HATCHERY, NURSERY, NUTRITION AND STOCK EVALUATION OF

REDCLAW CRAYFISH *Cherax quadricarinatus*

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee.

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HATCHERY, NURSERY, NUTRITION AND STOCK EVALUATION OF REDCLAW CRAYFISH *Cherax quadricarinatus*

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REDCLAW CRAYFISH *Cherax quadricarinatus*

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VITA

Antonio Garza de Yta, son of Carlos H. Garza Becerra and Cecilia de Yta de la Peña, was born on November 20, 1973, in Mexico City, Mexico. He graduated with a Bachelor of Science degree in Chemical Engineering from the Instituto Tecnologico y de Estudios Superiores de Monterrey, Monterrey, Mexico in 1997. He worked for American Standard in Mexico as a Procurement Manager from 1997 to 1999. He entered Auburn University in September 1999 to pursue a Master of Science degree in aquaculture from which he graduated in 2001. Upon completion, he returned to Mexico and founded Aquaculture Global Consulting for which he has consulted in several countries in aquacultural matters. He returned to Auburn University in January 2007 to pursue the degree of Doctor of Philosophy degree. In that same year he was appointed by FAO as external consultant for aquaculture production. He created the concept of the Certification for Aquaculture Professionals (CAP) program and is currently engaged in its development.

DISSERTATION ABSTRACT

HATCHERY, NURSERY, NUTRITION AND STOCK EVALUATION OF

REDCLAW CRAYFISH *Cherax quadricarinatus*

Antonio Garza de Yta

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The redclaw crayfish (*Cherax quadricarinatus*) aquaculture industry has developed rapidly in tropical and subtropical regions of the world during the last decade. Important advances have been made; however, technology for commercial production is still in its infancy and still faces major challenges. With the purpose of improving economic viability and overcome these challenges, a series of hatchery, nursery, nutrition and stock evaluation experiments were conducted. Research was conducted at the AGY hatchery property of Megar S.A. de C.V in Soto La Marina Tamaulipas, Mexico and at the E.W Shell Fisheries Center, Auburn University, Alabama between May 2007 and August 2008.

Nutrition studies demonstrate that soybean based diets formulated to contain 36% protein and 7% lipid maintaining an adequate amino acid profile can use fish meal, poultry by-product meal, distiller's dried grains with solubles or ground pea meal

indistinctly as alternative sources of protein without negative effects on growth, yield or survival of the crayfish.

Experiments also suggest that the addition of stargrass (*Cynodon nlemfuensis*) at a rate of 125 kg/ha/wk in combination with a properly balanced pelleted feed for juvenile redclaw can reduce feed inputs and, thereby, reduce feed costs for redclaw producers.

The hatchery-nursery techniques used in these experiments recommend that water volume has minimal effect on the production performance in a redclaw hatchery-nursery system. Surface area is the best way to account for juvenile production of redclaw. Juveniles from female densities between 4.2 and 6.9 females/m^2, averaging approximately 80 g/female, with a 30-day nursery period appear to provide the best results in a commercial hatchery-nursery, when compared to lower and higher densities of juveniles and 20 and 40-day nursery periods.

During the stock comparison studies, when comparing the best two stocks identified by Australian and Mexican farmers, results imply that there is no significant differences in growth performance between the Walkamin (Australia) and the Megar (Megar) stocks.

The experiments conducted in this study have increased the general knowledge and understanding of the redclaw crayfish, *C. quadricarinatus*.

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CHAPTER I

INTRODUCTION

Redclaw crayfish, *Cherax quadricarinatus*, has a natural range that encompasses most of the drainages along northeastern Australia, the Gulf of Carpentaria and the Timor Sea (Merrick and Lambert 1991). The species is also found in Papua New Guinea (Austin 1986). Redclaw is a gregarious species that tolerates broad environmental challenges, and relatively high stocking densities. In addition, it has favorable reproductive characteristics, including moderate fecundity, a simple life cycle and maternal incubation of eggs through to the hatching of highly developed juveniles (Jones 1995a, 1995b; Masser and Rouse 1997; Jones and Ruscoe 2000), which together make the redclaw a good aquaculture species. Commercial redclaw aquaculture was initiated in Queensland in 1987. It has since been introduced successfully to several countries in the world. Most redclaw stocks used in commercial farms have originated from very limited collections of wild stocks in North Queensland. Cultured stocks are often heterogeneous either because river populations were mixed to produce heterotic hybrids or because collectors consider them to be a single genetic resource (Macaranas et al. 1995).

Aquaculture of redclaw crayfish is an activity that has developed at an accelerated pace in the last decade in tropical and sub-tropical regions of the world. Although there have been major advances, the evolving industry still faces major challenges. To overcome these challenges, a better understanding of the biology and behavior of the species in intensive culture conditions is important.

Mature male redclaw have a distinctive reddish patch on the outside margin of the claws, females do not. However, the best way of identifying males from females is by examining the genital organs on the underside of the cephalothorax. Examination of the genital organs will also allow the identification of non-mature males from females. Males have a pair of small projections (genital papillae) at the base of the fifth pair of pereiopods. Females have a pair of genital pores at the base of the third pair of pereiopods. Sexual maturation is generally reached between 6 and 8 months at temperatures above 22ºC. Males will deposit a spermatophore (sperm sack) of 0.8 to 1.0 cm in diameter on the underside of the female between the third and the fifth pair of pereiopods. Twelve to 24 hours after mating the females will break the spermatophore, spawn or release their eggs, and external fertilization will occur while the eggs are being released. Eggs attach to the female pleopods. After hatching, approximately 30 to 40 days later, juveniles will cling to the female for another 7 to 10 days (Masser and Rouse, 1997).

In subtropical regions, redclaw usually spawn from spring to mid-autumn, with two to three reproductive cycles per year (Jones, 1990, 1995a). In tropical regions, reproduction may occur all year round, with as many as five spawns per year (Sammy, 1988). Spawning sequences usually are spawn-molt-spawn and spawn-spawn-molt (Barki et al., 1997). Spawning is dependent on temperature and photoperiod, with optimals being 26 to 28ºC and 14 L:10 D, respectively (Jones 1990, 1995a; King 1994; Yeh and Rouse 1994). Continuous spawning can be achieved during the previous conditions, but females need a period of rest after about 6 months of continuous spawning (Yeh and Rouse, 1994).

Clutch sizes (counts of pleopodally-attached eggs) are directly correlated to female size. Females spawn around 10 eggs per gram of body weight (Rouse and Yeh, 1995). Although egg production is dependent on nutrition and environmental factors, under laboratory conditions, the amount of eggs attached to the pleopods prior to hatching usually ranges from 4.5 to 8.7/ g body weight of female (King 1993b; Yeh and Rouse 1994; Jones 1995a; Austin 1998). Hatching rates of the eggs have been reported to be around 95% (Rouse and Yeh, 1995).

Redclaw eggs go through several stages during their developmental period (Jones 1990, King 1993a). Yeh and Rouse (1994) and Jones (1995a) give a detailed description of the developmental stages. The duration of the various developmental stages depends to a large extent on water temperature. Hatching time can range from 30 days at 28ºC to 45 days at 24ºC (Masser and Rouse 1997). At hatching, juvenile size is not correlated to female size (Barki et al. 1997).

In commercial operations, density of broodstock in earthen ponds ranges from 0.5 to 2 per meter square. Male:female ratios in earthen ponds, as well as in laboratory conditions vary between 1:1 to 1:5 without any difference in percentage of berried females (Jones 1995a; Yeh and Rouse 1995; Barki and Karplus 2000).

Providing shelter during the mating season is important as it offers protection during periods of vulnerability when molting, protects the broodstock against predation, and minimizes aggressive interactions (Jones and Ruscoe 2001). Jones and Ruscoe (2001) reported that pipe stacks or similar shelter proved to be the best for increasing the percentage of berried females. Special attention needs to be given to berried females when handling, since eggs can be dislodged from the pleopods as females react with violent movements of their tail when threatened (Huner and Lindqvist 1991; Masser and Rouse 1997; Austin 1998).

It is very important to provide shelter to juveniles to maximize survival during the nursery stage. Juveniles are able to discriminate between different shelter types and display clear preferences. Mesh bundles have been proven to have the best success on the survival of *C. quadricarinatus* juveniles (Jones 1995a, 1995b; Parnes and Sagi 2002). Precise production information from these systems is not possible; however, survival generally ranges from 5 to 10% and the harvest weight of the juveniles is extremely variable (Jones, 1995a).

Redclaw grow-out is generally based on a 2- or 3-phase system. The 3-phase system consists of a first phase where reproduction ponds are stocked with brood size females and males (1 to 4 females per male) at a stocking density around 1 to 3 brooders per m^2. The females spawn and the eggs hatch in the reproduction ponds. Juveniles of approximately one gram are collected from shelters made primarily of small mesh plastic bags and stocked in nursery ponds (2nd phase) until they are approximately 5 to 12 grams in size. After this nursery phase, ponds are drained and the surviving juveniles are stocked in grow-out ponds (3rd phase) and raised until they reach commercial size. Grow-out ponds can be stocked with males or females only (monosex culture) or with both males and females (mixed sex culture). In the 2-phase system there is no nursery ponds and juveniles are collected from the shelters in the reproduction ponds (1st phase) when they are around 5 to 12 grams and then stocked directly into the grow-out ponds (2nd phase).

Producers in Latin America are currently using commercial shrimp feeds with a protein content that ranges from 23 to 35%, with fish meal as the protein source and complemented with forage at a rate of 100 to 150 kg/ ha/ wk. Forage is believed to be an important factor for the crayfish development.

Auburn University has been a major contributor of the industry development in several countries. In Mexico, which started its commercial culture in 1996 in the state of Tamaulipas, Auburn University scientists have worked with local governments and farmers to create joint programs that have improved redclaw yields from 800 kg/ha in 2001 to 3,200 kg/ha in 2006. Further increases in production rates under the current conditions are considered doubtful. Producers around the world need to make their process as cost effective as possible to continue facing the challenges of a highly competitive market. After a broad investigation and conversation with several producers worldwide, four main objectives were identified to confront these challenges:

1. Evaluate the production performance of redclaw crayfish receiving artificial pelleted feeds with different protein sources.

2. Evaluate the contribution of dry hay to the production variables for juvenile redclaw crayfish and its implications on the partial replacement of artificial feed.

3. Evaluate the effects of water volume vs. bottom surface, female and juvenile stocking density, and nursery period length for redclaw crayfish under current commercial hatchery conditions.

4. Evaluate the growth performance of the most widely cultured redclaw crayfish stocks in commercial aquaculture under pond and tank culture conditions.

These series of experiments were conducted to broaden the understanding in diverse aspects of the culture of *C. quadricarinatus* in an effort to further develop the redclaw industry.

CHAPTER II

EVALUATION OF PRACTICAL DIETS CONTAINING VARIOUS PROTEIN SOURCES FOR JUVENILE AUSTRALIAN RED CLAW CRAYFISH

Cherax quadricarinatus.

Introduction

Aquaculture of redclaw crayfish (*Cherax quadricarinatus*) is an activity that has developed rapidly and acquired a commercial relevance over the last two decades in tropical and sub-tropical regions of the world. In many countries where its culture is feasible, scientists have worked with local governments and farmers to create joint programs that have improved redclaw yields and reduce costs of production. Although there have been major advances, the evolving industry still faces major challenges including the development of species-specific feeds.

Feed cost accounts for a big percentage of total cost in aquaculture. Redclaw feed cost is no exception. World production of fishmeal has averaged 6,500,000 tons per year (Hardy, 2006). Demand for fishmeal continues to increase. Evaluating alternative sources of protein other than fishmeal is required.

Seventy percent of the shrimp consumed in the world by people come from aquaculture (FAO 2009). Considerable effort has been exerted toward reducing the cost of shrimp feeds. Information on shrimp nutrition may shed light on improved redclaw diets. The first step in reducing feed costs is identifying the most expensive components, typically protein (Akiyama, 1992; Forster and Dominy 2006), and then identify alternative sources that might be cheaper.

There have been several feed trials conducted to grow crayfish with different diets. Karplus et al. (1995) used a commercial carp pellet with 25% protein to hold animals in a recirculation system under laboratory conditions. Pinto and Rouse (1996) used a commercial crayfish pellet with 25% protein in ponds to produce between 1,020 and 1,422 kg/ha of animals ranging between 38 and 48 grams in a 158-day period. Jones and Ruscoe (2000, 2001) used a crayfish diet (Athmaize ™) containing 17% protein, to grow redclaw in ponds up to 60 g in a 140-day period. Barki et al. (2006) used a 42% protein extruded fish pellet diet to grow animals in individual containers in a recirculation system. All the previous experiments had above 60% survivals.

Various levels of protein have been evaluated by comparing growth, survival, body composition and dress-out percentage of redclaw (Manomaitis 2001; Thompson 2004, 2005, 2006). Rodriguez-Gonzalez et al. (2006) also compared effects of protein on spawning and egg quality. There is an interaction between lipid and protein levels as both dietary components have been analyzed in redclaw studies. Protein levels between 24% and 40% and lipid levels between 4% and 8% were adequate for the performance of redclaw in culture conditions (Manomaitis 2001; Hernandez-Vergara et. al 2003), and within ranges contained by commercial shrimp feeds. Producers in Latin America are currently using commercial shrimp feeds with a protein content that ranges from 23 to 35% with fishmeal as the protein source. The lipid content is usually 8%.

The use of plant proteins such as soybean meal (Lim and Dominy, 1990; Tidwell et al., 1993; Sudaryono et al. 1995), solvent-extracted cottonseed meal (Lim, 1996), various legumes (cowpea, green mungbean, rice bean) and leaf meals (Eusebio, 1991; Eusebio and Coloso, 1998), papaya or camote leaf meal (Penaflorida, 1995), and co-extruded soybean poultry by-product meal (Samocha 2004) have been evaluated as replacements of fish meal in crustacean feeds. Plant proteins, because of their low price and consistent quality, are often an economical and nutritious source of protein. However, due to potential problems associated with insufficient levels of indispensable amino acids (e.g. lysine and methionine), anti-nutrients and poor palatability, their use is often limited (Davis and Arnold 2000). Sources of terrestrial animal protein, which are primarily rendered by-products such as meat and bone meal (Tan et al. 2005) and poultry by-product meal (Markey 2007), and a combination of soybean and poultry by-product meal (Amaya 2007) have also been evaluated to replace fish meal as the main source of protein. Davis et al. (2004) demonstrated that fishmeal can be removed from shrimp formulations if suitable alternative sources of protein and lipids are provided to meet the nutritional requirements of the animal.

Information about the use of alternative protein sources as replacements of fishmeal in *C. quadricarinatus* diets is limited. It has been reported that fish meal can be replaced by a number of plant proteins (e.g. brewer's grains with yeast and soybean meal) without detrimental effects on redclaw survival and growth (Webster et al., 2002, Muzinic et al., 2004, Thompson et al., 2005, 2006) and by poultry by-product meal (Saoud et al. 2008). The following study was designed to evaluate four soybean-based diets containing either fishmeal, poultry by-product meal, ground pea meal or distiller's dried grains with solubles as a protein source.

Materials and Methods

The research was conducted at the AGY Redclaw Hatchery, property of Megar S.A. de C.V. in Soto La Marina, Tamaulipas, Mexico. Juvenile redclaw crayfish *C. quadricarinatus* released during a 48-hr period were collected and placed in an indoor nursery tank. Juveniles were then harvested, weighed individually, eliminating large and small outliers, separated into groups of thirty six individuals and stocked into 20 rectangular tanks (2.4 x 1.2 x 0.1 m, 288 L volume, 2.88 m^2 bottom area) (12.5 m^{-2}). Average initial weight of the juvenile redclaw was 0.125 g ± 0.025 at stocking. Each tank received water continuously from an outside reservoir at approximately 20 L hr^{-1} for the duration of the experiment. Each tank contained 36 pieces of 5-cm diameter PVC pipe used for refuge and four submerged air diffusers for aeration and water mixing. Juvenile crayfish in four replicate tanks were randomly assigned to one of four practical diets. Test diets were soybean-based diets containing approximately 36% total protein. Diets contained either 10% of poultry by-product meal (Diet 1), fish meal (Diet 2), distiller's dried grains with solubles (Diet 3), or ground pea meal (Diet 4) (Table 1). The dietary treatments were formulated to provide equal protein and lipid levels while maintaining a minimum lysine and methionine plus cystine content of 5% and 3% of the total protein, respectively (Table 2). A full amino acid profile is presented in Table 3 for the four diets. A fifth treatment, which did not receive feed, was included to estimate growth from natural productivity.

