Japo Jussila

PHYSIOLOGICAL RESPONSES OF ASTACID AND PARASTACID CRAYFISHES (CRUSTACEA: DECAPODA) TO CONDITIONS OF INTENSIVE CULTURE

Doctoral dissertation

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ABSTRACT

Three freshwater crayfish species, noble crayfish (*Astacus astacus*), signal crayfish (*Pacifastacus leniusculus*) and marron (*Cherax tenuimanus*), were studied to assess their physiological responses to an intensive crayfish culture system (ICCS) and to describe the environmental characteristics of the system. The measures of physiological responses were growth rates, hepatopancreatic indices, carapace mineralisation, age and size at maturity immune system competence, and changes in total hemocyte numbers. Both indoor and outdoor ICCSs facilities and several different modifications were used in these studies. The ICCS was an environmentally controlled, high density rearing system (15 - 30 crayfish per m^2), where crayfish were held in individual compartments, water was recirculated and crayfish obtained up to 90 % of their nutrition from pelleted diets. Marron showed, at their best, similar growth rates in the ICCS as in semi-intensive farm ponds and experimental tanks (SGRs from 0.4 to 0.7). Nordic species, noble crayfish and signal crayfish, grew slowly (SGRs from 0.1 to 0.2), which together with relatively high mortality, resulted in low production in the ICCS. Highest growth rates and production were obtained in a system where marron were fed with both pelleted commercial diet and food items produced in the ICCS. For the other physiological responses, carapace mineralisation showed lower or similar concentrations in both calcium and magnesium for all three species compared to the wildstocks or semi-intensively reared co-species. In marron, hepatosomatic indices were lower in the ICCS reared compared to semi-intensively farmed ones and higher than in wildstocks, while hepatopancreas moisture content was higher in the ICCS reared compared to semi-intensively farmed and lower than in wild marron. Noble crayfish and signal crayfish had lower hepatosomatic indices and higher or similar hepatopancreas moisture concentration compared to the wild co-species. Noble crayfish, reared in the ICCS, were infected with several gram-negative bacteria and the infection prevalence increased throughout the experiment. Total hemocyte counts (THCs) were lower in the ICCS reared marron compared to the semi-intensively reared marron. The growth of marron in the ICCS was strongly affected by type and amount of feeds, compartment size (density), total water volume, total ammonia and diurnal temperature variation. Dissolved oxygen level and water temperature had a moderate affect on marron growth in the system. Based on these results it can be concluded that the ICCS, as used in these experiments, showed low commercial potential and the intensively reared crayfish possibly experienced severe stress causing loss of condition. The main reasons for the suboptimal rearing conditions were poor quality of artificial diets, temperature stress and system limitations due to poor infrastructure design.

Universal Decimal Classification: 595.3, 639.517
CAB Thesaurus: Crayfish; Aquaculture; Animal Physiology; Growth Rate; Hepatopancreas; Exoskeleton; Animal Nutrition; Sexual Maturity; Hemocytes; Immune Comptence; Parastacidae; Astacidae

'Prefiero morir de pie, que vivir siempre arrodillado'

'Parempi kuolla saappaat jalassa kuin elää ryömien'

Che Guevara

STYLE

Style is the answer to everything -
a fresh way to approach a dull thing or a
dangerous thing.
to do a dull thing with style
is preferable to doing a dangerous thing
without it.

Joan of Arc had style
John the Babtist
Christ
Socrates
Caesar
Garcia Lorca.

style is the difference,
a way of doing,
a way of being done.

6 herons standing quietly in a pool of water
or you walking out of the bathroom naked
without seeing
me.

Charles Bukowski

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Tuija, mä rakastan sua.

Perth, Western Australia, June 1997

Abbreviations

BOD_{24h} = Biological Oxygen Demand in 24 h, mg L^{-1}
cfu = Colony Forming Unit
D_m = Time of Molt, d
DO = Dissolved Oxygen, mg L^{-1}
HI_{dry} = Dry Hepatosomatic Index, %
HI_{wet} = Wet Hepatosomatic Index, %
ICCS = Intensive Crayfish Culture System
k = Space Factor
N = Number of Samples
P = Production, g m^{-2}
P - value = Expresses Significance Level in Statistical Analyses
S = Survival, %
SGR = Specific Growth Index
SGR_{df} = Difference between individual SGR obtained in the experiment and SGR in the farm ponds
T_{im} = Intermolt Period Length, d
W_m = Weight Increment at Molt, g
W_f = Final Weight, g
W_i = Initial Weight, g
W_{pre} = Premolt Weight, g
W_{post} = Postmolt Weight, g
W_{dh} = Dry Hepatopancreas Weight, g
W_{wh} = Wet Hepatopancreas Weight, g
y.o. = Years Old
AKFD = Association of Kuopio Free Droppers

LIST OF THE ORIGINAL PUBLICATIONS

This dissertation includes the following papers referred to in the text by their Roman numerals:

I Jussila J, Evans L H: Impact of sump tank size on growth and production of marron (*Cherax tenuimanus*) in an intensive system. J Appl Aquaculture 3(6): 23-31, 1996

II Jussila J: Carapace mineralisation and hepatopancreatic indices in natural and cultured populations of marron (*Cherax tenuimanus*) in Western Australia. Mar Freshwater Res 48(1): 67-72, 1997

III Jussila J, Evans L H: On the factors affecting marron (*Cherax tenuimanus*) growth in intensive culture. Freshwater Crayfish 11: 428-440, 1998

IV Jussila J, Evans L H: Marron (*Cherax tenuimanus*) growth and condition on water stable pelleted diets. Aquaculture Nutr. 4: 143-149, 1998

V Madetoja M, Jussila J: Gram negative bacteria in the hemolymph of noble crayfish (*Astacus astacus*) in an intensive crayfish culture system. Nord J Freshwater Res. 72: 88-90, 1996

VI Jussila J: Physiological responses of noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) (Astacid: Crustacea: Decapoda) to intensive culture. Manuscript.

This thesis also contains unpublished data.

Contents

1. INTRODUCTION

Crayfish have a strong status in European culture, both in fisheries and social life, dating back several centuries (Lehtonen 1975, Westman and Nylund 1984). The exploitation of natural stocks still plays a substantial role in rural economics in the areas where wild crayfish are abundant, for example in Finland (Jussila 1993, Jussila *et al.* 1995). However, recent reductions in natural stocks due to crayfish plague (*Aphanomyces astacii*) and devastation of crayfish habitat have resulted in declining catches (Westman *et al.* 1990) and stimulated the development of the methods for both stockling and market size crayfish culture in Europe (Gydemo 1989, 1992, Taugbøl *et al.* 1989). The Australian freshwater crayfish, marron (*Cherax tenuimanus*) in Western Australia, are important to recreational fisheries also with the annual catch of 50 to 150 tonnes caught by up to 25,000 licensed marroners (Anon 1988, Morrissy and Fellows 1990).

Potential markets for freshwater crayfish are well established both in Finland and Europe (Huner *et al.* 1987, Huner 1989, Nyström and Rönn 1990, Holdich 1993) as well as in Asia (Lee and Wickins 1992). The demand in European markets is high for market size crayfish, with estimated annual crayfish imports being in the order of 4000 metric tonnes (Ackefors and Lindqvist 1994). There are also local markets for stocklings in Finland and other Scandinavian countries. The Asian markets for live freshwater crayfish are currently partially supplied by crayfish farmers from Australia but the demand seems to exceed the supply. Prices for crayfish have traditionally been high both in Europe (Jussila 1993, 1995c) and Asia (Lee and Wickins 1992, Holdich 1993), which is encouraging farmers to start crayfish culture.

Recent changes in the Finnish agricultural system have forced farmers to seek new sources of income. One of the possibilities has been intensive, indoor farming of freshwater crayfish. Furthermore, to encourage research and development of crayfish culture methods suitable for Scandinavian conditions, several independent regional Crayfisheries Management Programs were carried out in Southern Finland's provinces in late 1980's (Jussila *et al.* 1990, Jussila and Mannonen 1995). However, farming methods suitable for Scandinavian conditions remain poorly developed and so far there has been no commercially viable systems for indoor culture of market size crayfish.

Crayfish culture in Finland has been focusing on production of juveniles for stocking purposes (Ackefors and Lindqvist 1994). Methods to produce juveniles have been steadily intensified and as a result crayfish eggs are incubated in a sophisticated, intensive and computerized system (Järvenpää and Ilmarinen 1995, Järvenpää *et al.* 1996). Juvenile culture (0+ y.o. stocklings) is intensive with stocking densities being around 100 juveniles m^{-2}.

Freshwater crayfish culture, at its present stage, is a combination of extensive and semi-intensive techniques (Lee and Wickins 1992, Ackefors and Lindqvist 1994, Mills *et al.* 1994). The level of intensity is determined by species' characteristics in different parts of its life cycle. The most technically advanced culture methods, developed in Finland (Järvenpää *et al.* 1996), resemble those used in the finfish culture (Ackefors *et al.* 1994), where eggs are hatched in intensive systems, juveniles are raised in intensive or semi-intensive systems (D'Abramo *et al.* 1985) and grow-out systems for market size animals are semi-intensive (Lee and Wickins 1992, Huner 1994).

Intensification has been a long term trend in both fish and crustacean aquaculture. Reasons behind intensive, and thus more complicated, methods are largely in land or water usage policy and the need to have more control over culture conditions (Lee and Wickins 1992). There has been a trend to overcome the short growth season in Nordic countries with attempts to use heated or indoor facilities in aquaculture. The development of an intensive freshwater crayfish culture system in Western Australia (O'Sullivan 1990) has shown some commercial potential (Jussila 1996a), but so far the intensive methods have been poorly developed and even most of the basic problems still remain unsolved (Lee and Wickins 1992). At the moment the intensive crayfish culture system (ICCS) developed in Australia is mainly used in the growth and nutritional studies rather than for commercial production. The ICCS offers control over the culture environment and a possibility to monitor behaviour and performance of individual crayfish.

The commercial and biological potential of a crayfish culture system can be evaluated in experimental conditions by assessing a range of physiological responses of potential culture species under practical rearing conditions. Such an approach could be useful in evaluating the possible development needs of the system, especially if the studied species do not show substantial production and the reasons behind inhibited growth or poor production performance are investigated. This is the approach which was used in these studies. The physiological responses or indicators selected for evaluation in these studies were growth, size at sexual maturation, carapace

mineralisation, hepatopancreas size and moisture content, changes in total hemocyte counts and the presence of gram-negative bacteria in the crayfish hemolymph.

Growth of crayfish, the most crucial physical response to ambient conditions from aquaculturists point of view, is a complicated process with protein synthesis and cellular proliferation during intermolt and a rapid increase in length and weight at molt (Aiken and Waddy 1992). Studies on crustacean growth have shown that acclimation, dissolved oxygen concentration, food supply, isolation, temperature, photoperiod, sexual maturity and age could have a selective effect on weight increment at molt or length of the intermolt (Chittleborough 1975, Aiken 1980, Hartnoll 1983, Botsford 1985). Stocking density or compartment size has been also shown to affect growth (Aiken 1980, Morrissy 1992a, Morrissy *et al.* 1995a)

Carapace mineralisation is dependent on the availability of calcium in the ambient water (Wheatly and Gannon 1995) and the first postmolt days are crucial for carapace mineralisation (Wheatly 1995, Welinder 1975) leaving a narrow window of opportunity for calcification. Normally, the calcium is in sufficient quantity in the ambient water for carapace mineralisation, and the calcium obtained in feeds acts as a supplemental source in later stages of intermolt (Greenway 1985).

The size and energy content of the hepatopancreas has been used as a condition index in crustaceans (Haefner and Spaargaren 1993, McClain 1995a, 1995b). Mannonen and Henttonen (1995) have argued that hepatopancreatic indices could be used to assess impacts caused by the changes in the environment or density on wild noble crayfish (*Astacus astacus*) populations.

The total hemocyte counts (THCs) have been show to be affected by various stressors and by the nutritional status of the crustaceans (Stewart *et al.* 1967, Bauchau 1981), but the exact THCs responses to the various simultaneous stressors still remain unclear. The hemolymph of a healthy crayfish is normally free of bacteria, but especially in culture conditions the healthy crayfish have been reported to be infected with bacteria (Scott and Thune 1986). The changes in THCs or hemolymph bacteremia could thus be taken as indicators of the suitability of the rearing conditions.

In this thesis I investigate the physiological responses of two Astacid (noble crayfish, *A. astacus*, and signal crayfish, *Pacifastacus leniusculus*) and one Parastacid (marron, *C. tenuimanus*) crayfish reared in the intensive system (ICCS). I also evaluate the potential of the ICCS as a freshwater crayfish culture environment based on the responses of crayfish to variable conditions in the intensive rearing.

2. REVIEW OF THE LITERATURE

This literature review focuses on the studies carried out on freshwater crayfish. Some of the studies on marine crustaceans are also cited, but only if there was not relevant information on freshwater crayfish or the information on the marine species was considered crucial to the present studies.

2.1 Growth process in crayfish

The growth cycle of crayfish consists of a series of molts and following intermolt periods. The growth in crayfish, increase in length and weight, is an stepwise process happening during the molt, while the resources for growth are gathered during intermolt period (Lowery 1988, Aiken and Waddy 1992). The increase in length is normally linear or even decreases in time while weight increase follows an increasing exponential trend. The growth, weight gain, takes place during the molt itself, when crayfish takes water into the tissues and thus increases in size, weight and volume. The weight gain at molt is normally between 30 and 60% of premolt weight. During intermolt the crayfish substitutes its water content with tissue growth, and the weight gain during the long intermolt is normally less than 5% of immediate postmolt weight. Also, the water content of hemolymph decreases during the intermolt (Sardá and Cros 1984).

In this section I review the growth process of crayfish, the molt cycle and its hormonal control, environments impact on growth and results of some crayfish growth studies.

2.1.1 Molt cycle

The overview on the different molt stages is based on the review article by Aiken and Waddy (1992) describing the growth process in crayfish. The molt stages are formally named as A, B, C and D with several substages within them (Drach 1944, Travis 1965, van Herp and Bellon-Humbert 1978, Lowery 1988, Aiken and Waddy 1992).

Stage A takes only 2% of the molt cycle (Drach 1944), and begins right after a crayfish has cast the exuvium. The stage is dominated by absorption of water, when a crayfish expands its volume up

to 50%. Tips of the cheliped, cutting edges of the mandibles and maxillipeds are hardened using the calcium stored in gastroliths during stage A.

The next stage is B, which is sometimes hard to separate from stage A. In stage B crayfish is immobile, while in stage A crayfish are already moving. Stage B occupies 8% of the molt cycle. The criterion for stage B is endocuticle deposition onset (van Herp and Bellon-Humbert 1978). Stage B ends when the chemical changes in the preexuvial layers (epicuticle, exocuticle) are completed (Drach 1939).

Stage C is the longest molt stage occupying up to 65% of the molt cycle, and it has several substages (C_{1-4}). Shell rigidity is a useful index to determine when the stage C starts, and in *Orconectes sanborni* the postorbital ridge and cervical groove change from flexible to rigid in the onset of stage C (Stevenson 1968). During stage C the exoskeleton is mineralized and it assumes the typical rigid form.

Stage D is the premolt period of molt cycle and it occupies roughly 24% of its length. During stage D crayfish prepares for the molt by severing pore canals, accumulating reserves and synthesizing the two preexuvial layers of the new cuticle. The stage D can be divided into five substages (D_{0-4}).

Stage E, or ecdysis, is the molt itself, which has sometimes not been considered as a part of classical intermolt stages. Ecdysis is often divided into active and passive phases. During the passive phase epidermal sutures are decalcified, water is absorbed and redistributed and as the pressure increases the thoracoabdominal membrane begins to bulge. Crayfish can lengthen the passive phase if conditions are not suitable for molting. The active phase commences when the hydrostatic pressure is strong enough to rupture the membrane between carapace and abdomen and the crayfish molts. The molt cycle is completed and crayfish enters stage A.

2.1.2 Endocrine control of molting

The molt cycle of crayfish is controlled and affected by several internal and external factors (Aiken and Waddy 1992). Hormonal control is based on the balance between molt-inhibiting hormone (MIH) and ecdysteroids, that can be taken as molt-promoting steroids. The control of

molting is relatively complex, and its components are fairly well know but the whole process is not fully understood (Aiken and Waddy 1992).

Molting inhibiting hormone (MIH) is secreted in the X organ sinus gland complex in the crustacean eyestalk (Aiken and Waddy 1992). The release is regulated primarily by serotonergic neurons and secondarily by ecdysteroid feedback and the environment. MIH secretion decreases in the immediate premolt in crustaceans (Spaziani *et al.* 1989). MIH release is mediated by the neurotransmitter 5-hydroxytryptamine and controlled by a reversible dose-dependent manner.

MIH itself controls the synthesis and release of ecdysteroids from the Y organ and regulates cholesterol uptake (D'Abramo *et al.* 1985, Naya *et al.* 1989). The Y organ is an endocrine structure of ectodermal origin located bilaterally beneath the epidermis of the anteriolateral carapace. The Y organ is maintained and its products are removed by superfusion of the hemolymph (Spaziani *et al.* 1989). MIH suppresses the Y organ on ribosomal level in the process which is mediated by cAMP in the crab (Mattson and Spaziani 1986a, 1986b). MIH could also modify target tissue responses to circulating ecdysteroids (Freeman 1980, Freeman and Costlow 1979, 1984). In addition to MIH controlling ecdysteroid excretion, there are indications that there is an alternative pathway involving biogenic amines in crustaceans (Luschen *et al.* 1988). Also, decapods have been observed to be able to control ecdysteroid level even though their eyestalks have been removed (Chang 1984), indicating that there could be organs other than Y organ producing ecdysteroids (Chang *et al.* 1976, Keller and Willig 1976, De Leersnyder *et al.* 1981, Jegla *et al.* 1983, Demeusy *et al.* 1988, Hopkins 1988, Taketomi *et al.* 1989).

Molting process in crustaceans is initiated by the secretion of ecdysteroids by the molting gland or Y organ (Aiken and Waddy 1992). Molting hormones are polyhydroxylated steroid hormones (ecdysteroids: ecdysone, 20-OH-ecdysone, and ponasterone A) that induce physiological and biochemical changes in the premolt. When the cAMP levels in the hemolymph decline due to declining MIH, the Y organs initiate a rapid uptake of cholesterols, convert them into to ecdysteroids and start the secretion. In *Orconectes* (Aiken and Waddy 1992) and *Astacus leptodactylus* (Durliat *et al.* 1988), the ecdysteroid levels are barely detectable right after the molt, levels then increase in late stage A and are fairly constant in stage B_2 and stage C. In stage D_0 the levels increase again to initiate apolysis and gastrolith formation. The peak happens in stages D_{1-2} (level in *A. leptodactylus* is 200 $\mu g\ mL^{-1}$), when the preexuvial cuticle is deposited, and the levels decrease again in stages D_{3-4}.

The exact role of different ecdysteroids secreted are still unknown, and some of them are bound to the target tissue receptors while others are necessary for molting (Aiken and Waddy 1992). Studies have revealed that the ecdysteroids synthesis occurs in series of pulses (Stevenson *et al.* 1979) and fluctuates even daily (Snyder and Chang 1991). The elimination of the ecdysteroids is rapid in late premolt. The sites for elimination are ovary, hindgut and epidermis. In the elimination, highly polar products and ecdysonic acids are formed (Lachaise and Lafont 1984).

2.1.3 Environment and growth

Temperature is the probably the most important factor regulating the growth rate in crayfish (Lowery 1988, Gydemo 1989), partly because of changes in food consumption with changing temperatures (Söderbäck *et al.* 1987, Seals *et al*. 1997). Growth is fastest within a species special optimum temperature range (Morrissy 1990, Jones 1990, 1995, Austin 1995). Temperatures below the optimum normally inhibits growth, and the temperatures above the optimum cause stress and result in increased mortality (Morrissy 1976a, Kartamulia and Rouse 1992, Jussila 1995a, Järvenpää *et al.* 1996). The optimum growth temperature for marron is reported to be 24°C (Morrissy 1990) and the optimum range, where the growth is more than 50% of the maximum growth, under different conditions and for different strains of marron, is from 17°C to 27°C (Morrissy 1990). Noble crayfish and signal crayfish juveniles grew fastest in temperatures from 20°C to 23°C (Huner and Lindqvist 1984, Järvenpää *et al.* 1996), while the optimum production temperature could be slightly lower, from 17°C to 21°C. The maximum production, a combination fast growth and high survival, is normally obtained in lower temperature than maximum growth (Järvenpää *et al.* 1996).

