under recirculation systems the biculture of crayfish and tilapia at densities between 30-50 orgm<sup>-2</sup>, where the main species is tilapia, and crayfish support sustainable use of water.

1.3.9.1.1 Response parameters

A experimental trial was done when different densities of culture where evaluated, survival (%), showed no significant differences between treatments and remained in a range between 67 and 85%, with the increase of survival were crayfish were kept at densities of 50 and 90 orgm<sup>-2</sup>, while the lowest occurred where the crayfish were kept at a density of 60 orgm<sup>-2</sup>, without a positive relation between survival and density (Table 3).

Treat.	SUP. (%)	IW (g)	FW (g)	IWG (gday <sup>-1</sup> )	WG (%)	SGR (%day <sup>-1</sup> )	IFC (gday <sup>-1</sup> )	FCR
T1	85.2±17	1.0±0.3	5.6±1	0.1±0	450±58.9	1.0±0.1	0.2±0	3.1±0.1
T2	67.5±16	1.0±0.3	5.6±0.6	0.1±0	507±207	1.1±0.2	0.2±0	3.4±0.7
T3	72.9±40	1.0±0.1	5.6±0.6	0.1±0	474±33.2	1.1±0	0.2±0	3.3±0.2
T4	80.4±10	1.0±0.3	5.3±1.2	0.1±0	463±166	1.0±0.2	0.2±0	3.5±0.8
T5	84.4±14	1.1±0.2	5.3±0.7	0.1±0	412±85.8	1.0±0.1	0.2±0	3.5±0.4
T6	75.0±30	0.9±0.2	5.3±0.6	0.05±0	504±62.1	1.1±0.1	0.2±0	3.5±0.1

T1=50, T2=60, T3=70, T4=80, T5=90, T6=100 (org/m2)

Treat.= Treatments (org/m<sup>2</sup>); SUP= Survival; IW= Initial Weight; FW= Final Weight; IWG= Individual weight gain; SGR= Specific growth rate; IFC= Individual food consumed and FCR= Feed Conversion rate

Table 3. Response parameters ± s.d of the crayfish was culture at different densities.

With respect to growth, the final weight (FW g), weight gain (WG%) and specific growth rate (SGR%/day) for all treatments was similar. The organisms in treatment 1, had a greater weight gain, during the first 30 days of culture maintained a growth rate similar to that of crayfish of the other treatments, then increased its rate of growth, although not differ significantly from other organisms under study, it was considered that the density did not significantly affect growth or survival of the crayfish. The juvenile kept it in treatment 5 and 6 had the lowest growth without significant differences between treatments (95% confidence).

### 1.3.9.2 Biculture

Actually research on the feasibility of polyculture systems including fish and crustaceans, has produced inconsistent conclusions, for that reason is difficult to determinate the potential of polyculture in recirculation system for sustainable aquaculture. In a study with red claw crayfish *C. quadricarinatus* and tilapia *Oreochromis niloticus* in a polyculture system in earthen ponds, Brummett & Alon (1994) reported positive results for the crayfish growth and survival; whereas using the same species combination Rouse & Kahn (1998) reported that competition for feed and space between species negatively affected survival in *C. quadricarinatus*. An alternative to the above system is the use of recirculation systems in crustacean/fish polyculture because water quality and feed supply can be controlled, and shelters can be provided for crustaceans to prevent territorial competition, allowing in both species a survive and grow properly (Karplus et al., 2001). Polyculture is particularly appealing since it makes extremely efficient use of resources and can increase production. It can be quite viable for producers as long as appropriate species are identified in terms of biology and market demand. Using a polyculture with common carp, grass carp, silver carp, tilapia, mullet and Malaysian prawn, Cohen et al. (1983) reported efficient water use and an increase in production from 3.5 to 11 tons/ha/year. In a polyculture system for carp (*Cyprinus carpio*) and crayfish (*Cambarellus montezumae*) growth in artificial ponds in which feeding was focused mainly on the carp, Auró et al. (2000) reported that these species could coexist and use the food in the system, making it a viable system.