Based on previous work, mean weights of the crayfish would be assumed to double during the first three weeks and, thereafter, have a weekly growth rate of 1 g/week. Based on the expected growth rates and a feed conversion of 1.5, a feeding table was built for the 8-week growth trial (Table 4). Daily ration was weighed every afternoon and offered to the crayfish in the evening (~1600 h). All practical diets were produced by Rangen® Inc. (Angleton, TX).

Dissolved oxygen (DO) concentrations and temperature were measured twice daily using a YSI 55 DO meter (Yellow Spring Instrument Co., Yellow Springs OH, USA). Ammonia was measured bi-weekly using a LaMotte® Freshwater Aquaculture kit (LaMotte Company, Chestertown MD, USA) (Nessler method).

At the conclusion of the growth trial crayfish were counted and individually weighed. Mean final individual weight, final biomass, food conversion ratio (FCR), and percent survival were calculated.

Statistical Analysis

Data from the feeding trial was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test to determine significant differences ($P<0.05$) among treatment means. All statistical analyses were performed using MINITAB software (version 15.1, MINITAB Inc., State College, Pennsylvania).

Results

Over the course of the 8-week growth trial water parameters were (mean ±SD): water temperature 27.8±0.8ºC; dissolved oxygen 6.3±0.5 mg/l; and total ammonia nitrogen (TAN) 0.1±0.1. Water quality conditions were consistent among experimental units. All parameters were within acceptable limits for indoor production of redclaw crayfish (Masser and Rouse 1997).

Analysis of variance was performed to assure that there were no significant differences in initial weight among groups. No significant differences were found among the mean initial stocking weights ($P > 0.05$).

Crayfish were counted and individually weighed at the conclusion of the growth trial. Weight gain, food conversion ratio (FCR), and percent survival were determined. Crayfish fed with one of the four test diets had mean final weights that ranged from 3.84 to 4.98 g/animal, average final biomass ranged from 119.3 to 158 g/tank, mean FCRs ranged from 2.10 to 2.70 and mean survival rates ranged between 86 to 90%. There was no significant difference among these treatments. There was a significant difference in final weight, final biomass and FCR between the fifth treatment that did not receive feed input and the four treatments that did receive feed ($P < 0.05$). Survival between the five treatments was not different (Table 5).

Discussion

Although it is difficult to compare growth rates, survivals and FCRs across different years, production systems and facilities, there is value in comparing data from other researchers using similar protein levels and sources of protein when crayfish are stocked at similar sizes. The results of this experiment were better than the 0.46 g reported by Jones (1995) in tanks at a high stocking density of $100/m^2$ in a 39-day period who used a combination of flake diet (40% protein) and zooplankton. Results were comparable to the ones reported by Manomaitis (2001) who individually stocked sixty

0.07 grams crayfish in a semi-recirculating system for a 7-week period providing a 42% protein pelleted feed to satiation and reported final individual weight of 2.65 g and survivals that ranged between 53 and 100%.. Results were comparable to the ones obtained by Hernandez-Vergara et al. (2003) stocking 12.5/m^2 in a flow through tanks system with a mean initial weigh of 4.2 grams, providing diets with 30% protein and a minimum lipid level of 4.2% resulting in an average growth rate of 1.96 g/wk in a 12 week growth trial. The result as well are comparable to the ones of Saoud et al. (2008), who in the same system as Hernandez-Vergara et al. (2003), fed various levels of fish meal and poultry by-product meal at 15% protein level and reported growth rates of around 11 grams starting with a 0.45 g animal in a 12-week growth trial with survivals above 83%. Results were also consistent results of previous trials at this site (Nabor Medina Vazquez, Megar S.A. de C.V. General Manager, Personal communication) and fed with a 30% protein commercial diet with fishmeal as the primary protein source.

Although fishmeal has high levels of available energy, exceptional amino acid profiles, is very digestible and generates excellent attractability and palatability, it is one of the most expensive ingredients in prepared fish and shrimp diets. Natural events also affect the supply of fishmeal, which affects the price. The predictions for its future availability are uncertain (Hardy 2006). Therefore, replacement of fishmeal diets for formulations that have ingredients different from the ones derived from the marine environment must be of high priority to ensure the development of sustainable and profitable aquacultural industries (Jones et al. 1996). Marine protein sources may be replaced by various terrestrial and animal sources.

Soybean meal (SBM) has been the most thoroughly evaluated and most frequently used replacement in commercial aquaculture diets. Several attempts have been successful in partially or totally replacing fishmeal (FM) with SBM in practical diets for *Litopenaeus vannamei* (Lim and Dominy 1990; Davis and Arnold 2000; Samocha 2004), *Penaeus monodon* (Sudaryono et al. 1995; Piedad-Pascual et al. 1993) and *Macrobrachium rosenbergii* (Tidwell et al. 1993). Piedad-Pascual et al. (1993) found no significant differences in weight gain of tiger shrimp fed different levels of SBM (up to 55% SBM) when completely replacing FM. Tidwell et al (1993) stated that variable percentages of SBM (25%, 15% and 26.5%) and 40% of distiller's dried grains with solubles (DDGS) partially or completely replaced FM in the diets of freshwater prawns (*M. rosenbergii*) grown in ponds. With *C. quadricarinatus*, Muzinic et al. (2004) in tanks and Thompson et al. (2006) in ponds reported success in raising juvenile redclaw with no adverse effects on growth or survival when FM was totally removed from diets containing protein levels ranging between 18 and 40% .

As more than one source of protein is recommended to formulate a practical feed to enrich the amino acid profile, various protein sources have been analyzed in combination with SBM as possible substitutes. One of the most important substitutes has been poultry by-product meal (PBM), because of its availability and low cost. A combination of co-extruded soybean and poultry by-product meal was used to replace FM in *L. vannamei* diets by Samocha (2004). Davis and Arnold (2000) also used PBM without any SBM for the replacement of FM in *L. vannamei* diets. However, it should be mentioned that Davis and Arnold (2000) did not test diets with 100% replacement of FM with PBM. Markey (2007), although trying to replace PBM for another source of plant protein, had excellent results in growth, final biomass and survival of *Litopenaeus vannamei* cultured under semi-intensive conditions and fed various combinations of SBM and PBM. Saoud et al. (2008) had no adverse effects on production parameters when partially or totally replacing FM with PBM in practical diets for *C. quadricarinatus*.

Pea meal has long been used in livestock feeds as a source of energy but has only recently been evaluated in feeds for aquatic species. Although data is limited, feed peas have been evaluated in diets for *Penaeus monodon* (Smith et al 1999, Bautista-Tereul et al 2003), *L. stylirostris* (Cruz-Suarez et al. 2001) and *L. vannamei* (Davis et al. 2002). Davis et al. 2002 concluded that when properly processed (extruded or micronized), pea meal is highly digestible and there appears to be no adverse effects on *L. vannamei* growth, survival or feed efficiency results for values tested between 5 to 20% of the total dry weight of the feed. Bautista-Tereul et al. (2003) concluded that feed peas could be included at a level of up to 42% in practical diets without any adverse effects on growth, feed intake, FCR or survival of juvenile *P. monodon*.

Distiller's dried grains with solubles (DDGS) have been analyzed recently as another source of energy for crustaceans. Tidwell et al. (1993) concluded that growth, survival, and pond yield of freshwater prawns (*M. rosenbergii*) were unaffected by either 50% or 100% replacement of FM with SBM and DDGS. Thompson et al. (2006) conducted experiments completely replacing FM with DDGS with no significant differences in FCR, survival, and total yield on *C. quadricarinatus*. The utilization of DDGS is an interesting and suitable option for replacing FM, as its availability might tend to increase if the use of corn for ethanol production becomes a major practice.

Roy et al. (2009) used similar diets as used in this study on *L. vannamei* reared in low salinity waters of west Alabama without significant differences in shrimp growth, weight gain, survival or FCR. The results of this study and that of Roy (2009) support the statements of Davis et al. (2004) that fishmeal can be removed from shrimp formulations if suitable alternative sources of protein and lipids are provided to meet the nutritional requirements of the animal.

The apparent cost reduction generated by replacing FM for other ingredient should be evaluated. Cost and quality of substituting FM for any other protein source will vary over time and location. Different locations have different ingredient availability, quality and cost. Dependability in the supply and quality should be considered in the same way cost is. Local feed makers might have different options for regional producers; so, more than one source of protein for replacing FM should be evaluated.

In addition, while PBM in combination with soybean is a plant-animal energy choice for replacement of FM, pea meal and DDGS are all-plant options. Emerging environmental and safety issues related to contaminated animal by-products have been a concern to the industry. The development of all-plant protein diets could also provide a niche market for crustacean producers, as some segments of the market are willing to pay a higher price for organisms produced without the use of proteins made from animal by-products (Samocha et al. 2004).

Results from this study confirm that fishmeal can be removed from diets for redclaw juveniles utilizing a plant-animal protein combination (PBM and SBM) or all-plant protein alternatives such as pea meal and DDGS. As well, the results of this study demonstrate four ways to produce diets that could be applied to various scenarios of cost and availability of ingredients, without having any negative effect on the production parameters of *C. quadricarinatus*.

Table 1. Ingredient composition (g/100g dry weight) of four practical diets utilizing soybean-based diets containing 10% poultry by-product meal, fishmeal, distiller's dried grain with solubles, or ground pea meal as a protein source.

Ingredient	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Soybean meal	55.12	53.71	58.01	58.00
Milo	24.81	26.19	16.34	15.33
Poultry by-product	9.99	-	-	-
Menhaden select	-	10.01	-	-
Distillers grains	-	-	10.00	-
Peas, ground	-	-	-	10.00
Corn gluten yellow	-	-	4.83	4.83
Dicalcium phosphate	2.90	2.90	3.38	3.42
Fish oil	5.08	5.09	48.3	58.2
Bentonite	1.50	1.50	1.50	1.50
Squid meal	-	-	0.50	0.50
Vitamin premix*	0.33	0.33	0.33	0.33
Mould inhibitor	0.15	0.15	0.15	0.15
Mineral premix*	0.08	0.09	0.09	0.09
Stay-c 35% (c)	0.02	0.02	0.02	0.02
Copper sulfate	0.01	0.01	0.01	0.01
Total	100	100	100	100

Diets were formulated to contain 36% protein and 8% lipid.

Diets were commercially manufactured by Rangen® (Angleton, TX, USA) using extrusion processing.

*Vitamin premix and mineral premix are proprietary products, thus their composition is not listed.

Table 2 Nutritional composition of four practical diets utilizing soybean-based diets containing 10% poultry by-product meal, fish meal, distiller's dried grain with solubles, or ground pea meal as protein sources.

Ingredient (%)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Moisture	8.85	8.11	8.43	8.39
Protein	35.90	35.50	38.20	36.10
Fat	8.16	8.26	7.66	7.41
Fiber	1.78	1.77	1.95	2.39
Ash	8.40	9.59	6.68	7.90

Diets were formulated to contain 36% protein and 8% lipid.

Diets were commercially manufactured by Rangen® (Angleton, TX, USA) using extrusion processing.

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ, USA.

Table 3 Amino acid profile (g/100g dry weight) of four practical diets utilizing soybean-based diets containing 10% of poultry by-product meal, fishmeal, distiller's dried grain with solubles, or ground pea meal as a protein source.

	Percent as is			
Amino Acid	Diet 1	Diet 2	Diet 3	Diet 4
Methionine	0.54	0.61	0.62	0.6
Cystine	0.53	0.54	0.62	0.61
Lysine	2.19	2.08	2.18	2.07
Phenylalanine	1.52	1.63	1.71	1.72
Leucine	2.65	2.78	2.91	2.99
Isoleucine	1.47	1.54	1.53	1.53
Threonine	1.31	1.33	1.35	1.34
Valine	1.27	1.33	1.3	1.29
Histidine	0.81	0.87	0.9	0.88
Arginine	2.42	2.41	2.54	2.51
Glycine	1.71	1.91	1.7	1.56
Aspartic Acid	3.61	3.8	3.9	3.83
Serine	1.72	1.72	1.82	1.86
Glutamic Acid	6.15	6.57	7.40	7.00
Proline	2.03	1.84	2.06	2.06
Hydroxyproline	0.24	0.15	0.15	0.07
Alanine	1.91	1.82	1.86	1.75
Tyrosine	1.08	1.1	1.2	1.21
Total	33.16	34.03	35.75	34.88

Diets were analyzed by New Jersey Feed Laboratory, Trenton, NJ, USA.

Table 4. Feed calculation for redclaw crayfish grown in flow through tanks receiving reservoir water, assuming a doubling in size during the first three weeks and thereafter a weekly growth rate of 1 g $week^{-1}$.

Juveniles per tank	36
Forecast growth wk 1. (g/wk)	0.125
Forecast growth wk 2. (g/wk)	0.25
Forecast growth wk 3. (g/wk)	0.5
Forecast growth wk 4-8 (g/wk)	1
Total forecast growth (8 weeks) (g)	5.9
Forecast FCR	1.5
Total feed (g)/individual	8.8
Total feed (g)/tank	317.3

Table 5. Mean production parameters for juvenile redclaw, *Cherax quadricarinatus*, stocked at an initial weight of 0.125±0.025 g and cultured for 8 weeks, fed four practical diets utilizing soybean-based diets containing 10% poultry by-product meal, fish meal, distiller's dried grain with solubles, or ground pea meal as a protein source and a negative control with no feed input during the growth trial.

Parameters[1]	Diet 1	Diet 2	Diet 3	Diet 4	No feed	PSE[2]	P Value
Final weight (g)	4.04^{a}	3.85^{a}	4.20^{a}	4.98^{a}	0.54^{b}	0.406	0.001
Growth (%)	3232.5^{a}	3076.2^{a}	3356.4^{a}	3987.1^{a}	428.4^{b}	325.0	0.001
Growth rate (g/day)	0.0699^{a}	0.0664^{a}	0.0727^{a}	0.0868^{a}	0.0073^{b}	0.0072	0.001
Final biomass (g/tank)	132.3^{a}	119.3^{a}	135.1^{a}	158.0^{a}	15.1^{b}	13.295	0.001
FCR[3]	2.51^{a}	2.71^{a}	2.40^{a}	2.10^{a}	0.00^{b}	0.222	0.001
Survival (%)	90.3	86.1	90.3	88.2	75.0	0.049	0.200

[1] Means within the same row with different superscripts are significantly different ($P<0.05$).

[2] Pooled standard error of treatment means. (n=4)

[3] Feed conversion ratio = Total feed offered / biomass increase.

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CHAPTER III

EVALUATION OF THE CONTRIBUTION OF DRY STARGRASS HAY (*Cynodon nlemfuensis*) TO THE GROWTH, SURVIVAL AND YIELD OF JUVENILE AUSTRALIAN RECLAW CRAYFISH (*Cherax quadricarinatus*) AND ITS IMPLICATIONS ON THE PARTIAL REPLACEMENT OF ARTIFICIAL FEED

Introduction

Crayfish aquaculture has depended on forage as a major food source in many parts of the world. Planted forages, mainly rice and to a less extent hay, have been used in the southern United States to support a detritus-based system that has proven to be reliable and cost effective for the nourishment and production of the red swamp crayfish (*Procambarus clarkii*) (Avault and Bronson, 1990; McClain et al., 1998). In Australia, the use of forage has been a common practice for the extensive and semi-intensive farms of the *Cherax* spp., with or without the use of artificial diets (O'Sullivan, 1992, Geddes and Smallridge, 1993). In Latin America, the commercial farms of redclaw (*Cherax quadricarinatus*) complement their feeds by adding around 500 kilograms of hay, usually stargrass (*Cynodon nlemfuensis*), per month as recommended by Masser and Rouse (1997).

