

A Review of the Culture and Diseases of Redclaw Crayfish *Cherax quadricarinatus* (Von Martens 1868)

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Abstract

The redclaw crayfish, *Cherax quadricarinatus*, is a freshwater decapod crustacean displaying a number of physical, biological, and commercial attributes that make it suitable for commercial aquaculture. Interest in redclaw crayfish, both for aquaculture and aquarium trade, has resulted in wide translocations of the species within Australia, south-east Asia, and Central/South America. The redclaw aquaculture industry has been growing rapidly since the mid-1980s in tropical and sub-tropical regions of the world. Redclaw aquaculture is done mostly in extensive pond systems, but interest in developing more intensive systems is increasing. The present manuscript reviews current knowledge and trends of redclaw aquaculture, and areas where further research is needed are identified. Nutrition and reproduction of redclaw were recently reviewed in other manuscripts and those are summarized here. The present manuscript emphasizes environmental tolerances, diseases, aquaculture techniques, and marketing.

Crustacean production for the year 2008 was 5 million tons with a market value of approximately US\$ 22.7 billion. Production was relatively evenly distributed among brackish water (2.4 million tons, or 47.7%), freshwater (1.9 million tons, or 38.2%), and marine water (0.7 million tons, or 14.1%) (FAO 2010). World production of crustaceans has increased yearly since the 1980s both in quantity produced and in number of species aquacultured. A late but nonetheless important new entrant is the Australian redclaw crayfish, *Cherax quadricarinatus*.

Cherax quadricarinatus (von Martens 1868) is a decapod crustacean (Decapoda: Parastacidae) endemic to freshwater habitats of northeastern Queensland, northern and eastern parts of the Northern Territory of Australia, and southeastern Papua New Guinea (Riek 1969; Holthuis 1986; Curtis and Jones 1995; Lawrence and Jones 2002). Potential economic importance of the species both for aquaculture

and aquarium trade has resulted in wide translocations within Australia (parts of Western Australia: Doupe et al. 2004, New South Wales: Coughran and Leckie 2007) and internationally into Mexico (Ponce-Palafox et al. 1999), Ecuador (Romero 1997), Costa Rica, Guatemala, Japan, Taiwan, Jamaica (Medley et al. 1994), China (Xiaoxuan and Edgerton 2001; He et al. 2012), Italy (D'Agaro et al. 1999), Greece (Koutrakis et al. 2007), Israel (Karplus et al. 1995), Caribbean Islands, USA, New Caledonia (Rubino et al. 1990; Wickins and Lee 2002), Spain (Gozlan 2010), Malaysia, Thailand, Singapore (Chang 2001; Alimon et al. 2003), Argentina (Vazquez and López Greco 2007), Zambia (Nakayama et al. 2010), Uruguay (Volonterio 2009), Indonesia and Vietnam (Edgerton 2005; Yuniarti et al. 2011), and Puerto Rico (Vazquez 2008). The species has established feral populations in South Africa (de Moor 2002), Mexico (Bortolini et al. 2007), Puerto Rico (Williams et al. 2001), Singapore, and Jamaica (Todd and D'Andrea 2003; Ahyong and Yeo 2007; Belle and Yeo

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2010), with the potential of establishing feral populations in Israel (Snovsky and Galil 2011), and specific thermal freshwater ecosystems in Europe (Jaklič and Vrezec 2011).

Redclaw display a number of physical, biological, and commercial attributes that make them suitable aquaculture candidates (Jones 1990; Holdich 1993; Masser and Rouse 1993; Webster et al. 2002a, 2002b; Edgerton 2005). They grow rapidly, reaching commercial size (40–200 g) in 6–9 mo at optimal conditions (Rouse et al. 1991; Wickins and Lee 2002). Other positive characteristics include gregariousness, non-aggressiveness, and non-burrowing behavior, straightforward production technology, and tolerance to relatively high stocking densities (Masser and Rouse 1997). The species is physiologically robust and can tolerate low oxygen concentrations (>1 ppm) as well as wide ranges of water quality conditions including, hardness and alkalinity (20 to 300 ppm), and pH (6.5 to 9) (Masser and Rouse 1997). Redclaw is classified as an eurythermal, mesohaline species (Meade et al. 2002) as it can survive wide ranges of temperatures and salinities, respectively (see also Austin 1995; Karplus et al. 1998; Meade et al. 2002; Nyström 2002). Moreover, *C. quadricarinatus* is a gonochoristic species exhibiting sexually dimorphic growth patterns. Sexual maturity is reached within 7 to 9 mo by both males and females under optimal conditions. They are multiple spawners (3 to 5 annual spawns) with moderate fecundity ranging from 100 to 1000 eggs/spawn depending on animal size (Jones 1995a; Masser and Rouse 1997). Development of redclaw is relatively direct as there is a lack of living planktonic larval stages. This factor eliminates the requirement for expensive and sophisticated hatcheries for larval rearing and makes production of juveniles much simpler (Curtis and Jones 1995; Jones 1995a; Masser and Rouse 1997; Jones and Ruscoe 2000; Jones et al. 2000; Rodgers et al. 2006). The species can also efficiently utilize a wide range of feed resources (Jones 1995b; Jones and Ruscoe 1996b, 1996c, 1996d; Masser and Rouse 1997). At present, redclaw have been successfully cultured, extensively

(in earthen ponds) or semi-intensively (in large tanks), in both tropical and sub-tropical regions (Lawrence and Morrisy 2000; Lawrence and Jones 2002). The present review synthesizes current knowledge and trends of aquaculture practices of the freshwater crayfish redclaw, *C. quadricarinatus*, and identifies areas where further research is needed.

Overview of Reproduction

The reproduction biology of *C. quadricarinatus* was recently reviewed by Saoud and Ghanawi (2012) and the important aspects are summarized here. Redclaw are gonochoristic species, yet the presence of few intersex individuals within a population is often observed. Males tend to be larger than females and can be distinguished by an important sexually dimorphic structure; a red patch of soft uncalcified thin red to whitish membrane on the propodus (Curtis and Jones 1995; Masser and Rouse 1997; Karplus et al. 2003a). Females have thin chelae with no red patches on them. Males and females can also be differentiated by locating the gonopores at the base of the third pereopod for females and at the base of the fifth pereopod in males. Often, intersex individuals have the red patch on the chelae, but the gonopores at the base of the third pereopod.

Reproduction of redclaw may occur year round with as many as three to five spawns per year in tropical regions (Sammy 1988; Jones 1990; Jones 1995a; Masser and Rouse 1997). Barki et al. (1997) reported a seasonal pattern of spring–summer breeding with spawning occurring throughout the year, confirming reports by Sammy (1988) of three spawning events during wet summers (26–29 C) and twice during cooler and dryer summers (21–22 C), decreasing to no spawning during winter. Similar annual spawning patterns were reported by Yeh and Rouse (1994) and Jones (1990, 1995a). Molting is not always necessary before spawning, thus the sequential pattern of spawning and molting during breeding season in redclaw could be spawn–molt–spawn or spawn–spawn–molt (Barki et al. 1997). Fecundity is moderate ranging from 100 to 1000 eggs/spawn (depending on female size).

Mating behavior in redclaw includes three consecutive phases; precopulation, copulation, and postcopulation (Barki and Karplus 1999). In brief, during precopulation female redclaw initiate mating interactions, followed by cooperative actions between male and female during copulation with a copulatory position of male-beneath-female. During postcopulation a dominant-subordinate relationship exists between the male and female, respectively (Barki and Karplus 1999). Similar mating behavior was noted by Sammy (1988) except that he reported males actively seeking mates and performing courtship behavior in which they adopted a courtship position stance against the female prior to copulation. Accordingly, identifying periods of female receptivity in *C. quadricarinatus* could be a useful tool for hatchery managers. Possibly, the level of shelter occupation by females could be a potential indicator; more females were noted outside shelters on mating day (Barki and Karplus 1999).

Nutrition

In natural ecosystems, crayfish have polytrophic feeding habits and have been described as predators, omnivores, and/or detritivores (Momot et al. 1978; D'Abramo and Robinson 1989; Jones 1990; Brown 1995; Mamot 1995; Nyström 2002), consuming a variety of macrophytes, benthic invertebrates, algae, and detritus (Brown 1995; Nyström 2002). Jones (1990) suggested that in general *Cherax* species are primarily detritivores, a statement supported by findings of Loya-Javellana et al. (1993) who reported that *C. quadricarinatus* demonstrates an ontogenetic shift from non-selective feeding on decayed plant material or zooplankton to a selective feeding on decayed plant material. The feeding behavior (omnivorous/detritivorous) of redclaw allows for the incorporation of a broad range of animal and plant-based ingredients into formulations of practical diets for aquaculture (Jones 1990; Campaña-Torres et al. 2005, 2006, 2008; Pavasovic et al. 2007a). This could be linked to the variety of digestive enzymes including proteases, lipases, and carbohydrases

that are found in the midgut gland (hepatopancreas) and gastric fluid of redclaw (Figueiredo et al. 2001).

