Arch. Biol. Sci., Belgrade, 66 (2), 579-586, 2014 DOI:10.2298/ABS1402579Q

EFFECT OF INTENSIFICATION IN ENVIRONMENTS WITH ZERO-WATER EXCHANGE ON THE REPRODUCTIVE POTENTIAL OF *CHERAX QUADRICARINATUS*

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Abstract - Reproductive parameters (number of eggs, fertility index, survival of juveniles) and the biochemical composition (proteins, carbohydrates, lipids) of *Cherax quadricarinatus* broodstock were examined in specimens with weights ranging from: (i) 30-45; (ii) 46-60; (iii) 61-75g; (iv) 76-90 g, in an intensive system (4 organisms/m^2) with zero-water exchange (maintained at 28±1°C and aerated continuously). Better reproductive efficiency was observed in specimens with smaller weights. This effect was also reflected in the biochemical parameters of the eggs and juveniles. The use of broodstock whose weight ranges between 45 and 60 g is recommended.

Key words: Cherax quadricarinatus; broodstock; reproductive efficiency; juvenile production

INTRODUCTION

Aquaculture worldwide has maintained a sustained annual growth of 8-10% over the last ten years (FAO, 2008). In Mexico, its development has been mostly limited to three species: Pacific white shrimp *Litopenaeus vannamei*, Japanese oyster *Crassostrea gigas* and some varieties of tilapia. The diversification of the aquaculture industry is important and the cultivation of different species of shellfish and fish is recommended (Villarreal, 2000).

The cultivation of freshwater crayfish has generated great interest worldwide because of its growth rate, size and acceptance by the consumer. The redclaw, *C. quadricarinatus*, is native to northwest Australia (Jones and Curtis, 1994), and was originally introduced into Mexico in the 1990s by the Ministry of Fisheries to evaluate its potential. Best practice semi-intensive culture techniques were developed to take advantage of the species resistance to environmental changes, ease of reproduction and good growth potential between 23 and 31°C (King, 1994; Jones, 1988). Redclaw can reach 70-100 g in 6-8 months of commercial rearing (Jones1990; Hutchings and Villarreal, 1996; Villarreal and Peláez, 1999). Nevertheless, development of a strong industry has been limited due to limited scientific research (Austin, 1992, Naranjo, et al., 2004). Information generated by the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in more than two decades has allowed for the development of an efficient technology that mitigates the impact on the environment by eliminating water exchange while increasing culture density (Naranjo et al. 2006). Current commercial production yields without water exchange are 5 tons/ha/cycle (Naranjo et al., 2009), which is double the reports for commercial farms in Australia (Jones, 1990) and elsewhere.

Garcia-Guerrero et al. (2003b) indicated that the species matures and spawns readily. Studies of the

embryonic development of *C. quadricarinatus* (e.g. Garcia-Guerrero et al., 2003a) and the production of vitellogenin during female maturation (Serrano-Pinto et al., 2004) have helped in understanding the reproductive behavior of the species in captivity. Yeh and Rouse (1995) found that redclaw can reproduce in temperatures exceeding 23°C, and that it reaches sexual maturity between 7 and 9 months of age (Rouse et al., 1991) or an average size of 15-20 g (Naranjo, 1999; Villarreal and Pelaez, 1999; Rodríguez-González, 2001). As current three phase systems (Villareal and Pelaez, 1999; Naranjo et al., 2006, Naranjo et al., 2009) use 25% of pond area for reproduction, intensification of this phase in zero-water exchange systems would benefit the economic viability of redclaw culture. The present trial discusses the effect of intensification on the reproduction efficiency of different-sized *C. quadricarinatus* breeders.

MATERIALS AND METHODS

Intensive rearing of C. quadricarinatus

Experimental organisms were selected from intensive monosex culture growout ponds without water exchange at CIBNOR in La Paz, B.C.S., Mexico. Juveniles (3±0.5 g) were stocked at 25/m^2 in outdoor 100 m^2 lined ponds at the beginning of April, when water temperatures averaged more than 23°C, and reared for 100 days. Average depth was 1.8 m. The ponds were aerated 24 h/day using a turbo blower and a grid of 1.3 cm diameter perforated PVC pipes placed in the pond bottom. Feeding was done twice per day with a 35% crude protein commercial pellet for shrimp, according to biomass, following Villarreal and Pelaez (1999). At harvest, organisms averaged 25±3 g and were sexed before being stocked in 100 m^2 ponds at a density of 10 organisms/m^2 for monosex culture during 120 days. Experimental males and females were selected at harvest.