Red swamp crayfish aquaculture is the largest commercial crayfish culture in the world. Ponds are usually stocked between April and May and drained over a 2- to 4-week period during May or June. Forage, usually rice, is planted between June and August while the crayfish live in borrows. Ponds are reflooded in October, which encourages adult and recently hatched juvenile crayfish to come out of their borrows. Harvest usually starts in November and lasts until May or June of the following year. Although some have recommended the use of artificial feeds to increase yields (Huner et al., 1975; McClain et al., 1992a, 1992b), the general practice still depends primarily on forage and the detritus-based system generated by its decomposition as the main source of food for red swamp crayfish.

Redclaw culture is generally based on a 2- or 3-phase system. The 3-phase system consists of a first phase where reproduction ponds are stocked with brood size females and males (1 to 4 females per male) at a stocking density around 1 to 3 brooders per m^2. The females spawn and the eggs hatch in the reproduction ponds. Juveniles of approximately one gram are collected from shelters made primary of small mesh netting and stocked in nursery ponds (2nd phase) until they are approximately 5 to 12 grams in size. After this nursery phase, ponds are drained and the surviving juveniles are stocked in grow-out ponds (3rd phase) and raised until they reach commercial size. Grow-out ponds can be stocked with males or females only (monosex culture) or with both males and females (mixed sex culture). In the 2-phase system, there is no nursery ponds and juveniles are collected from the shelters in the reproduction ponds (1st phase) when they are around 5 to 12 grams and then stocked directly into the grow-out ponds (2nd phase). All ponds are usually fed with pelleted diets and complemented with forage, generally dry stargrass hay (*C. nlemfuensis*), at a rate of 100 to 150 kg/ ha/ wk. Forage is believed to be an important factor for the crayfish development.

In both cases, juvenile crayfish are exposed to the same conditions as adults. Stomach content analyses from various Astacid and Cambarid species indicate that selective feeding occurs and that the diet changes during ontogeny. The relative proportion of plant and animal material taken as food is age (or size) related, e.g. juveniles may require more animal based food than adults (Goddard 1988; Jones 1990).

Loya-Javellana et al. (1993) found that decayed plant material in addition with their associated organisms is primary the food source chosen by the young and subadults of *C. quadricarinatus,* if given the opportunity to choose between decayed plants and zooplankton. However, zooplankton was as readily acceptable as the decayed plant to the early independent young if both foods were offered simultaneously. Their results imply that redclaw are primarily a detritivore but will feed opportunistically during free-living stages.

Several efforts have been made to find the adequate diets for juveniles of different crayfish species. Huner et al. (1975) offered an extruded, water-stable diet to red swamp crayfish (*P. clarkii*). Morrissy (1984) tested a variety of commercial crustacean feeds for juvenile marron (*C. tenuimanus*). Celada et al. (1989) evaluated several fresh and artificial diets for signal crayfish (*Pacifastacus leniusculus*). McClain et al. (1992a, 1992b) tested detrital rice, detrital hay and an artificial diet (Crawdeaux™) on juvenile red swamp. Jones et al. (1995) tried pelleted diets with different levels of protein (15, 30 and 52%) in combination with *Daphnia* spp. to feed yabbie crayfish (*C. albidus-destructor*). Gonzalez et al. (2008) tested *Artemia* spp. nauplii and two commercial replacements for juvenile signal crayfish (*P. leniusculus*).

For *C. quadricarinatus* juveniles, Jones (1995) evaluated fresh zooplankton and a high protein artificial flake diet in the presence and absence of a floating aquatic macrophyte (*Pistia stratoide*). As well, several trials have been conducted to raise juveniles in tanks with artificial diets only (Manomaitis 2001, Muzinic et al. 2004; Thompson et al. 2005; Saoud et al. 2008).

As the industry has developed, demand for quality juveniles has increased. To meet this demand, specific juvenile production technologies have been developed. The adequate feeding strategies to maximize growth, survival and yield of juveniles raised in ponds and hatcheries are of vital importance to the industry. In addition, with the objective to reduce production costs, semi-intensive redclaw farmers have shown interest in supplementing pelleted feed with the addition of forage in production ponds. The following study was designed to evaluate the contribution of dry stargrass hay (*Cy. nlemfuensis*) to the growth, survival and yield of juvenile redclaw crayfish (*C. quadricarinatus*) and its implications on the partial replacement of artificial feed.

Materials and Methods

The research was conducted at the AGY Redclaw Hatchery, property of Megar S.A. de C.V. in Soto La Marina, Tamaulipas, Mexico. Juvenile redclaw crayfish, *Cherax quadricarinatus*, released during a 48-hr period were collected and placed in an indoor nursery tank. Juveniles were then harvested manually, size sorted eliminating large and small outliers, separated into groups of thirty six individuals and stocked into 20 rectangular tanks (2.4 x 1.2 x 0.1 m, 288 L volume, 2.88 m^2 bottom area) (12.5 m^{-2}). Average initial weight was 0.125 g ± 0.025 at stocking. Each tank received water from an outside reservoir at approximately 20 L hr^{-1} for the duration of the experiment. Each tank contained 36 pieces of 5-cm diameter PVC pipe used for refuge and four submerged air diffusers for aeration and water mixing. Juvenile crayfish in four replicate tanks were randomly assigned to one of seven experimental treatments. Based on previous experience in the hatchery, mean weights of the crayfish were assumed to double during the first three weeks and thereafter have a weekly growth rate of 1.0 g $week^{-1}$. Based on the expected growth rates and a feed conversion of 1.5, a full (100%) feed ration was calculated for the treatments. Treatment A received only artificial feed input; the nutritional composition of the artificial feed is presented in Table 1. Treatment B received artificial feed produced by Rangen® Inc. (Angleton, TX) and hay at a rate of 150 kg/wk/ha. Treatments C, D and E received the same amount of hay but only 75, 50 and 25 percent of the full feed ration. Treatment F received only hay and Treatment G did not received any feed input during the length of the 8-week growth trial. Daily artificial feed rations were weighed every afternoon and offered to the crayfish in the evening (~1600 h). Dry hay was offered once a week at the start of every week. The feed input is shown in Table 2.

Dissolved oxygen (DO) concentrations and temperature were measured twice daily using a YSI 55 DO meter (Yellow Spring Instrument Co., Yellow Springs OH, USA). Ammonia was measured bi-weekly using a LaMotte® Freshwater Aquaculture kit (LaMotte Company, Chestertown MD, USA) (Nessler method).

At the conclusion of the growth trial crayfish were counted and individually weighed. Mean food conversion ratio (FCR), final individual weight and percent survival were calculated.

Statistical analysis

Data from the feeding trial was analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple range test to determine significant differences ($P<0.05$) among treatment means. Statistical analysis was performed using MINITAB software (version 15.1, MINITAB Inc., State College, Pennsylvania).

Results

Over the course of the 8-week growth trial water parameters were (mean ±SD): water temperature 27.8 ± 0.8 ºC; dissolved oxygen 5.8 ± 0.7 mg/l; and total ammonia nitrogen (TAN) 0.3 ± 0.1. These water quality conditions were within acceptable limits for indoor production of redclaw crayfish (Masser and Rouse 1997).

Analysis of variance was performed to assure there were no significant differences in mean initial weight among groups. No significant differences were found between the initial stocking weights.

Mean survival rate ranged between 70 to 90% (Table 3). There was no difference between any of the seven treatments. Final mean individual weight for the different treatments ranged from 0.53 to 5.11 g/ animal (Table 3). Average yield ranged from 5.05 to 57.67 g/m^2. There was a significant difference in final weight and yield among the treatments that receive feed input (A, B, C, D and E; final weight range from 4.01 to 5.11g; yield range from 41.50 to 57.67 g/m^2) and treatments F and G that only received hay and no input at all ($P < 0.05$). There was no significant difference among treatments that received feed input. There was also no significant difference between the treatment receiving only dry hay and the treatment receiving no feed input when compared to each other.

Mean FCR was calculated for the treatments receiving feed input only. Mean FCRs ranged from 0.5 to 2.5 (Table 3). There was a difference between treatments ($P < 0.05$). Treatment A was similar to treatments B, C and D, but significantly different from treatment E. Treatment B was similar to treatment C and D but significantly different to treatment E. There was no difference between treatments C, D and E.

Discussion

Growth and survival of redclaw in fed treatments (A to E) can be considered comparable to other trials with juvenile *C. quadricarinatus* fed artificial pelleted feeds (Manomaitis 2001; Hernandez-Vergara et al. 2003; Muzinic et al. 2004; Thompson et al. 2005; Saoud et al. 2008) with protein levels between 23% and 40% and with lipid levels between 4% and 8%.; and with previous trials conducted at Megar S.A. de C.V. (Nabor Medina Vazquez, Megar S.A. de C.V. General Manager, Personal communication) where juveniles were fed with a 30% protein, 8% lipid commercial diet. The results are also comparable with Jones (1995) who in recirculating tanks used a combination of flake feed (40% protein) and zooplankton. The treatments receiving dry hay only (F) and the treatment with no feed input (G) had considerably less growth, although their survival was comparable to the previous studies. The mortalities of the unfed group (Treatment G) were expected to be higher than the actual results due to cannibalism but similar with other *Cherax* spp. experiments. Morrissy (1984) reported up to 100% survival in tanks on an unfed control group over 100 days for *C. tenuimanus*.

FCRs in this experiment ranged between 0.52 and 2.51. Manomaitis (2001) reported FCRs that ranged from 1.14 to 1.61; Hernandez-Vergara et al. (2003) FCRs were between 1.26 and 1.41. Muzinic et al. (2004) failed to report FCRs as the system was cleaned by siphoning every other day to remove uneaten feed. Thompson et al. (2005) reported FCRs between 3.03 and 5.73. Saoud et al. (2008) had FCR results between 1.56 and 1.75. Although it is difficult to compare the FCR results of this experiment with others, it can be considered that Treatments A, B, C, and D had similar results to Manomaitis (2001), Hernandez-Vergara et al. (2003) and Saoud et al. (2008), and better than Thompson et al. (2005). FCR for treatment E was considerably lower than all the previous studies.

Similar results of growth with artificial feed or forage have been obtained with *Procambarus clarkii*. McClain et al. (1992a) evaluated the utilization by juvenile crayfish *P. clarkii* of green and degraded rice (13% protein, 2% lipid) as sole food sources in comparison to a commercially prepared diet (Dupont's Crawdeaux™ 31% protein, 13.8% lipid). Mean final weight of the animals fed with forage was significantly inferior (0.487 g) to the crayfish fed with the commercial feed (2.796 g). In another set of experiments with artificial diets, the final weight of *P. clarkii* was increased (6.908 g) if fed twice a day compared to only feeding once per day (4.915 g), both superior to the average of just feeding forage in different stages of decomposition (0.967 g). In the present experiment stargrass (*Cy. nlemfuensis*) was used as forage. The protein (10.85%) and lipid (1.45%) contents (Maya et al. 2005) are similar to those of the rice used by McClain et al. (1992a).

As presented by Loya-Javellana et al. (1993), juvenile redclaw spend similar amounts of time feeding on plant material and zooplankton. Several previous studies have indicated that decayed plant material plus zooplankton are important for juvenile crayfish (D'Abramo et al. 1985; Celada et al. 1989; Brown et al. 1992; McClain et al. 1992b). Zooplankton has been preferred over artificial feed by some authors, as juvenile crayfish diets (Jones 1995; Jones et al. 1995; Gonzalez et al. 2008). Water exchange throughout the experiment was approximately 166% per day. Water received from an outside reservoir was filtered with a 0.1 cm mesh filter on the intake, located 20 centimeters from the top of the water level, permitting phytoplankton and zooplankton to flow into the system. It is important to comment that even though zooplankton was not part of the treatment and no samples of it were taken, the contribution to the daily provision may be relevant. The combination of zooplankton and dry hay (Treatment F) would have been expected to give better results than the treatment that did not received any feed (G) and only accounted for natural productivity, but the contribution of nitrogen and phosphorous by the dry hay might have not been enough to increase natural productivity with the conditions of the experiment. The results of this experiment indicate that artificial feed or a combination of artificial feed and natural food generates the best performance in growth of juvenile redclaw crayfish. The results agree with Kondos (1990) who stated that to achieve the maximum levels of production of redclaw, it is imperative to supplement the natural food resources found in ponds with artificial feed.

The contribution of artificial feed to natural productivity is something that needs to be evaluated. The daily inputs of nitrogen and phosphorous contained in artificial feed, can be significant to promote natural productivity in culture water. Nevertheless, in this study with approximate 166% daily water exchange, the contribution from dissolved nitrogen and phosphorus might not be as significant as culture conditions where water is just added to compensate for losses due to evapotranspiration and seepage. Although, the results in treatment E (Mean FCR = 0.52) suggest that the contribution of a small amount of artificial feed (25%) to natural productivity is measurable, further studies to evaluate this contribution should be conducted.

Protein and lipid contents of artificial feed, as well as the amino acid profile in the feed, are probably the most important factors that influence growth of juvenile crayfish. Rice forage (13% protein, 2% lipid) (McClain et al. 1992a) and stargrass forage (10.85% protein, 1.45% lipid) (Maya et al. 2005) do not have the minimum protein levels (23% protein, 4% lipid) (Thompson et al. 2004; Hernandez-Vergara et al. 2003; respectively) recommended for the development of juvenile redclaw crayfish. Therefore, in aquaculture ponds with higher densities, neither rice nor stargrass forage are a complete diet for the redclaw juveniles, even when they are complemented by naturally produced zooplankton available in pond conditions. Most likely, crayfish in Treatment F fed with dry hay only did not receive adequate nutrition. In consequence, average weight at harvest was significantly inferior to all fed treatments.

The addition of aquatic plants in combination with artificial feed for raising juvenile crayfish has been previously documented. Celada et al. (1989) found that fresh aquatic plant material, when added to pelleted diets for juvenile signal crayfish, *Pacifastacus leniusculus*, improved growth and showed slight increases in survival. Jones (1995) on the other hand did not recommend the use of aquatic macrophytes for intensive juvenile production of *C. quadricarinatus*.

In grow-out pond conditions, Pinto and Rouse (1996) obtained good growth and survival with a FCR of 1.39 when using artificial feed (25% protein) with 500 kg/ha/month of a combination of hay and corn silage. In recent studies, Metts et al. (2007) achieved similar results (53 to 62 g average) when using artificial feed (13% protein) with 500 kg/h/month of dry alfalfa hay. Although Metts et al. (2007) used a reduced protein feed, she failed to report feed input or FCRs in her study and therefore the actual benefit is difficult to access.

All fed treatments in the present study had similar results in growth, yield and survival, but not FCR. Extrapolating the current results of Treatment E to production conditions, feeding a high quality artificial feed (35% protein and 7.1% lipid), and using a forecasted FCR of 0.52 in combination with 125 kg/ha/wk of dry stargrass hay could result in equivalent growth and survival rates to the ones currently obtained by producers in Latin America using a 1.5 forecasted FCR with or without the addition of the same amount of hay. This could imply a reduction in feed expenses of around 64% to the producers that are currently applying hay and of 45% to the producers that do not apply hay (Table 4).