Competition is increasingly high for good quality water sources for productive activities such as aquaculture. There is also a need to optimize space during production and to promote productive and sustainable alternative activities in rural areas. Under these conditions, the most adequate option for polyculture systems is to use recirculation systems, ensuring that the species in the system do not compete for resources and have established market niches (Kazmierczak & Caffey, 1995). With the objective to determine the feasibility of a crayfish *P. acanthophorus*/ tilapia *O. niloticus* polyculture and monoculture using a water recirculation system, as aquaculture sustainable alternative, a experimental research in outdoor facilities was conducted. Six plastic tanks (3 m diameter x 1.2 m depth) in a recirculation system with a biological and sand filter were used. During 90-day experimental period, three treatments were evaluated with two replicates per treatment in a completely random design. T1: crayfish monoculture ( $1.02 \pm 0.2$  g); T2 polyculture: crayfish ( $1.04 \pm 0.2$  g) and tilapia ( $2.99 \pm 0.1$  g); T3: tilapia monoculture ( $3.45 \pm 0.6$  g).

Survival in the T2 crayfish was significantly lower (34.7%) compared to that in the T1 crayfish (72%). In contrast, the tilapia in both T2 and T3 had similar survival (>95%) and growth rates (83-86 g) with no apparent effect from the presence of the crayfish (Table 4).

Treatment		S (%)	IW (g)	FW (g)	IWG (g)	WG (%)	SGR (%)
T1	Crayfish	72±12.7 <sup>a</sup>	1.02±0.2 <sup>a</sup>	4.8±0.4 <sup>a</sup>	0.042±0 <sup>a</sup>	373.5±55 <sup>a</sup>	68.4±3.3 <sup>a</sup>
T2	Crayfish	34.7±20.5 <sup>b</sup>	1.04±0.1 <sup>a</sup>	3.9±0.3 <sup>b</sup>	0.033±0 <sup>b</sup>	282.6±73 <sup>b</sup>	59.9±3.3 <sup>b</sup>
	Tilapia	98.7±0 <sup>a</sup>	2.99±0.1 <sup>a</sup>	88.5±9.9 <sup>a</sup>	0.95±0.1 <sup>a</sup>	2861.2±26 <sup>a</sup>	194.2±5.3 <sup>a</sup>
T3	Tilapia	93.7±9.9 <sup>a</sup>	3.45±0.6 <sup>a</sup>	84.3±10.7 <sup>a</sup>	0.89±0.1 <sup>a</sup>	2344.9±71 <sup>a</sup>	192.1±5.6 <sup>a</sup>

<sup>1</sup>Values in the same column with the same superscript are not statistically different (p>0.05)

S% = survival rate; IW = initial weight; FW = final weight; IWG = individual weight gain; WG% = percentage weight gain; SGR = specific growth rate.