Studies on the temperature show that the intermolt period is affected first, and the weight gain at molt tends to remain constant in *Austropotamobius pallipes* (Brown and Bowler 1979, Pratten 1980), while in *P. leniusculus* the weight gain at molt was affected by temperature while molting frequency remained constant (Shimizu and Goldman 1983). Also, the differences in growth among populations inhabiting different types of water bodies have been explained by the differences in the thermodynamics in their environment (Mees 1983). Temperature also affects survival and the fastest growth can be obtained in conditions where the survival is lowest (Mason 1979), which has to be taken into account when the temperature ranges for aquaculture are considered.

The population density inhibits growth in freshwater crayfish (Lowery 1988, Gydemo 1989, McClain 1995c), including marron (*C. tenuimanus*) (Morrissy 1975, 1992a, Morrissy *et al.* 1995a, 1995b, Whisson 1995). The mechanisms could be linked to the availability of nutrition, population hierarchical structures or stress caused by frequent encounter of the co-species. Morrissy (1975) has show that the difference in density (0.2-1.6 crayfish m^{-2}) affected the final OCL of marron (*C. tenuimanus*) grown in different environments, with the higher density resulting in slower growth. The density factor has to be considered in relation to the environment or the availability of substrate, while hides can alter the effects of density *per se* (Lowery 1988).

The density factor k, which describes the size of a compartment in individual rearing systems (Equation 6, Materials and Methods), has been evaluated as a tool to describe rearing systems. Du Boulay *et al.* (1995), Goyert and Avault (1978) and Geddes *et al.* (1988) have found that k values less than 50 cause growth inhibition in freshwater crayfish, while van Olst and Carlberg (1978) found that k less than 33 caused growth inhibition in lobster.

Photoperiod has been shown to have minimal affect on crayfish growth (Sáez-Royuela *et al.* 1996), while in some other studies both growth and survival have been improved when the photoperiod has increased (Westman 1973, Mason 1979, Taugbøl and Skurdal 1992).

The type and amount of feeds affects the production of the crayfish (Lowery 1988, Gydemo 1989, McClain 1995c, Barki *et al.* 1996) by altering the level of cannibalism and offering variety of nutrients required in the growth process. The nutrient profile affected both weight gain at molt and intermolt period in a study carried out by Jones *et al.* (1995) on yabbies (*Cherax destructor*). Furthermore, feeding ratio affected both intermolt period and weight gain at molt, though the influence on intermolt period was greater in the prawn (*P. elegans*) (Salama and Hartnoll 1992), and Barki *et al.* (1996) showed that feed ration and distribution affected growth, survival and competition in laboratory reared juvenile red claw (*C. quadricarinatus*).

Rouse *et al.* (1995) and Liu *et al.* (1995) suggested that the level of dissolved nitrites in water should be kept low, close to the minimum detectable concentrations, to minimize growth inhibition in crayfish, while Lourey and Mitchell (1995) concluded that both total ammonia and pH should be carefully controlled in intensive systems, with minimal inhibition on growth in un-ionised ammonia concentrations less than 0.01 mg L^{-1}.

Water calcium has to be over 5 mg L^{-1} to ensure proper exoskeleton mineralisation (Lowery 1988), but marron (*C. tenuimanus*) have been successfully molting in water bodies containing less

than 5 mg L^{-1} calcium (Noel Morrissy, Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, personal communication). Low pH inhibits calcium uptake amongst other things and thus inhibits growth also (Wheatly 1996). Dissolved oxygen saturation should be over 60% to ensure uninhibited growth, between DO 40-60% the growth is inhibited and below that mortality increases in the spiny lobsters (Chittleborough 1975).

2.1.4 Weight gain at molt and intermolt period

Noble crayfish's (*A. astacus*) weight increment at molt can be between 33% and 65% under laboratory conditions (Ackefors *et al.* 1995). Intermolt period has been reported to be in males 44 d and in females 40 d for approximately 9 cm TL noble crayfish (Henttonen *et al.* 1993) or from 58 to 93 d for juvenile noble crayfish weighing less than 4 g (Ackefors *et al.* 1995).

Westman *et al.* (1993) obtained a mean length increase at molt for signal crayfish to be 5.5 mm (0+ y.o.) and 4.8 mm (1+ y.o.) in males and 4.6 mm (0+ y.o.) and 4.5 mm (1+ y.o.) in females. They obtained the following length increments at molt in noble crayfish: 5.3 mm (0+ y.o.) and 4.7 mm (1+ y.o.) for males and 4.0 mm (0+ y.o.) and 3.0 mm (1+ y.o.) for females.

Intermolt period in juvenile marron (*C. tenuimanus*) has been shown to be between 15 and 45 d (Morrissy 1984), while the weight gain at molt was 52% of the premolt weight for animals weighing from 0.04 g to 120 g..

2.2 Crayfish culture

Crayfish farming in Europe, after the crayfish plague devastated wild populations, has been focusing on producing stocklings of native crayfish and recently also market size crayfish (Ackefors and Lindqvist 1994) using both native and translocated species. The crayfish farming in Southern USA has long been focusing on rearing red swamp crawfish (*P. clarkii*) to market size on large farms or rice fields (Huner 1994).

Huner and Lindqvist (1995) have discussed the physiological adaptations common to the freshwater crayfishes in commercial culture. The requirements were summarized as ability to burrow to some extent, capability of aerial respiration, polytrophism, rapid growth, high fecundity and

disease resistance. Of the studied species, marron (*C. tenuimanus*) fulfilled these criteria best, while noble crayfish (*A. astacus*) was known to grow slowly and was less resistant to diseases. Signal crayfish (*P. leniusculus*) has been shown to grow faster than noble crayfish but was almost as vulnerable to diseases as noble crayfish.

In the following section, I focus on describing the most commonly used semi-intensive crayfish culture methods, discuss the intensive culture as it has been studied and used, production in commercial freshwater crayfish farming and also nutrition under culture conditions.

2.2.1 Crayfish culture methods

Most of the present commercial market size freshwater crayfish production is derived from semi-intensive pond systems (Huner 1989, 1994). Largest producers in late 1980's were USA (60,000 tonnes), Spain (8,000 tonnes) and Sweden (1,000 tonnes), and the total production in Europe was 11,000 tonnes annually (Huner *et al.* 1987, Holdich 1993). These production figures are high compared to the commercial production of the Parastacid species, 113 tonnes in 1990, in Australia (O'Sullivan 1991, Mills *et al.* 1994).

The crayfish production in USA is obtained from polyculture type semi-intensive farms, where former rice fields have been converted into red swamp crayfish (*P. clarkii*) production units (Huner 1994), with sophisticated synchronized rearing of crayfish and the plants to supply food and nutrients for the crayfish (Huner 1995). Ponds are drain or trap harvested, and most of the catch is consumed locally. USA also exports large quantities of crayfish to European markets (Holdich 1990).

In Finland, Sweden and also elsewhere in Europe, the commercial freshwater crayfish production is based on semi-intensive monoculture, with the crayfish being normally the only species in the farm ponds or raceways (Ackefors and Lindqvist 1994). The broodstock crayfish are either trapped from the wild populations or more recently, reared and selected in the farms and held in special broodstock ponds in low densities (<10 crayfish m^{-2}, male:female ratio 1:3), where the crayfish mate following the natural cycle. The eggs are either artificially incubated in sophisticated intensive incubators with controlled water quality and temperature (González *et al.* 1993, Järvenpää and Ilmarinen 1995, Järvenpää *et al.* 1996) or in the ponds following the natural reproduction cycle (Pursiainen and Järvenpää 1981). The juveniles are reared in specially designed small (10 - 200 m^2) and shallow (0.5 m deep) ponds in high densities, 50-100 juveniles m^{-2}, for the first summer, and

transferred into the growout ponds to lower densities in the following autumn. The growout ponds (0.1 - 2 ha) for market size crayfish culture are typically shallow (2-3 m deep), stocked with 1-10 crayfish m^{-2}. A total production up 1,000 kg ha^{-1} to can be harvest in 3-5 years depending on the conditions in the ponds (Ackefors and Lindqvist 1994, Gydemo 1995). Under favorable conditions in Central Europe even 300 kg ha^{-1} can harvested annually (Keller and Keller 1995). Juveniles feed on natural production in the ponds, while the adults in the growout ponds could receive supplemental food, namely plants, vegetables or even fish.

Marron (*C. tenuimanus*) are farmed commercially in extensive or semi-intensive farm dams or ponds. The farm production is based on either natural reproduction and growth of marron in the water bodies (hobby farming) or more sophisticated semi-intensive single cohort farming method (Morrissy 1974, 1976a, 1976b, 1992b). In the latter system broodstock mate in August or September in special ponds (1-3 females m^{-2}), where females release the juveniles, in high densities (up to 1,000 III stage juveniles m^{-2}), in December or January. The adult marron are removed after the juveniles are released and the juveniles are reared six more months in the ponds grazing on the natural pond production supplemented by commercial marron pellets. After 6 mo the juveniles, from 3 to 40 g in size, are selected to be stocked in growout ponds in lower densities (3-10 crayfish m^{-2}). There are several modifications of this basic principle depending on the farm location and farmer's production expectations. Marron reach marketable size, from 80 to 300 g, at the age of 18 to 24 mo and annual production could be anything between 400 and 4,000 kg ha^{-1} (Morrissy 1979, Morrissy 1992a, Mills *et al*. 1994). Marron are fed with several types of commercial crayfish pellets (20% or 30% protein, 20% carbohydrate, 10% fat) and also with pelleted diets designed for fish or chicken. The ponds are normally aerated with air pumps, cascades, airlifts, paddle wheels, etc. First trials on commercial monosex culture of marron have been carried out with similar encouraging results (J Jussila, unpublished data) as has been obtained in prawns (*Macrobrachium rosenbergii*), where all-male populations gave the best production (Siddiqui *et al.* 1997). The total marron production in Western Australia has been less than 20 tonnes annually (Fotedar *et al.* 1996a, 1996b), indicating that the present commercial pond area (approximately 200 ha) has not been efficiently managed (Mills *et al.* 1994).

2.2.2 Intensive culture systems

The basic design of the intensive crustacean farming systems discussed here is as follows: one or more culture tanks, housed vertically one on top of the other, connected to a sump tank and, sometimes, a biofilter system. Water is normally recirculated (Heiken 1987), while some systems use flow through design. Crustaceans are reared in individual compartments to minimize social contacts and cannibalism, although some systems rely on a communal rearing. Crustaceans are fed with commercial pelleted diets or with naturally produced food items as a supplement to pelleted diets. A control over health, molting, water quality and feeding is crucial. Water quality, dissolved oxygen, temperature, pH, minerals, etc., are maintained to the optimum of the cultured species.

Taugbøl *et al.* (1989) point out quite clearly that the present knowledge on freshwater crayfish biology does not allow commercial applications of the intensive farming. They emphasize the construction and management costs as well as discuss the principle of the intensive culture systems for the cannibalistic and territorial freshwater crayfish. They suggest that the tank structures have to be vertical and conclude that if development is to proceed it should focus on improving systems for communal instead of individual rearing. Gydemo (1989) also points out the bio-economical problems in intensive farming and identifies high mortality as one of the factors limiting the production.

Lee and Wickins (1992) discussed the battery farming system, that has been trialled in Western Australia on marron (*C. tenuimanus*) (Morrissy 1984, Kowarsky *et al.* 1985, O'Sullivan 1990, Jussila 1996b) and South Australia with yabbies (*C. destructor*) (Geddes *et al.* 1988). Succesfull development of the battery culture relies on four crucial factors (Lee and Wickins 1992): minimum density inhibition in the system; cost-effective construction materials; design that allows high water quality; effective feeding. The conclusions were that the problems related to ineffective diets and high labor costs form the major tasks for the development and that the systems tested so far with freshwater crayfish have not been as successful as the ones tested for *Homarus* spp.

The Nardi system, as described by O'Sullivan (1990), and its modifications (Morrissy 1984, Morrissy *et al.* 1995a) have been tested and also used in small scale marron (*C. tenuimanus*) farming in Western Australia since early 1980's. There has been several attempts to develop the intensive system (Morrissy 1984, Kowarsky *et al.* 1985, Jussila 1996b), but no commercially viable intensive systems are operational. The results from the latest experiments show, that marron could be

raised in short, 4 mo, studies at similar growth rates that have been obtained in the semi-intensive ponds (Jussila 1996a).

The intensive culture systems for lobsters have been tested since late 1970's (Mickelsen *et al.* 1978, van Olst *et al.* 1980, D'Abramo and Conklin 1985, Waddy 1988) and the successful systems share following common features: horizontally stacked shallow trays with containers or compartments to house the crustaceans; feeding with live food or compounded feeds; efficient water flow through the system. Intensive culture requires special characteristics from the cultured species also, and they must be resistant to diseases and able to grow in high densities.

Intensive shrimp farming has been developed and studied mainly because of the same reasons as this thesis was initiated: climatic restrictions and the relatively high cost of land (Sandifer *et al.* 1987, Chen *et al.* 1989, Lee and Wickins 1992). The shrimp (*Penaeus vannamei*) was found feasible for intensive culture in southern USA by Sandifer *et al.* (1987), because it grew well under high stocking densities (up to 40 shrimp m^{-2}) and gave annual yields between 12 to 15 tons ha^{-1}. Red-tailed shrimp (*Penaeus penicillatus*) has been shown to produce similar yields in super intensive culture (densities from 170 to 290 shrimp m^{-2}), while causing increased ammonia levels in the ponds (Chen *et al.* 1988). The development of intensive shrimp culture is going on.

Softshell crayfish industry, viable in Louisiana, USA (Huner 1994), exploits intensive culture's principles. The final stage product, newly molted red swamp crayfish (*P. clarkii*), marketed either for fish bait or gourmet food, is cultured under intensive conditions (up to 250 crayfish m^{-2}) in a highly developed environment (Malone and Burden 1988, Huner and Barr 1991). The softshell crayfish production unit comprises of shallow tanks where the crayfish are reared communally, and an efficient biofilter. The production units, trays, are approximately 10 x 250 x 15 cm in size. Water levels are held low in the holding trays, less than 3 cm, while in the molting trays the water level is higher. Crayfish are collected from the semi-intensive ponds in their late premolt and placed to holding trays, and the ones preparing for molting are transferred from the holding trays to the molting trays. The molting related behavior, moving to the shallower water, ceasing to feed, and finally going with the current, is used as criteria in selection (Bodker 1984, Malone and Culley 1988, Robin *et al.* 1991). Water quality and temperature have to be maintained at optimal levels for molting. Both flow through and recirculating systems have been used (Huner 1988) and biofilters with several technical innovations have been used (Malone and Burden 1988, Malone *et al.* 1991, Robin *et al.*

1991). This type of crayfish production has so far been the only successful application of intensive culture of adult, market size freshwater crayfish.

2.2.3 Crayfish growth and production in the culture systems

It is relatively difficult to compare growth performance between systems in which animals are reared in individual compartments (e.g. the ICCS) and those in which animals are held communally in ponds. Furthermore, crayfish age, reproductive status, season, stocking density, and feeding regime are all known to influence growth rates in freshwater crayfish (Lowery 1988, Mills *et al.* 1994). From the point of view of commercial farming, it would also be preferable to examine the commercial biomass production of a culture system rather than the growth rates of the crayfish.

Marron growth rate, expressed as specific growth rate (SGR), under semi-intensive rearing has been from 0.5 to 1.1 (Morrissy 1979, 1980, 1990, 1992a, Jussila 1996a) and under intensive rearing from 0.4 to 0.7 (Morrissy *et al.* 1995a, Jussila 1996a). Juvenile marron (0+ y.o., mean weight 2.0 g) growth rates under constant temperature (18.5°C) in aquaria in low density were from SGR 0.6 to 1.1 (Fotedar *et al.* 1997). Marron growth in extensively reared, wild-like populations in Western Australia was estimated from Morrissy's (1974) data to be approximately SGR 0.5. It has been argued that marron growth rates should be SGR 0.6 or higher in a commercial farm over the 2 year farming period (Jussila 1995a).

Marron growth in the semi-intensive culture is variable as is also the production. O'Sullivan (1995) has reported the production from a successful marron farm as being from 500 to 2,000 kg ha^{-1} $crop^{-1}$ and suggested that there could be two crops a year. Morrissy (1979, 1992a, 1995), on the other hand, reports production being from 1,100 to 3,000 kg^{-1} ha^{-1} $year^{-1}$ of harvestable biomass. The growth of marron has been SGR 1.0 for the 1 y.o. and SGR 0.6 for the 2 y.o. marron in these studies. These growth rates have been obtained in the south western Western Australia, and even faster growth rates can be obtained in the farms in central western Western Australia. Australian commercial crayfish species, marron, red claw or yabbies, could give annual yields from 2,000 to 4,000 kg ha^{-1} according to Lee and Wickins (1992) and Morrissy *et al.* (1995).

Noble crayfish (*A. astacus*) have obtained growth rates from SGR 0.2 to 0.7 in semi-intensive rearing (Gydemo and Westin 1989, Taugbøl and Skurdal 1992), while signal crayfish (*P.*

leniusculus) has performed from SGR 0.3 to 1.5 in semi-intensive rearing (Westman and Nylund 1984, Celada *et al.* 1993, Tulonen *et al.* 1995).Tulonen *et al.* (1995) have shown that signal crayfish grew faster than noble crayfish in earthen ponds under semi-intensive rearing. The SGRs for signal crayfish and noble crayfish were at the age of 1+ y.o. 0.7 and 0.4, 2+ y.o. 0.4 and 0.3 and 3+ y.o. 0.4 and 0.2, respectively. Gydemo (1989) has observed fast growth in noble crayfish, which reached 7 cm TL in 1+ y.o. and 9 cm TL in 2+ y.o. in Island of Gotland, Sweden. In his experiments noble crayfish growth rates were similar to those observed in signal crayfish in Sweden.

Keller (1995) obtained decreased growth rates on noble crayfish (*A. astacus*) reared communally in high densities (10, 20, 40 crayfish m^2) for a period of 27 mo. The conclusion was that the type of intensive rearing studied could not have a commercial future, even though mortality in the system was low (no cannibalism). Keller and Keller (1995) have shown that the production of noble crayfish can be up to 300 kg ha^{-1} $year^{-1}$ and the growth close to SGR 0.3 (4+ y.o.) if proper management practices are followed in semi-intensive farming, while the production predictions based on small scale experiments (Keller 1988) were 'unrealistically high' 2,000 kg ha^{-1} $year^{-1}$. Ackefors *et al.* (1995) obtained a 40% mean weight gain at molt and a range from 40 to 80 d in the intermolt period for stage 7 or older juvenile noble crayfish in an experimental system. The mean size of the crayfish after 16 mo rearing was 2 g.

The biomass after 3 years of rearing was 1,089 kg ha^{-1} in signal crayfish and 470 kg^{-1} ha in noble crayfish ponds, with noble crayfish having a slight decreasing trend in biomass production due to high mortality (Tulonen *et al.* 1995). Wickins (1982) reports annual yields from 500 to 1,000 kg ha^{-1} for signal crayfish. The signal crayfish yield per meter of bank was 100-150 g in Lee and Wickins (1992) review. Yields as high as from 500 to 1,000 kg ha^{-1} $year^{-1}$ of signal crayfish could be expected in British waters (Wickins 1982). In addition, it was concluded that the crayfish alone could not provide commercial crops and polyculture would be necessary to secure incomes since under extensive conditions the yield of European freshwater crayfish are normally from 60 to 500 kg ha^{-1}. On the other hand, the annual production of narrow clawed crayfish (*A. leptodactylus*) can range from 200 to 400 kg ha^{-1} (Brodsky 1982).

Yabbies (*C. destructor*) have been shown to grow relatively fast in semi-intensive rearing (Geddes *et al.* 1988, Geddes *et al.* 1993, Jones *et al.* 1995). The mean SGRs between 3.2 and 10.6 have been reported over a period of 35 d for juvenile yabbies reared on a variety of feeds and mean SGRs of 1.9 for yabbies reared on commercial fish food. The survival has been high in these

studies, normally between 80% and 100%, resulting in relatively high theoretical production figures (300 g m^{-2} in 2 mo). A production of 1,500 kg ha^{-1} has been achieved in a yabbie farm in 6 mo (Mills and McCloud 1983), with an increase in crop with an increasing feeding rate but not with an increasing density. Lee and Wickins (1992) report that annual yield in yabbie farm can be 1,500-2,000 kg ha^{-1}, while Mills *et al*. (1994) suggest that the production in an extensive system is around 300 kg ha^{-1}, and with pond management the production can be up to 1,000 kg ha^{-1}, while semi-intensive systems can produce 1,200 kg in 6 mo.