The addition of forage to ponds or tanks can generate low dissolved oxygen and increase ammonia concentrations. Further studies, in tanks and ponds are needed to analyze the optimal amount of artificial feed inputs in combination with an adequate amount of forage that will result in the minimal FCR that still generates adequate growth and survival. It would be useful to determine how little feed would be needed when forage is not used, to still maintain good growth and survival. Unpublished data suggest that an FCR around 0.8 using an artificial pelleted feed with 23% protein can be achieved in juvenile redclaw ponds in similar conditions as the present study without any negative effect on growth or survival. The addition of forage is a common practice yet there is little data on the efficacy of this practice.

The results of the present study suggest that the addition of stargrass (*Cy. nlemfuensis*) at a rate of 125 kg/ha/wk in combination with a properly balanced artificial feed for juvenile redclaw (*C. quadricarinatus*) can reduce the current feed inputs and thereby reduce cost for redclaw producers.

Table 1 Nutritional composition of artificial feed offered to juvenile redclaw crayfish, *Cherax quadricarinatus*, stocked at an initial weight of 0.125±0.025 g and maintained for eight weeks in flow through tanks receiving reservoir water.

Ingredient (%)	Treatment diet
Moisture	8.11
Protein	35.50
Fat	8.26
Fiber	1.77
Ash	9.59

Diets were formulated to contain 36% protein and 8% lipid.

Diets were commercially manufactured by Rangen® (Angleton, TX, USA) using extrusion processing.

Table 2. Feed calculation for treatments assuming a doubling in size during the first three weeks and thereafter a weekly growth rate of 1 g week^{-1} for juvenile redclaw crayfish, *Cherax quadricarinatus*, stocked at an initial weight of 0.125±0.025 g and maintained for eight weeks in flow through tanks receiving reservoir water.

Treatment	A	B	C	D	E	F	G
Artificial feed	100%	100%	75%	50%	25%	0%	0%
HAY	NO	YES	YES	YES	YES	YES	NO
Juveniles per tank	36	36	36	36	36	36	36
Forecast growth wk 1. (g/wk)	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Forecast growth wk 2. (g/wk)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Forecast growth wk 3. (g/wk)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Forecast growth wk 4-8 (g/wk)	1	1	1	1	1	1	1
Total forecast growth (8 weeks) (g)	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Forecast FCR	1.5	1.5	1.125	0.75	.375	NA	NA
Total feed/ individual (g)	8.8	8.8	6.6	4.4	2.2	0	0
Total feed/tank (g)	317.3	317.3	237.9	158.6	79.3	0	0
Hay/tank (g/wk)	0	43.2	4.32	4.32	4.32	4.32	0
Total hay/tank (g)	0	345.6	345.6	345.6	345.6	345.6	0

Table 3. Mean production parameters for juvenile redclaw, *Cherax quadricarinatus*, stocked at an initial weight of 0.125±0.025 g, cultured in flow through tanks receiving reservoir water and fed different levels of artificial feed and/or dry hay for 8 weeks.

Treatment [1]	Final weight (g)	Growth rate (g/wk)	Survival (%)	Yield (g/m^2)	FCR [3]
A (100[4]%)	4.04[a]	0.49[a]	90.3	45.60[a]	2.51[a]
B (100% + hay)	5.11[a]	0.62[a]	90.3	57.67[a]	1.92[a]
C (75% + hay)	4.26[a]	0.52[a]	88.9	47.29[a]	1.76[a,b]
D (50% + hay)	4.73[a]	0.58[a]	70.1	41.50[a]	1.70[a,b]
E (25% + hay)	5.08[a]	0.62[a]	86.1	54.72[a]	0.52[b]
F (hay)	0.91[b]	0.10[b]	77.1	8.74[b]	NA
G (No feed)	0.54[b]	0.05[b]	75.0	5.02[b]	NA
PSE [2]	0.373	0.047	5.980	5.000	0.324
P Value	0.001	0.001	0.124	0.001	0.009

[1] Means within the same column with different superscripts are significantly different ($P<0.05$).

[2] Pooled standard error of treatment means (n=4).

[3] Feed conversion ratio = Total artificial feed offered / biomass increase. Analysis of variance was performed on the fed treatments only.

[4] Percentage of artificial diet fed one time daily.

Table 4. Forecast total feed cost (ha/wk) of a commercial redclaw farm in Latin America stocked at $6/m^2$, with a forecast average growth of 2 g/wk and fed using different forecasted FCR with or without the addition of stargrass forage at a rate of 125 kg/ha/wk.

Forecast FCR	1.5	1.5	1	0.75	0.52
Use of hay	No	Yes	Yes	Yes	Yes
Forecast growth (g/wk)[2]	2	2	2	2	2
Density (org/m2)	6	6	6	6	6
Artificial feed (kg/ha/wk)	180	180	120	90	62.4
Stargrass hay (kg/ha/wk)	0	125	125	125	125
Artificial feed cost ($/kg)[1]	0.56	0.56	0.56	0.56	0.56
Stargrass hay cost ($/kg)[1]	0	0.17	0.17	0.17	0.17
Total feed and hay cost/ha/wk ($)	100.80	122.05	88.45	71.65	56.19

[1]Cost provided by Megar S.A. de C.V. in Mexican pesos and converted to U.S. dollars using a 14 pesos: 1 dollar exchange rate.

[2]Based on typical growth over a production cycle

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CHAPTER IV

EVALUATION OF DIFFERENT HATCHERY-NURSERY PROCEDURES TO MAXIMIZE SURVIVAL, FINAL WEIGHT AND PRODUCTION OF JUVENILE AUSTRALIAN RECLAW CRAYFISH *Cherax quadricarinatus*

Introduction

In the last two decades, there has been an increasing interest in the development of hatchery and nursery procedures for freshwater crayfish. In Europe, decimation of native stocks by the crayfish plague have encouraged the establishment of restocking programs. Development of hatchery techniques for native crayfish such as the noble crayfish (*Astacus astacus*) in northern Europe (Huner and Lindqvist 1987), the white-clawed crayfish (*Austropotamobius pallipes*) in Spain (Perez et al. 1999), as well as for the introduced signal crayfish (*Pacifastacus leniusculus*) in several regions of the continent (Celada et al. 1989; Gonzalez et al. 2008) have been explored.

Although restocking of natural populations has been lately the major reason for developing crayfish hatchery and nursery techniques, aquaculture in other regions of the world has been an important contributor, specially motivated by the culture of the American red swamp (*Procambarus clarkii*) and the Australian *Cherax* spp. Even though, the southeastern United States has a substantial production of crayfish, intensive hatchery and nursery techniques suggested and investigated have been limited (Huner and Barr 1984, Trimble and Gaude 1988) as under the extensive methods applied for commercial farming of red swamp crayfish, the cost-effectiveness of producing juveniles in intensive systems has not yet proven feasible.

On the other hand, he culture of Australian crayfish, especially redclaw (*C. quadricarinatus*), in semi-intensive systems has continued to increase, not just in Australia but also in several other parts of the world. Production of juveniles has been achieved successfully by maintaining selected sexually mature redclaw in earthen ponds, from which the juveniles are periodically harvested. Precise production information from these systems is not possible; however survival is estimated to range from 5 to 10% and the harvest weight of the juveniles is extremely variable (Jones, 1995a). As the industry develops, the demand for quality juveniles will increase. To meet this demand, improved juvenile production technologies will be required. The need for proven rearing procedures that optimize the quantity and quality of the juveniles is a must if the industry wants to keep its pace in growth. A better understanding of the biology and behavior of the species in intensive nursery conditions is important.

Mature male redclaw have a distinctive reddish patch on the outside margin of the claws, females do not. However, the best way of identifying males from females is by examining the genital organs on the underside of the cephalothorax. Examination of the genital organs will also allow the identification of non-mature males from females. Males have a pair of small projections (genital papillae) at the base of the fifth pair of pereiopods. Females have a pair of genital pores at the base of the third pair of pereiopods. Sexual maturation is generally reached between 6 and 8 months at temperatures above 22ºC. Males will deposit a spermatophore (sperm sack) of 0.8 to 1.0

cm in diameter on the underside of the female between the third and the fifth pair of pereiopods. Twelve to 24 hours after mating the females will break the spermatophore, release their eggs (spawn), and external fertilization will occur while the eggs are being released. Eggs are then attached to the female pleopods. Approximately 30 to 40 days later the eggs hatch. Juveniles will cling to the female for another 7 to 10 days before total release (Masser and Rouse, 1997).

In subtropical regions, redclaw usually spawn from spring to mid-autumn, with two to three reproductive cycles per year (Jones, 1990, 1995a). In tropical regions, reproduction may occur year round, with as many as five spawns per year (Sammy, 1988). Spawning sequences usually are spawn-molt-spawn and spawn-spawn-molt (Barki et al., 1997). Spawning is dependent upon temperature and photoperiod, with optimals being 28 to 30ºC and (14 L:10 D), respectively (Jones 1990, 1995a; King 1994; Yeh and Rouse 1994). Continuous spawning can be achieved during the previous conditions, but females need a period of rest after about 6 months of continuous spawning (Yeh and Rouse, 1994).

Clutch sizes (counts of pleopodally attached eggs) are directly correlated to female size. Females spawn around 10 eggs per gram of body weight (Rouse and Yeh, 1995). Although egg production depends upon nutritional and environmental factors, under laboratory conditions the amount of eggs attached to the pleopods prior to hatching usually ranges from 4.5 to 8.7/ g body weight of female (King 1993b; Yeh and Rouse 1994; Jones 1995a; Austin 1998). Hatching rates of the eggs have been reported to be around 95% (Rouse and Yeh, 1995).

Redclaw eggs go through several stages during their development period (Jones 1990, King 1993a). Yeh and Rouse (1994) and Jones (1995a) gave detailed descriptions of the developmental stages. The duration of the various developmental stages depends to a large extent on water temperature. Hatching time can range from 30 days at 28ºC to 45 days at 24ºC (Masser and Rouse 1997). At hatching, juvenile size is not correlated to female size (Barki et al. 1997).

In commercial operations, density of broodstock in earthen ponds ranges from 0.5 to 2 per meter square. Barki and Karplus (2000) tried up to 60 broodstock per m^2 in laboratory conditions without any negative effect on mean survival, percentage of spawning or clutch size. Male:female ratios in earthen ponds, as well as in laboratory conditions vary between 1:1 to 1:5 without any difference in percentage of berried females (Jones 1995a; Yeh and Rouse 1995; Barki and Karplus 2000).

Providing shelter during the mating season is important as it offers protection during periods of vulnerability when molting, protects the broodstock against predation, and minimizes aggressive interactions (Jones and Ruscoe 2001). Jones and Ruscoe (2001) reported that pipe stacks or similar shelter proved to be the best for increasing the percentage of berried females. Special attention needs to be given to berried females when handling, since eggs can be dislodged from the pleopods as females react with violent movements of their tail when threatened (Huner and Lindqvist 1991; Masser and Rouse 1997; Austin 1998).

During the nursery period, crayfish densities should receive special attention. Although increased stocking densities are reported to have no effect on survival of juveniles when adequate nutrition and shelter are provided, they often have an inverse effect on final weight at harvest (Pinto 1993; Morrisy et al. 1995; Pinto and Rouse 1996; Austin et al. 1997; Jones and Ruscoe 2001; Manor et al. 2002; Naranjo et al. 2004; Barki et al. 2006).

It is very important to provide shelter to juveniles to maximize survival during the nursery stage. Juveniles are able to discriminate between different shelter types and display clear preferences. Mesh bundles have been proven to have the best success in maintaining good survival of *C. quadricarinatus* juveniles (Jones 1995a, 1995b; Parnes and Sagi 2002).

Although juveniles are less benthic than adults (Jones 1995b), normally juvenile *C. quadricarinatus* still spend most of the time on the bottom of the tank, leaving the water column virtually empty. Hatchery operators working with *Macrobrachium rosenbergii* have been able to increase production by supplying adequate substrate and increasing the water volume while keeping the same surface area (Tidwell et al. 1999; D'Abramo et al. 2000). Effects from increasing the water volume while keeping a constant area in redclaw nurseries has received little attention and warrants further research.

Whereas, *C. quadricarinatus* culture techniques in temperate and tropical areas of the world have developed differently, hatchery and nursery techniques have remained the same. The current 5 to 10% survival in reproduction ponds (Jones 1995a; Masser and Rouse 1997) is not space and resource efficient for producers. In addition, stocking recently hatched juveniles directly into grow-out ponds has resulted in poor and unpredictable survival rates. Redclaw growers prefer juveniles above 1g for stocking into grow-out ponds since they usually have higher survivals. For the redclaw industry to further develop, survival rates and average size at the hatchery-nursery phase have to increase substantially.

Several attempts for hatchery-nursery procedures have been described by Jones (1995a), Masser and Rouse (1997), and Parnes and Sagi (2002). Based on their experiences, a commercial redclaw hatchery-nursery was designed and built in 2006 in Mexico. Three experiments to maximize juvenile survival, final weight at harvest and production under commercial hatchery-nursery conditions are described. The objectives of these experiments were to evaluate: 1) bottom area and water depth relationships on juvenile production, 2) broodstock and juvenile densities, and 3) nursery duration.

Materials and methods

The research was conducted at the production ponds and AGY Redclaw Hatchery property of Megar S.A. de C.V. in Soto La Marina, Tamaulipas, Mexico.

Egg staging

Based on the stage development tables published by Yeh and Rouse (1994) and Jones (1995a) in addition to practical observations in culture ponds in Tamaulipas, a stage development table specific for the region was prepared by CRM International and has been used in the Megar facilities since 2002 (Table 1). The stages in this table were the ones used in this study.

Female selection

Female broodstock redclaw crayfish were selected from three 2,000-m^2 reproduction ponds that had been previously stocked at 1.5 females/ m^2 at a 3:1 female to male ratio the previous year. Ponds were prepared with 1 terracotta block per meter square placed evenly across the bottom. Each terracotta block had 4 cavities, which resulted in 4 individual hideouts per m^2. A mesh bundle constructed with plastic netting was also provided as additional refuge every 4 square meters. Water for the reproduction ponds was pumped from a local stream to replace the losses due to evapotranspiration, water intake was filtered with a 300 micron mesh. Stage 5 females were harvested with extreme caution to prevent egg dislodging and held in flow-through systems prior to stocking the nursery experiment. Females that did not have this specific egg stage were returned to the reproduction pond.

Egg count

A random sample of thirty stage 5 females was selected and individually weighed. Eggs were stripped gently with the aid of a brush and a pair of forceps and counted. Total eggs per female and eggs per gram of female were calculated. For production results, a 95% hatching rate was considered based on Yeh and Rouse (1994).

Experiment 1

One hundred and eight females with similar stage eggs were selected from the reproduction ponds. Each replicate group was individually weighed, so, that the production of juveniles per gram of female could be calculated and randomly stocked into one of 9 circular tanks (1.9 meter diameter, 2.84 m^2 bottom area). Each tank received water from an outside reservoir at approximately 20 L hr^{-1} for the duration of the experiment. Females were held in plastic mesh containers to allow juveniles to drop through the walls and bottom after release to prevent juvenile predation from the brooder. Tanks were also provided with four submerged air diffusers for aeration and water mixing. Females in three replicate tanks were randomly assigned to one of three experimental treatments. The first treatment was stocked at 9 females per tank and an average water depth of 10 cm (9-10). A second treatment was stocked at 9 females per tank and an average water depth of 20 cm (9-20). The third treatment was stocked at 18 females per tank and a water depth of 20 cm (18-20). Each tank contained onion mesh bundles to provide refuge for the juveniles. The amount of refuge was directly related to the volume of water to keep mesh per unit of water as a constant value. Once the females had spawned and the juveniles had released, the females were removed to minimize predation and decrease the biomass in the tanks. All juveniles released within a 96-hour period. When the last group of juveniles of each tank had released, the experiment began and the juveniles were nursed for a 31-day period. Juveniles were fed with ground commercial crayfish pellets (Aquaprofile: 30% protein, 8% lipid, Purina®, Mexico, MEX) at an initial rate of 10% their body weight. Feeding rates were later increased and adjusted periodically based on observations of uneaten feed. Uneaten feed was removed once a week via siphoning the bottom of the tanks.