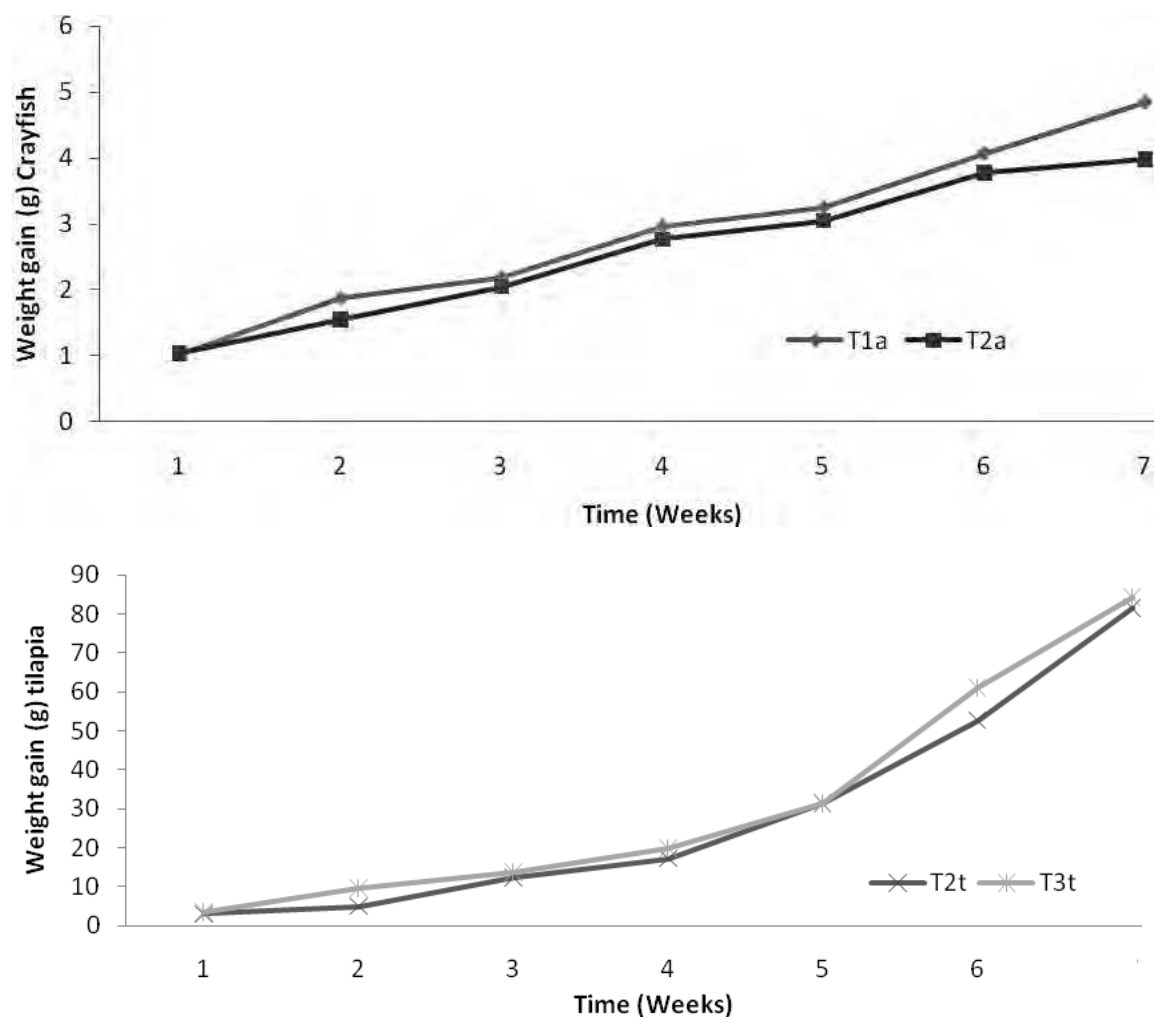
Table 4. Growth and efficiency parameters in crayfish and tilapia monocultures and crayfish/tilapia polyculture in a water recirculation system<sup>1</sup>.

The lower crayfish survival rate in T2 had no apparent effect on growth, as might be expected due to the density effect, since IWG and WF in T2 (0.033±0g; 3.9±0.3g, respectively) were significantly lower than in T1 (0.042±0g; 4.8±0.4g, respectively). Tilapia growth in T2 and T3 followed a steeply-sloped exponential curve whereas crayfish growth was constant but with a lesser slope, reflecting their lower growth rate (Figure 19).