Red claw (*C. quadricarinatus*) juveniles showed relatively high growth rates in individual rearing in high densities, 100-400 crayfish m^{-2} (Du Boulay *et al.* 1995), with the mean SGR being 1.3 over 235 d. The growth of the red claw was comparable to *Penaeus monodon* and faster than in most of the commercially cultured crustaceans. Mortality also remained low in the individual rearing.

Production of red swamp crayfish (*P. clarkii*) under semi-intensive conditions could be after the first year 500 kg ha^{-1}, and it can increase by 1.5-2.5 times for the second year (Huner 1994). Most of the sites have pre-existing populations and production after the first year is in many cases more than 1,000 kg ha^{-1}.

2.2.4 Nutrition in crayfish farming

Crayfish are opportunistic polytrophic (Momot *et al*. 1978, Ilheu and Bernardo 1993, Huner and Lindqvist 1995) and a variety of micro-organism enriched plant material as well as fresh plant and animal material can be found in their stomachs (O'Brien 1995). Freshwater crayfish diets have so far been inadequate due to lack of detailed nutritional information (Lee and Wickins 1992, Brown *et al.* 1995), especially under practical farming conditions, as in other crustaceans (Tacon 1996), but this has not caused substantial problems in extensive or semi-intensive farming since the crayfish get large proportion, up to 50%, of their nutrition in the pond environment from natural production in the ponds (Lee and Wickins 1992). The nutrients in the intensive farming conditions, on the other hand, are provided in the form of pelleted diets (Apud *et al.* 1983, Bordner *et al.* 1986), and so the nutritional requirements of the freshwater crayfish, if farmed intensively, have to be understood. The importance of vegetable matter in crustacean feeding is well established (Villarreal 1989, 1991, Aiken and Waddy 1992), and a balance between animal and vegetable protein and minerals has been shown to give best results in crustacean farming (Sommer *et al.* 1991). *Cherax* sp. use from 50 to 58% of the consumed energy for growth (Woodland 1967, Villarreal 1991), while growth efficiencies are lower in other freshwater crayfish.

2.2.4.1 Nutritional requirements

The protein requirements are reported to be between 20% and 30% for noble crayfish (*A. astacus*) (Ackefors and Lindqvist 1994) and red swamp crayfish (*P. clarkii*) (Huner and Meyers 1979), while Huner and Lindqvist (1984) found, with diets containing 30% or 40% protein, that the higher level increased glycogen and lipids reserves in the hepatopancreas, which was an indication of better condition. Tsvetnenko *et al*. (1995) observed highest growth rates in marron fed with 20% or 30% protein diets. Hubbart *et al.* (1986) found that protein level of 30% and energy level of 2,500 kcal kg^{-1} (protein to energy ratio 120) gave best growth and protein deposition in freshwater crayfish. Jones *et al*. (1997) found that yabbies (*Cherax albidus* or *C. destructor*) growth was improved when pellet protein level was 30%, while 15% resulted in slower growth and nutritional stress.

In addition to being a source of energy, protein is a supply of essential amino acids. They are generally same as those of other animal groups: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine (Claybrook 1983, D'Abramo and Robinson 1989). The amino acid profile of a crayfish diet could be based on the profile obtained in crayfish muscle tissue or on the whole body analyses, as suggested by Cowey and Tacon (1983) or Wilson and Poe (1985). The sources of protein in the artificial diets are normally blends of shrimp meals, fish meals and soyabean meals (Tarshis 1978, D'Abramo *et al*. 1985).

Freshwater crayfish require low levels of lipids in their diets (D'Abramo *et al.* 1985, Goddard 1988, Fotedar *et al*. 1997), with indications of growth inhibition when the levels exceeded 8% (Andrews *et al*. 1972, Foster and Beard 1973, D'Abramo 1979, Davis and Robinson 1986). The lipid content of 0.1 to 3% has been shown to be adequate for crayfish, if such sterols as cholesterol (0.4% dry weight), and fatty acids as linoleic (18:2 n-6), linolenic (18:3 n-3), eicosapentaenoic (20:5 n-3) and docosahexaenoic (22:6 n-3) are included (Zandee 1966, D'Abramo *et al.* 1985, Lee and Wickins 1992). In addition to the lipid profile in the diets the protein-to-energy (P:E) ratio (mg protein per kcal) and total energy has to be considered (Lee and Wickins 1992). There are indications that the shrimp require a P:E ratio of 63 to 117 at 25-35% protein level and 3-4 kcal g^{-1} energy level, while D'Abramo and Robinson (1989) suggested that in the crustacean diet P:E ratio should be close to 120 (30% protein and 2,500 kcal kg^{-1}). The common lipid sources in the crustacean diets are marine or plant oils and lecithin (Tarshis 1978, Huner and Lindqvist 1984).

Carbohydrate requirements of freshwater crayfish are somewhat unclear, but the crayfish readily utilize diets with high levels of carbohydrates and the commercial diets normally contain carbohydrate levels of 25% or more (D'Abramo and Robinson 1989). Common carbohydrate sources in crayfish diets are starch and other soluble polysaccharides, which are used as an alternative source of energy to protein (Hubbart *et al.* 1986).

Carotenoids are an essential part of crayfish nutrition (D'Abramo and Robinson 1989), and the lack of carotenoids causes changes in exoskeleton pigmentation and could also compromise growth in lobsters (Bordner *et al*. 1986), red swamp crayfish (*P. clarkii*) (Lochmann *et al.* 1992) and in marron (*C. tenuimanus*) (Sommer *et al.* 1991). However, Huner and Lindqvist (1984) did not find any effects of supplemental carotenoid on the condition of noble crayfish (*A. astacus*) and Tsvetnenko *et al.* (1995) obtained no differences in growth in marron fed with diets supplemented by carotenoids. A mixture of vitamins, for example 13 different vitamins in mixture BML-2 (

D'Abramo *et al.* 1985), have to be added to the crayfish diets (Lochmann *et al.* 1992), but the detailed information on the vitamin requirements is unclear (Goddard 1988). Calcium is the most important mineral to be added to crustacean diets (Gallagher *et al.* 1978), but normally the ash content of the diet is high enough to ensure adequate levels of other minerals (Goddard 1988).

2.2.4.2 Natural food items production

Crayfish are polytrophic animals and this nutritional flexibility is the basis of several commercially successful farming methods (Huner and Lindqvist 1995). Natural foods in the culture systems form an important supplemental source of nutrients (Tidwell *et al.* 1995, Mitchell *et al.* 1995, O'Brien 1995) and it has been estimated that, under semi-intensive farming conditions, crayfish can derive up to 50% of its nutrition from this alternative source (Apud *et al*. 1983, Lee and Wickins 1992). Furthermore, different species have different dietary preferences when choices of aquatic plants (Warner and Green 1995 or higher aquatic organisms (Covich 1977, Covich *et al*. 1980, Warner *et al.* 1995) are available. Avault and Brunson (1990) reported in their review that a substantial increase in the red swamp crayfish (*P. clarkii*) production (2,016 kg ha^{-1}) occurred when a combination of rise forage and pellets were used in comparison to production on pellets only feeding (881 kg ha^{-1}) or production on rice forage only (1,274 kg ha^{-1}). Jones *et al.* (1995) have shown that diets consisting mainly of zooplankton have given fastest growth rates in crayfish.

The nutrition in some of the extensive or semi-intensive pond culture systems relies on detritus based ecosystems which are created by growing plants in the ponds (Mills *et al*. 1994). This type of natural supplementation is practiced especially in southern USA (Avault and Brunson 1990) and also in yabbie (*C. destructor*) culture in Australia, even though the more common approach is to add alfafa or hay pellets to ponds for the basis of the detritus production when ponds are filled (Mills *et al*. 1994). Plant detritus has been mentioned to be the basis of also marron (*C. tenuimanus*) nutrition in the farms (Morrissy 1992b).

The European crayfish culture is based on the natural plankton, benthic animal, algae, plant, etc., production in the ponds, even though the diets are sometimes substituted with fish meat, fish pellets or vegetable matter (Ackefors and Lindqvist 1994). The nutrition in the juvenile ponds is normally totally based on the natural pond production.

2.2.4.3 Pelleted diets and other supplemental feeding

The lack of knowledge on detailed balanced diet of crayfish has largely prevented the development of intensive farming (Ackefors and Lindqvist 1994) and could also have delayed the spread of semi-intensive farming in some areas due to low and unpredictable production (Lee and Wickins 1992, Järvenpää *et al.* 1996). The growth of *Cherax* species on artificial diets has been reported to be poor (Morrissy 1989, Anson and Rouse 1996), while Castell *et al.* (1989) observed that several other crustaceans accepted artificial diets readily and growth was satisfactory. McClain (1995c) concluded, that even though the microbially enriched detritus forms the basic food delivery for crayfish, a supplemental feeding may be necessary.

The most important characteristic of the pelleted diets, together with the nutritionally optimal content, is the ability to resist leaching and the loss of nutrients after water immersion (Farmanfarmaian *et al.* 1982, Cuzon *et al.* 1994). The leaching has been minimized by using binders or ingredients with binding agents (Goldblatt *et al.* 1980, Heinen 1981, D'Abramo and Robinson 1989, Meyers 1991, Gadient and Schain 1994) and the possibility to bind the diets without costly ingredients has been recommended (Lee and Wickins 1992) and successfully developed (Chen and Yenn 1992, Jussila 1996c). The loss of nutrional value in the pellets is caused by both physical breakdown of the pellets and leaching by osmosis, which is based on the chemical characteristics of the nutrients. The former can be easily measured as weight loss, while the latter has more nutritional importance (Noel Morrissy, Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, personal communication).

The cost effectiveness has also to be considered when the ingredients are chosen (Jones *et al.* 1997). The cheap sources for protein, soybean meal or cottonseed meal, contain antinutritional factors and the expensive ones, like whole eggs, would jeopardize the farming economics. The closed systems, like ICCS, sets special requirements for the diets, and trace mineral supplements have to be added. Sterols also have to be added to the crayfish diets, since the external source of cholesterol is necessary (D'Abramo *et al.* 1985, Howell and Matthews 1991). Unfortunately the knowledge on freshwater crayfish nutritional requirements is limited, and the commercial diets used in the farming are normally based on other crustacean's nutritional requirements (Morrissy 1989).

Marron (*C. tenuimanus*) are fed with commercial pellets in the intensive or semi-intensive farming (Morrissy 1989). The commercial pellets consist of 30% of protein, 20% of carbohydrate and 10% of fat. The main ingredients in the diets are fish meal (18%), fish oil (2%), dehulled lupins (25%), maize (10%), oat flour (27%), shrimp meal (10%), and soyabean meal (3%). Other ingredients are dicalcium phosphate, fish premix, lecithin, limestone, spirulina and vitamin C (Mike Hoxey, 'Glen Forrest Stockfeeders Pty Ltd', personal communication). The P:E ratio of this diet is 30% of protein to 12.2 MJ kg^{-1}. A crayfish reference pond diet has also been used, and the formulation is fish meal (8%), meat meal (9%), blood meal (1%), wheat (55%), W.A. lupins (18%), tallow coater (5%), calcium lingosulphonate (2.5%), salt (0.3%), methionine (0.2%) and premix (vitamins and minerals, including choline) (1%) (Morrissy 1989). Also chicken pellets, lucern and sorgum are given as food in marron farming (Lee and Wickins 1992).

Morrissy (1984, 1989) observed that marron had longer intermolt period and less weight gain at molt when their only food source was pelleted diet compared to mixture of natural food items production and pelleted diet. Flint and Goldman (1975) and Anson and Rouse (1996) argued, that the bacteria produced in the culture system provides an important source of nutrition, and could explain possible differences in growth.

Geddes *et al.* (1988) stressed the importance of nutritionally adequate food for biomass production in the ICCS. In their study, under proper conditions and feeding, the yabbie (*C. destructor*) grew almost as fast as in farm ponds. Morrissy (1984) has similarly concluded that there is a need for more nutritionally adequate feeds, mainly because the growth of marron in his experiment was less than 30% of that obtained in semi-intensive pond culture.

Signal crayfish (*P. leniusculus*) are fed with vegetable matter, potatos, apples, etc., in pond farming (Lee and Wickins 1992). Also other types of supplemental feeds like cereals, par-boiled root vegetables, softleaved vegetation and fish have been used (Goddard 1988). Furthermore, commercial pellets are available in Europe, but their usage is limited (Järvenpää *et al.* 1996). The use of fertilizers to improve food items production has also been recommended as means to improve natural food items production in the ponds (Goddard 1988).

Red claw (*C. quadricarinatus*) were observed to obtained fastest growth rates when fed with combinations of *Artemia* and commercial shrimp or catfish diets (Anson and Rouse 1996), showing that in intensive conditions a combination of diets could provide adequate nutrition to promote growth. The benefits in growth were quite similar to those observed by Jones (1989) for using

zooplankton in feeding red claw. It was also noted by Anson and Rouse (1996) that deaths occurred at molt and were possibly caused by suboptimal nutrition. This has been described as 'molt-death syndrome' by Bowser and Rosemark (1981) in Homarid species and further studied by D'Abramo *et al.* (1985) in shrimp. The importance of bacteria, zooplankton or detritus is emphasised when raising juvenile crayfish, especially if the diets are only substituting nutrition derived from yolk sac (Anson and Rouse 1996).

The feeding rates given for supplemental pelleted feeds are 10 g m^{-2} wk^{-1} for juvenile marron (*C. tenuimanus*) (0-4 mo old) in the density of 5 crayfish m^{-2}, and after they reach 5 g in size the rate has to be increased to 25 g m^{-2} wk^{-1}. Feeding rates higher than 30 g m^{-2} wk^{-1} could cause increased BOD even with proper aeration (Morrissy *et al.* 1986).

The recommended feeding rates for fast growing freshwater crayfish juveniles are 1-4% wet weight d^{-1} and 0.3-1% wet body weight d^{-1} for adults (Avault and Huner 1985, Lee and Wickins 1992). The feeding rate depends on the population structure of the farmed stock as well as the farm pond dynamics.

Food conversion ratios vary largely depending on the species in question, feeding rate and type of the study. A 1:1 ratio has been calculated for red swamp crawfish (*P. clarkii*) diet (Huner and Meyers 1979), while ratios from 5:1 to 12:1 have been observed for yabbie (*C. destructor*) (Mills and McCloud 1983).

Pellet quality has been shown to enhance growth and production of crustacean (Lee and Wickins 1992), and the improvements in pellet stability have been shown to improve crustacean growth in the ICCS (Meyers 1991, Chen and Yenn 1992). The pellets have to be water stable to minimize the loss of the nutrients after water immersion (Lee and Wickins 1992).

2.3 Exoskeleton mineralisation and pigmentation

The exoskeleton is the only calcified hard tissue in crayfish, and provides the protection of the crayfish as well as has crucial role in feeding and mating of crayfish. The exoskeleton goes through a complicated series of changes throughout the crayfish molt cycle, changing from soft cuticle into hard exoskeleton to be demineralised prior to the molt (Lowery 1988). Here I discuss the process of exoskeleton mineralisation and pigmentation.

2.3.1 Calcium balance in crayfish

The predominant mineral in the crustacean exoskeleton is $CaCO_3$ (Zanotto and Wheatly 1995). Postmolt crayfish take calcium from the environment to calcify the new exoskeleton and the source of carbonate (CO_3^{2-}) is provided from metabolic processes or from the environment, possibly in the form of bicarbonate. The calcium (Ca^{2+}) and bicarbonate (HCO_3^-) then form calcium carbonate ($CaCO_3$) and a byproduct, hydrogen ions (H^+) (Zanotto and Wheatly 1995).

Crayfish regulate the total and hemolymph calcium (Ca^{2+}) concentrations during the molt cycle by complicated mechanisms based on changes in the extracellular osmolality and reabsorption of calcium (Wheatly and Gannon 1995). The calcium balance is dependent on the molt stage and the ambient water ion concentrations. Over the intermolt, calcium is slowly lost into the environment through the gills, the hemolymph calcium concentration peaks in the premolt, while the postmolt is characterized by an active uptake of calcium accompanied with other ions (Wheatly 1996). Calcium balance during the intermolt is negative (Greenway 1972), and it has been argued that this is beneficial since the calcium storage in the crayfish exoskeleton is virtually unlimited and the active uptake would require energy.

During the process of molting the calcium content of the crayfish varies highly due to losses of the mobilized exoskeleton calcium into the environment in the late premolt followed by the postmolt uptake. Crayfish calcium content is lowest in immediate postmolt (approximately 10% of that in intermolt), and the losses have to be compensated later either from the ambient water (Lowery 1988) or feeds (Greenway 1972).

Calcium balance, towards the late premolt, is most probably controlled by the same hormones that control the molt cycle (Greenway 1985). The stimulus for the formation of the gastroliths is a steroid, β-ecdysone (or compounds related to it), that peaks early in premolt and later more distinctly in the moltstages D_{3-4}. The β-ecdysone itself is a precursor of α-ecdysone that is secreted after the molting inhibiting hormones levels decrease. A peptide resembling calcitonin (CT) might also control calcium balance and it has been shown that the levels of CT have been inversely proportional to hemolymph calcium levels in shrimp *Paleomon serratus* (Arlot-Bonnemains *et al.* 1986). The control of postmolt calcium balance in crustacea has not yet been solved (Wheatly 1995).

Exoskelon mineralisation requires also CO_3^{2-}, which originates from internal HCO_3^-, and it is normally present in intermolt crayfish (Wheatly 1989). The mechanisms of HCO_3^- influx are poorly understood (Cameron 1989, Wheatly 1995).

2.3.1.1 Intermolt

The calcium balance in the intermolt crayfish is negative (Greenway 1972, Wheatly and Gannon 1995), resulting from unidirectional efflux of calcium in the gills which is not compensated by active reabsorption. Normally, the intermolt crayfish hemolymph calcium concentration is 10 mM, and the concentration is maintained on a steady level irrespective of the medium. Main gateway for calcium efflux are the gills, and a 15 g crayfish, *P. clarkii*, loses calcium at a rate of 30-40 μmol kg^{-1} h^{-1} (Greenway 1972, Wheatly 1989). The calcium losses are compensated from the substantial calcium reserve in the exoskeleton. The ion balance in the crayfish is largely based on the reabsorption of the electrolytes, Na^+, Cl^- and Ca^{2+}, from urine to compensate the rather low or negligible replacement of the lost ions from medium through the gills (Wheatly and Gannon 1995).

Antennal gland is the main site for ion reabsorption (Wheatly and Henry 1987, Wheatly 1990), and up 90% of the electrolytes including calcium are absorbed. The Ca^{2+} ATPase activity in antennal gland is double compared to that measured in the gills (Wheatly 1996). Urine calcium levels remain low throughout the intermolt (Greenway 1985) while the gut, in addition to the gills, could be one of the alternative pathways for calcium efflux. The reabsorption from the hemolymph requires less resources since the concentration gradient between hemolymph and urine is 1:10 while the

concentration gradient between crayfish hemolymph and ambient water could be as high as 1:290 (Wheatly and Gannon 1995). Calcium influx through the gills is negligible during the intermolt.

2.3.1.2 Premolt

The hemolymph calcium levels remain relatively constant or decline during the premolt period and are slightly elevated towards the end of premolt in the crustacea (Greenway 1985, Sardà and Cros 1984, Sardà *et al.* 1989), even though calcium translocation is substantial, highlighting the importance of extra cellular regulation during these processes (Greenway 1974b).

The calcium translocations result in net loss of 80% of the intermolt calcium, 40% in cast exuvia and 40% as efflux to the ambient water. Approximately a week before molt the net efflux rate is increased up to 800 $\mu mol\ kg^{-1}\ h^{-1}$ in juvenile crayfish, *P. clarkii* (Wheatly and Ignaszewski 1990), 25 times greater than in intermolt, involving active transportation mechanisms (Greenway 1974b). The transport mechanism may involve a $Ca^{2+} + HCO_3^-$ cotransporter or a $Ca^{2+}/2H^+$ exchanger in the crustacea (Greenway 1985). Also urinary calcium levels increase, but to a lesser extent.

Part of the intermolt calcium is stored in the paired gastroliths in some crustaceans (Sardà and Cros 1984), to be used as $CaCO_3$ stores for postmolt calcification, and the formation of the gastroliths commences 10-20 d before the molt (Willing and Keller 1973). The relatively small amount of calcium stored in gastroliths has been discussed in the literature and the possible reasons for the limited calcium reserves could be space limitations due to their location in the cephalothorax, the mobilization requirements (presence of certain tissues) at postmolt and low selective pressure for larger calcium reserves. Marine crustaceans, such as shrimp (*Aristeus antennatus*) (Sardà *et al.* 1989), on the other hand, can store calcium in the hepatopancreas and gastric epithelium.