At the conclusion of the nursery trial, juvenile crayfish were bulk counted and weighed. Total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight and survival of juveniles were calculated.

Experiment 2

Females with similar stage eggs were selected from the reproduction ponds as before and weighed individually, so, that juvenile numbers could be calculated. Each replicate group was individually weighed and stocked into one of 20 rectangular tanks (2.4x 1.2 x 0.1 m, 288 L volume, 2.88 m^2 bottom area). Each tank received water from an outside reservoir at approximately 20 L hr^{-1} for the duration of the experiment and were provided with four submerged air diffusers for aeration and water mixing. Each tank contained plastic mesh containers to hold individual females that allowed juveniles to drop through the walls and bottom after release to prevent juvenile predation from the brooder. Females in four replicate tanks were randomly assigned to one of five experimental treatments. Treatments consisted of stocking tanks with either 8, 12, 16, 20, or 24 female broodstock redclaw (2.8, 4.2, 5.6, 6.9 and 8.4 per m^2, respectively). After release, the juveniles were nursed for a 30-day period. Each tank contained onion mesh bundles to provide refuge to the juveniles. Once the females had spawned and the juveniles had released, females were removed. All juveniles released within a 96-hour period. When the last group of juveniles of each tank had released, the nursery period for each tank was considered to start. Juveniles were fed with ground commercial crayfish pellets (Aquaprofile: 30% protein, 8% lipid, Purina®, Mexico, MEX) at an initial rate of 10% of their body weight. Feeding rates were later increased and adjusted periodically based on observations of uneaten feed. Uneaten feed was removed twice a week via siphoning the bottom of the tanks.

At the conclusion of the nursery trial juvenile crayfish were counted and weighed. Total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight and survival of juveniles were calculated.

Experiment 3

Females with similar stage eggs were selected from reproduction ponds as in previous experiments and weighted individually for juvenile calculations. Each replicate group was individually weighted and stocked into one of 36 rectangular tanks (2.4x 1.2 x 0.1 m, 288 L volume, 2.88 m^2 bottom area). Each tank received water from an outside reservoir at approximately 20 L hr^{-1} for the duration of the experiment and were also provided with four submerged air diffusers for aeration and water mixing. Each tank contained plastic mesh containers to hold individual females that allowed juveniles to drop through after release to prevent juvenile predation from the brooders. Females in four replicate tanks were randomly assigned to one of nine experimental treatments. Treatments consisted in a 3x3 factorial design. Three treatment groups were stocked with either 8, 12, or 16 female redclaw broodstock (2.8, 4.2 and 5.6 /m^2, respectively). After release, juvenile groups were nursed for either 20-day, 30-day or 40-day nursery periods. Each tank contained onion mesh bundles to provide refuge to the juveniles. All juveniles released within a 96-hour period. When the last group of juveniles of each tank had released, the nursery period for each tank was considered to start. Juveniles were fed with ground commercial crayfish pellets (Aquaprofile: 30% protein, 8% lipid, Purina®, Mexico, MEX) at an initial rate of 10% body weight. Feeding rates were later increased and adjusted periodically based on observations of uneaten feed. Uneaten feed was removed twice a week via siphoning the bottom of the tanks.

At the conclusion of the nursery trial juvenile crayfish were counted and weighed. Total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight and survival of juveniles were calculated for the different nursery periods regardless of the stocking density of the females.

Water Quality

Dissolved oxygen (DO) concentrations and temperature were measured twice daily using a YSI 55 DO meter (Yellow Spring Instrument Co., Yellow Springs OH, USA). Ammonia was measured bi-weekly using a LaMotte® Freshwater Aquaculture kit (LaMotte Company, Chestertown MD, USA) (Nessler method). Values for water temperature were recorded. Values for DO and ammonia were only recorded if values were below 60% saturation or above 1 ppm, respectively.

Statistical analysis

Results were compared with one-way and two-way analysis of variance (ANOVA) and considered significant at $P<0.05$. Because of the potential for differential total production of juveniles and differences in average weight among the different nursery period lengths (Experiment 3), the data were analyzed by two-way ANOVA using number or females and days in the nursery as factors. The data was also sorted by nursery period length for juvenile production and average weight. Tukey's test was used to identify statistically significant differences among treatment means. All statistical analysis was performed using MINITAB software (version 15.1, MINITAB Inc., State College, Pennsylvania).

Results

Egg and juvenile production.

Average egg count (mean ±SD) per female at stage 5 was 7.8±2.15/g body weight. The expected production of juveniles per gram of female using a 95% hatch rate was 7.4 juvenile/ g of female.

Water quality

Throughout the duration of the different trials, water temperature ranged between 24.9 to 28.7ºC, with a mean temperature of 27.4ºC . Dissolved oxygen levels were not observed below 60% saturation and ammonia levels were never above 1 ppm. These water quality conditions were within acceptable limits for indoor production of redclaw crayfish (Masser and Rouse 1997).

Experiment 1

At the conclusion of the nursery trial, crayfish were counted and weighed by group (Table 2). Mean total juvenile production ranged from 1147 to 1516 juveniles. Juveniles per female ranged between 68 and 168. Juveniles per gram of female ranged between 1.11 and 2.12. Biomass ranged between 235 and 322 grams per meter square. Average weight of juveniles ranged from 0.55 to 0.65 grams. Survival ranged between 15 and 29%.

No significant differences were found among total juvenile production, juveniles per female, Juveniles per gram of female, biomass, average weight of juveniles or survival (Table 2).

Experiment 2

Total juvenile production (Table 3) ranged from 1419 to 2596 juveniles. There was a significant difference between groups ($P<0.05$). Juvenile production in the treatment with 8 (2.8 /m^2) females was different from the treatment with 20 females (6.9/m^2). However, there were no differences among other treatments.

Juveniles per female ranged between 59 and 181. Juveniles per gram of female ranged between 1.07 and 2.86. The treatment stocked with 24 females was significantly different from all other treatments in terms of juveniles per female and juveniles per gram of female ($P<0.05$).

Biomass ranged from 192.71 to 320.75 grams per m^2. The treatment stocked with 8 females (2.8/m^2) was different ($P<0.05$) than the treatments stocked with 16 and 20 females (5.6 and 6.9 /m^2, respectively), however it was not different from any other treatment.

Average weight of juveniles ranged from 0.38 to 0.52 grams. Treatments were not different ($P>0.05$).

Survival ranged between 14 and 36%. The treatment stocked with 24 females was different ($P<0.05$) than treatments with 8, 12 and 16 females, but similar to the treatment with 20 females. There were no other differences in survivals.

Experiment 3

Based on regression analysis, both nursery period and broodstock density were determined to be explanatory variables. Although, two-way ANOVA analysis showed that there was no significant interaction between factors (P value= 0.596). Been no interaction between factors and the effect of stocking density proven on the previous experiment, data was analyzed pooling the data by nursery period disregarding the broodstock density. Finding the best nursery period under the current culture conditions is considered of major importance by producers.

When data was grouped by nursery period, mean total production ranged between 1353 and 1853 juveniles. Mean juveniles per female ranged between 119 and 158. Mean juveniles per gram of female ranged between 1.93 and 2.65. Mean biomass ranged from 121.53 to 263.89 grams per meter square. Survival ranged between 24 and 35%. There was a significant difference between groups ($P<0.05$) Treatments nursed for 30 days were different from the ones nursed for 20 and 40 days. There was no difference between the 20 and 40 day nursery periods.

Average weight per juvenile ranged between 0.27 g for the 20-day nursery trial to 0.41 and 0.44 g for the 30-day and 40-day nursery trial, respectively. The 20-day treatment differed from the other two treatments. ($P<0.05$) (Table 4).

Discussion

Egg count

The results of 7.8 eggs/g of female for *C. quadricarinatus* is similar to the results reported by other investigators. King (1993a) reported 8.7 eggs/g of female, Yeh and

Rouse (1994) 7.6 eggs/g of female, Jones (1995a) 8.2 eggs/g of female and Austin (1998) 7.5 eggs/g of female. The results during this trial are higher than the ones reported by Sammy (1998) who had results of only 2.8 eggs/g of female and to the ones reported in a second experiment by Austin (1998) that had counts of 4.5 eggs/g of female.

Yeh and Rouse (1994) was the only publication where hatch rates were reported. Their 95% rate was the one utilized to calculate the amount of juveniles stocked in this series of experiments. The proper assumption of successful hatching is of major relevance to having accurate calculations of juvenile production if commercial hatcheries calculate production from female weight. It would be recommended to conduct further experiments under different conditions and specific locations to corroborate the accuracy of this assumption.

Experiment 1: Effects of water volume

The increase of water volume on the nursery tanks did not show any differences on any of the variables measured, total production of juveniles, juveniles per female, juveniles per gram of female, biomass, average weight of juveniles or survival

D'Abramo (2000) reported that weight gain was maximized through a simultaneous increase in volume and surface area for *Macrobrachium rosenbergii*. This experiment does not show an increase in body weight from increasing water volume in redclaw nurseries.

Parnes and Sagi (2002) providing a seaweed-like material as substrate to *C. quadricarinatus* juveniles reached densities of 6.5 juveniles/l with an average weight of 0.34±0.04 g after stocking females 75 days from the day females were found to be gravid.

The biomass per liter reported was approximately 2.21 g/l. Transforming data of this experiment, densities ranged between 2.02 and 5.34 juveniles/l with average weights from 0.59 to 0.65 g, resulting in a biomass ranging from 1.19 to 3.47 g/l. Parnes and Sagi (2002) results are between the ranges obtained in the current experiment. Results of both experiments can be considered similar, although Parnes and Sagi (2002) survival of 60% was much higher than survivals obtained in this trial, the final output of juveniles and biomass per liter is consistent.

Among the current treatments, mean total production, average weight and biomass were similar. Although there was no difference in survival, the trend among the observed means was that it will decrease as stocking density increases. It appears that the number of juveniles that can be nursed in indoor nurseries should be based on bottom surface areas not water depth or volume.

It was observed during the length of the experiment that although juveniles spend most of the time on the onion mesh bundles provided as refuge and which were above the bottom in the water column, they spend an important amount of time on the bottom of the tank. Jones (1995a) and Masser and Rouse (1997) provide results of juveniles per meter square, which should be considered, at least in the specific case of *C. quadricarinatus*, the correct format to report production data. Consequently, this study will state its results in units of bottom surface area.

From the results of this experiment, it can be inferred that increasing water volume has no significant benefit on the total production, biomass, average weight or survival of juvenile crayfish *C. quadricarinatus* under the present nursery conditions.

Experiment 2: Effects of female densities

The current hatchery-nursery procedure did not begin with a consistent stocking density of juveniles. Berried females were stocked into nursery tanks and their eggs were allowed to hatch and the young released as had been suggested by Jones (1995a), Masser and Rouse (1997), and Parnes and Sagi (2002). This has been a common practice by redclaw producers in Australia and Latin America. This technique reduces handling of newly hatched juveniles, which usually results in very poor survivals. Reasonable estimates of the stocking density of hatchlings can be calculated from the female weight (Masser and Rouse 1997) and estimated hatching rate (Yeh and Rouse 1994).

Calculated stocking densities of juveniles in this experiment ranged from 1317 to 3461 juveniles per m^2. Jones (1995a) used densities between 980 and 1842 juveniles per m^2. Masser and Rouse (1997) recommend that density should not exceed 270 juveniles/m^2 when using a static water nursery. All stocking densities in this experiment were significantly higher than the ones recommended by Masser and Rouse (1997). Stocking densities in the treatments stocked with 8 and 12 females per tank were similar to the ones used by Jones (1995a). Treatments stocked with 16, 20 and 24 females per tank were higher than any previously reported densities. It is important to comment that Masser and Rouse (1997) used a static water system, and the differences to this experiment with approximately 166% water exchange per day, and the one by Jones (1995a) with approximately 200% exchange per day, can be related to the differences between a static and flow-through systems. Mean survival in general in this experiment were inferior to the approximately 50% survival reported in previous studies (Jones 1995a, Masser and Rouse 1997). The higher stocking densities can be one explanation of why mean survival rate were poorer. Although, in the present experiment, only survivals in the tanks stocked with 24 females (14.2%) were different from the tanks stocked with 8 to 16 females per tanks (38.1 to 32.6%). Observed mean survivals decreased as stocking densities increased (Survival% = $(0.2476 + 0.06474(\text{females/m}^2) - 0.009145(\text{females/m}^2)^2)*100$). In commercial conditions, some decrease in survival would be acceptable as long as the total production increases and the average weight of the juveniles is not affected.

Total production of juveniles, which ranged between 493 and 901 per meter square, was superior to those projected by Masser and Rouse (1997) (135 to 202 juveniles per meter square), with a static system and lower stocking densities, and comparable to the ones reported by Jones (1995a) (80 and 1212 juveniles per meter square). In the present study, although only the treatment stocked with 8 females per tank was different from the others, a clear trend can be observed: total production constantly increased as density of females increased from 8 females/m^2 to 20 females m^2 and it suddenly decreased at a density of 24 females/m^2. (Total production = $4798 - 936.8 (\text{females/m}^2) + 80.91 (\text{females/m}^2)^2 - 1.988 (\text{females/m}^2)^3$). As commented before, 24 females per meter square gave a lower survival that can be explained by the cannibalistic behavior of the juvenile redclaw. Cannibalism is usually thought to occur mainly at the beginning of the nursery period when juveniles are competing for shelter areas. In the current design most of the water volume has shelter areas. The previous experiment proved that an increase in area through increasing water volume would not have increased the survival for the tanks stocked with 24 females. Although from the current results it can only be implied that stocking 20 females per tanks gives better results that stocking 8 females per tank. The trend data suggest that stocking between 12 and 20 (4.2 to 6.9/m^2) females per tank, with the conditions of the present experiment, would give the best results in total production of juvenile redclaw, *C. quadricarinatus*.

Average weight of juveniles, which ranged between 0.382 and 0.516 grams, is similar to the one obtained by Jones (1995a), who also nursed juveniles for around a 30-day period and had an average of 0.4 ± 0.2 g per juvenile. Masser and Rouse (1997) projected juvenile sizes of approximately one gram, but did not specify the nursery period length. In the present study, there were no significant differences in the average weight of juveniles.

Since observed mean biomass continued to increase with each increasing stocking density until the 20 female/tank density, it is probably that critical standing crop was not reached even at the highest density. Although is appears to be a positive correlation between biomass and stocking densities, at the highest density (24 female/tank) survival, and in consequence biomass, showed a significant decrease, which can be attributed to cannibalism as a consequence of competition for space.

The results of this study suggest that under the conditions of this experiment densities of 12, 16 or 20 females per tank would have the best results for a commercial hatchery-nursery of redclaw, *C. quadricarinatus*, as they maximize total production and survival and average weight of juveniles is not affected.

Experiment 3: Effects of nursery period

None of the previous studies with *C. quadricarinatus* have compared the nursery period to the total production, survival and average weight of juveniles. Jones (1995a) used a 23 to 50 day nursery period. Using a 30-day period seemed a good option as no other experimental data was available but duration of nursery period needed to be tested.

When comparing all the treatments, regardless of the female density, mean total production and survival and, as a consequence, biomass was higher in the treatment with a 30-day nursery period. When comparing the 30-day with the 40-day nursery period the results seem logical, as juveniles nursed for a longer time will cannibalize more on each other, resulting in a decrease in total production. Although, when comparing the 30-day with the 20-day nursery period, the results are not logical. The survival should have been similar, if not superior, for the 20-day nursery period. The tanks stocked with 16 females had an irregular behavior; when nursed for 30 days, mean survival was 30.9% and was 19.5% when nursed for 20 days, the poor results in this group could have reduced the total 20-day group average, and caused the groups to be different.