Mating

Twelve 1 500 l oval fiberglass tanks (area = 3 m^2) with filtered (5 micron) and aerated freshwater were used for mating. Temperature was maintained at 28±1°C, which is considered to be optimal in terms of energy efficiency for embryo development (Garcia-Guerrero et al., 2003). Following Villarreal and Pelaez (1999), two cylindrical PVC hiding places (7.6 cm diameter, 30 cm long) were used per adult crayfish as hiding places. The sex ratio was 3 females:1 male. Stocking density was 4 females/m^2 (Austin and Meewan, 1998), or 12 per tank, which is four times the normal stocking density in commercial ponds (Villarreal and Pelaez, 1999). Four experimental female broodstock size ranges were examined in replicate tanks: 30-45, 46-60, 61-75 and 76-90 g. Breeders were individually weighed (0.01 g) and marked with indelible ink on the exoskeleton. The selected males had corresponding weight ranges. Spermatophore attachment between the fourth and fifth pair of female pereiopods was evaluated daily following Rodríguez-González, et al. (2009). Two days after the females spawned (which allowed the eggs to glue properly to the pleopods), the total number of eggs (fecundity) and the number of viable eggs (fertility) was determined according to the methodology proposed by Harrison (1990). A sample of the eggs was taken for biochemical analysis.

Reproductive efficiency

The number of juveniles released (hatching percentage) was estimated (Garcia-Guerrero et al., 2003) by removing the females prior to juvenile hatching and individually placing them in 60 l aquaria with filtered and aerated freshwater at 28±1°C. The females were removed from the tanks after releasing the juveniles to avoid predation (Villarreal and Pelaez, 1999). Juveniles were counted to determine the rate of hatching. A sample (n= 30) was weighed, measured and used to determine biochemical composition (proteins and total lipids) for each treatment (Rodríguez-González, 2006). Once the hatch was counted and weighed, 200 juveniles from each size range were stocked in replicated 60 l aquaria and reared for 30 days in filtered and aerated freshwater at 28±1°C. The juveniles were fed 10% of initial biomass with a 40% crude protein micro particulate twice per day. Final average weight and survival were determined at the end of the trial.

Biochemical analyzes of eggs and juveniles.

Protein, carbohydrates and total lipids were determined according to the techniques described by Carreño (2000). Eggs and juveniles were homogenized in 1.2% NaCl saline solution. Protein concentration was determined by the Bradford (1976) method, using albumin as standard. The homogenate was first digested for 30 min at ambient temperature with 0.5 N NaOH. For carbohydrates, proteins were precipitated with 20% trichloroacetic acid and centrifuged at 3 000 rpm for 10 min at 10°C. Carbohydrates were then quantified from the supernatant by the Anthrone method (Van Handel, 1965), using glucose as standard. For total lipids, a modification of the method of Barnes and Blackstock (1973) was used; 0.1 ml of the homogenate was mixed with 1 ml of 76% H_2SO_4, and incubated at 90°C for 10 min in a water bath. The acid solution obtained was mixed with phosphosulphovanillin reagent. Concentration was calculated using a mixture of triacylglycerol (12 mg ml^{-1}) and cholesterol (8 mg ml^{-1}) as standard.

Statistical Analysis

Data were analyzed for normality and variance homogeneity before a one-way ANOVA (Sokal and Rohlf, 2000), and Tukey's test was used to establish differences between treatments (Zar, 1999) using STATISTICA 6®. Best-fit quadratic equations were adjusted to the data to establish reproductive efficiency.

RESULTS

There were no significant differences in total egg number produced by females ranging in size from 30 to 75 g. Fig. 1 shows that females over 76 g produced a significantly lower total egg number ($P < 0.05$). Fig. 2 shows an inverse relationship between fecundity index and size range, varying from 16.8 eggs/g for 30-45-g females, to 4.8 for the largest size range.