The control of calcium balance in premolt is partly hormonal. A decrease in molting inhibiting hormone (MIH), and, as a result, an increase in β-ecdysone and α-ecdysone levels in circulation, increased the total calcium concentration in hemolymph and also the proportion of protein bound calcium in hemolymph in crab *Carcinus maenas* (McWhinnie 1962). The unbound fraction of calcium decreased, possible slowing or minimizing the efflux.

2.3.1.3 Postmolt

Immediately after ecdysis, crayfish start to mineralize the newly formed exoskeleton by increasing the uptake of calcium through the gills (Greenway 1974a), consuming cast exuvia and mobilizing calcium from the gastroliths (Wheatly and Gannon 1995). The window of opportunity for successful calcification is up to 4 d after molt, with several studies suggesting that it can not be temporarily shifted (Wheatly 1995).

Postmolt calcium uptake in the crustacean is believed to be an analogy to fish Ca^{2+} ATPase mechanism (Wheatly 1990, 1995). The increased net uptake of calcium is evident 15 min after the molt in freshwater crayfish (Wheatly and Ignaszewski 1990), with the juvenile crayfish (*P. clarkii*) pumping in 1,150-2,500 $\mu mol\ kg^{-1}\ h^{-1}$ of calcium. This influx involves active uptake, and several electroneutral mechanisms have been suggested in crustaceans: $Ca^{2+}/2H^{+}$ exchange pump (Shetlar and Towle 1989, Ahearn and Franco 1990) or a basolateral Na^{+}/Ca^{2+} antiporter (Taylor and Windhager 1979). Ca^{2+} ATPase activity increased 2-3-fold in postmolt cuticular hypodermis, but remained unchanged in the gills or antennal gland, a possible indication of high calcium activity in cuticle during its hardening. The active intake of calcium is accompanied by basic equivalents, of which HCO_3^- is reported to be most important (Wheatly and Ignaszewski 1990).

The calcium stored in the gastroliths is mobilized within 48 h after the molt to harden the mouthparts, the gastric ossicles and the dactyls of the walking legs (Chaisemartin 1964). The cast exuvia can also be consumed to provide extra source of calcium.

The postmolt decline in hemolymph ecdysone hormone levels resulted in increased calcium uptake from the environment, causing a doubling of exoskeleton calcium in 12 h in crab *C. maenas* (McWhinnie 1962).

2.3.2 Carapace mineral concentrations

The studies on freshwater crayfish exoskeleton minerals show a wide range of calcium concentrations, depending on the environment where the crayfish have grown as well as the location of the exoskeleton sample (Huner *et al.* 1976, 1979, Mills and Lake 1979, Huner and Lindqvist 1985, Lahti 1988). This study also showed that the exoskeletons of Cambarid species were more heavily mineralized, with both calcium and magnesium, than Astacids'. Larger size crayfish are reported to be more heavily mineralized than their smaller co-species in some studies (Chaisemartin 1962, Greenway 1964, Mills and Lake 1976), while Huner *et al.* (1976) could not find such a trend, even though carapace density increased with size (age) of crayfish in the latter study. Sexual maturity and sexually active life stage also affects carapace mineralisation in Astacids, with a lower molting frequency due to maturity, especially in females, allowing more time for mineralisation (Huner and Lindqvist 1985).

Sardà *et al.* (1989) reported, after comparing life strategies of various crustaceans, that heavily calcified species were relatively more K strategists and weakly calcified species were r strategists. The criteria for heavy calcification in their study was 10-50% of calcium in carapace. The reasons for heavy calcification in K strategists were slower growth and longer intermolt periods.

The distribution of calcium in carapace could also be in relation to crayfish's environmental adaptations and behavior (Mills and Lake 1976), indicating that the analyses carried on the carapace mineral concentrations should be assessed bearing in mind the distinct differences between different species' behavioral requirements on exoskeleton hardening.

The mean calcium concentration in noble crayfish (*A. astacus*) carapace (branchiostegite, lateral part of the carapace) ranged from 21.6% to 23.5%, in signal crayfish (*P. leniusculus*) the mean was 23.9% and in red swamp crayfish (*P. clarkii*) the range was from 22.8% to 27.5% (Huner and Lindqvist 1985) in several wild populations. Chela palm calcium concentrations were from 21.9% to 24.1% in noble crayfish and from 24.8% to 28.0% in red swamp crayfish. Lahti (1988) found that noble crayfish mean carapace calcium concentrations were 25.1% in males and 27.1% in females and that the calcium concentration varied in different parts of the exoskeleton from 25.1% to 30.5% in males and from 25.0% to 27.1% in females. Jussila *et al.* (1995) observed carapace calcium concentrations between 22.4% and 28.0% in wild noble crayfish.

Mills and Lake (1976) reported that the carapace calcium concentrations of the *Parastacoides tasmanicus* and *Astacopsis fluviatilis* were lower than in other crustaceans, being on the average 8.3% and 10.4%, respectively. Carapace calcium concentrations in *O. virilis* were from 24.2% to 26.0%, in *Procambarus alleni* from 23.7% to 26.1% and in *P. clarkii* from 24.8% to 26.0% (Huner *et al.* 1976).

In addition to the individual minerals, also the total mineral content or exoskeleton ash concentration, has been studied (Welinder 1974, 1975, Huner and Lindqvist 1985, Huner *et al.* 1990) and used as an indicator of mineralisation or life strategy differences between freshwater crayfish species. The total mineral concentration in the exoskeleton, branchiostagites, varies from 67.6% to 72.9% in males and from 72.0% to 78.0% in females in intermolt freshwater crayfish in the above mentioned studies.

The sample processing seems to affect the results, especially in the total mineral analyses. The results obtained from ashed samples (Jussila *et al.* 1995) reveal lower levels than in some other studies. For example, Welinder (1974, 1975) reported high mineral concentrations (calcium salts 76-81%) in crustacean cuticle after demineralization with 5% acetic acid, 10% trichloroacetic acid (TCA) or 0.1 M EDTA, and the differences between those studies could be partly resulting from different demineralization methods.

2.3.3 Environmental and nutritional requirements for the exoskeleton mineralisation process

The distribution of freshwater crayfish can be limited in low calcium waters, less than 5 mg L^{-1} of calcium, because of inability to calcify exoskeleton rather than to maintain normal calcium metabolism (Wheatly 1995), while there is some evidence that *Cherax* sp. can complete mineralistation succesfully in even lower calcium waters (Bayly 1966).

External ion availability is of paramount importance to postmolt calcification. Up to 50% of Ca^{2+} uptake is at least partially Na^+, Cl^- and HCO_3^- dependent (Greenway 1974b, Wheatly and Gannon 1993, 1995). The first 4 d after molt are crucial for postmolt calcification, with evidence showing, that if calcium is not present in the aquatic media during this period the calcium uptake is decreased even if calcium is available later and, as a result, the exoskeleton becomes paper thin (Wheatly and

Gannon 1995). Low calcium in water, magnesium in a lesser extent, could affect, in addition to carapace mineralisation, also the ion balance in crayfish gills causing an efflux of ions (Kirscher 1994).

Ambient water calcium has to be higher than 5 mg L^{-1} to ensure normal exoskeleton calcification in freshwater crayfish in UK (Lowery 1988), however calcification is still effective if other conditions required for calcification, close to neutral pH, presence of Na^+, Cl^- and HCO_3^- (Wheatly and Gannon 1995), are favorable. Furthermore, there is evidence showing that crayfish (*Cherax*) and shrimp (*Caridina*) could inhabit waters in Queensland, Australia with calcium contents less than 1 mg L^{-1} (Bayly 1966, Bayly and Williams 1973), and the bicarbonate levels, rather than calcium, are used to explain the presence of crustacean in these waters. The low level of a few key ions in the ambient water, namely Na^+, Cl^- and Ca^{2+}, during the postmolt period could result, in addition to insufficient calcification, hemodilution (Wheatly 1995).

Water pH affects calcification directly, and the uptake of calcium was reduced by 60% at pH 5.2 (Zanotto and Wheatly 1993) and totally inhibited at pH 4.0 (Malley 1980). The effect of low pH is indirect and results from decreased availability of total ambient CO_2 or HCO_3^- or increased concentrations of the trace metals that become more soluble in acid conditions (Malley and Chang 1985, Zanotto and Wheatly 1990, 1995). The acid tolerance of different species is related to their evolutionary history (Wheatly 1995) and it has been shown, that under extreme conditions (low pH) *Orconectes* can mobilize exoskeleton calcium to regulate acid-base balance (McMahon and Stuart 1989). Furthermore, in extreme situations, a population collapse can result from acute changes in the water pH (Davies 1989).

Suboptimal conditions, low water calcium or pH, have been shown to result in decreased carapace mineralisation (Malley 1980, France 1985). Jussila *et al.* (1995) have shown that carapace calcium levels are dependent on both ambient water and sediment calcium, indicating the importance of aquatic sources of calcium for crayfish. Normally, the calcium is in a sufficient quantity in the ambient water for carapace mineralisation, and the calcium obtained in feeds acts as a supplemental source in later stages of intermolt (Greenway 1985).

The size of crayfish also affects the calcium metabolism: larger size individuals demineralisize their exoskeleton more effectively; premolt efflux is higher in larger crayfish; post molt calcium influx is lower in larger size crayfish; larger size crayfish have more time to complete exoskeleton

mineralisation due to longer intermolt; smaller size crayfish mineralize their carapace quicker; larger size crayfish are more dependent on calcium influx in later intermolt (Wheatly and Ayers 1995).

2.3.4 Exoskeleton pigmentation

The crayfish exoskeleton is normally dark brown or black (marron (*C. tenuimanus*)), dark greenish brown or dark brown (noble crayfish (*A. astacus*) or signal crayfish (*P. leniusculus*)). There are several variations in the exoskeleton pigmentation of wild crayfish, ranging from blue or red to totally white albino-like pigmentation. Exoskeleton pigments, astaxanthin, astaxanthin esters and keto-carotenoids (Wolfe and Cornwell 1965) are mostly derived from the feeds in the habitat, and the pigmentation in the wild crayfish, whatever the color is, could be taken as normal. Changes in exoskeleton pigmentation under farming conditions can lead to substantial financial losses due to reduced consumer acceptability (Lee and Wickins 1992), since the crayfish turn pale red after cooking if the astaxanthin level in exoskeleton is low.

Crayfish need an external source of β-carotenoids to complete exoskeleton mineralisation (Wheatly and Gannon 1995). They then convert the β-carotenoids into astaxanthin in a reaction where keto-carotenoids are the intermediate products (Thommen and Wackernagel 1964). Astaxanthin has also been found in the eggs and eyestalk of crayfish, suggesting that it has an important role in larval development and visual processes (Wolfe and Cornwell 1965).

In farming conditions, especially if the crayfish are reared in a simplified environment or intensively, insufficient nutrition may result in changes in the exoskeleton pigmentation (Nelis *et al.* 1989, Henttonen *et al.* 1993, Huner and Meyers 1979, Jussila 1996b) but the normal pigmentation can be achieved on diets including green plant material or carotenoid extracts (Huner 1984a, D'Abramo *et al.* 1985, Sommer *et al.* 1991, Tsvetnenko *et al.* 1995). The common change in exoskeleton is a pearly blue color, which normally initiates from the lateral parts of exoskeleton. This change in the exoskeleton pigmentation has been referred as 'blue shell disease' in shrimp (Baticados *et al.* 1987) and it has also been reported in crayfish (Huner and Meyers 1979, Huner 1984b). The phenomena is caused by β-carotenoid deficiency and can be relatively easily cured by adding astaxantin into the diets. In addition to causing pigmentation changes the β-carotenoid deficiency

might also inhibit growth (D'Abramo and Robinson 1989, Avault and Brunson 1990), although in one study on marron such an inhibition was not recorded (Tsvetnenko *et al.* 1995).

2.4 Hepatopancreas and crayfish condition

The hepatopancreas serves as the main digestive gland in crayfish responsible for both absorption of nutrients from the digestive track and excreting fluids enabling breakdown of the nutrients (Goddard 1988, Holdich and Reeve 1988). The hepatopancreas is also the main energy reserve in crayfish (Suprunovich *et al.* 1983, Huner *et al.* 1985) and the changes in its size and moisture content have been shown to be affected by diet (McClain 1995a, 1995b). The annual cycle of freshwater crayfish, growth and molting, can be characterized by changes in hepatopancreases energy content (Speck and Urich 1969, Huner *et al.* 1990).

Hepatopancreas wet and dry weight, relative to total body weight, and hepatopancreas moisture content have been used to indicate crustacean condition (McClain 1995a, 1995b, Mannonen and Henttonen 1995). This has shown to give additional information in studies on crustacean nutrition, growth and also when the impacts of waterways construction on aquatic ecosystems are evaluated.

In this section I review the role of hepatopancreas, as a condition indicator in crayfish.

2.4.1 Role of hepatopancreas in crustacean metabolism

The hepatopancreas has a range of functions, mostly controlled by the endocrine system (Loizzi 1971, Gibson and Parker 1979). The development of such a digestive system is argued to be based on the low selectivity of crayfishes' feeding habits and the resulting prolonged process of digesting (Vonk 1960). It has been shown (Francesconi *et al.* 1995) that marron (*C. tenuimanus*) hepatopancreas can act as a detoxifying organ for DDT, etc., in feed. Hepatopancreas serves as calcium storage in some crustaceans to allow rapid initial postmolt calcification (Sardà *et al.* 1989).

The hepatopancreas is located in the cephalothorax, occupying most of its space, above the midgut (Holdich and Reeve 1988). It is a trilobed structure with two lobes projecting forward and the third to the rear of the carapace (Huner and Barr 1991). The organ is in close contact to the hemolymph and is involved in digestive enzyme (proteinases, peptidases, lipases and amylases)

synthesis and secretion, lipid and carbohydrate metabolism, and storage of calcium and some heavy metals. The hepatopancreas is connected to the digestive track with tubes guiding the nutrients in and enzymes out of the organ. The tubes open to midgut, and the nutrients, after they have been carefully processed and converted into fine particulate matter in the complex gastric system, are actively transported into hepatopancreas by co-ordinated contraction of the muscle network investing the hepatopancreas.

The nutrients, entering the tubules of the hepatopancreas, are processed by the R, F or B cells (Stanier *et al.* 1966, Gibson and Parker 1979, Dall and Moriarty 1983). R cells absorb luminal nutrients, store and metabolize lipids and glycogen and absorb heavy metals from the hemolymph, F cells synthesize enzymes and pack them into vacuoles and absorb heavy metals before they enter hemolymph, absorb luminal nutrients and may even develop into B cells, while B cells are the storage and secretion bodies of the digestive enzymes (Loizzi 1971). E cells are earlier development stages of R or F cells.

Hepatopancreas acts as the main storage of lipids in crustaceans and lipid concentrations up to 92% of dry mass have been measured in the hepatopancreas of crayfish (Suprunovich *et al.* 1983).

2.4.2 Hepatopancreas response to changes in nutrition or stress

The energy and resources obtained from the environment in the form of nutrients are allocated between maintenance, growth, and reproduction (Tytler and Calow 1985). The growth rates and reproductive effort are largely a result of the amount of resources that organisms are capable allocating to these alternative metabolic pathways after the basic maintenance needs are fulfilled. Whether growth or reproduction are favored, depends on the life cycle strategies and life stage of the organisms. Normally, organisms store extra resources in specific tissues to be utilized later when the conditions and environmental clues favor or trigger either reproduction or growth. In crustaceans, the hepatopancreas or digestive gland serves as the main organ where the energy resources are stored.

The early stages of starvation involve depletion of protein, carbohydrate and lipid reserves in the crustaceans (Speck and Urich 1969, Hazlett *et al.* 1975, Regnault 1981) due to utilization of these reserves during nutritional deprivation. The short term starvation results in deprivation of the lipid droplets in hepatopancreas cells and changes in mitochondria size. Lipids are mobilized during the

first three days of starvation in the prawn (Vogt *et al.* 1985), and proteins are catabolised last of all in the shrimp (Schafer 1968). Schirf *et al.* (1987) did not discover any changes in hepatopancreas lipid or carbohydrate levels after 21 d starvation in red swamp crayfish (*P. clarkii*), a similar finding to that of Marsden *et al.* (1973) in the crab (*C. maenas*). Schirf *et al.* (1987) found, on the other hand, that the adenylate energy charge, based on ATP, ADP and AMP, declined and possibly indicated stressed condition of the red swamp crayfish after 21 d starvation.

The cells of the hepatopancreas react differently to starvation. Papathanassiou and King (1984) discovered that after 56 h of starvation both R-cells and F-cells had enlarged mitochondria and rough endoplasmic reticulum in the prawn (*Palaemon serratus*). The changes in R-cells, disturbances in absorption mechanisms and protein synthesis, were reflecting the hypoactivity of the cells, which might have been the initial modifications in fasting. Vogt *et al.* (1985) observed that R-cells clearly reacted to feeding on different diets and the changes in R-cells could be used as indicators of the nutritional value of the diets in the prawn (*P. monodon*). Prolonged starvation leads to loss of ability to metabolize food even if its availability is increased (Vogt *et al.* 1985).

Several studies show that the size and moisture content of the hepatopancreas in crustaceans, as a result of the metabolical changes described above, could be used to determine the condition of the crustacean (Huner *et al.* 1985, 1990, Evans *et al.* 1992b, Villagran 1993, Mannonen and Henttonen 1995, McClain 1995a, 1995b).

2.4.3 Hepatopancreatic indices as indicators of crustacean condition

Mannonen and Henttonen (1995) described the affects of peat mining on a wild noble crayfish (*A. astacus*) population by measuring hepatopancreas moisture and energy content and concluded that the indices could be used to evaluate the condition of the crayfish. They also added that other parameters from the environment, i.e. population density, food resources, water quality, etc., have to be taken into account when the results are interpreted. In their studies, noble crayfish had hepatopancreas moisture contents ranging from 70.4% to 75.7% in females and from 74.0% to 81.8% in males. Jussila and Mannonen (1997) have shown that the relationship between marron (*C. tenuimanus*) and noble crayfish hepatopancreas moisture content and total energy content is linear.

This gives an opportunity to estimate the total energy reserves of a crayfish by measuring its hepatopancreas moisture content.

Farmed crayfish are generally more intensively fed than wild marron with commercial pellets supplementing natural food production, resulting in increased energy reserves stored in hepatopancreas (Jarboe and Romaire 1995, McClain 1995b). McClain (1995a, 1995b) has shown that the availability of supplemental feed and population density both affected hepatopancreas moisture content and size in red swamp crayfish (*P. clarkii*). Prolonged starvation decreased hepatosomatic ratios in marron (Evans *et al.* 1992b), and in their report farmed marron had generally higher hepatosomatic ratio than wild marron, while there were no differences between sexes in hepatosomatic ratios. Stewart *et al.* (1967) have shown that 140 d starvation decreased lobster's (*H. americanus*) hepatopancreas mean weight from 5.2% of wet body weight to 2.6%, while their fed co-species hepatopancreas mean weight was 5.0%. Ackefors *et al.* (1997) have shown, that the fatty acid profile of hepatopancreas is affected by the type of food given to crayfish held in captivity, with the crayfish fed with marine fish showed high proportions of 20:5n-3, 22:5n-3 and 22:6n-3 in their hepatopancreases compared to crayfish reared on vegetable or freshwater fish diets.

Hepatopancreatic indices have been succesfully used to estimate the growth rates differences among wild West Coast rock lobster (*Jasus lalandii*) populations (Cockcroft 1997) and to describe the differences in southern rock lobster (*Jasus edwardsii*) condition under different feeding treatments (Musgrove 1997). In both studies hepatosomatic index and hepatopancreas moisture content, used together, were found useful to describe the condition of the lobsters.

Gu *et al.* (1996) have shown that starvation increases whole body water content in juvenile red claw (*Cherax quadricarinatus*) similarily to the changes in water content of hepatopancreas in red swamp crayfish (*P. clarkii*) fed with different diets (McClain 1995a, 1995b). In the previous study the water content of crayfish decreased when the feeding rate increased and crayfish were shown to catabolise tissue protein for metabolic requirements in starvation.