A comprehensive literature review on nutritional biology and dietary requirements of redclaw was conducted by Saoud et al. (2012), and will only be summarized here. Currently, there are few if any commercial feeds specifically formulated and manufactured for redclaw crayfish. Progress has been made over the past decade, but there are still knowledge gaps in relation to nutrient requirements of the species. Some of the areas that require further research include essential amino acid requirements, vitamin and mineral requirements, pelleting technology to produce a dry but malleable pellet, an estimation of optimal feed regimens, broodstock nutrient requirements, and formulations of diets using regionally available ingredients with least cost formulations.

Currently, diets for the commercial production of redclaw are based on formulations of other aquatic species, primarily penaeid shrimp feed but sometimes prawn and fish feed (Cortés-Jacinto et al. 2003, 2004, 2005; García-Ulloa et al. 2003; Thompson et al. 2003a, 2003b). Dietary requirements of some nutrients have been determined for rapidly growing juveniles only, with limited information for larger redclaw approaching market weight or for broodstock. Redclaw have the capacity to adapt their digestive physiology in response to changes in nutrient requirement, nutrient availability, and/or dietary profile (Pavasovic et al. 2007b) and consequently have been reared on a wide range of feed formulations. Redclaw diets could potentially be quite inexpensive to manufacture, considering that formulated diets with 20–30% crude protein and 5–10% lipids, based primarily on vegetable rather than animal ingredients allow for good survival and growth of the species (Cortés-Jacinto et al. 2004).

Saoud et al. (2012) recommended that semi-intensive farms use sinking diets containing 35% crude protein, 6% lipids, 18–20 MJ/kg digestible energy with crustacean vitamin and mineral premix supplement and a water stability of at least 30 min. Broodstock diets should also contain some fish oil and carotenoid pigments.

Environmental Requirements

Temperature and Photoperiod

Temperature and photoperiod are the two major environmental factors that govern the antagonistic processes of reproduction and molting in crustaceans (Daniels et al. 1994; Barki et al. 1997 and references therein). *C. quadricarinatus* is a “summer brooder”; it has a natural reproductive season from spring through summer (Reynolds 2002; Bugnot and López Greco 2009a) and its reproductive cycle is strongly modulated by temperature and photoperiod. Sperm count and weight of *vasa deferentia* rise in summer, whereas the weight of the testes increases in winter (Bugnot and López Greco 2009a). During the spring, the sperm cord becomes denser than during other seasons. Optimum temperatures for sperm production are between 26.5 C and 29.5 C (Bugnot and López Greco 2009a). Ovarian development (de Bock and López Greco 2010) as well as spawning rate are positively influenced by temperature (Yeh and Rouse 1995). Additionally, induction of vitellogenesis starts earlier in female juvenile redclaw at high temperatures (28 ± 1 C) (de Bock and López Greco 2010). In a recent study, Tropea et al. (2010) evaluated effects of long-term exposure to high temperatures (27–30 C) on survival, growth, and reproductive parameters (gonadosomatic index, mean oocyte diameter, proportion of mature versus immature testicular lobes and structure of *vas deferens*) of early juvenile to adult male and female redclaw crayfish. Results showed that high temperature (30 C) has a gender-dependent effect on growth parameters, exerting no influence on females and decreasing male somatic growth. However, high temperature (30 C) has an effect on both female and male reproductive parameters inducing spawning in the former and accelerating spermatogenesis in the latter (Tropea et al. 2010).

King (1993a) reported that breeding can occur all year if temperature is maintained at 25–26 C and photoperiod at 12L:12D. Jones (1995a) reported that exposing broodstock to constant conditions of 26 C and 14L:10D over

a period of 4 mo can stimulate spawning in the majority of females in a captive population. Similar results were also reported by Yeh and Rouse (1994) and Barki et al. (1997). Most spawning occurs during spring and summer conditions, and molting generally occurs after the breeding season, but sometimes is observed between spawns. Interestingly, Karplus et al. (2003b) exposed female redclaw crayfish to simulated winter photoperiod (gradual decrease from 14L:10D to 10L:14D), but maintained them at summer temperatures (27–29 C) and observed a threefold increase in spawning rate. Possibly, these environmental conditions emulate the summer period when temperature rises although days are getting shorter after the spring solstice.

Temperature also modulates other biological aspects of *C. quadricarinatus*. Higher temperatures resulted in shorter development duration, increased rate of energy consumption, and lowered survival during embryonic development of redclaw (Zhao et al. 2000; García-Guerrero et al. 2003). Apparently, survival and duration of development from egg extrusion to juvenile stage decrease with increasing temperature. Zhao et al. (2000) reported temperatures from 24 to 30 C as suitable for embryonic development of redclaw with optimal temperature from 28 to 30 C. King (1994) reported that the thermal tolerance of hatchling redclaw is 22–32 C with maximum growth rate at about 30 C.

Meade et al. (2002) reported that maximal weight gain and survival of redclaw juveniles is within the temperature range of 24–30 C with fastest weight gain at 28 C. Austin (1995) reported that in a 96-h survival trial, best survival for juvenile redclaw was between 10 and 30 C and growth was best at temperatures from 25 to 30 C during a 12-wk experiment. Similarly, Xiaoxuan et al. (1995) reported optimal growth at temperatures ranging from 25–30 C with maximum ingestion rate occurring at 30 C. Karplus et al. (1998) reported that redclaw can survive ambient winter temperatures in open earthen ponds in temperate zones, but exhibit complete growth arrest and lack of molting activity. Furthermore, thermal stress elicits production of heat shock proteins and changes the

protein profile in *C. quadricarinatus* (Cimino et al. 2002).

Ammonia, Nitrite, and Nitrate

As all aquatic animals, redclaw is susceptible to ammonia in the water. The acute toxicity (96-h LC₅₀) of unionized ammonia nitrogen (NH₃-N) to juvenile redclaw is 2.92 mg/L (Liu et al. 1995), suggesting that redclaw are quite resistant to aqueous ammonia, yet Meade and Watts (1995) found that ammonia toxicity (96-h LC₅₀) to juvenile redclaw was only 0.98 mg/L NH₃-N. Hatchlings, however, appear to be quite tolerant of ammonia, having a 96-h LC₅₀ of 4.4 mg/L (Rouse et al. 1995). Differences among reported tolerances could be attributed to differences in age, temperature, water pH, strain of animal used, or even physiological history of the animals used. Further investigations appear necessary before final conclusions on redclaw tolerance to ammonia are made. Nonetheless, all LC₅₀ values reported above are quite rarely encountered in well-managed aquaculture systems and thus aqueous ammonia is not a big threat to redclaw aquaculture.

Nitrite is generally more toxic than ammonia to freshwater animals. Nitrite 96-h LC₅₀ for juvenile redclaw was reported to be 4.7 mg/L NO₂-N (Liu et al. 1995). On the other hand, Meade and Watts (1995) reported much higher tolerance to nitrite toxicity by juvenile redclaw—96-h LC₅₀ of 25.9 mg/L of nitrite. A possible reason for this high nitrite tolerance level is the higher chloride concentration used in the study by Meade and Watts (1995). Lower values (1.09 mg/L nitrite-N) were reported for hatchling redclaw (Rouse et al. 1995). Apparently, after a 24-h exposure to 0.4 mg/L nitrite, growth of redclaw was decreased by 17% and survival by 5%. Exposure to 0.6 mg/L nitrite for 24 h decreased growth by 67% and survival by 48%.

No mortalities were reported for redclaw exposed to nitrate concentrations up to 1000 mg/L (Meade and Watts 1995).

Salinity

Adult redclaw appear to be more tolerant of saline waters than juveniles (Jones 1995c;

Prymaczok et al. 2008). Prymaczok et al. (2008) evaluated the ionic regulatory capacity of adult *C. quadricarinatus* in response to salinities ranging from 0 to 35 g/L for a period of 3 wk by studying growth and levels of Na⁺, K⁺, organic metabolites (glucose and lactate), and free amino acids in muscle and hemolymph. Growth performance and survival were reduced in redclaw exposed to salinities greater than 15 g/L for long periods. Glucose and lactate levels increased upon exposure to high salinity and hyper-regulation of both chemicals was observed up to a salinity of 15 g/L. Furthermore, the free amino acid content in the muscle increased concomitantly with hemolymph sodium. Interestingly, however, the flavor of redclaw flesh improved with water salinity so Prymaczok et al. (2008) recommended increasing water salinity for redclaw conditioning during the last weeks before harvesting. Jones (1995c) reported no effect of salinities up to 12 g/L on survival of adult redclaw, which corroborates findings by Prymaczok et al. (2008).

Maximum growth performance of juvenile redclaw was reported at salinities of 0 and 5 g/L (tested range 0–30 g/L), whereas mortality increased at salinities greater than 5 g/L (Meade et al. 2002). Although hatching rate of redclaw decreases with an increase in water salinity (range tested from 1.0 to 20.0 g/L) (Anson and Rouse 1994), no significant growth differences were reported for juveniles reared at salinities ranging from 0.3 to 14 g/L (tested ranges 0.3–40 g/L) for 12 wk (Austin 1995). Similar to juveniles, adult redclaw appear to be tolerant of brackish waters. Jones (1995a, 1995b) reported high survival for adult redclaw maintained at salinities up to 12 g/L for 3 wk whilst Austin (1995) observed high survival for individuals maintained for 12 wk at 14 g/L.