Average weight of juveniles was lower for the 20-day nursed juveniles (0.27 g), than the other two groups. Results are logical as they had less time to develop. Results between the 30-day period (0.41 g) and 40-day period (0.44) suggest that animals kept growing, although the growth rate slows down after a 30-day period at the present densities.

When analyzing the results of all densities combined, the 30-day nursery period gave the best results, as mean total production and survival were superior to the other two groups and average weight was superior to the 20-day period and similar to the 40-day period. Even if the results of survival would have been similar to the 20-day period, the 53% average weight difference makes the 30-day period a better option as bigger juveniles would have a better survival rate when stocked into another tank or pond phase (Masser and Rouse 1997).

An analysis of mean final production and average weight should be performed when deciding the procedure to follow in commercial conditions. Results of this experiment suggest that a 30-day nursery period will maximize the total production of juveniles without sacrificing the average weight output, as well as it would optimize the amount of times the tanks could be used throughout the year.

For commercial applications, hatchery procedures need to be optimized. Special consideration needs to be taken for egg staging and for handling of brooder females when transporting them from ponds to the hatchery. Incubation procedures of the eggs should be analyzed, as this will permit that juveniles from the same day are stocked in a nursery tank, which will result in less cannibalism and more uniformity at harvest. Successful hatching percentages need to be reevaluated as the survival results of this study could have been higher if in reality the hatching rate was lower.

A hatchery-nursery facility as the one used for the present study can produce approximately 1,400,000 juveniles per year, which when stocked at $4/m^2$ can fulfill the juvenile need for approximately 35 hectares and represent $140,000.00 USD dollars in sales for the owner of the hatchery. However, even these conditions, that could help the redclaw industry, are not enough and need to be improved. There is still a big window of opportunities for the redclaw industry to improve, and more practical experimentation is needed to reach maximum production.

Conclusions

The results of these experiments suggest that water volume has minimal effect on the production performance in a redclaw hatchery-nursery system. Bottom surface area is the best way to account for juvenile production of *C. quadricarinatus*. As well, densities between 4.2 and 6.9 females/m^2 with a 30-day nursery period would have the best results in a commercial hatchery-nursery, when conducted with similar conditions to these experiments, for juvenile redclaw, *C. quadricarinatus*, as they would maximize total production without a negative effect on survival or average weight.

Table 1. Morphological characteristics and duration (days) of successive stages of fertilized egg and larval development for *Cherax quadricarinatus*, during incubation at 26-28 ºC.

Egg stage	Morphological characteristics of egg	Approximate duration (d)
1	Olive green to khaki	10
2	Yellow	5
3	Orange	5
4	Red, eyes not visible	7
5	Red, eyes and pereiopods visible	7
6	Gray-released	5

Table 2. Total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight of juveniles and survival for juvenile redclaw, *Cherax quadricarinatus,* nursed at different densities and with different volumes for a 31-day period.

Parameters	9-10	9-20	18-20	P. Value	PSE[2]
Area (m^2)	2.84	2.84	2.84		
Depth (cm)	10	10	20		
Number of females stocked	9	9	18		
Number of females/m^2	3.17	3.17	6.35		
Total weight of females (g)	721	591	1101		
Average weight of females (g)	80.19	65.74	61.2		
Estimated juvenile/ gram of female	7.4	7.4	7.4		
Estimated juveniles hatched	5340	4378	8152		
Nursery period (days)	31	31	31		
Total juvenile production	1516	1147	1225	0.528	230.709
Juveniles per female	168.48	127.48	68.07	0.082	25.518
Juveniles/g/female	2.12	1.95	1.11	0.174	0.350
Biomass (g)	322	235	235	0.218	35.596
Average weight of juveniles (g)	0.65	0.59	0.55	0.744	0.098
Survival %	29	26	15	0.174	4.731

[1] No significant differences were found between treatments (P>0.05).

[2] Pooled standard error of treatment means (n=3).

Table 3. Mean total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight of juveniles and survival for juvenile redclaw, *Cherax quadricarinatus,* hatched in tanks from 8, 12, 16, 20 or 24 females and nursed for a 30-day period.

Parameters					
Females	8 ($2.8/m^2$)	12 ($4.2/m^2$)	16 ($5.6/m^2$)	20 ($6.9/m^2$)	24 ($8.4/m^2$)
Total weight of females (g)	512.75	686.75	940.75	1136.25	1347
Average weight of females (g)	64.09	57.23	58.80	56.81	56.13
Estimated juveniles hatched	3794	5082	6962	8408	9968
Biomass of females (g/m^2)	178.04	238.45	326.65	394.53	467.71
Total Juvenile Production	1445^{a}	$1846^{a,b}$	$2269^{a,b}$	2596^{b}	$1420^{a,b}$
Juveniles per female	181^{a}	154^{a}	142^{a}	130^{a}	59^{b}
Juveniles/g of female	2.86^{a}	2.69^{a}	2.41^{a}	2.27^{a}	1.07^{b}
Biomass (g/m^2)	$192.71^{a,b}$	$293.40^{a,b}$	305.56^{b}	320.75^{b}	$233.51^{a,b}$
Average weight of juveniles (g)	0.382^{a}	0.454^{a}	0.403^{a}	0.381^{a}	0.516^{a}
Survival %	38.1^{a}	36.3^{a}	32.6^{a}	$30.9^{a,b}$	14.2^{b}

[1] Means within the same row with different superscripts are significantly different (P<0.05).

Table 4. Mean total juvenile production, juveniles per female, juveniles per gram of female, biomass, average weight of juveniles and survival for juvenile redclaw, *Cherax quadricarinatus,* hatched in tanks and nursed for a 20-day, 30-day or 40-day period.

Parameters			
Nursery period (days)	20	30	40
Total weight of females (g)	721	713	764
Average weight of females (g)	60.21	60.04	63.28
Estimated juveniles hatched	5335	5279	5654
Biomass of females (g/m^2)	250.35	247.71	265.31
Total juvenile production	1354[a]	1853[b]	1362[a]
Juveniles per female	119[a]	159[b]	119[a]
Juveniles/g of female	1.99[a]	2.65[b]	1.93[a]
Biomass (g/m^2)	121.53[a]	263.89[b]	203.85[a]
Average weight of juveniles (g)	0.270[a]	0.413[b]	0.444[b]
Survival %	25.4[a]	35.1[b]	24.1[a]

[1] Means within the same row with different superscripts are significantly different ($P<0.05$).

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CHAPTER V

COMPARISON OF GROWTH PERFORMANCE BETWEEN THE AUSTRALIAN WALKAMIN AND THE MEXICAN MEGAR STOCKS OF AUSTRALIAN REDCLAW, *Cherax quadricarinatus*, IN EARTHEN PONDS AND TANKS

Introduction

Redclaw crayfish, *Cherax quadricarinatus*, has a natural range that encompasses most of the drainages along the northeastern Australia, the Gulf of Carpentaria and the Timor Sea (Merrick and Lambert 1991). The species is also found in Papua New Guinea (Austin 1986). Redclaw is a gregarious species that tolerates broad environmental challenges, and relatively high stocking densities. As well, it has favorable reproductive characteristics, including moderate fecundity, a simple life cycle and maternal incubation of eggs through to hatching of highly developed juveniles (Jones 1995a, 1995b; Masser and Rouse 1997; Jones and Ruscoe 2000), which together make the redclaw a good aquaculture species. Commercial redclaw aquaculture was initiated in Queensland in 1987, It has been introduced successfully to several countries in the world, including Mexico, which started its commercial culture in 1996 in the state of Tamaulipas. Most redclaw stocks used in commercial farms have originated from very limited collections of wild stocks in North Queensland. Cultured stocks are often heterogeneous either because river populations were mixed to produce heterotic hybrids or because collectors consider them to be a single genetic resource (Macaranas et al. 1995).

Genetic selection is somewhat implemented by the aquaculture industry whether intended or not. Some species (common carp, Atlantic salmon, rainbow trout, channel catfish, Nile tilapia, and the Pacific oyster) have received concentrated breeding efforts, while other major cultured species (Chinese and Indian carps and the giant tiger shrimp) have received relatively limited attention. A few species (Yesso scallop, blue mussel, white Amur bream and milkfish) have apparently not been genetically improved at all (Hulata, 2001). Although the number of breeding programs, both in private sectors and national levels is increasing, and the use of stocks genetically improved through selective breeding is gaining popularity, this trend seems to be stronger for finfish and weaker for crustaceans (Benzie 1998). Freshwater crayfish are one of the three groups of crustaceans cultured on a commercial scale and its genetic information is very limited. The *Cherax* species are not an exception.

Henryon et al. (1999) analyzed the objectives that a breeding program for marron crayfish, *C. tenuimanus*, should target. He analyzed the benefits of enhancing growth rates of carapace, tail and claws, survival, food conversion efficiency, reproductive rate, and fecundity. Results demonstrated that growth rate of the tail was between 30 and 7900 times more important than any of the other factors. Other scientists emphasize that selection should be focused on growth performance for all the *Cherax* spp. cultured in Australia: marron, *C. tenuimanus*, (Lawrence and Morrissy 2000), yabbie, *C. albidus* and *C. destructor,* (Lawrence et al. 2000a; Jerry et al. 2005) and redclaw, *C. quadricarinatus*, (Jones et. al. 2000; McPhee et al. 2004). Faster growth will provide a shorter production period or the option of culturing organisms to a larger size over a fixed period; any of the previous options will increases profitability.

It has been empirically known by farmers and proved by scientists that for *Cherax spp*. the monoculture of separate sexes and in a larger proportion of males-only bring superior results than the culture of mixed sexes (Curtis and Jones 1995; Lawrence et al. 2000a; Manor et al. 2002; Rodgers et al. 2006). Due to the laborious nature of manual sexing males and females, monosex culture has been practiced in Mexico and Ecuador where labor is less costly, but has proven to be unsuitable for Australia where labor cost is considerably higher.

In order to achieve the monoculture of males-only, the hybridization of *C. rotundus* and *C. albidus* to consistently produce only male progeny was successfully achieved (Lawrence et al. 2000b; Lawrence 2004). Unfortunately, for *C. quadricarinatus* the investigations for producing a male-only progeny is still in its infancy stage and there is currently no technique applicable in commercial aquaculture.

Genetic variation in wild populations of redclaw was found by Macaranas et al. (1995). Baker (2006) confirmed the variation not only of wild populations but of cultured stocks as well. Gu et al. (1995) demonstrated a substantial genetic component of growth performance variation for *C. quadricarinatus* and observed a heritability component. These findings originated selection programs for faster growth for all the *Cherax* spp. in Australia (McPhee and Jones 1997; Jones et al. 2000; Lawrence and Morrissy 2000; McPhee et al. 2004; Jerry et al. 2005).

For *C. quadricarinatus* a breeding program was performed at the Freshwater Fisheries and Aquaculture Center at Walkamin in North Australia. In 1993, a strain evaluation project was undertaken, which was followed by experimental (1994) and commercial-scale (1997) selective breeding programs (McPhee and Jones, 1997; Jones et al. 2000). Two selected lines were derived from the Flinders and Gilbert Rivers in Queensland and a selected and a control line were established. From 1999 to 2003, the previous project was continued on a larger scale and a genetic improvement program for increasing growth rates of redclaw and to generate a breeding stock for transfer to the aquaculture industry was conducted. After 4 generations, the selected line had approximately 25% better growth performance than the control line (McPhee et al. 2004). Several farmers in Australia started using this stock for commercial culture.

In Tamaulipas, Mexico, after recognizing that negative selection had been performed for 3 years as the larger organisms were sold before selection of broodstock was performed, a practical breeding program was initiated in 2001. The breeding program consisted of selecting between 10 and 15% of the largest crayfish, assuming that they had the fastest growth, before any organism was harvested from grow-out ponds for later stocking of reproduction ponds. Redclaw arrived to Mexico in 1996 most probably from the Hutchings stock (Baker 2006), and as there are not natural stocks in Mexico, inbreeding was a major concern. Samples were sent to the Queensland University of Technology in Brisbane, Australia, in 2002 for inbreeding analysis. In that same year, Baker (personal communication) reported, and later published (Baker 2006), that no significant differences in diversity indexes were found between the Australian (various stocks) and the Mexican stocks. To reduce inbreeding risk, broodstock males were exchanged between farms. The same selection process has continued regularly in Megar S.A. de C.V. Approximate a 23% increase in growth performance has been reported by Nabor Medina (Megar. S.A. de C.V. general manager, personal communication), but the farm had no control group to which to compare the selected stock. Farm records are the only means of comparison. Most of the recently developed farms in Mexico have acquired their broodstock from the Megar stock.

As producers in Mexico have noticed the reports of substantial growth performance enhancement that the Walkamin stock has had in Australia and the benefits that a faster growing redclaw could bring to them, an increasing interest arose to compare the growth performance of both stocks. The objective of this study is to analyze the growth performance of the Walkamin and the Megar stocks in similar pond and tanks conditions, as well as its commercial implications.

Materials and Methods

The research was conducted at the E. W. Shell Fisheries Center, Auburn University, AL. Two separate experiments were conducted:

Experiment 1.

Two different stocks of redclaw were received at Auburn University for evaluation. The stocks were from Australia (Walkamin stock. Source: Cherax Park, Theebine Queensland, Australia) and Mexico (Megar stock. Source: Megar S.A. de C.V, Soto La Marina Tamaulipas). Upon arrival, the animals were stocked into holding tanks with plenty of onion mesh provided as refuge and with aeration. Animals were held for five days to determine mortality caused by transportation.

After this period the animals were group weighed and stocked into twelve, 200 m^2 earthen ponds at 4 crayfish/m^2. Average weight at stocking was 24.49 grams (21.23 to 29.09 g range). Each pond contained two hundred 5 cm diameter x 20 cm long PVC pipes provided as refuge to reduce predation. Animals in three replicate ponds were randomly assigned to one of four experimental treatments. Australian and Mexican crayfish had two treatments each where males and females were stocked separately.

Based on previous work, crayfish were assumed to have a weekly growth rate of 2.0 g $week^{-1}$. Based on the expected growth rates and a feed conversion of 1.5:1, a feeding table was built for the 16-week growth trial. Daily ration was weighed and offered to the crayfish every evening (~16:00 h). (35% protein, Rangen® Angleton, TX, USA)

At the conclusion of the grow-out trial, crayfish were group weighed and counted. Growth, yield and survival were calculated.

Experiment 2.

Broodstock from the previous experiment were overwintered in indoor tanks and prepared for spawning the following spring. After spawning, juvenile redclaw crayfish released during a 72-hr period were collected from the Walkamin and Megar stocks. Crayfish were separated into groups of ten individuals and stocked into one of 8 circular tanks (600 L volume, 0.8 m^2 bottom area) (12.5 m^{-2}). Each tank contained 10 pieces of 5-cm diameter PVC pipe used for refuge and two submerged air diffusers for aeration and water mixing. Four replicate tanks were randomly assigned to one of the two different stocks. Thirty individuals were randomly collected and weighed to calculate the average initial weight at stocking (0.05 g).

Based on previous work, mean weights of the crayfish were assumed to double during the first five weeks and thereafter have a weekly growth rate of 2.0 g $week^{-1}$.

Based on the expected growth rates and a feed conversion of 1.5:1, a feeding table was developed for the 17-week growth trial. Daily ration was weighed and offered to the crayfish every evening (~16:00 h). (35% protein, Rangen® Angleton, TX, USA)

At the conclusion of the grow-out trial, crayfish were weighed and counted individually. Average weight, yield and survival were calculated.

Genotyping

A sample of 30 males and 30 females of each stock were sent to the Aquatic Microbiology Laboratory in Auburn University to analyze the amount of genetic variability between stocks.