Female noble crayfish deplete energy reserves from hepatopancreas during ovarian maturation process (Huner *et al.* 1985). This was observed as high hepatopancreas moisture content, up to 80%, compared to reproductively inactive females, that had 60-70% moisture levels (Huner *et al.* 1990). Lindqvist and Louekari (1975) have shown that mature females had lower wet hepatosomatic indices than smaller sized immature noble crayfish. Their conclusion was that the

hepatopancreas serves as an energy reserve for the maturation of the gonads and it has been argued by Niskanen *et al.* (1997) that the level of energy stored before precopulatory molt determines whether egg development or spermatoforesis initiates. Huner *et al.* (1988) observed no differences in hepatosomatic indices between sexes in noble crayfish in a study, where crayfish were collected in August and differences due to oogenesis should have been evident.

2.5 Crayfish hemocytes

Crayfish hemocytes, the equivalent to blood cells, form three distinctly different types, hyalinocytes, granylocytes and semigranulocytes, which differ in morphology and function (Bauchau 1981, Martin and Hose 1992). The numbers of circulating hemocytes in crayfish have shown to react to different stressors and diseases (Söderhäll *et al.* 1988) and could be used as indicators of either crayfish condition or environmental stress. Here I review the role of hemocytes in the crayfish and discuss their use as condition indicators.

2.5.1 Role of hemocytes in crayfish

Crustacean hemocytes are normally produced in the hematopoietic tissues. These are located in dorsal anterior part of thorax and on top of the cardiac stomach in lobsters (Hose *et al.* 1992). The maturing hemocytes (stem and differentiating cells) are organized in small lobules within the hematopoetic tissue in blue crab (Johnson 1980). Stem cells line the apical border of the lobules from where the maturing hemocytes are moving towards the hemal space and the young hemocytes are liberated first into adjacent hemal space and then into circulation.

Hematopoiesis is regulated in crustaceans probably by several physiological processes, for example molting, reproduction, and diseases, and also by environmental conditions (Hose *et al.* 1992). Stress induces hematopoiesis, as well as chronic infections and diseases likely to cause death (Johnson 1980).

There are three basic types on hemocytes in most crustacean species, hyalinocytes, granulocytes and semigranulocytes (Table 1), while some authors refer to two types of hemocytes, hyalinocytes and granulocytes (Bauchau 1981). Three hemocyte populations have been identified in freshwater

crayfish (Söderhäll *et al*. 1988), lobsters (Aono *et al*. 1993, Sequiera *et al*. 1995), crab (Söderhäll and Smith 1983, Clare and Lumb 1994) and shrimp (Martin and Graves 1985, Hose *et al*. 1987, Tsing *et al*. 1989).

Table 1. Different crustacean hemocyte types according to Bauchau (1981)

	Hyalinocyte	Semigranulocyte	Granulocyte
Shape	round or oval	oval or spindle	oval
Nucleus	central, round, large	central or eccentric, oval, lobed	eccentric, kidneyshape
Endoplasmic reticulum	smooth, rough, scarce	smooth, rough, abundant	smooth, rough, moderate
Free ribosomes	present	abundant	moderate
Golgi	0 or 1	1 or more	0 or 1
Granules	0 or few	moderate	abundant
Lysosomes	-	present	present
Mitochondria	moderate	abundant	abundant

All three hemocyte types contain variable amounts of antioxidant enzymes, which act in host defense mechanisms (Bell and Smith 1995). Hemocytes take part in the proPO activation system (see section '2.6.4 Immune system and prophenoloxidase activation system') (Söderhäll *et al*. 1988), and contain phenoloxidase (PO), prophenoloxidase (proPO), bactericidins and lectins (Takahashi *et al*. 1995).

Hyalinocytes can initiate or contribute to hemolymph clotting in crustaceans (Hose *et al*. 1990, Aono and Mori 1996), a procedure that has been questioned (Aono *et al*. 1993, 1994a, 1994b). Hyalinocytes also contribute to formation and hardening of cuticle at molt (Vacca and Fingerman 1983), since crosslinking of endocuticle protein matrix is dependent on diphenolic metabolites carried or synthesized in hyalinocytes. There is also some phagocytotic activity in hyalinocytes (Söderhäll *et al*. 1986). Hyalinocytes show no prophenoloxidase (proPO) activity (Sequiera *et al*. 1995).

Granulocytes are responsible for host defense mechanisms in shrimp (Hose and Martin 1989), with large granule granulocytes capable to encapsulate large particles like metazoan parasites while

small granule granulocytes phagocytose bacteria and other small particles in decapods (Hose *et al*. 1990). Large granules in granulocytes also contain effective L-cystain, that acts actively against invading microbi in crabs (Agarwala *et al*. 1996). Saito *et al*. (1995) found a defensin-like substance in both small and large granules in granulocytes, and demonstrated strong antibacterial activities in this substance against both gram-negative and gram-positive bacteria. Granulocytes, especially large granule granulocytes, show proPO activity in the granules (Söderhäll *et al*. 1986). There is evidence showing that granulocytes take part in hemolymph clotting (Söderhäll *et al*. 1988, Aono *et al*. 1993, 1994a, 1994b), especially if stimulated by invading microbi. Granulocytes take also part in cuticle hardening by producing proteins to be crosslinked with diphenols and granulocytes may also have an important role in tanning, basement membrane formation and wound healing (Vacca and Fingerman 1983), which further emphasis their role in early postmolt. Small granule granulocytes mature to large granule granulocytes also in peripheral circulation, in addition to hematopoietic tissue, and it is also argued that the line of hemocytes finally senescence as large granule granulocytes (Hose and Martin 1989)

Semigranular cells also have some phacytogic activities, and they are reported to specialize in encapsulation (Persson *et al*. 1987b). Semigranulocytes show proPO activity (Söderhäll *et al*. 1988). These cells, together with granular cells, granulate spontaneously and participate in cellular defense reactions (Söderhäll and Smith 1983).

2.5.2 The total hemocyte counts and factors affecting circulating hemocytes

The total hemocyte counts (THCs) have been analyzed in lobsters using either manual counting (hemocytometer and microscope) or particle counters, such as Coulter Counter or flow cytometer (Sequiera *et al.* 1996).

Evans *et al.* (1992) found marron THCs to be from 0.5 to 10×10^6 mL^{-1} in farmed and wild populations. Stewart *et al.* (1967) found *Homarus americanus* THCs to be 16.8×10^6 mL^{-1} (manual) and 18.1×10^6 mL^{-1} (Coulter counter), a comparable level to both Cornick and Stewart (1978) observation, THCs between 12.7 and 28.6×10^6 mL^{-1} in *H. americanus*, and Yeager and Tauber (1935) observation of THCs 18.7×10^6 mL^{-1} in *H. americanus*. In the latter study, THCs in other crustaceans were of the same magnitude ($5.1 - 54.1 \times 10^6$ mL^{-1}) as in lobsters. Flow cytometer

has been used to analyse THCs also, with the possibility to distinguish between different development stages in hemocytes (Sequiera *et al.* 1996).

Stewart *et al*. (1967b) noted that the THCs in lobster declines in presence of bacteria or when animals are starving, while Evans *et al*. (1992) did not find significant differences between marron reared in aquaria on a commercial diet, starved for 52 or 55 d or reared in a commercial farm. Field and Appelton (1995) found, that in the presence of dinoflagellate parasite, there was an increase in the combined number of parasites and hemocytes in the hemolymph which was due to an increase in the relative proportion of dinoflagellates and suggested a reduction in numbers of hemocytes. In presence of bacteria or mitogenic stimulation, spiny lobster hemocytes have a capacity to proliferate *in vivo*, and the increase in proliferation frequency can be up to six-fold compared to non-infected individuals (Sequeira *et al.* 1996).

The shrimp THCs are highly sensitive to the presence of water pollutants (Smith *et al.* 1995) and the THCs declined under pollution stress. A chronic (60 d) sublethal environmental stress, exposure to 0.2-1.0 mg L^{-1} of lead chloride, caused crab granulocytes to produce intensive granulation, hypertrophic nucleus and increased proliferation in the hemolymph (Victor 1994). In addition, hyalinocytes lysed resulting in hemolymph clotting causing, all together, a decline in THCs.

In infected *Saduria entamon*, the THCs increased three times as high as for the healthy animals (Hryniewieckaszyfter and Babula 1996). In the presence of degeneration and necrosis of cardiac muscle hemocytes have been reported to infiltrate muscle fibers (Wada *et al.* 1994).

The proportion of hemocytes in peripheral circulation varies in different molt stages in crustaceans. The production of hyalinocytes peaks around ecdysis while granulocytes are more numerous in intermolt (stage C) or early premolt (stages D_{1-2}) in shrimp *Sicyona ingentis* (Hose *et al.* 1992) or even in late premolt stages D_{3-4} (Tsing *et al*. 1989). Sequiera *et al*. (1995) reported sex related variations in hemocyte populations associated with molt cycle in spiny lobster, *Panulirus japonicus*, with hyalinocyte proportion being highest in postmolt (stage B) in both sexes and premolt (stage D_1) in males. Semigranulocytes were fairly constant in males and highest in premolt (stage D_1) in females. Granulocytes were highest in intermolt (stages C and D_0) in both sexes.

Persson *et al.* (1987a) have shown, that the changes in the THCs could affect immune competence in signal crayfish, *P. leniusculus*. In their study signal crayfish, infected with *Aphanomyces astaci*, was exposed to three different additional immune system activators and those crayfish, whose THCs increased after the treatment survived, while those, whose THCs declined

could not survive submortal fungal infection. The results indicate, that the decline in THCs under the normal THCs range could be taken as indication of worsening condition in crayfish.

2.6 Bacteria and crayfish

Normally, crayfish hemolymph is free of bacteria (Alderman and Polglase 1988), but under poor conditions, e.g. high density, lack food or poor water quality, bacterial infections in otherwise healthy crayfish may occur (Vey 1977). The bacterial infections can be taken as indications of decreased immune resistance and thus poor condition of the crayfish (Alderman and Polglase 1988). In this section I discuss the presence of bacteria in the aquatic environment and crayfish, and interactions between bacteria and crayfish.

2.6.1 Bacteria in aquatic environment

Bacteria form an important part of the aquatic ecosystem, and their role in the decomposing processes (Ackefors *et al.* 1994), as a source of nutrition (Momot *et al.* 1978) and finally in biofiltration (Malone and Burden 1988) has been established. In a balanced ecosystem, the bacteria have a controlled and a positive role, while a simplified aquaculture ecosystem with a tendency for efficiency can result in mass growth of bacteria which could result in increased mortality (Alderman and Polglase 1988).

Aeromonas sp. is one of the most common aquatic bacteria (Rhodes and Kator 1994), and Pseudomonads, which are very common in nature, can be isolated from variety of natural materials (O'Leary 1989). Both of these bacteria have been reported to infect crayfish and cause increased mortality in the farming conditions (Alderman and Polglase 1988)

Tap water is normally free of human pathogenic bacteria and the number of total bacteria is low (Miettinen *et al.* 1993). *Aeromonas hydrophila* is common in natural waters (Pathak *et al.* 1988) where the levels of *Aeromonas* sp. can reach 100 cfu mL^{-1} (Havelaar *et al.* 1990, Rhodes and Kator 1994). In the beginning of 90's the concentration of *Aeromonas sp.* in Finnish lakes during the summer was, on the average, 23 cfu mL^{-1} and total water bacteria (TWB) 60,000 cfu mL^{-1} and in

the winter season 2.3 cfu mL^{-1} and 34,000 cfu mL^{-1} respectively (Leena K. Korhonen; National Public Health Institute, Kuopio, Finland; personal communication).

In aquaculture conditions, *Pseudomonas* sp. can cause severe septicemia to crayfish and the bacteria can normally be found also in the hemolymph (Alderman and Polglase 1988). *Aeromonas* sp., on the other hand, causes infections mostly to fish (*Aeromonas salmonicida*) and are most common in salmonid fish farms. Thune (1994) mentioned that *Aeromonas* sp. and *Pseudomonas* sp., which are potential bacterial pathogens, are part of crayfish bacterial flora and can cause infections in cultured crustaceans. Finally, both species are indicators of polluted water and can also be linked to fecal pollution (Araujo *et al.* 1989).

Vibrio sp. has been shown to cause severe losses due to septicaemia in crayfish farming (Thune *et al*. 1991, Thune 1994). *Vibrio* sp. has also been found in freshwater crayfish environments in Western Australia (Noel Morrissy, Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, personal communication).

Ammonia is converted in an aquatic environment first to nitrite and then to nitrate by bacteria of the genera *Nitrosomonas* and *Nitrobacter* (Ackefors *et al.* 1994). These bacteria have to be present in any intensive aquaculture system, and special biofilters are normally built as an internal part of the recirculating systems.

2.6.2 Bacteria in crayfish

Normally, healthy crayfish are free of the bacteria (Bang 1970), although various research groups have isolated several genera of bacteria from the hemolymph of healthy crustaceans (Cornick and Stewart 1966, Amborski and Amborski 1974, Haskell *et al*. 1975). Scott and Thune (1986) also isolated significant levels of bacteria from the hemolymph of pond cultured crayfish (for example *Acinetobacter, Aeromonas, Arthrobacter, Vibrio, Bacillus, Corynebacterium, Pseudomonas*).

Three main categories of bacterial disease have been found in crayfish: bacteraemias involving the blood and internal organs of crayfish; infections of the exoskeleton by chitinoclastic bacteria; and gill infections by filamentous bacteria. Representatives of these categories are found in crayfish populations on a regular basis throughout Europe and it is expected that this is the case also world-

wide (Alderman and Polglase 1988). According to Unestam (1974) the specificity of bacteria is the same in the hemolymph as in the cuticle.

Potential bacterial pathogens of crayfish include species in the genera *Aeromonas, Bacillus, Corynebacterium, Mycobacterium, Flavobacterium, Pseudomonas, Vibrio, Edwarsiella,* and *Acinetobacter* (Amborski *et al.* 1974, Scott and Thune 1986, Thune 1994). All of these are gram-negative except *Corynebacterium* and *Mycobacterium*. Non sporulating gram-negative bacteria (genera *Pseudomonas, Aeromonas* and *Vibrio*) cause typical septicaemic infections in crayfish and freshwater shrimps (Toumanoff 1965, 1966, 1967, 1968, Vey *et al*. 1975, Boemare and Vey 1977, Vey 1986).

Gram-negative bacteria (e.g. *Aeromonas* and *Pseudomonas* sp.), have peptidoglycan cell wall covered with a lipid-rich outer coat containing hydrophobic proteins (Lehninger 1982). The gram-positive bacteria, on the other hand, have several concentric layers of peptidoglycan around the cell. This explains mostly why the gram-negative bacteria are not as sensitive to drugs as gram-positive bacteria and are easier to control in culture systems.

Bacteria can grow rapidly under certain conditions (i.e., pH close to neutral, organic particles in solution, temperature in the mesophilic range and good supply of DO), even when there is a shortage of complex growth factors that otherwise regulate their predominance (Krieg and Holt 1984). *A. astacus* hemolymph pH is normally between 7.5 and 7.6 (Järvenpää 1986), and water temperature and DO favourable to bacterial growth in farming conditions. The hemolymph of *A. astacus* may have also contained organic particles (Fetter and Graham 1976). These reasons could explain the presence of bacteria in the hemolymph of cultured freshwater crayfish.

2.6.3 Interactions between bacteria and crayfish

The bacteria, as a possible pathogen, when invading crayfish causes several different responses. Phagocytosis is the most common component of the cellular defense system in crustaceans, but little is know of this mechanism (Söderhäll and Cerenius 1992). In phagocytosis, hemocytes take up the bacteria from the hemolymph, perhaps triggered or accompanied by the activation of proPO system (see section '2.6.4 Immune system and prophenyloxidase activating system') that produces opsonin (Söderhäll *et al*. 1986). Opsonin has not yet been isolated.

Nodule formation is the second step, if the phagosytosis has not effectively removed micro-organisms. The micro-organisms are trapped inside several layers of hemocytes and the whole package becomes heavily melanised (Smith and Ratcliffe 1980a, 1980b). The bacteria are then quickly removed from the hemolymph to the hepatopancreas or the gills. What happens then to these particles is unclear. There are also other possible sites for bacteria clearance from the hemolymph (Martin *et al.* 1996).

Lectins bind to the surface structures of foreign particles and thus act as part of the recognition system and might represent a primitive immune response system. They are present in the hemolymph and capable in binding to both pathogens' and hemocytes' surface structures, quite like opsins (Söderhäll and Cerenius 1992). A protein that can bind to fungal β-1,3-glucans has been isolated from signal crayfish, *P. leniusculus* (Duvic and Söderhäll 1990).

The total hemocyte counts (THCs) are affected by the bacteria. Infections caused an increase in *Saduria entamon* THCs (Hryniewieckaszyfter and Babula 1996), while the THCs of signal crayfish (*P. leniusculus*) either increased or decreased in the presence of bacterial or fungal particles (Persson *et al.* 1987a). The THCs response could thus be related to the condition of the infected crayfish.

Finally, bacteria are capable to activate the proPO system, that is the main mechanism in crayfish immune system (Söderhäll and Cerenius 1992).

2.6.4 Immune system and prophenoloxidase (proPO) activating system

The prophenoloxidase (proPO) activating system has been reported to be an important component of arthropoda and especially crayfish immunological recognition system (Smith and Söderhäll 1986, Söderhäll *et al*. 1988). The activation of the proPO system produces phagosytosis, nodule and capsule formulation, hemocyte locomotion, and melanin synthesis in nodules and capsules (Lanz *et al*. 1993). The proPO system has been intensively studied in freshwater crayfish, *P. leniusculus* for the past 20 years (Cerenius and Söderhäll 1995) and recently also in red swamp crayfish, *P. clarkii* (Lanz *et al.* 1993).

The proPO activation system is a cascade like series of reactions (Söderhäll and Smith 1986), initiated either actively by presence of micro-organisms or passively by low Ca^{2+} concentration in crayfish hemolymph (Söderhäll *et al*. 1988). Inactive proPO enzymes are stored in hemocyte granules or vesicles. The schematic steps in activation chain are:

1) Inactive proPO enzymes are discharged from granulocyte or semigranulocyte granules into the hemolymph. The discharge is initiated by lipopolysaccharides (LPS) from gram-negative bacteria or β-1,3-glucans from fungi or calcium ionophore or degranulating factor (released into plasma from hemocytes!).
2a) LPS or β-1,3-glucans catalyze serine protease S activation into serine protease (proPO-activating enzyme) or
2b) Low hemolymph Ca^{2+} (at least *in vitro*) catalyze serine protease S activation into serine protease (proPO-activating enzyme) and
3) Active serine protease (proPO-activating enzyme) catalyze proPO activation into PO.

PO is an active enzyme causing oxidation of phenols to quinones, which further polymerize to form melanin.

The proPO activation system consists of several enzymes and proteins that are located within hemocytes in crayfish (Söderhäll *et al*. 1988). All the enzymes appear as inactive proenzymes and are activated by proteolytic cleavage or, as Sugumaran and Nellaiappan (1991) suggest, by alternative pathway involving lipids such as lysolectin in lobsters. The regulatory molecules co-exist either in the hemocytes or plasma. The activation of proPO system is proceeded by degranulation or lysis of either semigranulocytes or granulocytes.

The crayfish immune system can be severy compromised, and thus weaker, under conditions of multiple stressors, and then even otherwise harmless infestations can cause increased mortality as has been shown in noble crayfish and signal crayfish infested with either crayfish plague (*Aphanomyces astacii*) and *Psorospermium haeckeli* or both (Cerenius *et al.* 1991, Söderhäll and Cerenius 1992, Gydemo 1996).

2.7 Sexual maturation in crayfish

Sexual maturation is a process requiring energy, which either has to be extracted from the environment or reallocated from the existing resources to oogenesis or spermatoforesis after sexual maturation (Tytler and Calow 1985). Normally, organisms grow as fast as possible to reach the size of sexual maturation and later, depending on the effort required in the reproduction, allocate the resources between maintenance, growth and reproduction. There are several strategies for energy allocation. The freshwater crayfish studied here seem to put their effort into survival in case of limited resources, which means that even an ovum that has been already produced can be reabsorbed if the conditions in the environment could decrease future survival (Huner and Romaire 1979, Huner and Lindqvist 1986). In this section I discuss aspects in relation to the process of sexual maturation in crayfish.

2.7.1 Process of sexual maturation

Freshwater crayfish have to go through certain number of molts, between 11 and 15, to reach sexual maturity (Suko 1953, Black 1966, Pratten 1980, Henttonen *et al.* 1993). Normally, resources available in the environment or the intrapopulation competition result in differences in the age or the size at sexual maturation as well as the proportion of sexually active mature crayfish in the population (Järvenpää *et al.* 1996).