Production Technology and Practices

Broodstock

Hatchery operations for redclaw crayfish are relatively uncomplicated and often performed outdoors in earthen ponds. Brood animals are stocked in ponds at densities ranging from 0.5 to 2/m² with a male to female ratio between

1:1 and 1:5 (Jones 1995a; Curtis and Jones 1995). In indoor hatcheries or broodstock holding tanks, densities ranging between 6 and 25/m² with sex ratios of 1:1–1:5 have been suggested (Yeh and Rouse 1994, 1995; Jones 1995a; Austin 1998). Barki and Karplus (2000) even reported that breeding redclaw females over a range of densities (20, 40, and 60/m²) at a 1:5 male to female sex ratio does not affect spawning rate, fecundity, and survival of females. They suggested that broodstock densities greater than 60 crayfish/m² is convenient in indoor hatchery conditions. However, it is difficult and costly to have indoor, environmentally controlled broodstock holding facilities as that would require large holding facilities for animals that live on a two-dimensional surface rather than in the water column. Yet, as the industry grows into temperate areas, indoor wintering, hatcheries, and nurseries will become necessary and possibly can be provided by adding vertical substrate to holding tanks.

Hatchery Techniques

Recently, interest in the development of hatchery and nursery protocols for intensive production of juvenile freshwater crayfish has been increasing (see Verhoef and Austin 1999; Manor et al. 2002). Various hatchery–nursery protocols were described by Jones (1995a), Masser and Rouse (1997), Parnes and Sagi (2002), and de Yta (2009) but no unified set of procedures have been empirically tested or described. As the redclaw aquaculture industry develops, demand for quality juveniles will increase and the need for proven rearing protocols that optimize the quantity and quality of juveniles will become a necessity if the industry wants to maintain its present rate of growth.

Intensive larval and juvenile redclaw production is hindered by two main factors: the benthic nature of the species and cannibalism during early juvenile stages (Jones 1995a; Parnes and Sagi 2002). Although juveniles are less benthic than adults (Jones 1995b), juvenile *C. quadricarinatus* still spend most of the time on bottom, leaving the water column virtually

empty. Hatchery operators working with *Macrobrachium rosenbergii* have increased production by supplying adequate shelter substrate and increasing the water volume while keeping the same surface area (Tidwell et al. 1999, D'Abramo et al. 2000). Furthermore, during early juvenile stages redclaw crayfish are subject to cannibalism of newly molted individuals, and larger individuals preying on smaller individuals. The fact that juveniles molt frequently, and that competition for resources leads to size hierarchy among individuals, only exacerbates the problem. Consequently, precise predictions of juvenile production in pond-based hatchery–nursery systems are not possible because of poor survivals (5–10%) and size variability among congeneric juveniles (Jones 1995a; Masser and Rouse 1997). Although enough production of juveniles to support limited demand has been achieved by maintaining sexually mature redclaws in earthen ponds from which juveniles are periodically harvested, the model is land and labor intensive and is not the most efficient way to supply juveniles to farms in a sustainable manner. This problem cannot be solved by stocking younger individuals directly into growout ponds because survival would remain low and production thus becomes more difficult to predict. Consequently, controlled environment hatcheries will soon become necessary although ease of reproduction in ponds has delayed their advent.

The main impediment to controlled environment broodstock holding facilities is the need for space and shelter to hold animals that live on bottom on a two-dimensional surface instead of occupying the water body as most fish would. Also, providing shelter during the mating season such as pipe stacks of adequate diameter is important as it offers protection during periods of vulnerability such as molting, protects the broodstock against predation, and minimizes aggressive interactions (Jones and Ruscoe 2001).

Nursery

Although many commercial operations find redclaw nurseries unnecessary, we believe that

they help in controlling survival in growout ponds and improve estimates of production biomass. However, because redclaw are benthic and carnivorous organisms, nurseries need to have large surface areas and survivals are low at high stocking densities. The first problem is solved by adding lots of substrate to nursery tanks. The second problem is partially mitigated by reducing stocking density. Naranjo-Páramo et al. (2004) evaluated effect of stocking densities (5, 6, 8, 11, and 20 juveniles/m²) on survival and growth performance of redclaw reared in gravel-lined nursery ponds of a commercial farm in Ecuador. Stocking at approximately 11 crayfish/m² or less allowed for a desirable minimum size of 25 g to be obtained over an 80-d trial. Although Du Boulay et al. (1993) found no significant relationship between survival and stocking density of juvenile redclaw reared at 100, 200, 300, and 400 crayfish/m², they did observe high levels of mortality in trials where no refuge or substrate was provided.

Growout

Survival and Growth. Survival rates of redclaw cultured in ponds vary. Reports range from 19–24% (Rouse and Kahn 1998), 49.5–65.4% (Salame and Rouse 2000), 58.3–73.8% (Metts et al. 2007), 60.9–70.1% (Thompson et al. 2006), 46.1–61.1% (Thompson et al. 2004), 32.7–47.5% (Webster et al. 2004), 76.6–87.5% (Jones and Ruscoe 2000) to 15.1–75.1% (Jones and Ruscoe 2001), depending on experimental conditions, such as stocking size and density, diet, and water quality parameters. Good survival rate (65–70%) of redclaw reared in a recirculating system was reported by Rodríguez-Canto et al. (2002).

In general, growth of aquacultured juvenile and early adult freshwater crayfish is exponential (see Evans and Jussila 1997 and references therein). Thus, Evans and Jussila (1997) recommend the use of the specific growth rate (SGR) as a standard for reporting growth rate in freshwater crayfish. The authors also recommended that growout studies should include, if possible, information on age, nutrition, stocking density, and various environmental parameters such as

temperature as these parameters affect growth rate of crayfish.

Webster et al. (2004) stocked redclaw (initial weight of 8.1 ± 3.5 g) into earthen ponds at various densities (12,000, 18,000, and 24,000/ha) and reported average final individual weight ranging from 53.47 to 64.65 g over a 70-d period. Salame and Rouse (2000) reported final weights ranging from 30 to 34 g of juvenile (initial weight of 1–2 g) redclaw stocked at a rate of 4/m² in ponds for 90 d. Thompson et al. (2006) offered various practical diets to juvenile (5.75 ± 3.3 g) redclaw stocked at a rate of 25,000/ha over 97-d period and reported final weights from 51.71 to 62.35 g. Thompson et al. (2004) reported final weights ranging from 70.47 to 70.47 g of juvenile (initial weight of 4.6 ± 2.2 g) stocked in ponds at a rate of 25,000/ha. Metts et al. (2007) stocked juvenile (6.25 ± 3.0 g) redclaw at a rate of 27,170 redclaw/ha and reported final weights ranging from 49.0 to 68.1 g after 113 d of culture. Reported SGR (%/d) in some outdoor studies ranges from 1.83 to 2.10 (Metts et al. 2007), 2.33–2.43 (Thompson et al. 2004), 2.78–3.40 (Webster et al. 2004), 0.63–1.58 (Jones and Ruscoe 2000), and 0.54–0.63 (Jones and Ruscoe 2001). The reported growth rates vary considerably but reasons cannot be identified as they may include one or several variables such as temperature, feed, strain of crayfish, stocking density, water quality, and so on. Rodríguez-Canto et al. (2002) reported fair growth (SGR: 0.96–1.88) of redclaw reared in an indoors recirculating system. Possible suggested factors causing slower growth in tanks as compared to redclaw reared outdoors include inadequate nutrition, stocking density, and pheromone release. Several studies emphasize that natural nutrient sources influence redclaw growth and even when offered formulated diets, consumption of naturally occurring food items is likely to occur (Jones 1995b; Jones and Ruscoe 1996c; Hernández-Vergara et al. 2003). For example, natural productivity contributed 26% to the growth of redclaw reared in an outdoor flow-through culture system (Hernández-Vergara et al. 2003).

Stumpf et al. (2010) evaluated the nutritional vulnerability of Stage III juvenile redclaw based on the estimation of point-of-reserve-saturation (PRS) and the point-of-no-return (PNR), and evaluated their compensatory growth capacity when fed intermittently. Results showed that 50% of the juveniles were able to successfully molt to the following stage (PRS₅₀) after 3.53 feeding days and failed to molt to the following stage (PNR₅₀) after 4.28 starvation days. Moreover, Stage III juveniles are unable to molt to the following stage without feeding, requiring at least 2 feeding days to molt and survive. Three days of initial feeding were necessary to reach a weight comparable to that of the continuous feeding control. Full compensatory growth occurred during the restriction period in the initially fed group and during the re-feeding period in the initially starved group. The authors suggested that the capacity to fully compensate may lead to reduction in the amount of food supplied and thus decrease production costs and improve water quality.

Similarly, Calvo et al. (2011) reported PRS₅₀ for Stage III juveniles to be 2.05 d representing 30% of the stage duration (6.85 d). According to these results 2 feeding days are required for 50% of Stage III juveniles to molt to Stage IV. However, these molted individuals were not in optimal conditions based on growth and presence of hepatopancreatic abnormalities. PRS₅₀ for 1-g juveniles was estimated at 9.19 d; about 45% of the stage duration (21.08 d), suggesting that juveniles of 1 g need more food to accumulate sufficient reserves for molting to the next stage than Stage III juveniles.