In terms of egg biochemical composition, Figs. 3 and 4 show that mean protein and lipid levels were significantly higher ($P<0.05$) for the 46-60 g females.

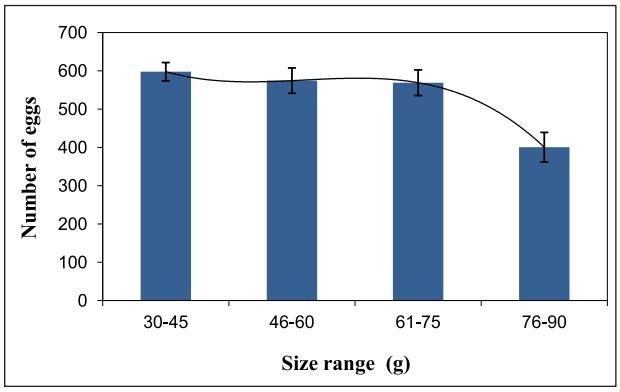

Fig. 1. Total egg number produced by different-sized *C. quadricarinatus* females reared in an intensive system without water exchange. (y = -30.148x3 + 189.83x2 – 381.8x + 819.78. $R^2 = 1$).

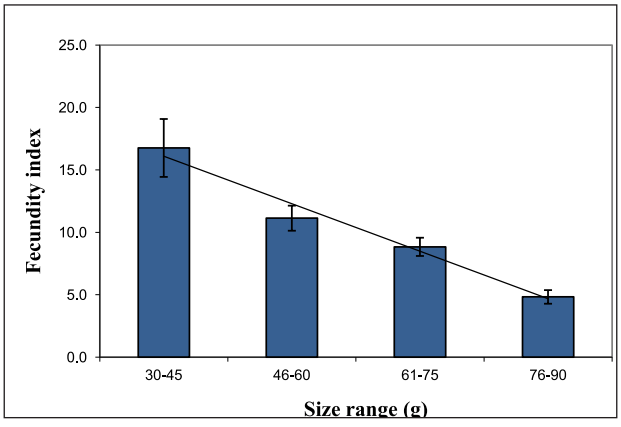

Fig. 2. Fecundity index of different-sized *C. quadricarinatus* females reared in an intensive system without water exchange. (y = -3,809x + 19,912. $R^2 = 0.9741$).

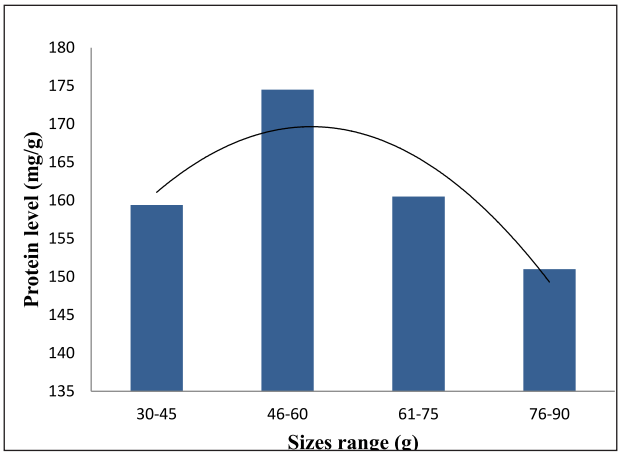

Fig. 3. Protein concentrations in *Cherax quadricarinatus* eggs from females reared in an intensive system without water exchange. The relationship between size and protein level is represented by the equation y= -6.15x2 + 26.83x + 140.4. $R^2 = 0.8016$.

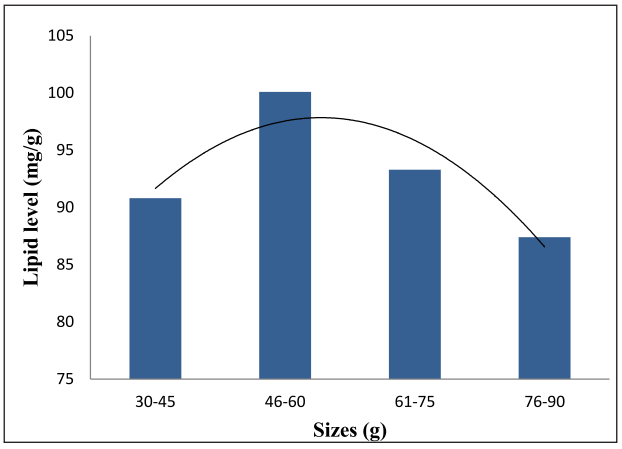

Fig. 4. Lipid concentrations in *Cherax quadricarinatus* eggs from females reared in an intensive system without water exchange. The relationship between size and lipid concentration is represented by the equation y= -3.8x2 + 17.3x + 78.15. R^2 = 0.8333

