



## HERITABILITY AND RESPONSE TO SELECTION FOR GROWTH IN THE F<sub>1</sub> GENERATION OF CRAYFISH *Procambarus acanthophorus*

[HEREDABILIDAD Y RESPUESTA A LA SELECCIÓN PARA EL CRECIMIENTO EN LA F<sub>1</sub> DEL ACOCIL *Procambarus acanthophorus*]

Lorenzo Díaz-Jiménez, Carlos I. Pérez-Rostro\* and Martha P. Hernández-Vergara

Instituto Tecnológico de Boca del Río, División de Estudios de Posgrado e Investigación. Laboratorio de Crustáceos Nativos. Km. 12 Carretera Veracruz Córdoba, CP. 94290. Boca del Río, Veracruz, México.

\*Corresponding Author: ivandna02@hotmail.com

### SUMMARY

The crayfish *Procambarus* (*A.*) *acanthophorus* has ideal characteristics for aquaculture, except for its small size. This study evaluated the growth, the response to selection in the F<sub>1</sub> generation, the and heritability ( $h^2$ ) of a crayfish population. Of 2135 specimens (weight  $4.1 \pm 1.79$  g) that were captured, 10% with the highest body weight (140 females [ $5.62 \pm 1.97$  g] and 48 males [ $6.02 \pm 1.9$  g]) were selected to parent the selection line (SL). The control line (CL) included 140 females and 48 males captured randomly from the population. Thirty full-sib families were obtained per line (F<sub>1</sub>), and were cultivated individually for five months in a recirculation system. Heritability, in the broad sense, was estimated each month, using a full-sib design based on components of variance. Growth was compared between lines in the F<sub>1</sub>. Mean  $h^2$  for weight after five months was  $0.27 \pm 0.11$  g for CL and  $0.34 \pm 0.12$  g for SL. The F<sub>1</sub> offspring was 9.6% higher in SL than in CL, with 84% and 88% survival rate, respectively, at the end of the study. These results indicate that it is possible to implement a genetic improvement program based on selection for size for this species.

**Key words:** Native crayfish; genetic selection; crayfish cultivation.

### INTRODUCTION

There are hundreds of species of crayfish in the world, but only a few are cultivated because there is little or no biological information available (Rodríguez, 1999), due to a lack of interest in these species by aquaculture producers or the market. The state of Veracruz, in Mexico, offers a wide variety of native crustaceans whose biological characteristics are suitable for aquaculture production, as in the case of the crayfish

### RESUMEN

El acocil *Procambarus* (*A.*) *acanthophorus* tiene características ideales para la acuicultura, excepto su pequeño tamaño. Este estudio evaluó el crecimiento, la respuesta a la selección en la F<sub>1</sub> y la heredabilidad ( $h^2$ ) en una población de acociles. De 2135 organismos (peso  $4.1 \pm 1.79$  g) que fueron capturados, el 10% con mayor peso (140 hembras [ $5.62 \pm 1.97$  g] y 48 machos [ $6.02 \pm 1.9$  g]) fueron seleccionados como progenitores de la línea de selección (SL). La línea control (CL) incluyó a 140 hembras y 48 machos capturados al azar de la población. Treinta familias de hermanos completos se obtuvieron por línea (F<sub>1</sub>) y se cultivaron individualmente durante cinco meses en un sistema de recirculación. La heredabilidad en sentido amplio se estimó cada mes utilizando un diseño de hermanos completos sobre la base de los componentes de varianza. El crecimiento se comparó entre líneas en la F<sub>1</sub>. La  $h^2$  media para peso después de cinco meses fue  $0.27 \pm 0.11$  para CL y  $0.34 \pm 0.12$  para SL. La descendencia (F<sub>1</sub>) en la SL fue 9.6% mayor que en CL, con 84 y 88% de supervivencia, respectivamente, al final del estudio. Estos resultados indican que es posible implementar un programa de mejoramiento genético basado en la selección de tamaño para esta especie.