Morrissy (1974) described the ovarian development of female marron (*C. tenuimanus*) as a process with three different stages. Type I ovary contains no visible ova under low power magnification (up to 12x), Type II ovary contains unpigmented or lightly pigmented ova with a mean length of the major axis of the ovum of 1 mm and Type III ovary contains large (a mean length of 4-5

mm), heavily pigmented ova. Type I ovary is immature, while Type II is a resting stage, from which the Type III develops. The development of Type III ovary normally initiates in February.

2.7.2 Size or age at maturation in marron

Normally, female marron reach sexual maturity at the age of 2+ y.o., but if the conditions are favorable for fast growth, i.e. low population density and excess food, fast growing females may mature at the age of 1+ y.o. If the nutrition is plentiful in the farming conditions, the fastest growing females mature at the of 1+ y.o., and could spawn every year from that on providing the conditions remain good (Morrissy 1992b).

Morrissy (1974) has reported that farm reared female marron (*C. tenuimanus*) reached Type III ovary development stage at the age of 2+ y.o. when their orbit carapace length (OCL) exceeded 7 cm. The 1+ y.o. female marron in that study had reached type II ovarian development when their OCL exceeded 4.5 cm. Immature marron were always younger than 2+ y.o. and less than 7.5 cm in OCL.

In the wild populations, Morrissy (1970) observed that female marron were producing ova for spawning from 3 cm CL on, and the age of the marron was estimated to be 2+ y.o.

2.7.3 Conditions necessary for sexual maturation

Population density affects age and size at sexual maturity (Huner and Lindqvist 1986). The crayfish, as well as other aquatic animals, try to optimize both present survival and future reproduction by growing as fast as possible to the size or age, when they mature (Tytler and Calow 1985, Henttonen *et al.* 1993) and then, especially in case on females, put high effort on producing offspring (Lowery 1988).

Studies on the wild noble crayfish (*A. astacus*) populations in Finland (Huner and Lindqvist 1986) have shown that the resources, in most cases the availability of food, triggers maturity and also determines whether once matured crayfish spawn every year, every alternate year or follow other reproductive strategies. Furthermore, the length of the growing season, which is also the length of the time for gathering resources, affects reproduction strategies.

Marron (*C. tenuimanus*) have been shown to reach sexual maturity in farming conditions and in the wild at the age 2+ y.o. or when they reach a minimum CL of 3 cm (Morrissy 1970, 1976b). The conditions, that determine and influence the age or size of sexual maturity, are population density and availability of food (Morrissy 1992b).

Environment, availability of nutrients and other resources, length of the growing season, population density, etc., has been reported to have a major role in the timing of sexual maturation of red swamp crawfish (*P. clarkii*) (Huner and Romaire 1979), *Orconectes* (Momot 1988), signal crayfish (*P. leniusculus*) (Hogger 1986a, 1986b), noble crayfish (*A. astacus*) (Brewis and Bowler 1982) and *Austropotamobius* (Laurent 1988). Furthermore, Laurent (1988) emphasizes that the individual characteristics could be exaggerated by the variations in climate to cause further differences in the timing of sexual maturation on population level.

Size or age at sexual maturation has been suggested to be an indicator of the state of the environment where the crayfish live (Wenner *et al.* 1974). This is based on the assumption that the maturation process and its timing actually reflects the ability of the crayfish to allocate normally limited resources to processes that decrease its possibilities to survival in the future.

3. AIMS OF THE STUDIES

The aims of these studies were to:

1) investigate, as the primary physiological response, growth and molting of marron (*Cherax tenuimanus*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) reared intensively in the ICCS,

2) assess some other physiological responses: exoskeleton mineralisation (carapace calcium, magnesium and total mineral concentrations) and pigmentation, attainment of sexual maturity, and crayfish condition (hepatopancreatic indices, total hemocyte counts (THCs) and hemolymph bacteremia) of the three freshwater crayfish species reared intensively in the ICCS and

3) study the intensive system as a crayfish culture environment and define factors affecting growth in the ICCS.

4. MATERIALS AND METHODS

This section summarises the experimental designs as they were in the subpublications and additional experiments, while further details of the experiments are given in the attached subpublications. The roman numerals in brackets refer to subpublications, as listed in the beginning of the thesis.

4.1 Experimental crayfish

Juvenile (0+ or 1+ y.o.) marron (*C. tenuimanus*) of mixed parentage for studies of growth, exoskeleton mineralisation and pigmentation, condition and nutrition were provided from commercial farms in central western and south-western Western Australia (I, II, III, IV). Marron were randomly divided into experiment groups and placed in the experimental systems for one week to acclimate before the experiments. Mean (±SE) initial individual weights were 4.5±0.12 g (I, II, III), 10.2±0.4 g or 13.3±0.5 g (III), and 11.5±0.3 g (IV). Mean initial specific growth rate, SGR (formula for SGR is given in section '4.3 Growth and production indices'), obtained in semi-intensive farms, for the marron in these experiments were ranged 0.68±0.1 to 0.97±0.1. The birthday for these marron was presumed to be 1 January and their birth weight 1 g.

Wildstock marron for carapace mineralisation and crayfish condition studies (II) were trapped in April 1995 from three rivers, the Warren River, the Donnelly River and the Gardiner River, in south-western Western Australia, where they are considered to be native (Morrissy 1978). Marron for these studies were also collected from the semi-intensive farms in April and May, 1995 and in January, 1996, and from the experimental systems (I) at the Curtin University field trial area in November, 1994. All sampled marron were in intermolt stage C_4 (whole cuticle calcified; Lowery 1988). The molt stage was defined by hardness of the carapace, time from last molt (at least 3-4 wks), and absence of gastroliths or new cuticle.

Noble crayfish (*A. astacus*), for studies on growth, carapace mineralisation, crayfish condition (VI) and hemolymph bacteria (V), originated from Lake Suur-Lauas, Valkeinen Pond or Valkealampi Pond in Kuopio Province in Central Finland and their mean weights (±SE) were 21.2±0.5 g (V, VI). They were reared communally at the Kuopio University Fish Farm for a period up to 1 year before the experiments. Signal crayfish (*P. leniusculus*), that were used in the later

ICCS growth studies (VI) (mean weight±SE: 31.5±0.9 g), were farmed and provided by The Finnish Game and Fisheries Research Institute's Research Station in Evo, Finland.

4.2 Intensive crayfish culture system (the ICCS)

Crayfish were cultured in an intensive crayfish culture system (ICCS or Nardi System; O'Sullivan 1990), comprising of 2 or 4 experimental culture tanks connected to a sump tank (I, II, III, IV, V, VI; Fig. 1). From now on, the ICCS refers to the system as described by O'Sullivan (1990), and constructed by Nardino Sorbello, Cohunu Wildlife Park, Gosnells, Western Australia, who provided the systems used in marron (*C. tenuimanus*) studies or the slight modification used in the noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) studies. Systems that were used in the studies with marron had two or four raceway type culture tanks (H x W x L: 21 x 40 x 360 or 520 cm) housed in racks, with water inlets on both ends and an outlet in the middle, connected to a sump tank (either 250 or 2500 L). Water was recirculated by the pump from the sump tank to the culture tanks and was let flow freely back to the sump tank.

The ICCS in noble crayfish and signal crayfish experiments comprised of two culture tanks (H x W x L: 20 x 80 x 200 cm), housed one on the top of the other, and the lower tank was connected to a sump tank (200 L). Water was recirculated by the pump from the sump tank to the upper culture tank, then led to flow to the lower and finally back to the sump tank again.

Individual crayfish (marron, noble crayfish or signal crayfish) were reared in separate compartments, area from 150 to 1200 cm^2, covered with a pale-colored perforated lid of polyvinylchloride (PVC) or plastic coated metal mesh (I, III, IV, VI) or in plastic cages (300 cm^2; noble crayfish) (V, VI). Water flowed from one cage to next through perforated cage walls. Initial stocking densities in the experiments ranged from 10 to 24 crayfish m^{-2}. Experimental growth tanks in these studies were made of either stainless steel (marron experiments; I, II, III, IV) or PVC (noble crayfish and signal crayfish experiments; V, VI).

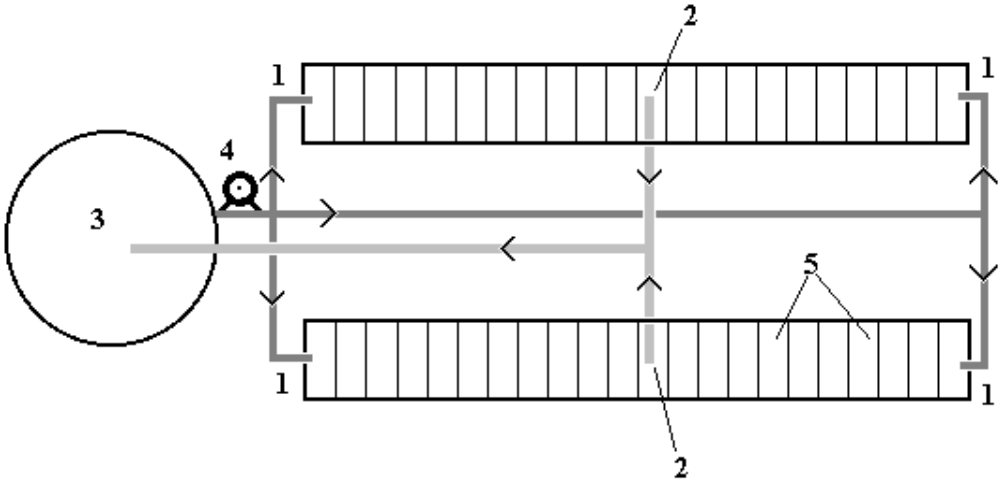

Figure 1. An upper view sketch of the ICCS as used in the marron (*C. tenuimanus*) experiments. Numbers refer to 1 = water inlets; 2 = water outlets; 3 = sump tank; 4 = pump; 5 = individual compartments in the culture tanks. Arrows indicate direction of the water flow (figure printed with permission from the Journal of Applied Aquaculture).

PVC tubes were used as hides in the compartments. Shell grit (1 cm in diameter) was used in compartments as a growth surface for epiphytic organisms and as a source of calcium in some of the marron studies (I, II, III). Some other studies on marron (III, VI) were carried out without substrate in the system. Noble crayfish and signal crayfish were reared without substrate (V, VI). The ICCS was exposed to natural light regime in marron studies (I, II, III, IV) while the indoor system, where studies on noble crayfish and signal crayfish were carried out, was under artificial photoperiod (V, VI; day:night, 14:10 h).

Marron were fed with commercial marron pellets (Marron Pellets by 'Glen Forrest Stockfeeders Pty Ltd', Perth, Western Australia; 30% protein, 10% lipid, 20% carbohydrate (IV)) every day or every second day at the rate of 5-7% of wet body weight per week. The diet varied in the experiment where the effects of the pellet water stability and supplemental pelleted fish diets were studied (IV) and the feeding rate was altered when the factors affecting growth of marron in the ICCS were studied (III). Noble crayfish and signal crayfish were fed with combinations of peas, potato, frozen alder leafs, frozen finfish (roach, *Rutilus rutilus*) and commercial marron pellets or salmon pellets (Vextra Mini 2, 'Suomen Rehu OY', Finland; 48% protein, 22% lipid (IV)) 6-10% of the body weight per week. Food was given every second day and the uneaten food was removed after 24 h and by siphoning the system once a week.

Temperature was maintained between 22 - 24ºC with thermostatic heaters (optimum temperature range for marron growth; Morrissy 1990) in the experiment testing water volume effect on marron growth (I), while other studies on marron were carried out without temperature control. Water was circulated at the rate ranging from 4 to 10 L min^{-1} per tank in marron experiments and at the rate ranging from 2 to 4 L min^{-1} per tank in noble crayfish and signal crayfish experiments. Water level in the tanks was from 9 to 10 cm.

4.3 Growth and production indices

The growth rates were estimated as specific growth rate (SGR, equation 1), weight increment at molt (W_m, equation 2) and intermolt period length (T_{im}, equation 3). SGR_{df} estimated the difference between individual SGR obtained in the experiment and SGR obtained in the farm ponds (Jussila 1996b).

$$SGR = ((\ln(W_t) - \ln(W_i)) \times 100) / t \quad \text{(Equation 1)}$$

where: W_t = weight at time t, g; W_i = initial weight, g; and t = time, d

$$W_m = (W_a - W_b) \times 100 / W_b \quad \text{(Equation 2)}$$

where: W_a = weight after molt, g; and W_b = weight before molt, g

$$T_{im} = T_{n+1} - T_n \quad \text{(Equation 3)}$$

where: T_n = date of n molt; and T_{n+1} = date of n+1 molt

Production (P, equation 4), survival (S, equation 5) and space factor (k, equation 6; Aiken 1980) were estimated as indicated below. Production was estimated on the surviving biomass (surviving production) after the study period, excluding crayfish that died during the experiments.

$$P = (B_t - B_i) \quad \text{(Equation 4)}$$

where: B_t = biomass after t days, g; B_i = initial biomass, g

$$S = (N_f / N_i) \times 100 \quad \text{(Equation 5)}$$

where N_f = final number of crayfish, N_i = initial number of crayfish

$$k = A / C^2 \quad \text{(Equation 6)}$$

where: C = carapace length, cm; and A = compartment area, cm^2

4.4 Hepatopancreas analyses

Marron (*C. tenuimanus*) in the studies on the hepatosomatic indices (II, IV) or carapace mineralisation (II) and noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) in the studies on physiological responses to intensive rearing (VI) were dissected within 5 h of capture, the hepatopancreases were removed, placed in foil cups and weighed. Whole hepatopancreases were dried at 80°C for 24 h and weighed. Results are expressed as wet hepatosomatic index (equation 7), dry hepatosomatic index (equation 8) and hepatopancreas moisture content (equation 9).

$$HI_{wet} = W_{wh} \times 100 / W_t \quad \text{(Equation 7)}$$

where: W_{wh} = weight of wet hepatopancreas (g); and W_t = total weight of marron (g)

$$HI_{dry} = W_{dh} \times 100 / W_t \quad \text{(Equation 8)}$$

where: W_{dh} = weight of dry hepatopancreas (g); and W_t = total weight of marron (g)

$$HM = (W_{wh} - W_{dh}) \times 100 / W_{wh} \quad \text{(Equation 9)}$$

4.5 Carapace mineral and pigmentation analyses

Carapace samples (1 cm^2) were cut from the left posterior side of the carapace. Samples were cleaned with distilled water and then dried at 60°C overnight (dry weight) and later ashed at 500°C for 24 h (ashed weight, measures total inorganic matter). Ashes were dissolved into 2 mL of Suprapur® (65% HNO_3) and diluted with distilled water and 0.5% lantane (magnesium 1:50 and calcium 1:500). Samples were analyzed with an AAS Perkin-Elmer 372 and the results were expressed as calcium or magnesium concentration in the dry carapace (mg kg^{-1}), proportion of inorganic matter in dry carapace (%, later referred to as carapace ash content), and calcium or magnesium concentration in ash (%). Carapace density was expressed indirectly as the weight of a 1 cm^2 sample.

Exoskeleton pigmentation was estimated using a three step index: 1 = perfect exoskeleton color; 2 = changes in the lateral parts of the exoskeleton; 3 = whole exoskeleton discolored. The typical change in the exoskeleton pigmentation was a light pearly blue color proliferating from the lateral parts of the carapace.

4.6 Hemolymph sampling

Hemolymph (200 µl) was sampled for the total hemocyte counts (THCs) analyses from intermolt marron (*C. tenuimanus*) at least 3-4 wks after molt and, in case of pond reared marron, crayfish with hard carapace and no evidence of molt preparations (no gastroliths or formation of cuticle under carapace) were sampled. Hemolymph was taken from ventral abdominal artery (from I or II pleonite) using 1 ml syringe and 24G needle. Clotting was prevented by filling syringe with 200 µl anticoagulant (EDTA based) before sampling. The anticoagulant formula was: 2.63 g NaCl, 1.12 g NaEDTA, 0.55 g citric acid and 0.29 g EDTA made into 100 mL of distilled water in pH 6-7. Solution was then added to 1.6 ml 0.2% Gentian violet, and carefully mixed. The reagents were precooled in ice and hemolymph samples were kept in ice (below 5°C) until analyzed.

Hemolymph samples for the hemolymph bacteria analyses (100 µl) were taken from ventral abdominal artery with syringe and 23G needle (V). Tail membrane was disinfected with 96% ethanol prior to inserting the syringe. Anticoagulants were not used for this procedure.

4.7 Total hemocyte counts (THCs)

The hemolymph samples were counted within 60 min after the crayfish were bled. A Neubauer Enhanced Line counting chamber was used, and four cornering fields (one field is formed of 4 x 4 squares, volume of each field was 0.1 mm^3) were counted. Each sample was counted twice from the two separate 4 x 4 fields in the counting chamber and the mean hemocyte count obtained from these two counts was used to estimate the THCs in 1 mL as expressed in equation 10.

$$THC = (HC \times D \times C) / 0.4 \qquad \text{(Equation 10)}$$

where HC = mean hemocyte count from counting chamber; D = hemolymph sample dilution factor (10); and C = conversion factor from 0.1 mm^3 to 1 mL (1000)

4.8 Hemolymph bacteria analyses

Hemolymph (approximately 0.1 ml) was spread on bovine blood agar plates. Plates were incubated at room temperature (20 - 25°C) in aerobic conditions for 7-14 d. Representative colonies were reisolated for characterisation (GSP, MERCK® and McConkey -agar) and identified with standard bacteriological methods (Baron and Finegold 1990) with the API 20E and API 20NE test kits (Analylab Products). API-test instructions were followed with one exception: incubation temperature was +20°C for three days instead of +37°C for three days. API test results were processed with APILAB Plus V3.2.2B. GSP-agar plates (MERCK®, *Aeromonas* and *Pseudomonas* sp. selective agar) were used for the genera *Aeromonas* and *Pseudomonas* identifications (Kielwein 1971).

The APILAB Plus shows the percentage of identification (%id): acceptable %id > 80.0, good %id > 90.0, very good %id > 99.0 and excellent %id > 99.9. APILAB Plus produced also a T -value, which estimates how closely the profile corresponds to the most typical set of reactions for each taxon ($0 < T < 1$).

4.9 Aquatic bacteria analyses

The bacterial level in the culture system water in the two experiments carried out on noble crayfish (*A. astacus*) lasting 22 and 26 wks (experiment 1 and 2, VI) (total water bacteria (TWB), *Aeromonas* sp. and *Pseudomonas* sp.) was monitored by taking water samples once a week (V). TWB, *Aeromonas* and *Pseudomonas* sp. cfu were analyzed in the Laboratory for Human Consumption in Kuopio Province (Kuopion kaupungin elintarvikelaboratorio).

4.10 Assessment of sexual maturition

The size at sexual maturaty for marron (*C. tenuimanus*) reared in the ICCS was estimated by sampling 90 marron from the clean system (III, IV). Marron (1+ y.o.) were weighed and dissected in November 1996 and the sexual maturity was estimated based on the presence of ovaries with visible eggs in females (Type II ovary with small size (1-2 mm) pigmented ova; Morrissy 1974) or testes with sperm in males. The size at sexual maturity was estimated by plotting the percentage of mature individuals in selected size classes (Ricker 1975), fitting a curve to the plots. The size of sexual maturity was defined as the approximate weight where 50% of the population had reach sexual maturity.

The sample was divided into 11 size classes each of which was 5 g in size range. The smallest size class was 10-15 g marron and the largest was over 60 g marron. The analyses was carried out separately for both sexes and for a pooled sample.

4.11 Other analyses

Psorospermium sp. infestation was checked in all growth experiments (I, II, III, IV, VI) using the method described by Evans *et al.* (1992a).

4.12 Data processing

Data was processed with SPSS/PC+ v5.01. Statistical analyses were carried out with EXAMINE, T-Test, ANCOVA, MANOVA and ONEWAY LSD. Results are expressed as mean±SE unless otherwise indicated. Correlation significance denoted by * = $P<0.01$ and ** = $P<0.001$ and the strength of correlation is expressed as strong ($r>0.9$ or $r<-0.9$), moderate ($0.6<r<0.89$ or $-0.89<r<-0.6$) and weak ($0.4<r<0.59$ or $-0.59<r<-0.4$).

4.13 Summary of experiments performed

The experimental designs are summarised in Table 2 (following page). A detailed description of the experiments is in the following sections.