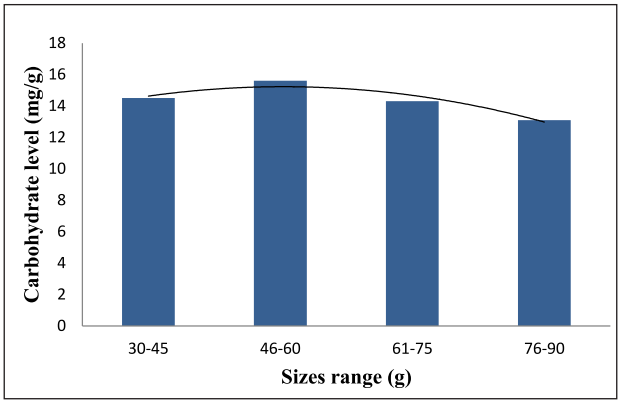

Fig. 5. Carbohydrate concentrations in *Cherax quadricarinatus* eggs from females reared in an intensive system without water exchange. The relationship between size and carbohydrate concentration is represented by the equation y = -0.575x2 + 2.325x + 12.875. R^2 = 0.9007.

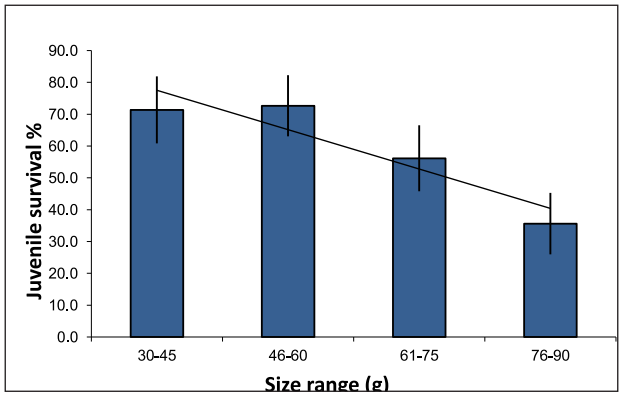

Fig. 6. Survival of *Cherax quadricarinatus* juveniles, from different-sized females, after 30 days of rearing. The relationship between size and survival is represented by the equation y = -12.372x + 89.88, with a R^2 = 0.8562.

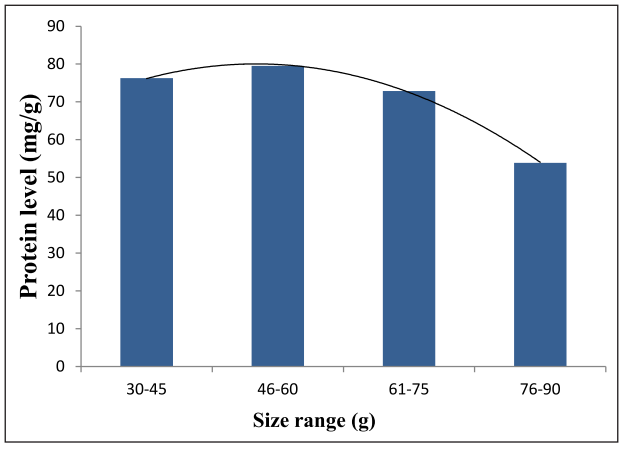

Fig. 7. Protein levels in *Cherax quadricarinatus* juveniles from females reared in an intensive system without water exchange. This trend line is represented by the equation y = -5.55x2 + 20.37x + 61.35, with a R^2 = 0.9991.

This would appear to be the optimum size range for first egg release for the species. As expected, carbohydrate values were not significantly different between treatments (Fig. 5).