Gu et al. (1996) evaluated the effects of feeding level and starvation on growth, water content, and protein content in juvenile *C. quadricarinatus*. Results of their study demonstrated that redclaw can tolerate relatively long periods of starvation by catabolizing energy stores such as protein and can reach water and protein contents similar to control after they had been fed for 6 d following deprivation of food for 12 d. The authors noted that the ability to readily recover from fasting and nutritional stress after access to food is of

adaptive significance; in the wild, redclaw are subject to sporadic food supply.

Genetically improving redclaw's growth rates would benefit the industry and attract investment. Faster growth rate or larger size for age will provide a shorter production period, or alternatively, the option of growing stock to a larger size over a fixed period, thus increasing profitability. Hinton and Jones (1997) and Jones et al. (2000) reported that genetic improvement of growth rate of redclaw is the most important characteristic for increasing economic returns, and that can be performed through selective breeding. Gu et al. (1995) provided evidence that body weight at specific post-release ages varied among stocks of redclaw from various Australian rivers, thus demonstrating the presence of a strong genetic component for growth performance in redclaw. Jones and Ruscoe (1996a) undertook a strain evaluation project followed by experimental selective breeding program (see McPhee and Jones 1997) for increased growth in redclaw. Accordingly, McPhee et al. (2004) undertook a cost-effective genetic improvement program for increasing growth rate of redclaw and to generate a breeding stock to transfer to the aquaculture industry. They suggested that a practical selection program could include periods of closed line selection alternating with test crossing with outside stocks to replenish genetic variation.

Although genetic research on redclaw has focused mainly on selective breeding, another stock improvement attempt consisted of chromosome manipulation (e.g., polyploidy manipulation, hybridization, and related genetic engineering). These were facilitated by Tan et al. (2004) who provided detailed information on the chromosome number and karyotype of redclaw.

Stocking Density. In typical redclaw farms, juveniles weighing 20–25 mg are stocked in ponds at densities ranging between 1 and 50 m² (see Jones 1995a). Jones and Ruscoe (2000) demonstrated that by increasing stocking density from 3 to 15 crayfish/m² and increasing stocking size 17 g, SGRs decline,

survival is unaffected, and economic return and yield of redclaw is increased. The authors suggested stocking densities of 9–15/m² for semi-intensive conditions that include supplemental feed. Weight of redclaw crayfish at harvest is inversely related to stocking density, and yields are generally correlated to density (Pinto and Rouse 1996; Jones and Ruscoe 2000). However, Webster et al. (2004) found no significant differences in final weight, SGRs, feed conversion ratios, survival, yield, and economic return of redclaw stocked at various densities. D'Agaro et al. (2001) found that final mean body weight and feed conversion ratio of redclaw stocked at 25 or 50/m² in recirculating system for 100 d were not affected by stocking densities, whereas SGR decreased and mortality increased significantly with increase in stocking densities. Budiardi et al. (2008) reported no significant difference in growth and survival of juvenile redclaw reared in recirculating system with densities ranging from 20 to 50/m². The authors thus report that the optimal stocking density for redclaw is 50/m². However, García-Ulloa et al. (2012) reared juvenile redclaw at various stocking densities (66, 89, 111, 133, and 156 crayfish/m²) in a closed recirculating system and reported no significant differences in length and SGR among treatments although final weight and daily weight gain were greatest at 66/m².

Barki and Karplus (2004) reported that social interactions in redclaw play a significant role in development of size variation in redclaw and small juveniles have the potential to grow rapidly when cultured in individual compartments. Karplus and Barki (2004) then investigated the effect of social interactions on growth and elucidated involvement of sensory modalities in redclaw. Their study demonstrated that social interactions in redclaw can reduce juvenile growth by 50% even when feed is provided *ad libitum*. Visual and/or chemical cues did not affect growth of neighboring redclaw, whereas growth inhibition in smaller individuals was noted whenever there was tactile contact between conspecifics (Karplus and Barki 2004). The same laboratory team (Barki et al. 2006) evaluated growth

performance of male redclaw reared in individual compartments using three-dimensional units as a means of overcoming social-dependent limitations in order to increase yields per unit area in intensive culture. Results indicated adverse effects of social interactions on growth performance of redclaw.

Karplus et al. (1995) reported that presence of shelters did not have influence on survival of redclaw, but Karplus and Barki (2004) suggested that providing shelters or substrates can potentially minimize interactions among communally cultured redclaw. Jones and Ruscoe (2001) assessed several shelter types on growth performance of redclaw cultured in earthen ponds and found that providing shelter, particularly mesh bundles, significantly improves survival without affecting growth in communally reared redclaw although Jones (1995a) had previously observed insignificant effects of two kinds of nursery shelter on survival. Viau and Rodríguez (2010) evaluated survival and growth of redclaw juveniles in aquaria with four different substrates (plastic mesh, small stones, fine sand, and bare glass) and found that the type of substrate significantly affects growth. In contrast, the substrate type did not seem to affect the growth of advanced juveniles. Survival, however, was not affected by type of substrate.

Aquaculturists generally try to distribute feed over large proportions of ponds in order to decrease possible intraspecific competition. However, spatial distribution of feed had marginal effects on growth and survival, but feed ratio does affect growth and survival of juvenile redclaw (Barki et al. 1997). They found that survival was lower when ratio was higher probably because of increased cannibalism due to higher molting frequencies. Nonetheless, Karplus and Barki (2004) suggested distributing feed over the entire pond or culture unit in order to prevent dominant individuals from monopolizing the food.

Polyculture. Polyculture, concurrent culture of several species, can considerably improve farm production yields by optimizing use of available resources (Bardach et al. 1972; Landau 1992;

Ponce-Marbán et al. 2006). Several trials have been attempted in the USA and Israel at communally culturing tilapia and redclaw with poor results. Brummett and Alon (1994) assessed the potential of polyculture of Nile tilapia (*Oreochromis niloticus*) and *C. quadricarinatus* in earthen ponds and reported reduced growth performance in tilapia and no effect of tilapia on growth performance of redclaw. Other studies resulted in better growth performance of tilapia, but reduced growth performance of redclaw. For example, Rouse and Kahn (1998) reported reduced growth and yield of redclaw when reared in tilapia ponds. The authors suggested that non-aggressive behavior of redclaw might be a reason for their reduced growth as compared to a more aggressive feeding behavior of tilapia. Similarly, Karplus et al. (1995) observed reduced growth performance of redclaw when cultured with tilapia and carp. Barki et al. (2001) evaluated the effectiveness of spatially and temporarily separating the feed for red hybrid tilapia and redclaw as a means of decreasing interspecific competition and possibly increasing growth performance of both species raised communally. Results indicated better growth performance of tilapia in intensive culture with redclaw than when monocultured. However, growth performance of redclaw was overall better in monoculture than when cultured with tilapia. The authors suggested that a temporal feeding strategy, redclaw fed at night whereas tilapia fed during the day, might be appropriate for intensive polyculture (preferably small tilapia and large redclaw), where tilapia is the primary species of interest. Then again, providing shelters for redclaws in polyculture with tilapia improves growth rates (Karplus et al. 2001) and might justify the additional effort. Ponce-Marbán et al. (2006) used a bio-economic model to analyze the economic feasibility of redclaw and tilapia culture in Yucatan, Mexico. Results of their simulations indicated that Nile tilapia polyculture with redclaw crayfish improves economic profitability when farms adopt a polyculture strategy over a 5-yr period. Moreover, polyculture of the two species shortens investment return time and buffers risk-related changes in tilapia sale price.

Optimum outcome would be achieved at densities of 33/m³ tilapia to 10/m² redclaw crayfish.

Harvesting, Processing, and Marketing

Harvesting

Most harvest of redclaw can be accomplished by using either baited crayfish traps, flow-traps, or by draining the pond (Masser and Rouse 1997). Flow-traps are very successful in trapping redclaw because the species responds strongly to a current of water (see Masser and Rouse 1997; Wickins and Lee 2002). The main problems we have encountered is that flowtraps have to be monitored or else predatory birds or otters and raccoons attack the crayfish that congregate on the ramp of the trap. Additionally, no trapping is 100% efficient and ponds have to be emptied during the cool early hours of the day and stragglers collected by hand from the mud.

Transportation

In general, juvenile crayfish are shipped in double plastic bags (placed in cardboard boxes with cooling packs) containing water (100–150 juveniles/10 L), some prewashed coconut fiber or onion sack mesh and oxygen (see Wickins and Lee 2002).

Transported juveniles should be acclimated to receiving waters before stocking. Romero and Murillo (1997) described procedures for transportation and acclimation of juvenile redclaw after a transportation time of 24 to 48 h. Prior to shipping and packing, juveniles should be starved and purged for 48 h. Acclimation tanks should have enough bottom area and plastic onion bag mesh. Oxygen should be maintained above 6 ppm and temperature increased at a rate of not more than 1 C/h. When these procedures were followed, the authors observed survival rates of 88.7% and 72.7% at 126 d after shipping for juveniles that had a transport time of 24 and 48 h, respectively.