4.13.1 Experiments in the subpublications

4.13.1.1 Marron

(I)

In the first experiment the effects of different water volumes on marron (*C. tenuimanus*) growth and production in the ICCS were studied(I). Two independent systems were used, with two identical culture tanks connected either to a 2500 L or a 250 L sump tank. The difference in total water volume between the two systems in this experiment was 5-fold. The study lasted 6 mo from May to November 1994. Standard water quality measurements, dissolved oxygen (Jenway DO_2 meter 9071), pH (CyberScan 100 pH), temperature (electronic present-minimum-maximum meters, 0.1°C), total ammonia (Merck® NH_4 kit nr 8024 (colorimetric method), or Aquaquant® ammonium kit nr 14428 (colorimetric method)), were taken.

(II)

Studies on farmed and wild marron (*C. tenuimanus*) carapace mineralisation and hepatopancreatic indices (II) were carried out from January 1994 to May 1995 using samples collected in the previous experiment (the ICCS sample, I), from four commercial farms in central western to south-western Western Australia and from three wild populations in south-western Western Australia.

Table 2. The experimental desings in the studies included in this thesis. The design of the experiments are explained in further detail later.

Experiment	Species	Density # m^{-2}	Feeds	Length	Treatment
I	marron	25	Marron pellets, natural food items	6 mo	Effect of total water volume on marron growth
II	marron	25	Marron pellets, natural food items	-	Carapace mineralisation and condition in farmed and wild marron
III	marron	14-25	Marron pellets, natural food items	1-6 mo	Factors affecting marron growth in the ICCS
IV	marron	25	Marron pellets, natural food items	4 mo	Marron growth on different pelleted diets
V	noble crayfish	14	Potato, peas, alder leafs, fish	2 mo	Gram-negative bacteria in the noble crayfish hemolymph
VI	noble crayfish, signal crayfish	14	Salmon pellets, marron pellets, fish	2 mo	Physiological responses of noble crayfish and signal crayfish in the ICCS
	marron	1-25	Marron pellets, natural food items	-	Marron THCs under different conditions
	marron	50	marron pellets	6 mo	Marron choice feeding
	noble crayfish	14	Potato, peas, alder leafs, fish	2 mo	Aquatic bacteria in the ICCS

(III)

Studies on the factors affecting marron (*C. tenuimanus*) growth in the ICCS (III) were carried out from April 1994 to May 1995. The studies consisted of three different growth studies carried out in Curtin University of Technology field trial area and at the Cohunu Wild Life Park's (Gosnells, Western Australia) experimental ICCS. Studies focused on describing the ICCS as an crayfish culture environment and on analysing the factors affecting marron growth in the system. In addition to routine water quality monitoring (DO, pH, temperature and total ammonia), diurnal temperature and DO variation, sessile organisms production (diatometer, see below), BOD_{24h} (clear bottle method, see below), and nitrate (Merck® nitrite kit nr 8025 (colorimetric method)) were analyzed, and the effect of compartment size (density) and feeding rate was monitored. A diatometer comprising a rack with seven vertical slides were placed in an empty compartment by the growth tank outlet for 2, 4, 8, 16 and 28 d of exposure. Slides were then dried for 72 h in room temperature and dry weight of the sessile organisms grown on the surface of the slides was estimated as difference between clean slide weight and dirty slide weight. BOD_{24h} was measured in the sump tanks in both in the clean (no food items production) and dirty (substantial food items production) ICCS during April-May 1995 to determine the amount of dissolved oxygen used by pelagic aquatic organisms and chemical reactions (Rump and Krist 1992). Three replicates were taken at each sampling time.

(IV)

The effect of pellet water stability and different commercial marron and fish diets on marron (*C. tenuimanus*) growth and condition in intensive and semi-intensive tank rearing (IV) was studied in an experiment that lasted until all marron in the ICCS treatment groups had molted twice (125 d). Two commercial pelleted marron diets and three pelleted fish diets were used. The results were expressed as crayfish growth (SGR, weight gain at molt and intermolt period) and condition (hepatopancreatic indices). In addition to routine water quality analyses, the production of food items in the systems were analyzed using a diatometer.

4.13.1.2 Noble crayfish and signal crayfish

(V)

The gram-negative bacteria in intensively reared noble crayfishes' (*A. astacus*) hemolymph (V) was analyzed during a growth study (experiment I, VI) in the Kuopio University indoor culture system. The study lasted 22 wks and the samples for the bacterial analyses were taken three times during the study (in the beginning of the experiment, after 16 wks and at the end of the study period). The hemolymph samples were cultured on bovine blood agar and the representative colonies were identified using API 20E and API 20NE standard bactereological kits ('Analylab Products').

(VI)

The physiological responses of noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) to intensive rearing (VI) were studied in three independent experiments lasting 22 and 26 wks (noble crayfish; experiments 1 and 2, respectively) and 46 wks (signal crayfish; experiment 3). Experiments were carried out using mature male crayfish. Physiological responses were described as growth (SGR, weight gain at molt and intermolt period), carapace mineralisation (carapace calcium, magnesium and total mineral concentrations) and crayfish condition (hepatopancreatic indices). Routine water quality analyses were made during the study.

4.13.2 New experiments

4.13.2.1 Marron

Total hemocyte counts (THCs) were studied to evaluate their usage as indicators of marron (*C. tenuimanus*) condition. THCs were analyzed in the hemolymph sampled from the marron reared in the ICCS, communally in tanks or in semi-intensive farm ponds (IV). Samples were also collected from two commercial farms in central western and south-western Western Australia. A minimum of 10 marron were sampled in each group. The THC analysis is explained in section '4.7 Total hemocyte counts (THCs)'.

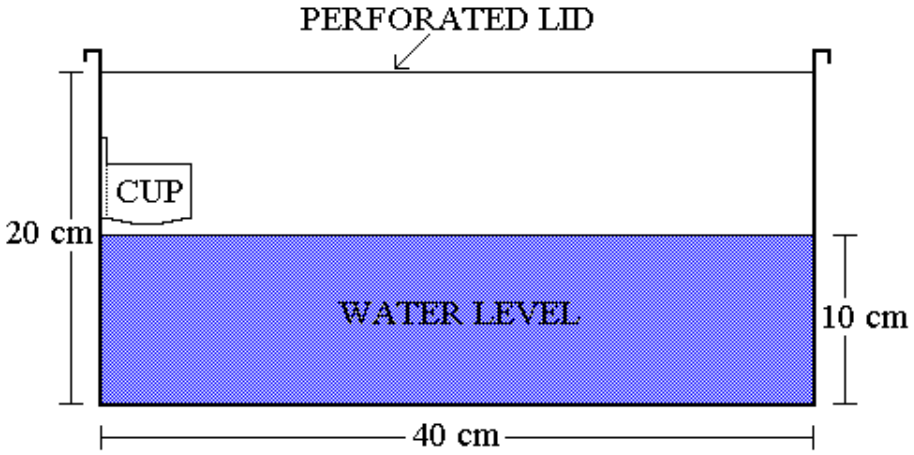

Figure 2. A sideview cross section sketch of the individual compartment with the feeding cup. The dimensions given here were the general dimensions of the compartments in the ICCSs used in the marron (*Cherax tenuimanus*) growth studies (I, III, IV).

The effect of crayfish choice feeding system on marron (1+ y.o.; mean weight (±SE): 25.9±1.4 g) growth in the ICCS was studied in the experiment carried out in Cohunu Wild Life Park, Gosnells (cf. Jussila *et al.* 1996). The study lasted 6 mo, from July to December, 1996. Marron were randomly divided into four groups (N=16) and placed in the ICCS. Half of the compartments (N=32) were provided with small PVC cups (25 mm diameter and 20 or 45 mm deep) attached 15 mm above water level (Fig. 2). Two groups, reared on two different commercial diets (Marron Pellets by 'Wesfeeds Pty Ltd', Perth, Western Australia (22% protein) and Allpet Pellets by 'Allpets Pty Ltd', Perth, Western Australia (33% protein)), administered in excess into the cups three times a week, were used as treatment groups. Two groups that were fed by immersing pellets in compartments (feeding rate 7% wet body weight per week) with the same diets were used as controls. The amount of ingested food (in grams), compartment cleanliness (three step index: 1=clean, 2=pellets and faeces or 3=faeces) and marron growth (SGR) were monitored. Routine water quality measurements were also carried out.

4.13.2.2 Noble crayfish

Two studies were carried out to evaluate water bacteria levels in the ICCS during the growth studies on noble crayfish (*A. astacus*) (experiments 1 and 2, V) in 1993-1994. Two separate tank systems were used in both studies (50 crayfish in each) to evaluate water born bacteria concentrations - total water bacteria (TWB), *Aeromonas sp*. and *Pseudomonas sp.*. One tank system was treated with bath chemicals (bactericides) once a week and the other was used as a control. A combination of formalin (37.5% formaldehyde stabilized with 10-15% methanol; 25 ppm) (Piper *et al.* 1982, Fulks and Main 1992) and methylene blue (raw powder dissolved in diluted water; 8 ppm) (Post 1987, Fulks and Main 1992) were used in the first study and potassium permanganate (8 ppm) in the second in the treatment system in 24 h baths once a week. Water inlet was then turned off for 24 h and reopened to flush the chemicals away. Treatments started after 21 d and were repeated every 7 d afterwards. Experiments lasted 22 and 26 wks.

Crayfish were fed in excess of consumption with frozen finfish (roach, *Rutilus rutilus*) once a week and with vegetables (potato and peas) twice a week. Remains of feed were removed after 24 h. The culture systems were cleaned once a week by siphon. Tap water was used in the culture units and it was recirculated without a filtering system (less than 50 ml min^{-1} fresh water added).

TWB, *Aeromonas* and *Pseudomonas* sp. cfu was analyzed by the Laboratory for Human Consumption in Kuopio Province (Kuopion kaupungin elintarvikelaboratorio) from weekly samples.

5. RESULTS

5.1 The environment

5.1.1 Water quality

The mean water temperature was high in marron (*C. tenuimanus*) experiments (Table 3), and exceeded 30°C occassionally in every experiment. The mean water temperatures in noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) studies were below 20°C and always below 25°C. Dissolved oxygen (DO) levels were high, with few exceptions, mainly during the high temperature peaks. Normally, the high temperature peaks correlated with low DO, but low DO did not occur during all of the high temperature peaks. Water pH remained below pH 9, with some exceptions (III) and water pH was generally higher in marron experiments compared to noble crayfish or signal crayfish experiments.

Table 3. Dissolved oxygen (DO), pH and temperature (T) in the water in the ICCS during the growth experiments (range of the means (minimum-maximum observed during the experiments)

Species	DO, mg L^{-1}	pH	T, °C
Marron (I)	7.1-7.4	8.1-8.6	25.5-26.7
	(5.7-11.3)	(7.4-8.8)	(14.0-32.1)
Marron (III)	6.0-7.8	7.0-8.3	21.5-26.7
	(4.5-10.4)	(7.3-9.1)	(20.7-32.9)
Marron (IV)	6.8	8.0	24.1
	(3.6-9.2)	(7.5-8.6)	(18.4-30.9)
Noble crayfish (VI)	6.8-7.4	7.3-7.5	18.2-18.6
	(4.3-9.4)	(6.7-8.0)	(15.1-21.3)
Signal crayfish (VI)	8.5	7.4	19.3
	(7.7-9.3)	(6.9-7.9)	(15.4-24.3)

The total ammonia levels were always lower than 0.1 mg L^{-1} and the nitrite levels lower than 0.05 mg L^{-1} in the marron experiments (III, IV). The total ammonia was lower than 0.2 mg L^{-1} in the noble crayfish and signal crayfish experiments (VI). The total unionized ammonia, estimated on the basis of daily pH and temperature values, was always lower than 0.01 mg L^{-1}.

Water calcium levels ranged from 8 to 34 mg L^{-1} in the marron experiments (I, II, III, IV) and were close to 18 mg L^{-1} in noble crayfish and signal crayfish experiments (VI). Other chemicals analyzed in the Curtin University of Technology field trial area tap water, which was used in the ICCS, were: Ca^{2+} 34-42 mg L^{-1}, Cl^- 110-165 mg L^{-1}, HCO_3^- 105-110 mg L^{-1}, K^+ 4.5-6.0 mg L^{-1}, Na^+ 75-100 mg L^{-1}, SO_4^{2+} 30-35 mg L^{-1}. The analyses of the University of Kuopio tap water, used in the ICCS, showed the following ion concentrations: Al^{3+} 0.07±0.01 mg L^{-1}, Ca^{2+} 18.8±2.6 mg L^{-1}, Cu^+ 0.01±0.001 mg L^{-1}, Fe^{2+} 0.07±0.02 mg L^{-1}, K^+ 5.0±0.1 mg L^{-1}, Mg^{2+} 6.5±0.2 mg L^{-1}, Mn^{2+} 0.03±0.01 mg L^{-1}, Na^+ 14.9±0.4 mg L^{-1}, P^{3-} 0.05±0.0001 mg L^{-1}, S^{2-} 12.5±0.2 mg L^{-1}, Si^{4-} 7.1±0.1 mg L^{-1}, N^{3-} 0.25±0.02 mg L^{-1} and C^{4-} 3.1±0.5 mg L^{-1}.

5.1.2 Aquatic bacteria

High levels of total water bacteria (TWB) were present in the ICCS during the first growth experiments on noble crayfish (*A. astacus*) (V) (Table 4). There were no differences in the TWB or *Aeromonas* sp. levels between experiments 1 and 2, but *Pseudomonas* sp. levels were significantly lower in experiment 1 compared to experiment 2. The TWB levels were observed to rise once the temperature exceeded 20°C.

Table 4. Bacterial levels in culture tank water during the experiments. Means in the same column with different superscripts are significantly different.

	TWB cfu ml^{-1}	*Aeromonas* sp. cfu ml^{-1}	*Pseudomonas* sp. cfu ml^{-1}
Experiment 1	78,900±13,800^a	14±4^a	11±4^b
Experiment 2	86,300±30,900^a	12±4^a	29±7^a

5.1.3 Pellets and supplemental feeding

The commercial marron pellets disintegrated within minutes after water immersion (unstable pellets), while the recently developed commercial marron pellets have been shown to remain their texture up to 22 h (IV). The proximate content of these commercial diets is 30% protein, 20% carbohydrate, 9% fat, 3% fiber.

Marron (*C. tenuimanus*) fed with the stable pelleted diets grew significantly faster than marron fed with similar unstable diets (IV). There were no significant differences in the hepatopancreatic indices (IV), indicating similar condition among the groups. Upon addition to the compartments the unstable pellets were observed to disintegrate, leaving a mound of fine particle matter (IV, Jussila 1996b). The stable pellets, on the other hand, remained intact after immersion. Marron were observed to grasp the intact pellets and direct them into mandibles. This behaviour was not observed in marron fed with unstable pellets, the fine particle matter being largely ignored by the marron.

The commercial marron pellets, when used as the only source of nutrition, resulted in substantial change in marron exoskeleton pigments (VI, Jussila 1996b). The marron developed syndromes described as a 'blue shell disease' (D'Abramo and Robinson 1989).

Marron were found to be capable of learning to collect food out of the water when their food was placed in the cups above the water level (Jussila *et al.* 1996; Leonie Higgins, South West Aquaculture & Environment Centre, Collie, Western Australia, personal communication). The growth rates of the marron fed with cups above the water level were comparable to those fed by placing food into the compartments (SGR 0.17-0.20 and 0.19-0.20, respectively). Marron fed more readily from the shallow (depth 1 cm) compared to deep cups (depth 3 cm) and the mean SGR was higher in the groups feeding from the shallow cups. Cup feeding resulted in cleaner compartments than feeding through pellet immersion into water.

It was shown that frequent feeding, preferably daily, enhanced marron growth (III). Also, the commercial marron pellets, which disintegrated quickly, could not be administered at a rate of more than 1% wet body weight a day, since even this low level of feeding resulted in problems with uneaten food growing fungi, possibly *Saprolegnia*, and poor water quality.

Noble crayfish (*A. astacus*) were fed on a diet combining vegetable matter (peas, potatos), fish (roach, *Rutilus rutilus*) and commercial fish pellets. The growth of noble crayfish was poorer when the fish in the diet was substituted with the commercial fish pellets (VI). Signal crayfish (*P. leniusculus*) were reared on commercial marron pellets ('Glen Forrest Stock Feeders Pty Ltd', Perth, Western Australia) and even though the growth was slower, survival was high and exoskeleton pigmentation was normal (VI).

5.1.4 Natural food items production

Natural food items production in the ICCS was considered as either sessile organism growing on culture system surfaces or floating planktonic organisms. There was no attempt to identify these organisms. The amount of natural food items production was measured as accumulation of dry matter on surface of slides and BOD_{24h} (Rump and Krist 1992).

The production of food items in the ICCS was variable. One ICCS, used in the nutrition and compartment size studies on marron (*C. tenuimanus*) (III, IV), produced a very low level or no detectable level of food items (clean system; III, IV), while the other system produced a substantial amount of sessile organisms (dirty system; III). The production of food items in the dirty ICCS was comparable to the food items production in the communal Aquaplate® tanks, that were used in the nutritional studies (IV). The ICCS used for noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) studies did not produce detectable levels of food items.

The level of food items production had a strong positive correlation with SGR and a similar strength, but negative correlation with the intermolt period in marron (III).

5.2 Growth, molting and production

Growth, expressed as SGR, ranged from 0.4 to 0.8 with marron (*C. tenuimanus*) reared in the ICCS (I, III, VI; Table 4). The growth of marron was significantly faster compared to noble crayfish (*A. astacus*), in which SGR ranged from 0.1 to 0.2 or signal crayfish (*P. leniusculus*), in which SGR was 0.1 (VI). The Scandinavian species, noble crayfish and signal crayfish, grew at similar slow rates in these experiments (VI).

There were no differences in growth rates between the sexes of immature marron, nor were there any differences in growth rates between the sexes during the first months after sexual maturation. Studies on mature marron were not conducted. There was a weak negative correlation ($r = -0.05^*$) between SGR and the distance between compartment and water inlet (III).

The production of marron in the ICCS ranged from 160 to 360 g m^{-2} during the experiments, being higher than in semi-intensive tanks or in farm ponds (Table 5). The production of noble crayfish or signal crayfish was low, because of the high mortality and nil or even negative growth during the experiments.

Table 5. Growth and production of marron (*Cherax tenuimanus*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) in the ICCS in the present (I, III, IV and VI) and in other studies.

	SGR	Production g m^{-2}	Density # m^{-2}	Reference or subpublication
ICCS				
Marron	0.7-0.8	160-240	24	I
Marron	0.4-0.8	-	10-24	III
Marron	0.5-0.7	220-360	24	IV
Marron	0.4-0.7	20-110	24	Jussila (1996a)
Marron	0.4-0.5	380-760	15-60	Morrissy *et al.* (1995a)[1)]
Noble crayfish	0.1-0.2	-	14	VI
Signal crayfish	0.1	-	12	VI
Signal crayfish	0.4-0.5	-	-	Ackefors *et al.* (1992)[1)]
Semi-intensive tanks				
Marron	0.7-0.8	20-30	5	Jussila (1996a)
Marron	0.6-0.7	60-80	5	IV
Semi-intensive ponds				
Marron	0.8-1.1	10-300	1-3	Morrissy (1979, 1980, 1990)[1)]
Marron	0.5-0.7	110-300	5-10	Morrissy (1992a)[1)]
Noble crayfish	0.5-0.7	-	64-350	Gydemo and Westin (1989)[1)]
Noble crayfish	0.2-0.3	-	50-100	Taugbøl and Skurdal (1992)[1)]
Signal crayfish	1.5	-	-	Westman and Nylund (1984)[1)]
Signal crayfish	0.5-0.9	-	50	Celada *et al.* (1993)[1)]

1=SGR estimated from the published data

Table 6. Survival rates (%) in the growth experiments in the ICCS.

Experiment	Treatment group	Survival (%)
Marron (I)	Large sump tank group	83
	Small sump tank group	71
Marron (IV)	Stable marron diet groups	100
	Unstable marron diet groups	90
	Fish diets groups	89-90
	Unfed group	89
Noble crayfish (VI)		66-70
Signal crayfish (VI)		84

Marron did not show significantly different growth rates (SGRs from 0.17 to 0.22) when food was given into the compartments three times a week compared to feeding from the cups above the water level at their choice.

The survival of marron in the clean ICCS was higher than in the dirty system (I, IV, Table 6). In the experiments on noble crayfish or signal crayfish the survival was slightly lower than in marron in dirty system, with signal crayfish having higher survival rates than noble crayfish.

Table 7. Weight increment at molt ($W_{m1..2}$), and intermolt period (T_{im}) in marron (*Cherax tenuimanus*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) growth studies (I, III, IV, VI). The figures given here are the ranges of the treatment group means observed in the experiments.