Table 1 shows that newly hatched juveniles had significantly higher initial weight for the 76-90 g female size range. However, at the end of the 30-day trial, there were no differences with juveniles from the 30-45 or 46-60 female size ranges. The mean final weight for the 61-75 g specimens was significantly lower than for the other treatments. There was an inverse correlation (y = -12.372x + 89.88, R^2 = 0.8562, $p < 0.05$) between female size and final survival (Fig. 6). This is consistent with the differential use of energy resources favoring growth over reproduction.

Figs. 7, 8 and 9 show a clear trend in which the protein, lipid and carbohydrate concentrations peaked in juveniles from the 46-60 g females. As expected, the main source for embryo development was the lipids. The values shown in Fig. 8 are about 90% lower than those for the egg.

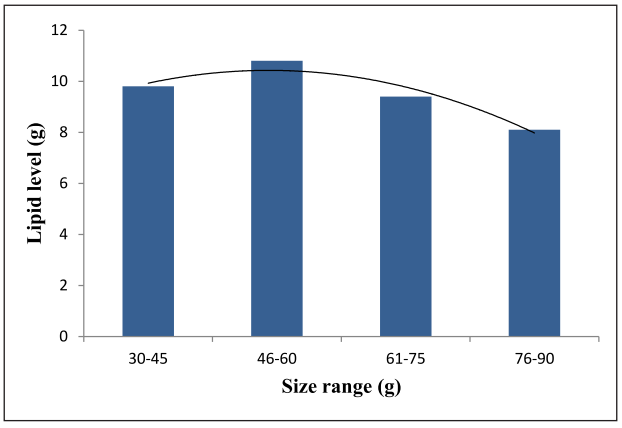

Fig. 8. Lipid levels in *Cherax quadricarinatus* juveniles from females reared in an intensive system without water exchange. This trend line is represented by the equation y= -0.575x2 + 2.225x + 8.275, with a R^2 = 0.9166.

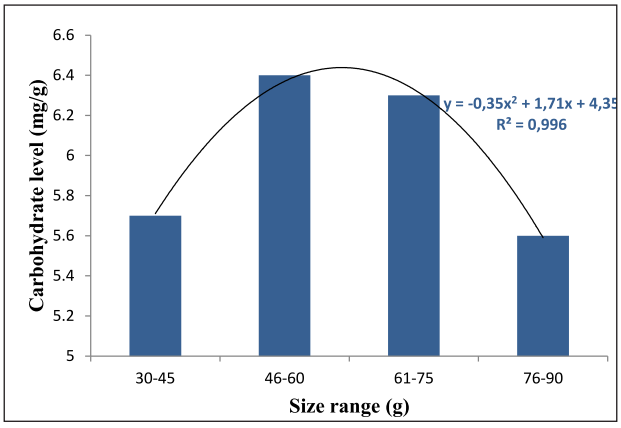

Fig. 9. Carbohydrates levels in *Cherax quadricarinatus* juveniles from females reared in an intensive system without water exchange. This trend line is represented by the equation y = -0.35x2 + 1.71x + 4.35 with a R^2 = 0.996.

Table 1. Mean initial weight (± SD), 30-day mean weight (± SD) and final survival of newly hatched juvenile *Cherax quadricarinatus* from different-sized females, originally reared in intensive ponds without water exchange. (Initial number = 200).

Female size range (g)	Initial weight (g)	30-day weight (g)	Survival (%)	Final Biomass (g)
30-45	0.071± 0.014a	0.46± 0.006b	71.4c	55.56b
46-60	0.084± 0.010a	0.38± 0.04b	72.7c	43.23b
61-75	0.080± 0.013a	0.24± 0.012a	56.2b	17.58a
76-90	0.109± 0.018b	0.42± 0.03b	35.6a	22.25a

DISCUSSION

For the first spawning of the species, the results show that females weighing between 30 and 75 g produced a similar number of eggs, which is larger than those produced by the 76-90 gram females. This was also consistent with the individual fertility index. In decapod crustaceans, an inverse relationship between female size and energy diverted to egg production is common. Gametogenesis requires a continuous transfer of energy reserves from the hepatopancreas to the gonads (Rodríguez-González, 2006). For redclaw, this transfer is efficient and Rodriguez et al. (2009) showed that the gross biochemical composition (i.e., protein and lipids) of the egg did not change, even when primary dietary nutrients were restricted, concluding that egg quality would probably be unaffected. In the present trial, survival, juvenile growth and total biomass after 30-day rearing were significantly higher for the smaller size ranges, thus indicating differences in reproductive efficiency. Consequently, in terms of energy efficiency, it would be advantageous to select smaller initial sizes for reproduction in commercial farms to exploit this high reproductive potential. However, the impact on long-term growth performance would still have to be determined, as it has been shown that breeder selection for reproduction affects growth and vice versa (Palacios et al., 2000; Arcos et al., 2004).