**Palabras clave:** Acociles nativos; selección genética; cultivo de acocil.

*Procambarus* (= *Austrocambarus*) *acanthophorus*, which is harvested and sold regionally. In recent years, studies have been conducted to determine the potential for cultivation and the requirements of lipids and proteins in its diet (Cervantes *et al.*, 2010a), for the production of juveniles under laboratory conditions, and the optimal female:male ratio for breeding and egg viability (Cervantes *et al.*, 2010b). Before commercial production is possible, greater understanding of growth and survival is required to make this crayfish

competitive with the large crustaceans on the market, such as prawns, shrimp, and freshwater lobsters. Some technological tools that can be applied to enhance growth of crayfish are techniques derived from quantitative genetics, such as directional mass selection, which estimates the response to selection and amount of genetic variability available for exploitation, expressed as heritability ( $h^2$ ) of the character.

This study estimated the heritability in the broad sense, from a full-sib design in the crayfish *P. acanthophorus*, in order to estimate the genetic variability available to implement a program of selection for weight gain to make this crayfish competitive in the market, by cultivating it under farm conditions.

## MATERIALS AND METHODS

### Experimental specimens

Two parental stocks were formed from crayfish captured in temporary water bodies around “La Mixtequilla” (18° 44' NL and 95° 56' WL) in the state of Veracruz, Mexico. After taking two samples at the beginning of the rainy season (July–August), 2135 crayfish were harvested. The average weight was  $4.1 \pm 1.79$  g. The 10% of the heaviest individuals of each sex were selected, resulting in 140 females ( $5.62 \pm 1.97$  g) and 48 males ( $6.02 \pm 1.9$  g), to maintain a 3:1 female:male ratio (Cervantes *et al.*, 2010b), to form the progenitor generation in the selection line (SL). The control line (CL) was formed with 140 females and 48 males taken at random from the initial population of organisms.

Lots of parents (CL and SL) were reproduced separately to obtain 30 full-sib families for SL and 30 families for CL, to obtain the filial 1 ( $F_1$ ) generation. The study lasted seven months, starting when the parents of CL and SL were bred, until obtaining the  $F_1$  organisms and all the families reached five months of age.

### Experimental system for reproduction of parents

For maintaining conditions for reproduction of the parental generation (CL and SL), what was used was a recirculation system with four fiberglass tanks (two per line; 2.4 m  $\times$  1.0 m  $\times$  0.25 m high), with a water column of  $\sim 0.15$  m, mechanical and biological filters, in a container with capacity of 500 L, driven by a 0.5 HP centrifugal pump (BYFP1006B®, Boyu Aquarium Industries, China), and an output flow of 3 L  $\text{min}^{-1}$  for each tank. Constant aeration was supplied with a 2.5 HP aerator (Sweetwater®, S11K, Baldor Electric, Fort Smith, AR). Also, PVC tubes ( $\frac{3}{4}$  and 1 inch diameter, 10 cm long) were placed in each tank in a 1:1 ratio for

each female crayfish (PVC tube:female crayfish) to avoid cannibalism and provide protection for ovigerous females.

### Maintenance of $F_1$ progeny

The broodstock of both lines remained in reproduction stage for three months. Every two weeks, the broodstock were checked to identify ovigerous females, which initially were placed in individual shelters constructed of PVC pipe (1½ inch diameter and 10 cm long) covered with 5 mm plastic mesh. These females were transferred to a closed system with 48 plastic tanks (0.4 m in diameter containing 70 L water), where they were individually maintained until hatching of larvae and formation of the families. Once hatching was completed, females were removed from the tanks and the larvae remained in the system to continue their growth for two months.

After the first month of age, 30 juvenile crayfish with the greatest weight in each family were used to form the high-growth line (SL). To form the control line (CL), 30 juvenile crayfish were selected at random. For the second phase of the study, the juvenile crayfish were raised for five more months. In this phase, a recirculation system was used, which included 60 rectangular plastic tubs (54  $\times$  37  $\times$  22 cm) distributed in three levels, with a constant-flow water supply (1 L  $\text{min}^{-1}$ ), a settling tank, mechanical filter, biological filter (biospheres) and a 500 L reservoir under constant aeration supplied through hoses and silicon air stones.

To maintain a constant population density because of differential mortality in the two family lines, the density was standardized at 11 crayfish per family to continue growing. Females and males were extracted at random from the CL group, and the largest crayfish in the SL group formed the  $F_1$  broodstock that were used to obtain the  $F_2$  generation for a further study.