Processing

Thompson et al. (2004) reported that male redclaw grow larger, have larger chelae, and more tail muscle compared to females.

However, no significant differences were observed in the proximate composition of the tail muscle of male and female redclaw. Typical redclaw flesh contained 81.0% moisture, 16.46% protein, 0.16% lipid, 0.1% fiber, and 1.42 % ash on a wet weight basis (Thompson et al. 2004).

In general, crayfish are purged in clean, running freshwater and marketed as whole live or frozen (cooked and uncooked), hard-shelled, soft-shelled, or fresh or frozen tails (Wickins and Lee 2002; Thompson et al. 2004). Marketing or processing characteristics of crustaceans generally include amount of tail meat, or percentage of edible muscle in relation to body weight. Meat yield in redclaw is about 22% of body weight (Jones 1990). Thompson et al. (2004) reported tail muscle weight (% of body weight) to be 27.4–27.9% and Metts et al. (2007) reported tail meat yield ranging from 23.2 to 24.9%.

Chelae weight comprises a larger proportion of male body weight than female body weight (16.2%–22.6%, 12.1%–14.2%, respectively) (Gu et al. 1994; Thompson et al. 2004). However, differences in total proportion of meat production between male and female redclaw was insignificant. Accordingly, females could be preferable to males since meat is only obtained from the tail (Gu et al. 1994). In contrast, Thompson et al. (2004) suggested that since male redclaw grow larger, have larger chelae, and higher tail muscle weights compared to females, it might be economically advantageous to stock all-male populations of redclaw in ponds to achieve maximum production. Such an advantage of stocking all male redclaw was demonstrated by Rodgers et al. (2006).

Redclaw Meat Quality Attributes and Meat Storage Stability

Production of successfully sustainable and economically feasible redclaw market is dependent on consumer's acceptability of the redclaw meat quality (Chen et al. 2010). Redclaw raw meat deteriorates rapidly *postmortem* due to a range of biochemical and microbial degradation

mechanisms (Tseng et al. 2002, 2003, 2005; Kong et al. 2006, 2007; Chen et al. 2007; Benjakul and Visessanguan 2010). Storage at low temperatures significantly reduces the growth of microorganisms (Chen et al. 2007; Benjakul and Visessanguan 2010), but meat quality is still susceptible to deterioration resulting from autolytic biochemical or physical processes and lipid oxidation (Flick et al. 1992; Mackie 1993; Tseng et al. 2003, 2005; Chen et al. 2007; Benjakul and Visessanguan 2010).

Several studies have evaluated various preservation techniques on redclaw meat quality (Tseng et al. 2002, 2003, 2005; Chen and Xiong 2008; Chen et al. 2007, 2010; Kong et al. 2006, 2007). Tseng et al. (2002) found that redclaw muscle was susceptible to lipid oxidation, but resistant to proteolytic degradation and protein denaturation during short-term storage (<7 d) at 0°C. However, iced storage did not prevent losses in cooking yield and tenderness of the redclaw tail muscle. Tseng et al. (2002) suggested that if redclaw tails are to be kept on ice, as in retail situations, the storage time should be limited to less than 7 d. Tseng et al. (2003) then evaluated quality changes in redclaw crayfish muscle exposed to multiple freezing–thawing cycles. They found that repeated freezing–thawing up to six cycles had detrimental effects on cooking yield and texture of redclaw muscle because of a series of protein changes, including denaturation, aggregation, and degradation, as well as lipid oxidation. They suggested not more than three freezing–thawing cycles for good eating quality frozen redclaw meat held for retail, wholesale, or home use. Dipping shell-on redclaw meat into antioxidant (tocopherols, propyl gallate, or rosemary extract) solutions effectively inhibits lipid oxidation and texture deterioration in muscle tissue when stored at –20°C for up to 6 mo; however, this did not prevent texture softening during frozen storage (Tseng et al. 2005).

Chen et al. (2007) investigated microbiological and physicochemical properties of redclaw crayfish tail meat stored in three different package systems (modified atmosphere packaging [MAP], polyvinylchloride [PVC], and vacuum

packaging [VP] at 2 C). They observed that redclaw meat was susceptible to microbial spoilage during refrigerated storage, attributable to the high pH of muscle tissue and abundance of nutrients. Of the three packaging systems, the MAP stored redclaw developed less off flavors and no rancidity within the experimental period of 14 d with some cooking loss and reduced meat tenderness observed. Similarly, Chen and Xiong (2008) reported that MAP is effective in inhibiting microbial growth while minimizing oxidative and textural changes in precooked shell-less refrigerated redclaw crayfish tails. Chen et al. (2010) compared the effect of vacuum packaging and air-permeable polyvinylchloride film wrap packaging on the quality of redclaw muscle during frozen conditions. The combination of vacuum and freezing at -20 C was more effective than non-vacuum freezing for inhibition of microbial growth. Moreover, vacuum packaging did not appear to have a significant effect on physicochemical attributes of proteins affecting meat texture of frozen redclaw flesh.

Kong et al. (2006) reported that spawning and gender influence redclaw crayfish meat during refrigerated storage (2 C). They found that spawning female redclaw have an inferior meat quality (i.e., reduced cooking yield, a higher shear force, and a lower sensory tenderness, juiciness, and overall acceptability) compared to male and non-spawning female redclaw. Kong et al. (2007) then found that spawning status was a more significant factor than gender in affecting the *postmortem* protein stability and enzyme degradation of redclaw meat and associated quality changes during refrigerated conditions (2 C). Spawning female redclaw muscle proteins were more heat stable and less susceptible to proteolytic breakdown than muscle proteins from non-spawning female redclaw and male redclaw.

Economics and Marketing

Redclaw production and sales are mainly in northeastern Australia. Although the production of redclaw is developing fast in south-east Asia and Central/South America, it is still small scale

and scattered (Medley et al. 1994; Jones and Ruscoe 1996a; Jones and Ruscoe 2001; Villarreal and Peláez 2000; Thompson et al. 2003a, 2003b, 2005, 2006; García-Guerrero et al. 2003; Campaña-Torres et al. 2008). According to Jones and Ruscoe (2001) the major difficulty the redclaw industry faces is lack of production volume and therefore an inability to exploit lucrative export market opportunities. An economic assessment by Hinton and Jones (1997) found that redclaw bioeconomics are sensitive to growth rate (i.e., time to reach market size) and consequently, growth rate is the most important characteristic to be genetically improved for better economics. Similarly, Jones and Ruscoe (2001) suggested that genetically improving redclaw growth rates would benefit individual enterprises and attract greater industry investment because of increased profitability. However, information on genetic selection of redclaw for growth or other attributes is sparse and profitability still depends on marketing and price offered for various size organisms. Some state governments in Mexico attempted to find markets for redclaw farmers with limited success.

Diseases

Diseases are a major constraint to the sustainable development of crustacean aquaculture, causing substantial animal and economical losses (see Lightner and Redman 1998; Wickins and Lee 2002; Walker and Winton 2010). Diseases caused by infectious pathogens including viruses, bacteria, protozoa, and fungi as well as nutritional deficiencies and bad water quality can occur during all life stages of crustaceans (Lightner and Redman 1998; Wickins and Lee 2002; Edgerton et al. 2002; Longshaw 2011). Redclaw crayfish are susceptible to a number of pathogens but none have been linked to widespread epizootics (Edgerton 1999). However, a growth in the crayfish industry is likely to be associated with increased occurrence of disease outbreaks (Edgerton et al. 2002). A number of general reviews on diseases of freshwater crayfish and a non-refereed review of redclaw diseases by Edgerton (1999)

have been published (see Edgerton et al. 2002 and references therein). Recently, a systematic review of parasites, pathogens, and commensals of freshwater crayfish was performed by Longshaw (2011). The nomenclature of diseases in this article is based on those of Longshaw (2011).

Brief Overview of Redclaw Immune System

The defense mechanisms of redclaw against infection are similar to those of other crustaceans. The first line of defense against microbial invasion is the cuticle that contains inhibitors against enzymatic attack (Wickins and Lee 2002). If the pathogen is able to penetrate the first line of defense an immediate recognition of the “non-self” material takes place by hemocytes and plasma proteins. The hemocytes can then mount phagocytic, cytotoxic, and inflammatory responses against invading pathogens (bacterial, fungal, and protozoan) (Wickins and Lee 2002). Unfortunately, viral pathogens are often less likely to be recognized because many viruses have surface molecules similar to those of the host’s cells.

Crustaceans in general lack adaptive immune systems and rely solely on an innate immunity that defends invertebrates from invading bacterial, fungal, and viral pathogens (Söderhäll and Cerenius 1998; Iwanaga and Lee 2005). The innate immune system is composed of a number of defense components (e.g., phenoloxidases, clotting factors, complement factors, lectins, protease inhibitors, antimicrobial peptides, toll receptors, and other humoral factors) mainly found in hemolymph plasma and hemocytes of crustaceans (Iwanaga and Lee 2005). One of the most studied innate defense mechanisms in crustaceans is the production of antimicrobial peptides as a response to parasite invasion. Foreign particles are identified using recognition molecules found in the blood (hemolymph) of invertebrates. Recognition molecules initiate the activation of the prophenoloxidase (proPO) system and may also induce activation of other defense systems. Upon activation of the proPO system, the associated proteins gain biological activity and participate in the cellular defense

reactions of the host species (see Söderhäll and Cerenius 1992, 1998 for a more thorough explanation).