In *C. quadricarinatus* eggs proteins are the most abundant component, followed by lipids and carbohydrates. This proportion is common in crustacean eggs (Holland, 1978; Whyte et al., 1990, Clark, 1992 and Garcia-Guerrero, 2003b, Luo et al., 2004). The carbohydrate contribution was limited and did not

show any differences between treatments. However, a clear trend was established for the nutrients, where mean protein, lipid and carbohydrate levels in the egg were consistently higher in the 46-60 g size group. Garcia-Guerrero et al. (2003b) demonstrated that energy consumption from spawning to hatching varied according to temperature in redclaw, and that lipids were the main source of energy. There were drops in protein, lipid and carbohydrate concentrations recorded for 30-day-old juveniles. Similar to the report by Garcia-Guerrero et al. (2003a), the drop of lipid concentration during embryo development was significant. A similar trend was observed in juveniles, where the mean protein, lipid and carbohydrate concentrations were higher for the 46-60 g size group. The biochemical profiles of the eggs point to the biochemical composition of juveniles. This can also serve as an indicator of early survival and growth in juveniles.

The results showed that intensification during nursery and growout in zero-water exchange systems did not affect the ability of *Cherax quadricarinatus* broodstock to reproduce. Biochemical parameters recorded were similar to previous works (García-Guerrero et al., 2003b; Rodríguez-González, 2006), where the organisms were reared in semi-intensive systems.

Although spawns from 30-45 g organisms resulted in the largest mean juvenile biomass at 30 days, it was not significantly different to that of the 46-60 g group, which had a better biochemical component concentration. As market sizes favor over-60 g animals for the species (Hutchings and Villarreal, 1996), it must be elucidated whether smaller size breeders affect long-term growth, diverting too much energy to reproduction, extending culture time to market size or preventing the organism to attain the desired size. Naranjo-Páramo (2009) showed that slow-growing organisms during the nursery phase do not exhibit compensatory growth during monosex culture and are unable to reach 60 g. These are colloquially called runts (Hutchings and Villarreal, 1996). In crustaceans, the initial phase of development is normally associated to somatic growth not reproduction (Osse et al., 1997), as the reproductive organs are still undeveloped. When maturity is reached, metabolic demand for reproduction increases significantly (Guadagnoli, 2005). *Cherax quadricarinatus* has the ability to spawn multiple times per year (Rodríguez-González, 2006) if conditions are adequate. Thus, broodstock size selection is critical for commercial operations. The present work shows that 46-60 g organisms produce good quality spawns with acceptable survivals for early juvenile growth. The biochemical composition of the egg appears to be a good predictor of early juvenile performance. Large-size organisms, which consume more energy for growth and therefore have less available energy for gonad development, showed less efficient juvenile production. Previous work has shown that the species is capable of developing healthy spawns even when nutrients are limited (Rodriguez et al., 2009). Perhaps this is performed by increasing the total amount of food consumed. Nevertheless, the impact on fast-growing individuals of limited nutrient availability, which may occur in intensive systems, must be analyzed. We conclude that intensive rearing of these species in zero-water exchange systems did not affect their reproductive potential.

Acknowledgments - The authors wish to thank Sandra de La Paz, Mayra Vargas, Jesús Aguilar, and Gilberto Gonzalez for technical support during the experimental phase, Roberto Hernandez for his support in biochemical analyses, Julio E. Felix for his help in data analysis, and Teresa Sicard for experimental advice. Armando Monge-Quevedo is also grateful to the National Council of Science and Technology (CONACYT) for the scholarship (no. 33713) to conduct his doctoral studies; the authors acknowledge the support of FINNOVA-CONACYT and the Aquaculture Program at CIBNOR for the grants awarded to Dr. Humberto Villarreal to conduct this research.

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