### Diet and water quality

During the reproduction stage of both lines and maintenance of their  $F_1$  progeny, the crayfish were fed *ad libitum* twice daily with commercial shrimp feed (Silver Cup®, Group El Pedregal, Toluca, Mexico) with 35% protein and particle size suitable for consumption by crayfish at each stage of development. Water temperature and dissolved oxygen in the water were recorded daily at 12:00–13:00 h, using a probe (EcoSense® DO200, YSI, Yellow Springs, OH). Siphons were used to exchange 50% of the water every 15 days after observing ovigerous females. Every two weeks, ammonia, nitrites, nitrates and phosphates levels, as well as pH, were determined through colorimetric test kits (Hagen Nutrafin®, Hagen Baie d’Urfé, Quebec, Canada).

## Biometrics

Weight gain (g) and length (cm) of each family in both groups (F<sub>1</sub> generation) were measured monthly during the five months of cultivation. Prior to weighing, each crayfish was placed on a dry cloth to remove excess of water. The length was measured from the tip of the rostrum to the tip of the telson, and the weight was determined using a digital scale (Scout Pro<sup>®</sup>, Ohaus, Pine Brook, NJ). Survival in each family was recorded at each monthly measurement.

A linear regression analysis was performed to identify possible relationships between weight gain, temperature, and survival of both groups, using Microsoft Excel<sup>®</sup> sheets.

### Estimate of heritability from full-sib design

Estimates of heritability ( $h^2$ ), in the broad sense, were obtained for both groups (SL and CL), using the formulas described by Roff (1997) (Table 1).

Table 1. Analysis of variance for full sibs, designated when no effect is linked to the environment .

Source of variation	Degrees of freedom	Mean squares	Expected mean squares
Among families (EF)	$N-1$	$MS_{EF}$	$V_{EP} + kV_{EF} = V_G$
Among progeny (EP) or "error"	$T-N$	$MS_{EP}$	$V_{EP} = V_e$

N = number of families, T = Total number of individuals, k = Size of the family.

Therefore:

$$h^2 = \frac{2V_{EF}}{V_{EF} + V_{EP}} = \frac{2(MS_{EF} - MS_{EP})}{MS_{EF} + (k-1)MS_{EP}} = V_G / V_f$$

And the standard error (Roff, 1997):

$$SE(h^2) = 2 \left( 1 - \frac{h^2}{2} \right) \left( 1 + (k - 1) \frac{h^2}{2} \right) \left( \frac{2}{k(k-1)(N-1)} \right)^{1/2}$$

All the analyses were performed using the software Statistica<sup>®</sup> 7.0 (StatSoft, Tulsa, OK) using general linear models with the Restricted Maximum Likelihood (REML) procedure.

### Performance of the SL and the CL groups

Development was compared between the SL and CL during each month of cultivation, using a multifactorial analysis of variance, using Statistica 7.0 software, with significance set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Estimates of heritability ( $h^2$ ) for growth

The correlation analyses made to identify the influence of temperature on growth and survival, expressed as weight gain, indicated a positive relationship between temperature and crayfish development (Table 2). This was observed when increasing water temperature from 20 to 24 °C. The growth rate decreased at temperature above 26 °C, which also interfered in the heritability

estimates, adding an error or environmental factor to the final estimates. Similar observations were reported in shrimp *Penaeus monodon*, *Penaeus stylirostris*, and *Penaeus vannamei* (Benzie *et al.*, 1997; Goyard *et al.*, 2002, Perez-Rostro, 2002, Perez-Rostro and Ibarra, 2003), which had increased heritability estimates from the effect of common environment variables (differences in density, survival, and changes in water quality within the same experimental unit).

The stocking density had no direct effect on growth and survival (50 to 100%) of crayfish per family because there was no relationship between these variables. Therefore, the increase in size (weight and length) of crayfish in families and lines could be attributed to both environmental factors (temperature *vs.* weight gain) and the genetic component of the species. This is similar to the results described by Lutz and Wolters (1989) and Kitcharoen *et al.* (2012) in *Procambarus clarkii* and *Macrobrachium rosenbergii*, respectively, of variations in phenotype within families of the same age after their genetic selection for growth of appendages and carapace.