Water quality

Tanks were provided with constant aeration. Ponds received mechanical aeration from dusk to dawn (12.5 hp/ha). Dissolved oxygen (DO) concentrations and temperature were measured twice daily using a YSI 55 DO meter (Yellow Spring Instrument Co., Yellow Springs OH, USA). Values for water temperature were recorded. Ammonia, hardness and alkalinity were measured bi-weekly using a LaMotte® Freshwater Aquaculture kit (LaMotte Company, Chestertown MD, USA) (Nessler Method).

Statistical Analysis

Data from Experiment 1 was analyzed using one-way analysis of variance (ANOVA) and Tukey's test to determine significant differences ($P<0.05$) among treatment means. Data from Experiment 2 was analyzed using Student-Neuman-Keuls multiple range test (α=0.05). Statistical analyzes were performed using MINITAB software (version 15.1, MINITAB Inc., State College, Pennsylvania).

Results

Experiment 1.

Throughout the length of the 16-week trial the mean ±SD water quality parameters were: water temperature 29.48 ± 2.93 ºC; dissolved oxygen 7.56 ± 0.97 mg/l; alkalinity and hardness 67.29 ± 11.82; and total ammonia nitrogen (TAN) 0.13 ± 0.06 ppm. These water quality conditions were within acceptable limits for production of redclaw crayfish (Masser and Rouse 1997).

Growth ranged between 36 and 77 grams per organism. Yields ranged between 553 and 1570 kg/ha. Survival ranged between 24 and 42%. There was no significant difference ($P>0.5$) for yield or survival. There was a significant difference ($P<0.05$) in growth between Australian males and females (Table 1).

Experiment 2

Throughout the length of the 17-week trial the mean ±SD water quality parameters were: water temperature 28.69 ± 2.91 ºC; dissolved oxygen 7.38 ± 0.60 mg/l; and total ammonia nitrogen (TAN) was consistently under 0.1 ppm. These water quality conditions were within acceptable limits for production of redclaw crayfish (Masser and Rouse 1997).

The Walkamin Australian stock averaged 3344 kg/ha yield, 33.75 g average weight and 80% survival. The Megar Mexican stock averaged 2965 kg/ha yield, 34.49 g average weight and 73% survival. There was no significant difference (P>0.05) between the two stocks for any parameters (Table 2).

Genotyping

The results provided by the Aquatic Microbiology Laboratory in Auburn University (Appendix 1) show that there is no differentiation between populations. Females tend to be more similar between stocks than males, which tend to form two separate groups, but individuals of both stocks appear indistinctly in both groups. It appears that the genetic variability of individuals within groups is larger than the variability between groups.

Discussion

Experiment 1

The average growth of the Walkamin females (36.23 g) was similar to the growth reported by Pinto and Rouse (1996) for unselected mixed sexes, under similar densities in earthen ponds. Average growth was between 35 and 45 grams at 5 and 3 org/m^2 respectively. The Average growth of the Walkamin males (77.43 g) and both Megar males (63.88) and females (51.05) were significantly higher. It is important to comment that Pinto and Rouse (1996) started their trial with organism of 10 grams, and even though the relative growth of small animals is higher, they had a lower absolute growth.

Manor et al. (2002) when analyzing average growth of unselected male redclaw stocked into individual cells at 23 g, reported average growth around 46 grams in 30 weeks, which are considerably inferior to the growth of both Walkamin and Megar males of 63.88 and 77.43 g in 16 weeks.

Jones et al. (2000), with an initial stocking weight of 10 grams, reported final weights of 57.7 g for males and 51 g for females in a 7-month trial. McPhee et al. (2004) reported final weights of 64.8 g for the Walkamin line stocked at an average weight of 16.4 g over a 180-day period. Although growth performances of both trials seem inferior, both research teams stocked animals with mixed sexes at higher densities and presented separate results for males and females.

Monosex culture has been proven to increase growth of males and females compared to mixed sex culture (Curtis and Jones, 1995; Manor et al., 2002; Rodgers et al., 2006). As well, growth performance of the male-only culture has been reported to be consistently higher than female-only culture. Results from this study are consistent with such remarks. Megar males and females, even when males showed a higher growth rate trend, did not have any significant differences between sexes or when compared to the Walkamin males or females. Walkamin male growth was substantially higher than that observed for the females of the same stock. Slower growth during the trial of the Walkamin females could be explained by high reproduction rates that was reported from their treatment ponds. Even though sex selection was performed at an advance stage and with a high level of accuracy, there were no physical barriers between treatment ponds, and reproduction during the trial can be attributed to the migration of males from adjacent ponds. Reproduction was present at the Megar female ponds, but to a lesser extent. Small crayfish that were assumed to be product of reproduction (weighing 25% or less of each pond average weight) were not accounted for in survival or yield calculations at the end of the trial.

Average yield results of the four treatments were comparable to the yields reported by Pinto and Rouse (1996) and Rodgers et al. (2006). Although the final weight of the organisms was higher, survival was considerably lower than the previous studies. Low survival was present in all treatments as predation in the ponds by raccoons and otters was unavoidable. High mortalities, with the resulting lower densities, could have also enhanced the growth performance of the surviving individuals.

Although growth performance of the Walkamin males and Megar males and females appear to be similar between each other and higher than that reported in previous studies, there is not absolute certainty as no trial with monosex culture and selected animals has been performed. In addition, analyzing results of different facilities, years, stocking densities, environmental conditions, growth periods, stocks and mixed or monosex culture is extremely difficult. During the study, initial stocking weights were similar, but age for both stocks might have been different as no proof of the sources was available. Besides, sexual maturity of the animals was reached, unwanted reproduction occurred, and survival were not consistent. In consequence, there is no definite information to compare the growth performance of the Walkamin and Megar stocks. However, the results suggest that there does not appear to be a difference between stocks.

Experiment 2

In Experiment 2, animals were stocked at the same age and had been produced from broodstock cared for under the same conditions. Even though they were cultured with males and females present, sexual maturity was not reached during the 17-week growth period. The conditions of this study eliminate the confounding variables of the previous study and provided a direct comparison between the Walkamin and Megar stocks.

No difference in growth was observed between stocks. As well, no significant differences were observed in yield or survival. Achieving similar survival rates during the growing cycle was crucial since different survival rates could have affected density, which affects growth rates (Pinto 1993, Pinto and Rouse 1996; Austin et al. 1997; Jones and Ruscoe 2001; Manor et al. 2002; Naranjo-Paramo et al. 2004; Barki et al. 2006). Survival results confirm that there was not a density effect on the growth of the crayfish, and a direct comparison of growth performance is applicable. Results from the current experiment suggest that there is no significant differences in growth performance between the Walkamin and the Megar stocks.

Conclusions

The fact that there was no difference in the two independently selected lines suggest that either both selection programs were working to the same level of efficiency or that neither program was working. The fact that there had been some measure of success recorded in Australia, suggests that both programs were working at a similar level.

Selecting processes as straightforward as the one performed by Megar are simple to achieve in a well managed farm and could have a direct impact on profitability for most of the aquaculturists that are producing their own juveniles. Nevertheless, it would be advisable to sustain a planned crossbreeding program from different stocks to maintain the loss of genetic material to a minimum and to continue moving toward improved genetic lines.

Table 1. Growth, yield and survival for males and females of the Australian Walkamin and the Mexican Megar stocks, stocked in ponds at an average initial weight of 24.49 g, stocked at 4 crayfish /m^2 and raised for a 16-week period.

Treatment[1]	Growth	Yield (kg/ha)	Survival (%)
Walkamin Females	36.23[a]	553.00	23.50%
Walkamin Males	77.43[b]	1108.00	28.96%
Megar Females	51.05[a,b]	1066.00	31.88%
Megar Males	63.88[a,b]	1570.00	42.25%
PSE	7.23	454.37	12.50
P Value[2]	0.02	0.51	0.759

[1] Means within the same column with different superscripts are significantly different ($P<0.05$).

[2] Pooled standard error of treatment means (n=3).

Table 2. Average weight, yield and survival for crayfish of the Australian Walkamin and the Mexican Megar stocks, stocked in tanks (n=4) at an average initial weight of 0.05 g, at a density of 12.5/m^2 and raised for a 17-week period.

Parameter[1]	Walkamin Stock	Megar stock	P. value
Average weight (g.)	33.75±1.3	34.50±4.4	0.882
Yield (kg/ha)	3344.38±177	2965.31±154	0.168
Survival %	80.00%±7.1	72.50%±10	0.575

[1] Based on Student-Neuman-Keuls multiple range test (α=0.05), no significant differences (P>0.05) were found among treatment.

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CHAPTER VI

SUMMARY AND CONCLUSIONS

The redclaw crayfish, *Cherax quadricarinatus*, industry has experienced a rapid expansion during the last decade as a crustacean aquaculture activity in tropical and subtropical regions. Currently the industry is facing economic challenges due to competition with wild fisheries and other crustacean species, as well as depressed prices. In order to ensure long-term viability of the industry, production efficiencies and costs of production must be improved. Options to improve production cost effectiveness is to reduce feeding costs and improve feeding strategies to minimize the amount of pelleted feed utilized. Optimization of pond usage and quality of juveniles through the establishment of hatcheries and the use of the best growth performance animal are also ways to improve economic viability.

During the series of experiments conducted in this study, results confirm that fish meal can be removed from diets for redclaw juveniles. Plant-animal protein combinations (poultry by-product meal and soybean meal) or all-plant protein alternatives such as pea meal and distiller's dried grain with solubles can be utilized. Findings of this study suggest that the industry can replace fish meal utilizing a wide range of protein sources, that might be easier to find in regional markets at a lower costs. This replacement is possible as long as the protein and lipid levels remain at acceptable levels and the amino-acid profile remains balanced. Cooperation between redclaw growers and local feed producers is essential to achieve cost reduction while maintaining quality.

Results of the present study suggest that the addition of stargrass (*C. nlemfuensis*) at a rate of 125 kg/ha/wk in combination with a properly balanced pelleted feed for juvenile redclaw can reduce the feed inputs and, thereby, reduce cost for redclaw producers. Further studies are needed to analyze the optimal amount of pelleted feed inputs in combination with an adequate amount of forage that will result in the minimal FCR and still generate good growth and survival. It would be useful to determine how little feed would be needed when forage is not used, to still maintain good growth and survival. It would not be advisable for producers to change their feeding strategies immediately. A progressive reduction of feed inputs with direct comparison with control ponds and farm records is recommended.

The hatchery-nursery techniques used in these experiments suggest that water volume has minimal effect on the production performance on a redclaw hatchery-nursery system. Surface area is the best way to account for juvenile production of redclaw. As well, densities between 4.2 and 6.9 females/m^2 with a 30-day nursery period appear to have the best results in a commercial hatchery-nursery, when conducted with similar conditions to these experiments. Although the current experiments set were the first such trials conducted under commercial conditions, improvements at the same facilities have already increased as management practices have been improved and standardized. Their results suggest that facility design can further increase production. Modified designs using area as the limiting factor may improve the total production per facility area. Current recommended female densities might be increased using a higher water exchange rate. Methods of nursing redclaw from 1 g to 5 g average individual weight and optimizing survival so separate sexes can be stocked into ponds are still required. There is still a wide window of opportunities for hatchery-nursery improvement in general and a close relationship between producers and scientists is necessary so the issues can be attended as soon as possible.

When comparing the best two stocks identified by Australian and Mexican farmers, results suggest that there is no significant difference in growth performance between the Walkamin (Australia) and the Megar (Megar) stocks. It would be advisable to continue further selection programs that focus on growth performance and identify other important characteristics such as late sexual maturity which. It is advisable for redclaw producers to plan crossbreeding programs from different stocks to keep the loss of genetic material to a minimum, specially for producers outside Australia that cannot refresh their genetic material from the wild.

The experiments conducted in this study have increased the general knowledge and understanding of the redclaw crayfish, *C. quadricarinatus*. Follow-up of the actual studies and interaction between scientists, producers, service providers and local authorities are necessary to avoid the interruption of the continuous improvement program established since 2001. This is the right moment to act and consolidate the redclaw industry; so, that it can surpass the challenges that every nascent industry faces.

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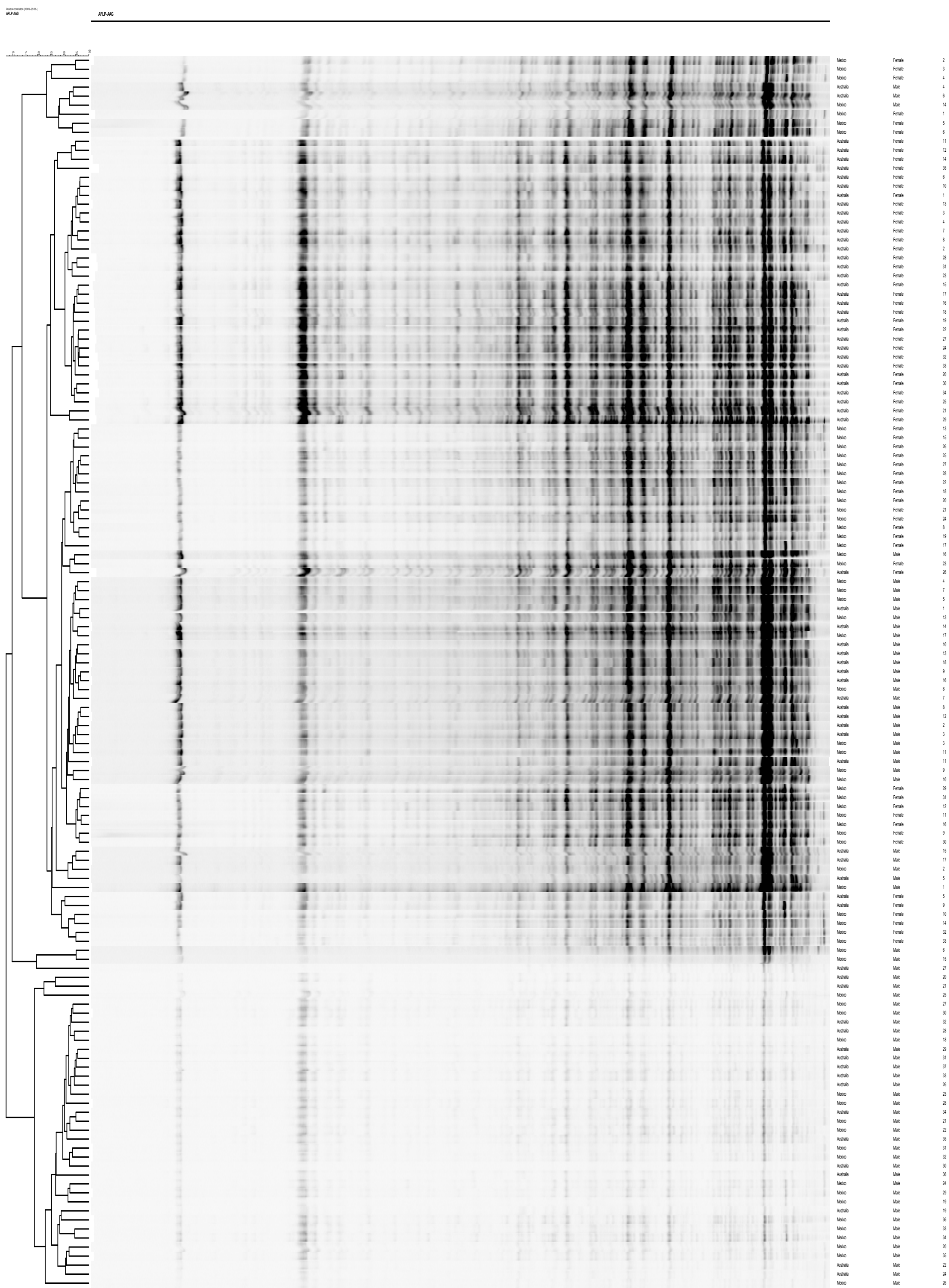
APPENDIX 1

GENOTYPIC VARIABILITY OF THE MEGAR AND WALKAMIN AUSTRALIAN REDCLAW CRAYFISH (*Cherax quadricarinatus*) STOCKS AS REPORTED BY THE AQUATIC MICROBIOLOGY LABORATORY AT AUBURN UNIVERSITY