Water quality parameter values during the trial were within the ranges tolerated by tilapia fingerlings and crayfish culture. Dissolved oxygen (DO) concentration was 3.8 mgL<sup>-1</sup> throughout the experimental period, lower than the 5 mg/L recommended for optimum growth in *P. acanthophorus* (Cervantes-Santiago et al., 2007). This level coincides with the >3 mgL<sup>-1</sup> DO level recommended for *P. clarkii* (Huner, 1994), and suggests that *P. acanthophorus* can adapt to environmental variations during cultivation. Like crayfish, tilapia can also tolerate low DO levels (<2 mgL<sup>-1</sup>), although levels greater than 3 mgL<sup>-1</sup> are recommended for good growth (El-Sayed & Abdel-Fattah, 2006). This DO level also favors proper functioning of the biological filter in the recirculation system and prevents the death of nitrifying bacteria (Yousef et al., 2003). Increased temperature during the experimental period improved growth in the tilapia and crayfish, although efficient growth in *P. acanthophorus* is reported to occur at temperatures <28°C. Optimum growth in tilapia occurs at 28°C, even though the species can tolerate a range of 15-35°C. Apparently, environmental parameters are no impediment to polyculture of Nile tilapia and *P. acanthophorus*. Higher temperatures (23-33°C) have also been reported to increase growth in a polyculture of *C. quadricarinatus* and tilapia (Rouse & Kahn, 1998). In addition, the temperature tolerance exhibited by *P. acanthophorus* coincides with overall temperature tolerance (10-38 °C) among *Procambarus* genus crayfish (Holdich, 2002). Of course, individual species have specific optimum temperature ranges for growth; for instance, *P. clarkii* prefer temperatures from 22-30°C (Holdich, 2002) with optimal levels around to 20-25°C (Huner & Gaude, 2001). This tolerance for a wide range of environmental temperatures highlights the potential for crayfish cultivation in commercial systems.

Water pH levels were adequate for proper growth in both tilapia and crayfish, although both can tolerate pH from 3.5 to 12, another advantage for polyculture of these species (Huner, 1994; El-Sayed & Abdel-Fattah, 2006). Ammonium, N-nitrite and N-nitrate (mgL<sup>-1</sup>) values were below sublethal and lethal levels for the two cultured species (Huner, 1994;





T1: Crayfish, T2: Crayfish/Tilapia, T3: Tilapia

Fig. 19. Weight gain in crayfish and tilapia monocultures and crayfish/tilapia polyculture in a water recirculation system.

El-Sayed & Abdel-Fattah, 2006; Cervantes, 2008), indicating proper biological filter functioning.

This is the first report of a crayfish *P. acanthophorus* / Nile tilapia *O. niloticus* polyculture. The feasibility of this species pair in polyculture as a productive alternative when a recirculation system to increase water efficiency use was evaluated, because both tilapia and crayfish are readily available, and the *Procambarus* genus is diverse around the world, similar than tilapia. The polyculture system was successful in that both species developed properly during the experimental period, although survival among the *P. acanthophorus* (37.4%) was significantly lower than in the crayfish monoculture (72%), and crayfish IWG and FW were lower in the polyculture than in the monoculture. Presence of the tilapia in the same tanks was apparently the main potential cause of this overall lower growth performance in the polyculture since all other variables were within proper ranges for crayfish. There may have been interspecies competition for space and/or feed, or predation by the tilapia of the crayfish during molting partially due to a substantial size difference between species. The latter possibility was not evaluated in the present study since the same

initial stocking sizes were used in all treatments: average initial stocking sizes were  $1.03 \pm 0.77$  cm (crayfish) and  $3.15 \pm 1.04$  cm (tilapia), giving a clear growth advantage to the tilapia. The tilapia did not necessarily need to be antagonistic for them to prey on the much smaller crayfish in T2. Different types and quantities of shelters could help to protect the crayfish from the larger tilapia, and manipulating initial stocking size of the different species might help to reduce predation.