Water quality indicators were within the optimal range reported in previous crayfish studies (Holdich, 2002; Cervantes *et al.*, 2010a, b): dissolved oxygen ( $6 \pm 0.82$  mg L<sup>-1</sup>), ammonium ( $0.08 \pm 0.03$  mg L<sup>-1</sup>), nitrite ( $0.1 \pm 0.007$  mg L<sup>-1</sup>), nitrate ( $5 \pm 0$  mg L<sup>-1</sup>), phosphate ( $5 \pm 0$  mg L<sup>-1</sup>), and pH ( $8.3 \pm 0.4$ ). The recommended levels in these studies are: dissolved oxygen (>5 mg L<sup>-1</sup>), ammonia (<1.0 mg L<sup>-1</sup>), nitrite (<0.5 mg L<sup>-1</sup>), nitrate (<10 mg L<sup>-1</sup>), phosphate (<10 mg L<sup>-1</sup>), and pH (6.5 to 8.5).

Table 2. Values of multiple correlation between temperature–weight and survival–weight during each month and in each selected line of crayfish *Procambarus acanthophorus*.

Age (month)	Multiple correlation coefficient			
	Control Line (CL)		Selection Line (SL)	
	weight/survival	weight/temperature	weight/survival	weight/temperature
2	0.20	0.64*	0.20	0.72*
3	0.05	0.16	0.18	0.35
4	0.33	0.77**	0.05	0.69**
5	0.22	0.78**	0.09	0.56**

\*Values with significant differences ( $P < 0.05$ ) are positive correlations. \*\* Highly significant difference ( $P < 0.01$ ) of negative correlation. Values without an asterisk have no significant correlation in variables.

Heritability ( $h^2$ ) estimates of progeny ( $F_1$ ) started with high values for SL and CL lines (0.48 and 1.1, respectively). These data are related to the intensity of selection used for SL compared with CL, the latter selected randomly. Families ( $F_1$ ) of SL had significant variations between them, caused by the seed density during the first month, which had a significant difference in the number of offspring produced by female (52 to 334 offspring), resulting from the larger size and variations in gonadal status of females (Arcos, 2004; Cervantes *et al.*, 2010b). The CL family weights were more homogeneous as a result of the random selecting for  $F_1$  progeny. In the second month, there was a significant decrease of  $h^2$  in CL related to differential mortality, compared with SL, and also the CL populations had a higher proportion of females. Rossel *et al.* (2009) mentioned that males grow faster than females. This was reflected in the sex proportions in the two lines evaluated, since SL had a higher number of males because specimens of greater height

were selected. Also, there was a reduced coefficient of variation (0.48 to 0.29 and 0.44 to 0.31 for CL and SL, respectively), indicating that populations become homogeneous with each other, directly reducing the value of  $h^2$  (from 0.48 to 0.08 for CL and 1.10 to 0.6 for SL). In the fourth month (after adjusting population density in the third month),  $h^2$  values changed again, setting the number of crayfish in the families of both lines to form the new  $F_1$  broodstocks to reduce the error caused by environmental variation in density between families (Hetzel *et al.*, 2000; Pante *et al.*, 2002). In this case, the coefficient of variation decreased only in CL (25 to 22%;  $P > 0.05$ ) because this population remained more homogeneous than SL, with a decrease in  $h^2$  from 0.23 to 0.22. In SL, the variation increased from 19 to 20%, indicating that the readjustment of populations (capturing more organisms of larger size by family) caused an increase in the size variation between families, with an increase in  $h^2$  from 0.27 to 0.58 (Table 3).

Table 3. Estimates of heritability ( $h^2 \pm E.S.$ ) and coefficient of variation (CV) for each month in the control line (CL) and the select line (SL) of crayfish *Procambarus acanthophorus*.

Age (month)	$h^2$ LC (CV) (n = 30)	k	$h^2$ LS (CV) (n = 30)	k
1	0.48 $\pm$ 0.11 (0.48)	30	1.10 $\pm$ 0.14 (0.44)	30
2	0.08 $\pm$ 0.04 (0.29)	25	0.61 $\pm$ 0.12 (0.31)	26
3	0.23 $\pm$ 0.07 (0.25)	22	0.27 $\pm$ 0.08 (0.19)	23
4	0.20 $\pm$ 0.10 (0.22)	10	0.58 $\pm$ 0.14 (0.20)	10
5	0.27 $\pm$ 0.11 (0.21)	7	0.34 $\pm$ 0.12 (0.18)	9

The heritability ( $h^2$ ) values indicate that this crayfish has a positive response to selection, as observed in other crayfish (Dean *et al.*, 2002, Cameron *et al.*, 2004, Dean *et al.*, 2005), demonstrating that it is

feasible to design breeding programs to produce larger crayfish for making them more desirable for the regional and national market and can compete with other commercially important crustaceans.