Pathogens

Viruses. Freshwater crustacean viruses have not been purified sufficiently to permit further characterization beyond preliminary descriptions of their morphology, pathology, and epidemiology (Edgerton et al. 2002). This paucity of knowledge of viruses is attributed to lack of sensitive diagnostic tools for viral detection and the establishment of a crustacean cell line for *in vitro* replication and analysis. Although the successful transfection of Australian redclaw crayfish cells with human cancer genes that was recently conducted by Claydon and Owens (2008) is a significant advance to achieving immortal crustacean lines, still the point where viral infection of crustacean cell lines is a common procedure is not reached. The lack of appropriate cell lines thus remains a serious obstacle to the development and ultimate commercial production of drugs and control agents for viral diseases of crustaceans.

A number of viruses including *C. quadricarinatus* bacilliform virus (CqBV) (Anderson and Prior 1992), *Cherax giardiavirus*-like virus (CGV) (Edgerton et al. 1994), spawner-isolated mortality virus (Owens and McElnea 2000), a putative gill parvovirus and a reo-like virus (Edgerton et al. 2000), and *C. quadricarinatus* parvo-like virus (CqPV) (Bowater et al. 2002) have been reported to infect cultured *C. quadricarinatus*. Some unidentified viral infections in *C. quadricarinatus* have also been reported in less detail (reviewed by Longshaw 2011).

Intranuclear Bacilliform Viruses. Crustacean non-occluded intranuclear bacilliform viruses (IBVs) are double-stranded DNA viruses (Longshaw 2011). There is a lack of specific knowledge of IBVs of redclaw (Claydon et al. 2004a) and these viruses are unclassified because of lack of specific molecular, immunological, and biochemical data. In freshwater crayfish IBVs appear to be restricted to the hepatopancreas and the gut. Improved viral

detection techniques would provide more material for viral purification, development of molecular diagnostic tools for detection, and allow for better phylogenetic analysis of the virus (Claydon et al. 2004a).

Anderson and Prior (1992) described the first bacilliform virus (CqBV) (non-occluded rod-shaped nuclear virus) infection among wild and farmed freshwater crayfish in *C. quadricarinatus* in Australia. The infected hepatopancreatic epithelial cells had nuclei containing amorphous, eosinophilic intranuclear inclusions. Virions length ranged from 172 to 220 nm and nucleocapsids were cylindrical with square ends and an average size of 34×154 nm. The authors noted that the virus infection did not cause any disease or mortalities and was observed in all ages of crayfish. However, Edgerton and Owens (1997) reported that the age at which *C. quadricarinatus* is first susceptible to infection by CqBV is 2 wk after juveniles molt into Stage III with susceptibility to the virus at all subsequent ages. CqBV was also detected in redclaw farms in Ecuador (Romero and Jiménez 2002). Although the virus was not benign, its high prevalence suggested it was not highly pathogenic in both Australia and Ecuador (Edgerton et al. 1995; Edgerton and Owens 1999; Romero and Jiménez 2002).

Groff et al. (1993) detected non-occluded baculovirus in farmed redclaw in California, USA. Although no external or internal signs of disease were observed in the infected crayfish, histological studies revealed eosinophilic to amphophilic intranuclear inclusions within the tubular epithelial cells of the hepatopancreas. Electron microscopy revealed numerous loosely enveloped, rod-shaped baculoviruses. The enveloped virions had a length of 292 ± 15 nm and a diameter of 102 ± 7 nm ($M \pm SD$). The cylindrical nucleocapsids often possessed squared ends and were 216 ± 13 nm in length by 47 ± 3 nm in width. There was no evidence of occlusion body formation similar to that known for certain other crustacean baculoviruses (Groff et al. 1993). Similarly, Hauck et al. (2001) described the pathology and extension range of CqBV strain in the hepatopancreatic epithelium of redclaw crayfish in Utah,

USA. No signs of clinical disease were reported but a small number of hypertrophic nuclei were detected. Rod-shaped virions had mean size of 224 ± 14 nm \times 74 ± 4 nm and cylindrical with square ends nucleocapsids measured 180 ± 9 nm \times 38 ± 3 nm. Although some disagreements about the size of CqBV are found in literature, this could be explained by the possible existence of several strains of CqBV (Edgerton 1996). The virus was also reported in *C. quadricarinatus* farmed in Chile (Bateman et al. 2005).

Pathogenicity of CqBV is unclear; some reports do not associate CqBV infections with disease or mortality (Anderson and Prior 1992; Hauck et al. 2001), whereas others linked CqBV with reduced growth (Groff et al. 1993; Edgerton et al. 1995) or low-grade mortality that occurs in association with bacterial infections. Hauck et al. (2001) points out that although pathogenicity of CqBV is unclear, there are potential risks of cross-species infections. Redclaw affected by CqBV are lethargic, have weakened tail-flick response, and are unable to turn themselves when placed on their back (Edgerton et al. 2002).

Parvoviridae. The *Parvoviridae* family, subdivided into two subfamilies: *Parvovirinae* (five genera) and *Densovirinae* (four genera), comprises small non-enveloped icosahedral viruses with linear single-stranded DNA genomes (Fauquet et al. 2005; Longshaw 2011). Edgerton et al. (2000) reported a parvo-like virus (*CqPIV*) infection among farmed *C. quadricarinatus* in Australia that was linked to chronic mortalities. Gill epithelial cells had hypertrophic nuclei with marginated chromatin and peripheral nucleoli. Virus-like particles of approximately 20 nm in diameter were noted within the nucleus of affected gill cells. Bowater et al. (2002) reported another parvo-like virus causing mass mortalities in juvenile and adult *C. quadricarinatus*, also in Australia. The affected crayfish were weak, anorexic, lethargic, had soft shells, and weakened tail-flick response. Intranuclear inclusion bodies with marginated chromatin were noted in a number of tissues including gills, cuticular epithelium,

foregut, midgut and hindgut epithelium, and connective tissues. Lower numbers of inclusion bodies were noted in the antennal gland, hematopoietic tissue, epithelial cells of seminiferous tubules, and interstitial tissue of ovaries, but no inclusion bodies were found within hepatopancreocytes, neurons, or the heart. Hexagonal parvo-like particles with an average diameter of 19.5 nm were found within infected cells.

Following reports by farmers of reduced tolerance to stress and reduction in yield and final harvest of *C. quadricarinatus*, Owens and McElnea (2000) investigated possible causative agents. They found positive *in situ* hybridization reactions using a spawner-isolated mortality virus in the nuclei of a number of tissues including hepatopancreas, midgut and associated glands, epithelium of seminal ducts, and follicle cells surrounding oocytes and to a lower extent in the heart, hemocytes, connective tissue, and subcutis. However, the crayfish did not exhibit the typical symptoms of the disease such as hemocyte infiltration and necrosis of affected tissues.

La Fauce and Owens (2007) challenged (orally and by inoculation) juvenile *C. quadricarinatus* with purified *Penaeus merguensis* densovirus (*Pmerg*DNV) and evaluated the effect of stocking density on the expression of the disease. Crayfish challenged with the virus showed signs of the disease (e.g., lethargic, weakened tail-flip response, lying in their sides, and reduced appetite) less than a week after the start of the challenge. Mortalities were reported in less than 24 h. No histopathological changes were noted in challenged crayfish; however, other opportunistic infections (bacteria, fungi, *Coxiella cheraxi* [rickettsia], and reovirus) were reported. Overcrowding had a significant effect on mortality rates. The authors concluded that *C. quadricarinatus* are potential carriers of *Pmerg*DNV and stress is an immunosuppressant allowing other opportunistic infections to be expressed.

Other Viruses. Reoviridae: members of the *Reoviridae* family viruses have a genome consisting of double-stranded RNA (see Longshaw 2011). A case of reovirus infection was

reported by Edgerton et al. (2000) in one moribund *C. quadricarinatus* in Australia. The crayfish had eosinophilic cytoplasmic inclusions in the hepatopancreocytes. The infected nuclei contained non-enveloped virions with regularly shaped hexagonal and pentagonal forms of diameter 35–40 nm. Hayakijosol and Owens (2011) challenged juvenile redclaw with purified reovirus in an attempt to determine the susceptibility of redclaw to hepatopancreatic reovirus. The infected redclaw showed reduced appetite, and appendages and mouthparts were reddened. Cytoplasmic inclusion bodies of reovirus were found in the hepatopancreatic tubules and the reovirus was non-enveloped and icosahedral in shape with diameter of 55 nm.

Totiviridae: Edgerton et al. (1994) reported *Cherax* Giardia-like virus in Australia. The infections were in the hepatopancreocytes with mildly hypertrophic nuclei. Non-enveloped virions, hexagonal or pentagonal in shape suggesting icosahedral shape measured approximately 25 nm (cross-sectionally). The infection was highly prevalent among juvenile *C. quadricarinatus* (Edgerton et al. 1994; Edgerton and Owens 1997; Edgerton and Owens 1999) and is thought to be a significant pathogen among juvenile redclaw.