Primers: M-CTC with E-AAG



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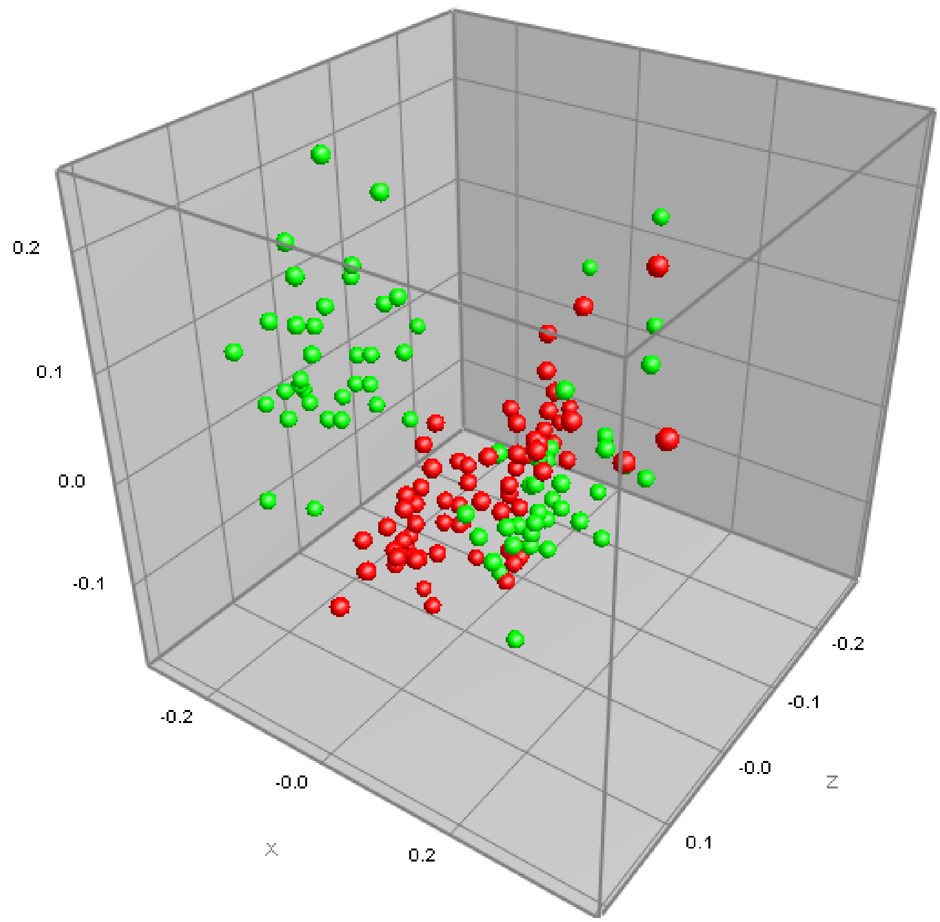
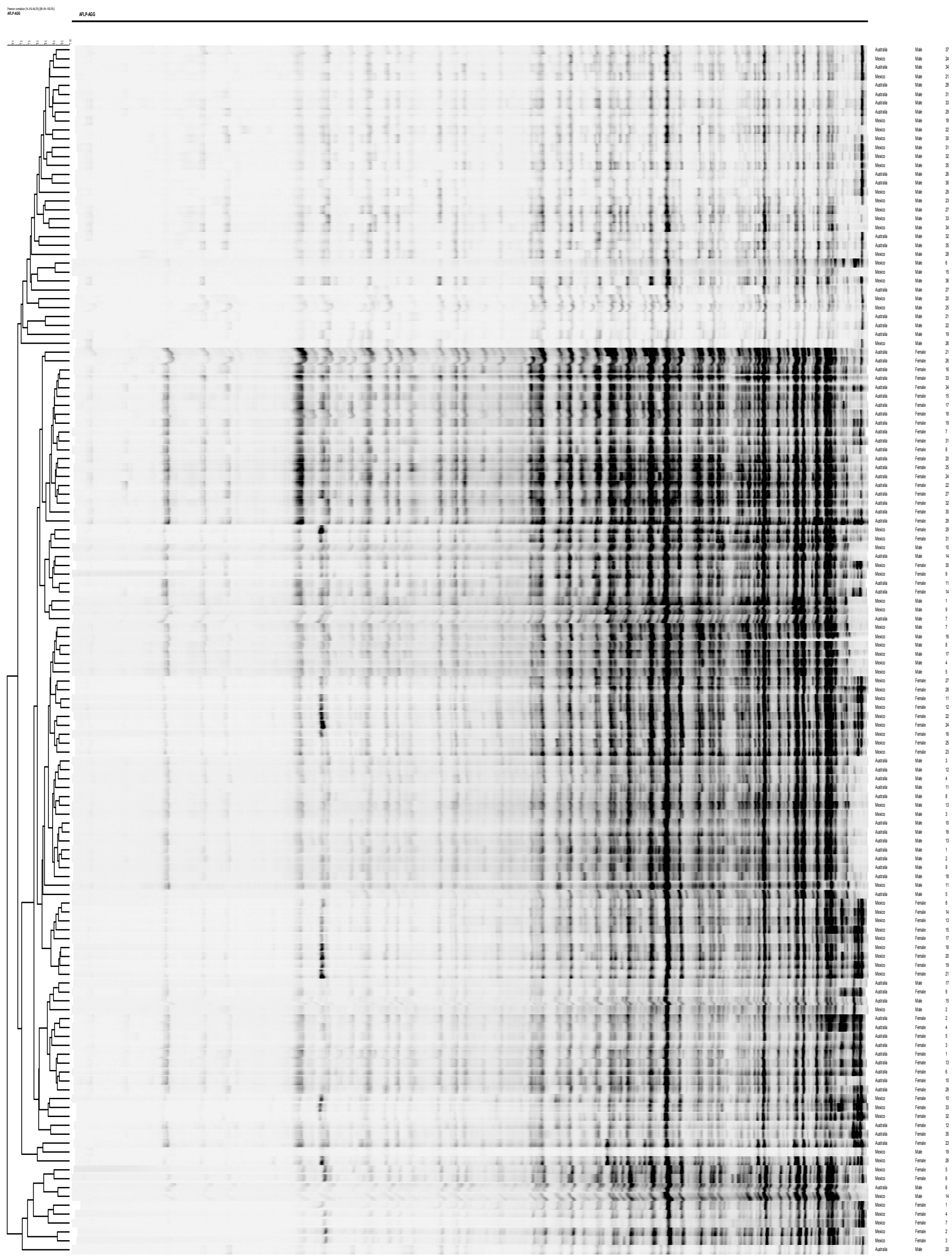


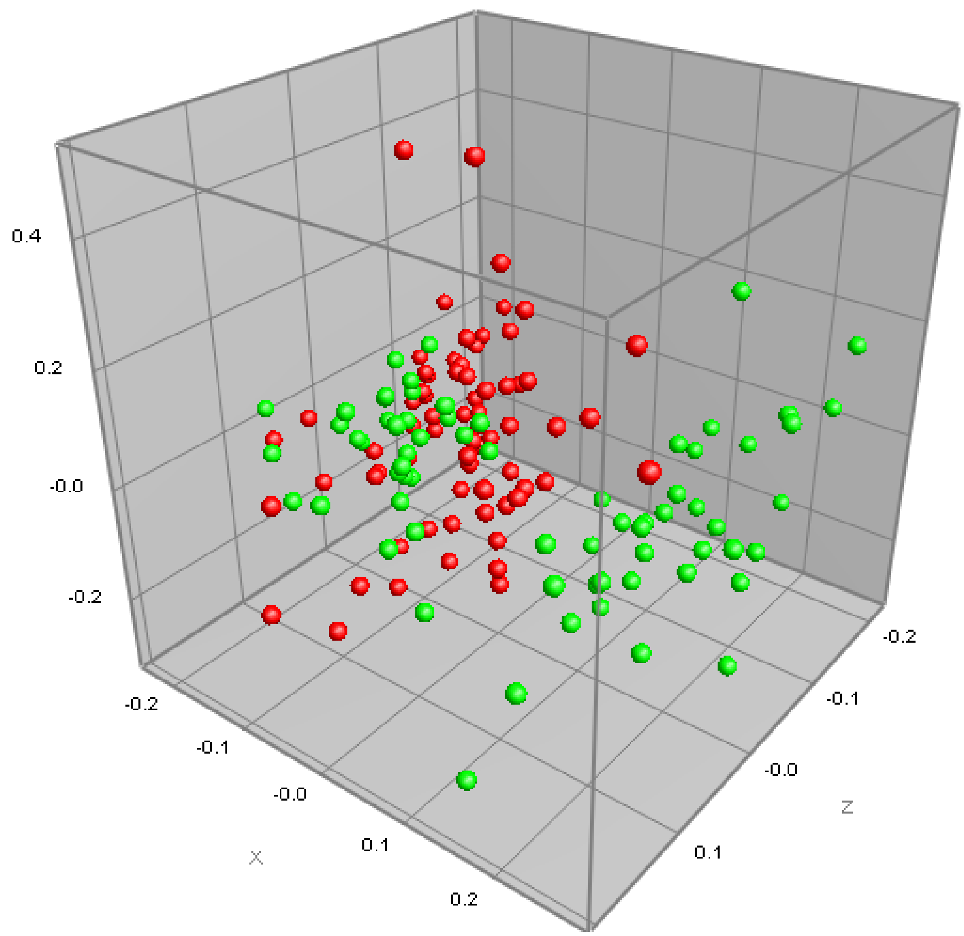
Green= male

Red = female

Primers: M-CTC with E-AGG



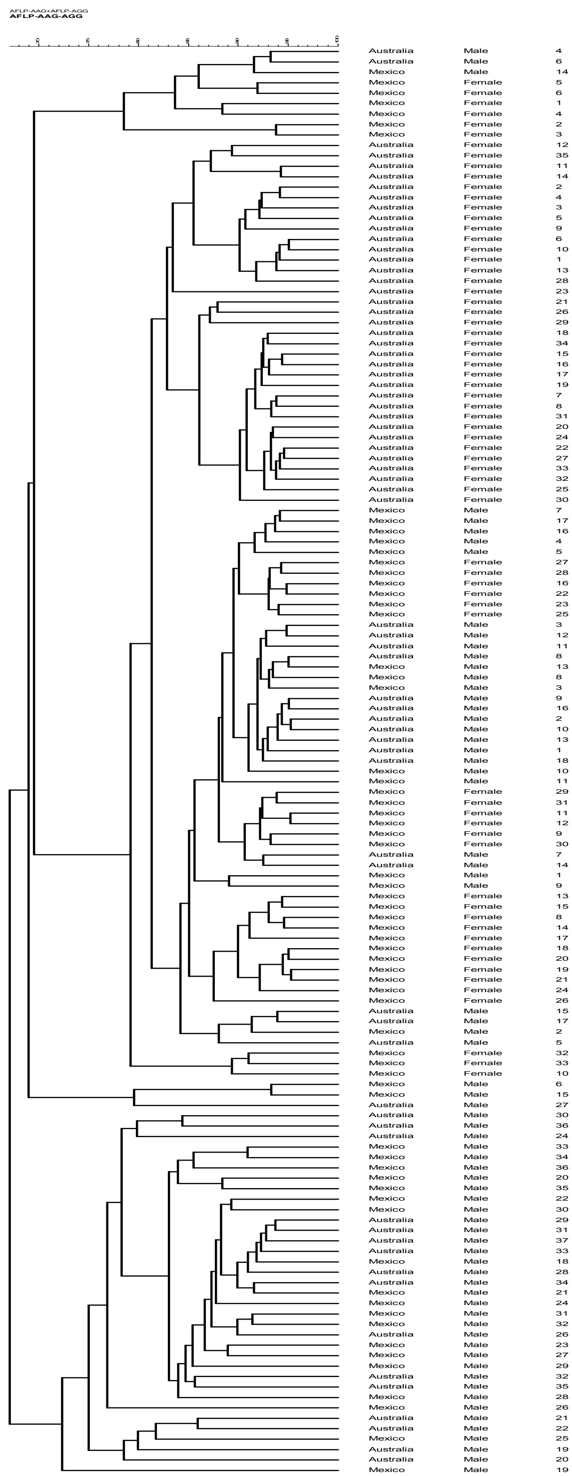
Primers: M-CTC with E-AGG



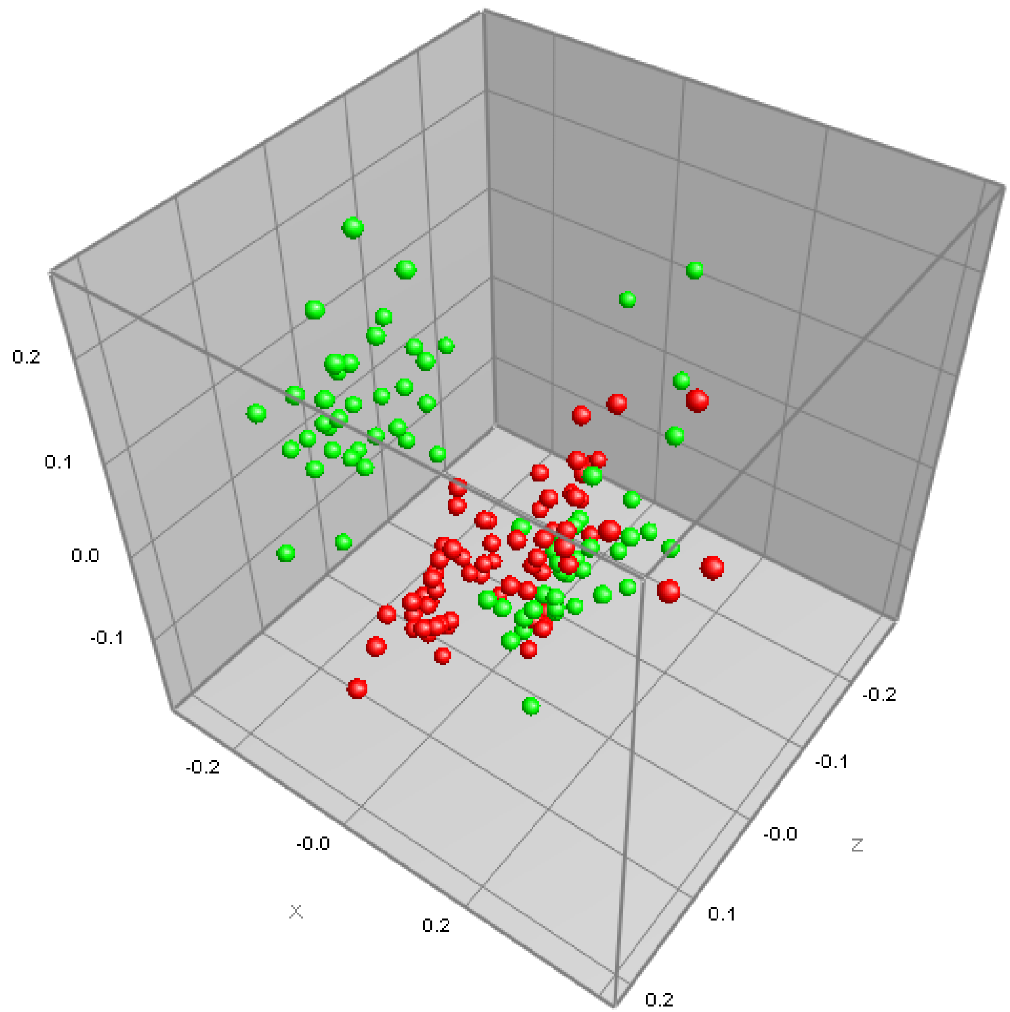
Green= male

Red = female

Composite set: E-AAG+E-AGG



Composite set: E-AAG+E-AGG



Green= male

Red = female