In addition to the  $h^2$  estimates, the final mean weight of CL ( $2.26 \pm 0.47$  g) was significantly lower (by 9.6%), than SL ( $2.5 \pm 0.44$  g). These differences were

first observed after the third month, indicating an increase in weight from selection and a positive response to the breeding program (Figure 1).

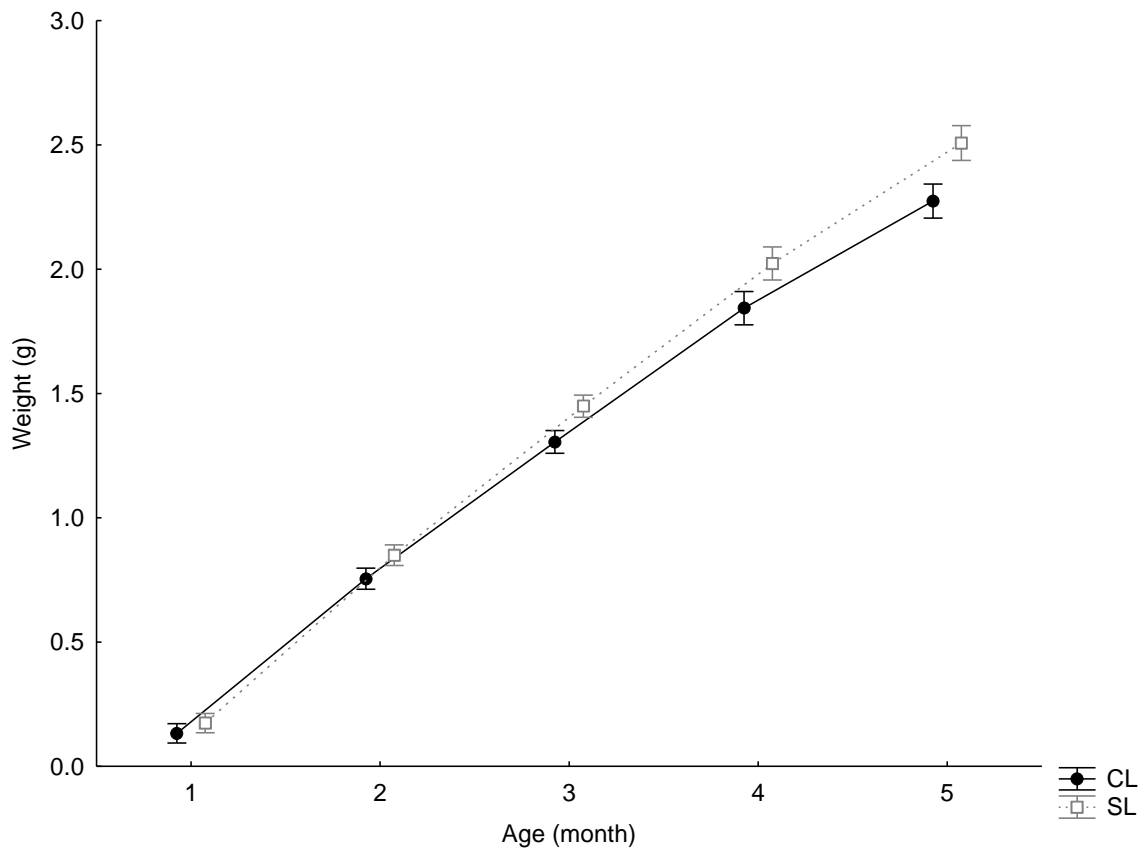


Figure 1. Monthly increase in weight (mean  $\pm$  S.D.) of the selected line (SL) and control line (CL) in the crayfish *Procambarus acanthophorus*.

### CONCLUSION

Heritability for weight after five months of growth was 9.6% in the selection line from a full-sib design. This demonstrates that the crayfish *Procambarus acanthophorus* responds positively to selection and genetic variation available for exploitation to increase its market weight.