Auró et al. (2000) highlighted the feasibility of crayfish/fish polyculture in a study using carp *Cyprinus carpio* and crayfish *Cambarellus montezumae* in artificial ponds. The species coexisted and had enough food resources at densities of up to  $50 \text{ Org m}^{-3}$  for both species under good water quality conditions. The crayfish *P. acanthophorus* has excellent potential for use in aquaculture systems, because it can be culture to high densities, tolerates handling, adapts to variable environmental conditions and accepts different kinds of artificial diets in captivity, although it does not reach sizes as large as other crustaceans, such as *C. quadricarinatus* (Cervantes, 2008; Cruz-Ordoñez, 2009). Its use in sustainable rural aquaculture production systems is promising under monoculture, and possibly under polyculture conditions after further research into optimum initial stocking size, densities and crayfish shelter type and quantity. Greater production of alternative protein sources using aquaculture in rural areas is an important step towards increasing food availability and diversity.

In addition to the above, the biculture system may be associated to aquaponic production of herbs (cilantro, basil and aquaponic green fodder) with satisfactory results.

### 1.3.9.3 Genetic improvement

An experiment was designed to estimate genetic variability of growth as heritability ( $h^2$ ) of the weight at different ages. For which was captured 2135 organisms ( $4.1\text{g} \pm 1.79$ ) from its natural medium (G0) of which 10% heavier were selected ( $i = 1,755$ ) for each sex: 140 females ( $5.62\text{g} \pm 1.97$ ) and 48 males ( $6.02\text{g} \pm 1.9$ ) to form the progenitors of the line selection (LS), while the control line (LC) consisted of organisms take it at random. These organisms were maintained for reproduction in two rectangular fiberglass tanks of  $2.4 \text{ m}^2$  and  $0.15 \text{ m}$  deep (one tank per line), and a density of  $49 \text{ orgm}^{-2}$ , and relation females: males (3:1). Biweekly organisms were reviewed to identify gravid females From these organisms 30 full-sib families per line (LC and LS) were obtained (F1), and grown individually for five months, tracking them individually in a recirculation system consisting of 60 rectangular plastic tubs ( $54 \text{ cm} \times 37 \text{ cm} \times 22 \text{ cm}$ ) distributed in three levels with mechanical and biological filtration under laboratory conditions and fed 2 times day with balanced feed for shrimp (35% protein). Once the organisms in lines arrived at three months old, and due to differential mortality in the families and in order to reduce the effect of the environment, the density was standardized to 11 organisms per family ( $55 \text{ orgm}^{-2}$ ) to continue growth in the same. Heritability for growth in broad sense ( $h^2$ ) and in each age, for both, the control (LC) and selection line (LS) was estimated from variance components (ANOVA method REML) using a full-sib design from the formulas described by Roff (1997). Growth between the lines in F1 was also compared for each age.

Table 4 shows estimates of heritability from full-sib design for F1. The value of  $h^2$  estimates for LC was 0.48 initially and decreased until the fourth month of age, not to suffer variations due to the rearrangement of the population in the third month compared to LS which began



Heritabilty estimate (h²) ± E.S.		
Age (month)	LC	LS
1	0.48 ± 0.11	1.10 ± 0.14
2	0.08 ± 0.04	0.61 ± 0.12
3	0.23 ± 0.07	0.27 ± 0.08
4	0.20 ± 0.10	0.58 ± 0.14
5	0.27 ± 0.11	0.34 ± 0.12

Table 4. Estimates of heritability (h²) ± E.S in F1 at the different ages (months), in selection line (LS) and control line (LC)

with a value of 1.1 reducing the third month (0.27) have to rise again in the fourth month (0.58). Due to the differential survival and in order to reduce the effect of common environment which is reported in previous studies with crustaceans (Benzie et al., 1997; Hetzel et al., 2000; Pérez-Rostro & Ibarra, 2003), the density of families in the third month were standardized, leaving 11 organisms per family in F1.

Estimates of heritability (combined males and females) in F1 at the end of the trial was similar for both lines (0.27 ± 0.11 and 0.34 ± 0.12, LC and LS, respectively), coinciding with results obtained by Pérez-Rostro & Ibarra (2003) in white shrimp *Litopenaeus vannamei*, where values of 0.20 were obtained at 17 weeks of culture as well and those of Cameron et al. (2004) in the red claw crayfish *C. quadricarinatus*, where a decrease in the value of heritability of 0.38 for the first year to 0.13 after 4 years.