Nimaviridae: White spot syndrome virus (WSSV), belonging to the family *Nimaviridae*, is a double-stranded DNA virus with elliptical to rod-shaped virions that measure 80–120 × 250–380 nm (Edgerton et al. 2002; Longshaw 2011). WSSV is considered as the most serious viral pathogen in cultured penaeid shrimp (Soowannayan and Phanthura 2011), and associated with epizootic mortality in prawn aquaculture (Edgerton et al. 2002). Shi et al. (2000) infected crayfish with WSSV isolated from *Penaeus chinensis* and concluded that WSSV can develop and express pathogenicity in redclaw and therefore the species is considered a potential carrier of WSSV. However, Edgerton et al. (2004) noted that the species studied by Shi et al. (2000) was not *C. quadricarinatus*, but *Procambarus clarkii*. Although the infectivity of redclaw by WSSV is not certain, Liu et al. (2011) isolated differentially expressed genes related to WSSV

from redclaw, suggesting WSSV pathogenesis in redclaw. This suggestion is supported by reports that redclaw is susceptible to infection by WSSV and can transmit WSSV to the giant tiger shrimp, *Penaeus monodon* (Soowanayan and Phanthura 2011). This question was resolved by Wang et al. (2012) who found that WSSV infection in redclaw caused changes in gill enzyme activity and markedly damaged gill epithelium, suggesting that the pathology of the virus in redclaw exists and is multi-faceted.

A number of diagnostic tests to detect WSSV, based on hybridization assays using probes derived from cloned pieces of the viral genome, have been developed (see Shi et al. 2000; Longshaw 2011). The World Organization for Animal Health (formerly the Office International des Epizooties [OIE]) recommends the use of a nested polymerase chain reaction (PCR) approach using primer sets developed by Lo et al. (1996) designed for the detection of WSSV. However, Claydon et al. (2004b) noted that false-positive results for WSSV associated with the primer sets of Lo et al. (1996) can lead to incorrect quarantine and unnecessary destruction of animals.

Nodaviridae: *Macrobrachium rosenbergii* nodavirus (MrNV) or white tail disease is a newly reported disease in crustaceans in western Queensland, Australia (Hayakijkosol et al. 2011). MrNV is a small, non-enveloped RNA virus with a genome consisting of two linear ssRNA fragments (see Hayakijkosol et al. 2011). Hayakijkosol et al. (2011) evaluated experimental infection of redclaw with *Macrobrachium rosenbergii* nodavirus and reported that redclaw have low susceptibility to the virus and are limited carriers of white tail disease. Recently, Hayakijkosol and Owens (2012) reported finding a sequence specific dsRNA against protein B2 produced RNAi (RNA interference; an innate immune response effective against viral infections) that was able to functionally prevent and reduce mortality in MrNV infected redclaw.

Bacteria

Bacterial diseases are common in cultured crayfish (Longshaw 2011). Although many

harmful bacteria are generally present in the aquatic environment, it is usually under stressful environmental conditions that these bacteria cause pathogenicity in cultured organisms (Edgerton et al. 2002). The main known bacterial pathogens of redclaw appear to be *Vibrio* and *C. cheraxi*.

Vibrio: Eaves and Ketterer (1994) reported two cases of mortality in cultured redclaw crayfish in Australia, both linked to *Vibrio mimicus* infection. *V. mimicus* is an opportunistic pathogen causing systemic infections following stress from overcrowding or poor water quality (Eaves and Ketterer 1994). *V. mimicus* can cause systemic disease in commercially cultured redclaw Wong et al. (1995) and has been associated with gastroenteritis in humans through the ingestion of improperly cooked crayfish (Eaves and Ketterer 1994).

Coxiella cheraxi: A number of reports have isolated rickettsia or rickettsia-like organisms (RLOs) from cultured redclaw. RLOs are small, pleomorphic, rod-shaped coccoid prokaryotes, most of which are Gram-positive intracellular organisms (Edgerton et al. 2002; Longshaw 2011). However, Edgerton and Prior (1999) reported basophilic, Gram-negative, microcolonies of rickettsia-like organisms in the hepatopancreatic tubule epithelium in one moribund *C. quadricarinatus*, in Australia. Basophilic inclusions similar to rickettsia-like microcolonies were reported earlier in *C. quadricarinatus* by Ketterer et al. (1992) and Owens et al. (1992). Systemic intracellular bacteria, similar to rickettsia-like organisms, causing chronic mortality have been observed in farms in Ecuador (Jiménez and Romero 1997; Romero and Jiménez 2002). Romero and Jiménez (2002) regarded the disease as the most important and virulent redclaw pathogen in Ecuador. These rickettsia-like organisms reported by Jiménez and Romero (1997) and Romero et al. (2000) were probably closely related to *C. cheraxi* (Longshaw 2011). Reports of mortality of aquacultured redclaw caused by RLOs identified as *C. cheraxi* by Tan and Owens (2000) working in Australia corroborated previous reports in Ecuador.

Intracellular Prokaryotes

Jiménez et al. (1998) reported the presence of prokaryotic intracellular organisms in the cuticular epithelium of cultured redclaw in Ecuador. The authors assumed that these organisms were most likely mollicute-like because of their lack of cell wall and pleomorphic morphology and presence of terminal structure. These intracellular prokaryotes were again found in redclaw in Ecuador by Romero and Jiménez (2002), but they did not appear to be highly pathogenic. Additional work on their pathogenesis, geographical distribution, and infectivity to other species of crustaceans is needed.

Fungi

Microsporidia. The microsporidian parasite *Thelohania* spp. has been reported in wild populations of redclaw by Herbert (1988), but is not thought to be a threat to redclaw culture in Australia (Edgerton and Owens 1999). Infected animals tend to be sluggish with a weak tail-flick response (Herbert 1987, 1988). An unidentified microsporidian infection was also reported by Edgerton and Owens (1999) in CqBV-infected claws of redclaw. Microsporidian spores were observed in the connective tissue near the hepatopancreas with an intense hemocytic and melanistic response linked to the spores. Other fungal infections belonging to classes Oomycetes and Sordariomycetes have also been observed in redclaw (Herbert 1987; Edgerton et al. 2002; Longshaw 2011), but their effects on aquaculture are yet to be properly evaluated.

Mesomycetozoa. Mesomycetozoa are a monophyletic group of organisms at the animal–fungi border (Longshaw 2011). Herbert (1987) reported *Psorospermium* sp. in the gills, connective and neural tissues and occasionally in ovary membranes, cardiac and skeletal muscle of *C. quadricarinatus* with no pathologies observed. Edgerton and Owens (1999) also reported *Psorospermium* infection in *C. quadricarinatus*, but in the connective tissue of the subcutis, hepatopancreas, antennal gland and gill and rarely in the skeletal muscle, and

neural and hemopoietic tissues. No reports of mortality of aquacultured redclaw caused by Mesomycetozoa are available in the literature.

Protista

Aspostomate ciliate and protozoan epibionts were reported in redclaw in Australia by Herbert (1987) and Ketterer et al. (1992) and in Ecuador by Romero and Jiménez (2002). *Epistylis* sp. was common in some farms and linked to occasional mortality due to heavy infestation. We have often observed *Epistylis* on large adult redclaw in our ponds at Auburn University, but never associated such infestations with mortality. They just tend to decrease market value of the animals as reported by Herbert (1987). However, some ciliates such as *Tetrahymena pyriformis* do cause lethargy, weakened or failed tail-flick responses and a loss of the ability to right themselves in *C. quadricarinatus* (Edgerton et al. 1996).

Temnocephalida

Temnocephalans are mainly in freshwater crustaceans ectosymbiotic flatworms in the phylum Platyhelminthes (Longshaw 2011). Herbert (1987) reported finding *Diceratocephala* sp. on the ventral abdominal surface of redclaw and Cannon (1991) described four species (*Temnocephala rouxii*, Merton 1913; *Notodactylus handschini*, Baer 1945; *Diceratocephala boschmai* Baer 1953; *Decadidymus gulosus*) of turbellarian temnocephalan symbionts on the body exterior and locomotory legs of *C. quadricarinatus* in Australia. *D. boschmai* preferentially occupies specific sites on the ventral surface and the interorbital-rostral region of *C. quadricarinatus* and is able to complete its life cycle without leaving the host (Jones and Lester 1992). Apparently, *D. boschmai* do not harm the host, but sometimes up to 90% of external shell surfaces can be covered by temnocephalan eggs (Herbert 1987), which can reduce the commercial value of the crayfish due to the strongly adherent eggs that resist boiling (Herbert 1987).

Populations of *D. boschmai* on crayfish are influenced by two main factors, grooming and

molting (Jones and Lester 1996). Crayfish grooming using walking legs can dramatically reduce the numbers of *D. boschmai* and molting dramatically reduces the numbers of eggs and worms on the live animals. Unfortunately, *D. boschmai* was recently reported to have been introduced into Uruguay redclaw farms (Volonterio 2009).