### REFERENCES

Arcos, O.G.F. 2004. Análisis fisiológico y genético del desempeño reproductivo del camarón blanco (*Litopenaeus vannamei*). Tesis Doctoral. Centro de Investigaciones Biológicas del Noroeste, S.C. CIB. Baja California Sur, México.

Benzie, J.A.H., Kenway, M., Trott, L. 1997. Estimates for the heritability of size in juvenile *Penaeus*

*monodon* prawns from half-sib matings. *Aquaculture*. 152:49-53.

Cameron, P.M., Jones, C.M., Shanks, A.S. 2004. Selection for increased weight at 9 months in redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture*. 237:131-140.

Cervantes, S.E., Hernández, V.M.P., Pérez, R.C.I., Olvera, N.M.A. 2010a. Protein lipid ratio for the growth and survival of juvenile crayfish *Procambarus acanthophorus* (Hobbs 1972) reared under controlled conditions. *Aquaculture Research*. 41:1-10.

Cervantes, S.E., Hernández-Vergara, M.P., Pérez-Rostro, C.I., Olvera-Novoa, M.A. 2010b. Reproductive performance of the crayfish *Procambarus acanthophorus* (Austrocambarus) Villalobos 1948 under

- controlled conditions. *Aquaculture*. 308:66-70.
- Dean, R.J., Purvis, I.W., Piper, L.R. 2002. Genetic differences in growth among wild populations of the yabby, *Cherax destructor* (Clark). *Aquaculture Research*. 33:917-923.
- Dean, R.J., Purvis, I.W., Piper, L.R., Chris, A.D. 2005. Selection for faster growth in the freshwater crayfish *Cherax destructor*. *Aquaculture* 247. *Genetics in Aquaculture*. VIII:169-176.
- Goyard, E., Patrois, J., Peignon, J.M., Vanaa, V., Dufour, R., Viallon, J., Bedier, E. 2002. Selection of better growth of *Penaeus stylirostris* in Tahiti and New Caledonia. *Aquaculture*. 204:416-468.
- Hetzel, J.S.D., Crocos, J.P., Davis, P.G., Moore, S.S., Preston, C.N. 2000. Response to selection and heritability for growth in the Kuruma prawn, *Penaeus japonicus*. *Aquaculture*. 181:215-223.
- Holdich, D.M. 2002. *Biology of Freshwater Crayfish*. Blackwell Science. USA. pp. 155-158.
- Kitcharoen, N., Rungsin, W., Koonawootrittrion, S., Na-Nakorn U. 2012. Heritability for growth traits in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879) based on best linear unbiased prediction methodology. *Aquaculture Research*. 43:19-25.
- Lutz, G.C., Wolters, R.W. 1989. Estimation of heritabilities for growth, body size and processing traits in red swamp crawfish, *Procambarus clarkia* (Girard). *Aquaculture*. 78:21-33.
- Pante, R.M.J., Gjerde, B., McMillan, L., Miszta, I. 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 204:383-392.
- Pérez-Rostro, C.I. 2002. Parámetros genéticos para caracteres del crecimiento, metabólicos y de respuesta a estrés en larvas, juveniles y adultos de camarón blanco (*Litopenaeus vannamei*). Tesis de Doctorado. Centro de Investigaciones Biológicas del Noroeste, S.C. Baja California Sur, México.
- Pérez-Rostro, C.I., Ibarra, A.M. 2003. Quantitative genetic parameter estimates for size and growth rate traits in Pacific White shrimp, *Penaeus vannamei* (Boone 1931) when reared indoors. *Aquaculture Research*. 34:1-11.
- Rodríguez, S.M. 1999. Biología y sistema de los cambáidos del sudeste de México y su potencial aprovechamiento en acuicultura. Tesis de Doctorado. Universidad Autónoma Metropolitana. México.
- Roff, D.A. 1997. *Evolutionary Quantitative Genetics*. Editorial Chapman & Hall. USA. pp. 41-42.
- Rossel, M.T., Hernández, V.M.P., Cervantes, S.E., Díaz, J.L. 2009. Evaluation of the monosexual and mixture growth and survival of juveniles freshwater crayfish *Procambarus (Austrocambarus) acanthophorus* in culture conditions. Abstract World Aquaculture Society Meeting. Veracruz, México.

*Submitted March 21, 2012 – Accepted June 18, 2012*  
*Revised received July 31, 2012*