When growth gain between the lines was compared, it was seen that LS was significantly heavier (9.6%) than the control line (Figure 20).

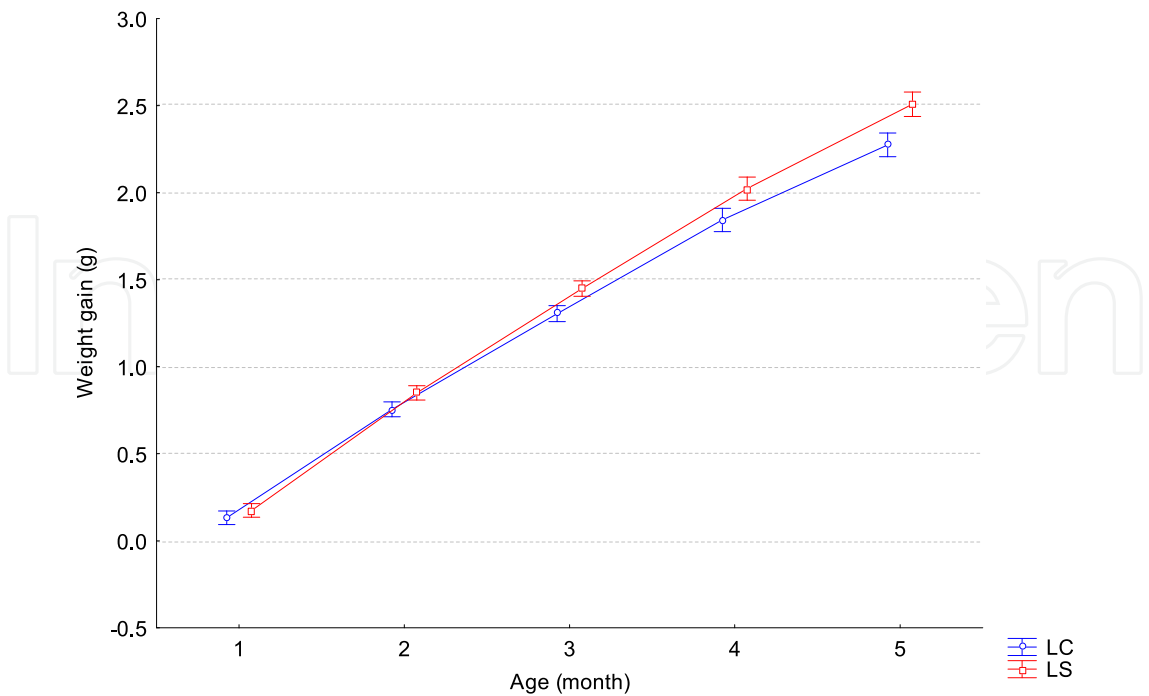


Fig. 20. Weight gain between selection line (LS) and control line (LC) during the five months of culture.

The values of heritability indicate that the species has a positive response to selection, so they can continue to implement a screening program to improve the growth of the species and thus promote commercial cultivation.

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## **Aquaculture**

Edited by Dr. Zainal Muchlisin

ISBN 978-953-307-974-5

Hard cover, 390 pages

**Publisher** InTech

**Published online** 27, January, 2012

**Published in print edition** January, 2012

This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Martha P. Hernández-Vergara and Carlos I. Pérez-Rostro (2012). Advances in Domestication and Culture Techniques for Crayfish *Procambarus acanthophorus*, Aquaculture, Dr. Zainal Muchlisin (Ed.), ISBN: 978-953-307-974-5, InTech, Available from: <http://www.intechopen.com/books/aquaculture/advances-in-domestication-and-culture-techniques-for-crayfish-procambarus-acanthophorus>

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