Idiopathic Conditions

Often, idiopathic lesions in the exoskeleton, antennal gland, mandibular organ, hemolymph vessel endothelium, and enteric tissues are observed on redclaw collected from farms in Australia (Edgerton et al. 1995; Edgerton 1996; Edgerton and Owens 1999). Additionally, black spots are often observed on redclaw exoskeletons (Jiménez and Romero 1997; Edgerton 2000). Edgerton (2000) suggested that these black spots in redclaw may be a result of melanization in the cuticle in response to a biotic or possibly abiotic irritant. Other factors such as nutritional and infectious etiologies should also be considered. Presence of black spots that persist after cooking is of concern because they reduce the commercial value of the animals (Edgerton 2000).

Various other idiopathic conditions have been reported in redclaw. Edgerton et al. (1994) noted iron granules in the F-cells of the hepatopancreas of redclaw infected with *Cherax Gardiavirus*-like virus. Similarly, iron granules were observed in the cuticle and hepatopancreas (mainly in R-cells and F-cells) of redclaw in Ecuador (Jiménez and Romero 1998; Romero and Jiménez 2002). Jiménez and Romero (1998) noted that presence of iron deposits might affect feeding habits or metabolism of crayfish thus reducing growth. Hemocytic enteritis was reported by Edgerton (2000) and associated with necrosis of the midgut and associated ceca, but neither in the foregut nor hindgut. Some possible causes for hemocytic enteritis include ingestion of blue-green algae, filamentous and/or Gram-negative bacteria, toxins from abiotic sources, or poor water quality (Edgerton 2000). Other idiopathic lesions reported in *C. quadricarinatus*

include needle-shaped crystals in the nephridial canal and giant cells in the labyrinth epithelium. These giant cells were possibly caused by chemical toxicity (Edgerton 2000). Furthermore, Romero and Jiménez (2002) reported nodules and granulomas in the hepatopancreas of redclaw without much discussion of their causes or effects.

Other Conditions

Abnormalities in the Reproductive System of Redclaw. Although no diseases affecting reproductive performance of redclaw have been reported, Bugnot and López Greco (2009b) observed progressive structural alterations of the *vas deferens* and spermatophore in male reproductive system of redclaw cultured in Argentina during autumn and winter. The authors noted similarities between “male reproductive tract degenerative syndrome” in shrimps and the abnormalities found in male redclaw, and linked said abnormalities to stressful low temperatures.

Algal Toxins in Ponds. Algal blooms are common in freshwater aquaculture ponds (Rodgers 2008). The most common type of potentially harmful algal blooms is cyanobacterial. These cyanobacterial blooms occur more often in areas where optimal conditions for growth, such as warm water temperatures, limited vertical mixing of the water column, and high nutrient concentrations are favorable (Saker and Eaglesham 1999; van Apeldoorn et al. 2007). They can be responsible for the production of a wide range of toxic compounds that are potentially harmful to wild/domestic animals, birds, and humans (Saker and Eaglesham 1999; van Apeldoorn et al. 2007). Saker and Eaglesham (1999) reported that redclaw harvested from an aquaculture pond infested by the cyanobacterium *Cylindrospermopsis raciborskii* accumulated, but did not metabolize the toxic alkaloid cylindrospermopsin. Crayfish exposed to pure cultures of *C. raciborskii* under experimental conditions also accumulated cylindrospermopsin mainly in the hepatopancreas and muscle tissues. The suggested mechanisms for

accumulation of the toxin in tissues of redclaw are ingestion of cyanobacterial cells and/or direct uptake of the toxin in the water. Such accumulation could potentially be harmful to consumers.

Metal Accumulation. Heavy metal contaminants are a significant health issue in areas of rapid agricultural and industrial development (Nakayama et al. 2010). As such, there has been increased risk to both humans and wildlife from consuming aquatic organisms collected in contaminated waters. Nakayama et al. (2010) reported heavy metal accumulation in tissues of *C. quadricarinatus* from lakes in Zambia. They believe crayfish are an ideal biological indicator of heavy metal pollution because they live in direct contact with sediments where metals accumulate in their muscles, exoskeleton, and hepatopancreas (Kouba et al. 2010; Nakayama et al. 2010). Unfortunately, that also makes crayfish a dangerous species to consume if source waters are not properly tested.

Pesticide Exposure. Glyphosate-based herbicides are widely used in controlling growth of weeds and grasses in agricultural, industrial, urban, forestry, and aquatic environments (Giesy et al. 2000). Redclaw are often cultured in ponds dug in the ground where there is high likelihood of contamination of the ponds with herbicides used in agricultural production. Frontera et al. (2011) evaluated the effects of glyphosate and polyoxyethylenamine (POEA) on growth and energetic reserves in redclaw over a 50-d exposure and found that exposure to low and high concentrations of glyphosate-POEA mixture (3:1) resulted in decreased growth and correlated to decreased muscle protein levels. Possible effects on consumers were not evaluated.

Concluding Remarks and Future Research

Redclaw aquaculture is relatively still in its infancy and is yet to reach critical mass, after which growth of the industry becomes logarithmic. The industry needs better market penetration, a more reliable seed supply, a

species-specific diet, and better disease management. The fact that redclaw is a benthic, warm freshwater organism means that it can be farmed in irrigation ponds that undergo periodic water column reduction, as long as the ponds are not completely emptied. The main impediment to global redclaw aquaculture should have been the fact that it is an exotic species in most parts of the world. However, now that it has been introduced to most tropical and temperate regions, commercial production should increase. Finally, the main barriers to cross are the development of species-specific diets formulated using sustainable ingredients and suitable vitamin and mineral inclusion levels, and sex reversal of females into males to take advantage of the sexual size dimorphism of this species.

Nutritional requirements of redclaw crayfish have been extensively studied during the past 10–15 yr (reviewed by Saoud et al. 2012). However, much of this research was narrowly focused on formulation of nutritious and economical practical diets and the assessment of suitability of various ingredients. Although this is understandable given the novelty of interest in redclaw aquaculture, the result is that we have a limited understanding of amino acid, vitamin, and mineral requirements of the species. Furthermore, these requirements should be appraised for the various life stages of redclaw as they may change as the organism grows. Information on the digestibility of ingredients is also lacking. Such information would greatly assist in formulating species-specific diets that producers could use to improve production.

Another area of related research would be to determine optimal feeding regimes for various sizes of redclaw using information generated through the various nutritional studies. As redclaw appear to be nocturnal in their habits, would there be advantages to feeding during the night compared to daytime? Would there be any advantages to feeding through a 24-h cycle as opposed to feeding at various time intervals during the day? Would there be advantages to feeding frequently compared to longer time periods between feedings?

In addition to developing species-specific diets, aquaculturists should also evaluate culture protocols in order to understand which method to use depending on farm site, water availability and properties, and diet used. Redclaw are adaptable to various culture systems, including pond and confined tank systems. In pond culture, the relationship between dietary inputs and stocking density need to be refined; research on assessment and quantification of natural productivity and biota as they relate to stocking density and dietary input needs to be conducted. Also, an evaluation of how dietary inputs and stocking density affect water quality and effluent quality is essential for intensive, pond-based culture systems. Research directions for culture using recirculating system technologies include (1) evaluating dietary formulations that will provide optimal growth and health of redclaw and yet maintain water quality parameters; (2) determining maximum density for profitable, sustainable production; (3) evaluating various unique culture-system designs that would allow for three-dimensional water usage, such as the use of substrate or habitats in the culture tanks in order to increase production and revenue; and (4) evaluation of biofloc technologies in culture of redclaw which could reduce diet costs and increase profitability and sustainability.

In conjunction with investigations of using alternative ingredients and new diet formulations for redclaw, the influence of extrusion processing methods on pellet quality will need to be elucidated. Production of pellets with consistently high quality and good digestibility must be ensured. In future nutrition studies, detailed description of pellet production parameters and physical attributes and properties of diets must be given so that a database of methods can be compiled allowing for producers, nutritionists, and feed mills to ascertain the interactions among ingredients, processing methods, pellet quality, and digestibility. Furthermore, innovative methods of pellet production may need to be used so that nutrient leaching is minimized and pellet stability maximized. This could reduce feed conversion ratios, increase growth, minimize or eliminate adverse water quality, reduce diet costs, and increase profits.

Many feed manufacturing technological variables, such as ingredient particle size during grinding, mixing methods, temperature, amount of added moisture, screw configuration, screw rotation rate, amount and timing of added lipid to the diet, and size of die may all influence pellet quality, nutrient availability, and utilization and should thus be described.

The present review describes various health problems that could affect redclaw, but few cures are also available. Accordingly, farmers should consider prevention rather than treatment. Research with many aquatic species is increasingly demonstrating the benefits of various prebiotics and probiotics in health management. However, information on gut microbiota of crustaceans, particularly redclaw, is extremely limited and the effects of added prebiotics and probiotics on organism health are inconclusive. Use of pre and probiotics in diets have led to a variety of results depending upon species, duration of probiotic feeding, culture conditions, and type of probiotic offered. Use of pre or probiotics in redclaw diets to evaluate growth and immunological impact is thus necessary. Coupled with investigation of prebiotics and probiotics should be the use of proteomic analysis for genome sequencing as well as analysis of gut microbiota to determine if the use of supplements results in changes in gut microbiota ecology and if these changes impart beneficial immunological parameters to the organism.

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