	W_{m1} (%)	W_{m2} (%)	T_{im} (d)
Marron			
Sump tank experiments (I, III)			
Small sump tank	23.4 - 31.6	21.9 - 30.6	41 - 42
Large sump tank	29.2 - 32.1	23.2 - 41.6	32 - 34
Compartment size experiment (III)			
Large sump tank	27.3 - 33.7	36.3 - 41.7	52 - 62
Pellet stability experiment (IV)			
Marron pellets	29.7 - 39.5	37.1 - 45.2	43 - 57
Fish pellets	36.1 - 41.6	34.3 - 38.1	44 - 58
Noble crayfish			
Intensive culture study (VI)	25.7 - 32.6	-	-
Signal crayfish			
Intensive culture study (VI)	38.5	21.8	101

The differences in growth rates between the treatments and experiments in marron were primarily caused by differences in the intermolt period rather than in weight gain at molt (III, Table 7). Signal crayfish had higher weight gain at first molt compared to noble crayfish (VI; Table 7), both being low or comparable to marrons' weight gain at first molt. Signal crayfish had significantly lower weight gain at second molt compared to the first molt (VI). The intermolt period in signal crayfish, and also in noble crayfish, was significantly longer than in marron. Noble crayfish molted only once during the 22 or 26 wks experiments (VI) and the mean intermolt period could not be measured.

Marron held in the ICCS with larger total water volume grew faster than marron held in the ICCS with smaller water volume (I). The difference in the water volume was 5-fold. This finding was verified in the later studies (III). In addition to the volume-density effect, marron growth was faster when the individual compartment size was larger (III), with a significant decrease in SGR occuring when the density factor (k) was less than 45.

Marron grew faster when fed with water stable pelleted diets both in the ICCS (density: 25 crayfish m^{-2}) and communal semi-intensive (density 5 crayfish m^{-2}) tank rearing (VI). Marron growth was increased with increasing feeding frequency (III), and the most efficient feeding rate was feeding at least once a day. The results of these studies (I, III, IV) indicate, that marron growth can be faster in the ICCS that produces a substantial amount of food items, i.e. dirty ICCS.

Intermolt period was the more sensitive growth component when the different treatments had an effect on marron growth (III, IV), while weight gain at molt was affected mainly by differences in nutrition (IV) and less by environmental factors (III, Table 8).

Table 8. Correlations between growth indexes, weight increment at molt (W_m), intermolt period (T_{im}) and specific growth rate (SGR), and parameters in the ICCS environment based on four experiments on marron (*Cherax tenuimanus*) growth (III). Only the parameters with at least one significant correlation are included. Correlation significance presented as * = P<0.01 and ** = P<0.001 (Reprinted with permission from IAA).

	W_m	T_{im}	SGR
Sessile production (mg)	ns	-0.9**	+0.9**
DO (mg O_2 L^{-1})	ns	-0.8**	+0.8*
Diurnal DO variation	-0.8*	ns	ns
Compartment size (cm^2)(1	ns	ns	+0.9*
Total ammonia (mg L^{-1})	ns	+0.9**	ns
T (°C)	ns	ns	+0.7**
ΔT (°C)	ns	+0.9*	-0.9**

1 = correlation estimated for four treatment groups

In marron, the SGR correlated strongly with sessile organisms production, compartment size and diurnal temperature variation and had a moderate positive correlation with dissolved oxygen and temperature (III, Table 8). Intermolt period correlated strongly with sessile organisms production, total ammonia and diurnal temperature variation and had a moderate negative correlation with dissolved oxygen. Diurnal dissolved oxygen variation had a moderate negative correlation with weight gain at molt.

5.3 Sexual maturation in marron

Marron (*C. tenuimanus*) (N=90) matured in a clean ICCS at the age of 1+ y.o. when they reached a size of approximately 16 g in males, 23 g in females (Table 9) and 16 g in the sample with sexes pooled (Fig. 3). A minority of the mature females, 20%, had reabsorbed their eggs by the end of November. In another study, where marron (N=30) were fed on both commercial pellets and food items produced by the system (dirty ICCS), the smallest size of mature males was 18.3 g and females was 26.6 g, both sexes being at the age of 1+ y.o.

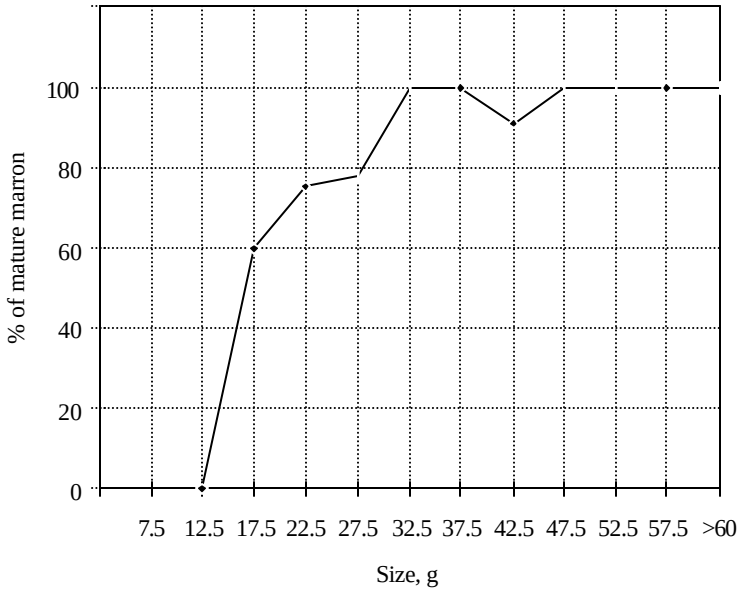

Figure 3. Proportion of sexually mature, intensively reared marron (*Cherax tenuimanus*), sexes pooled, in different size classes.

Maturation in the ICCS followed the natural oogenesis cycle, where glair glands appear in May and ovaries and testis are fully developed by August.

There were no attemps to study marron mating in the tanks. Previous experience showed that mating is partially inhibited under tank rearing conditions even though the ovaries and testis are normally developed (J Jussila, unpublished data), while marron have been reproducing under communal tanks conditions in commercial farms and in other experiments (Louis H. Evans, Aquatic Science Research Unit, Curtin University, personal communication).

Table 9. The proportion (%) of sexually mature ICCS reared marron in different size classes (N=90)

	10-15 g	15-20 g	20-25 g	25-30 g	30-35 g	35-40 g	40-45 g	over 45 g
Male	0	60	100	67	100	90	100	100
(N=40)	(N=1)	(N=5)	(N=6)	(N=3)	(N=2)	(N=10)	(N=7)	(N=6)
Female	0	-	0	83	100	100	100	100
(N=50)	(N=2)		(N=2)	(N=6)	(N=9)	(N=10)	(N=11)	(N=10)

5.4 Hepatopancreatic indices

Marron (*C. tenuimanus*) reared in the ICCS had wet hepatosomatic indices comparable or higher to wild marron populations but lower or comparable to those of semi-intensively or tank reared marron (II, IV; Table 10). Dry hepatosomatic indices were slightly higher in the ICCS reared marron compared to wild marron and lower compared to semi-intensively or tank farmed marron. Hepatopancreas moisture content in the ICCS reared marron was higher than in the semi-intensively reared marron and lower than in the wildstock.

Noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) had smaller wet and dry hepatosomatic indices than wild co-species (VI; Table 10). The hepatopancreas moisture content was higher in noble crayfish and similar in signal crayfish compared to wild populations of these species.

Marron reared in the smaller total water volume had smaller, less moist, hepatopancreases than marron from the larger water volume (II, Table 10). The hepatopancreatic indices in marron reared

in small sump tank system were similar to those of wild marron (II). Marron reared on six different commercial crayfish or fish diets in the ICCS (IV) did not show significant differences in hepatosomatic indices, even though the trends were evident. Also, the groups reared in communal tanks in lower densities showed significantly larger wet and dry hepatosomatic indices and lower hepatopancreas moisture content (IV) than groups reared in the ICCS on a similar pelleted diet. The group fed with high lipid and protein diet (D3, IV) in the ICCS showed similar hepatopancreatic indices as communally reared groups but grew significantly slower (IV).

Table 10. Hepatopancreatic indices in farmed and wild marron (*Cherax tenuimanus*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) populations (I, II, IV, VI). Figures given here are ranges of the treatment group means from the original experiments.

	Hi_{wet}, %	HI_{dry}, %	HM, %
Marron (II)			
ICCS, large sump group	5.8	2.2	67.0
ICCS, small sump group	4.9	1.7	62.3
Semi-intensive farms	5.6 - 6.0	2.2 - 3.3	41.9 - 62.3
Wildstock	4.5 - 5.4	1.3 - 1.5	71.6 - 71.9
Marron (IV)			
ICCS, marron pellets	5.1 - 5.9	1.6 - 1.7	66.6 - 73.0
ICCS, fish pellets	4.5 - 6.0	1.6 - 2.1	63.2 - 66.0
Communal tank, marron pellets	5.8 - 6.0	2.3 - 2.5	58.0 - 60.4
Noble crayfish (VI)			
ICCS	4.6 - 4.8	1.1 - 1.2	76.0 - 77.1
Wild population	6.7	1.7	75.1
Signal crayfish (VI)			
ICCS	4.0	1.0	75.7
Wild population	6.6	2.4	63.1

Noble crayfish reared in the ICCS did not show any difference between the experiments 1 and 2 (VI) in hepatosomatic indices, and they were similar to those of the ICCS reared signal crayfish. The hepatosomatic indices of both species were lower in the ICCS reared compared to their wild co-

species (Table 10), while hepatopancreas moisture contents were similar in the ICCS reared and wild noble crayfish and higher in the ICCS reared compared to wild signal crayfish.

In marron, there was a significant correlation between hepatosomatic indices and SGR in the pellet stability experiment (IV) (Table 11, Fig 4), while in the water volume experiment (I) SGR did not correlate with the hepatopancreatic indices. The previous results were obtained combining several treatment groups fed with different diets (IV), while the latter analyses was carried out within treatment groups (I).

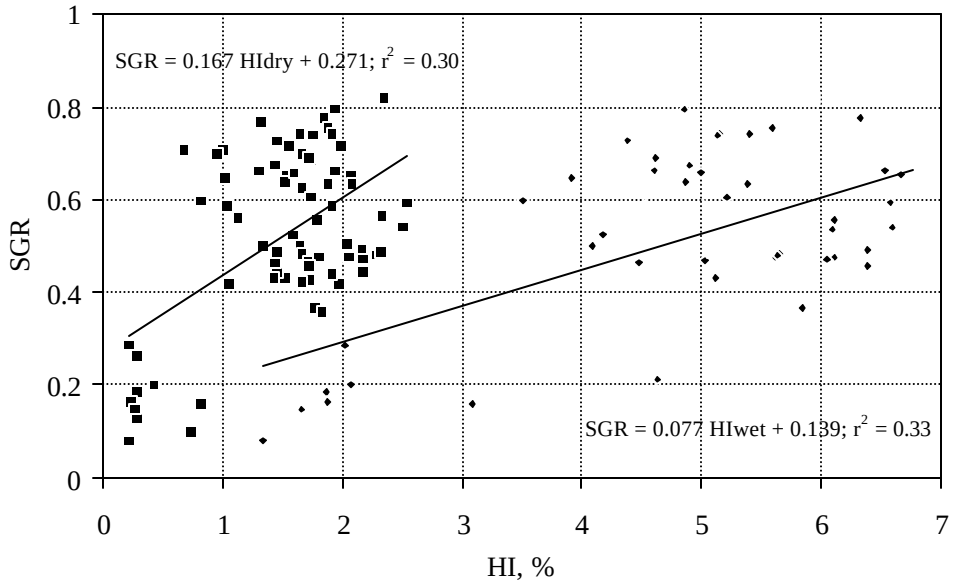

Figure 4. The relationship between growth, expressed as SGR, and the hepatosomatic indices in intensively reared marron (*Cherax tenuimanus*) fed with several different diets (IV) (■=HI_{dry} and ◆= HI_{wet}).

The regression between SGR and hepatopancreatic indices in signal crayfish was significant (Fig. 5) with coefficient of determination (r^2) close to 0.50, while in noble crayfish only hepatopancreas moisture content had a weak correlation with SGR (Table 11). The trends in signal crayfish data were reverse to trends in marron data and were obtained within one treatment group.

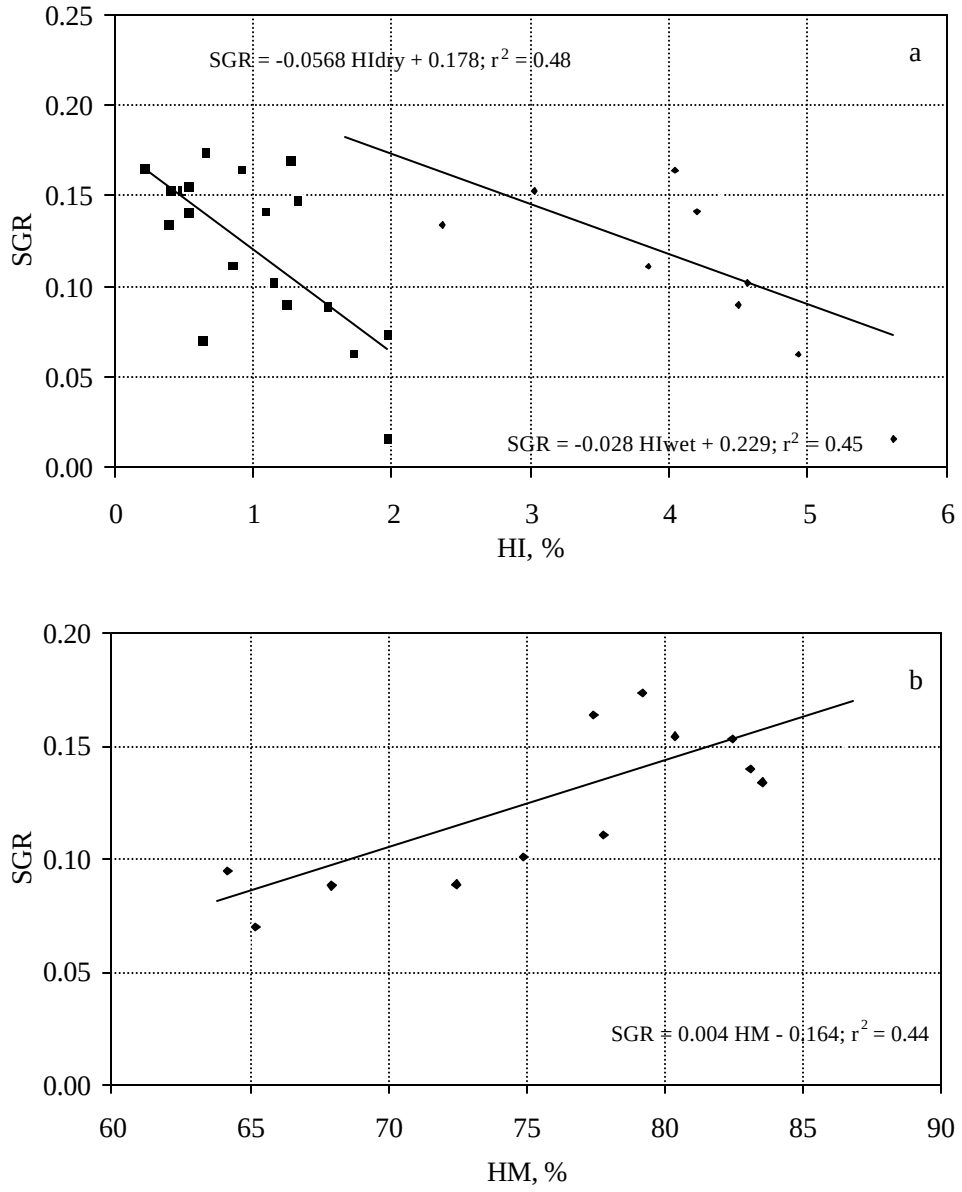

Figure 5. Relationship between growth, expressed as SGR and (a) hepatosomatic indices or (b) hepatopancreas moisture content in intensively reared signal crayfish (*Pacifastacus leniusculus*) (VI) (Symbols in Fig. 5a are ■=HI_{dry} and ◆= HI_{wet}).

Table 11. Correlation coefficients (r) between SGR and hepatopancreatic indices in intensively reared marron (*Cherax tenuimanus*), noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) (I, IV, VI).

	HI_{wet}	HI_{dry}	HM
SGR			
Marron (IV)	0.6***	0.5***	NS
Marron (I)	NS	NS	NS
Noble crayfish (VI)	NS	NS	-0.4***
Signal crayfish (VI)	-0.7***	-0.7***	0.7***

5.5 Carapace mineralisation and pigmentation

The ICCS reared marron (*C. tenuimanus*) had intermediate carapace calcium concentrations compared to semi-intensively reared marron (II; Table 12). Farmed marron, either in the intensive or semi-intensive rearing, had lower carapace calcium concentrations than wild marron. Noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) had carapace calcium concentrations comparable to wild marron (Table 12). The ICCS reared marron (II) and noble crayfish (VI) had comparable carapace magnesium concentrations, and they were significantly lower than in semi-intensively reared or wild marron. The carapace ash concentrations were higher in noble crayfish and signal crayfish than in marron. There were no evident trends between wild, semi-intensively or intensive farmed marron.

Signal crayfish had significantly higher carapace density, a thicker carapace, than either noble crayfish or marron (Table 11). Marron reared in the ICCS had the thinnest carapace and the semi-intensively farmed 1+ y.o. ones had the thickest carapace in these studies.

Fast growing young marron (0+ y.o.) had significantly thinner carapaces than 1+ y.o. marron from the same farm (II). Otherwise carapace mineral concentrations were similar in both year classes.

Marron showed changes in carapace pigmentation, when reared in the clean ICCS on commercial marron pellets or pelleted fish diets (IV, Jussila 1996b). The changes in carapace pigmentation were more distinct in the groups reared on commercial marron diets compared to fish diets. Also, marron reared in the ICCS that produced food items (I) or were reared in the

communal semi-intensive tanks with similar type of food items production, did not show major changes in carapace pigmentation.

Table 12. Calcium and magnesium levels in carapace, carapace ash levels and carapace density in farmed and wild crayfish populations (II, VI). Figures given here are the ranges of the means obtained in the original experiments.

	Calcium ($mg\ kg^{-1}$)	Magnesium ($mg\ kg^{-1}$)	Carapace ash (%)	Carapace density ($mg\ cm^{-2}$)
Marron (II)				
ICCS	164,090	970	30.3	24.4
Semi-intensive farms				
1+ y.o. or over	141,960 - 182,090	1420 - 1880	22.7 - 28.3	38.2 - 47.7
0+ y.o.	167,050	1420	28.3	29.5
Wildstock	182,320 - 213,140	1550 - 1710	31.1 - 35.3	26.8 - 35.9
Noble crayfish (VI)				
ICCS	183,170 - 200,205	605 - 763	36.6 - 38.8	23.3 - 36.1
Signal crayfish (VI)				
ICCS	211,470	-	51.4	61.1

5.6 Total hemocyte counts (THCs)

The total hemocyte counts (THCs) were similar in all four groups fed with commercial marron diets in the ICCS with low production of natural food items (Table 13). The THCs in the ICCS reared, fed groups was slightly lower than in marron (*C. tenuimanus*) populations from two commercial semi-intensive farms and significantly lower than in marron reared communally in tanks. Rearing conditions were different in the ICCS and communal tanks, with test animals being in were lower densities in the tanks (5 crayfish m^{-2} compared to 25 crayfish m^{-2} in the ICCS) and having a more varied diet due to higher levels of algae and zooplankton growing in the tanks compared to the ICCS (IV).

Starved marron, either for 3 d or 4 mo, showed significantly lower THCs than fed groups (Table 13). The starved group THCs increased to a similar level to other ICCS groups within three days

after feeding was recommenced. Furthermore, a group, fed with unstable pellets and algae, reared in the ICCS producing substantial amount of food items (dirty system, III) showed low THCs, comparable to starved group's THCs.

Table 13. Total hemocyte counts (THCs) in marron (*Cherax tenuimanus*) under various culture conditions. The means with different superscripts are significantly different. The figures given here are mean±SE.

	Total hemocyte counts (10^6 mL^{-1})	Food type	Density (# m^{-2})	Growth, SGR
ICCS				
	5.3±0.6^{c,d}	Unstable pellets	25	0.54±0.04^{d}
	4.3±0.5^{d,e}	Stable pellets	25	0.57±0.04^{d}
	4.3±0.4^{d,e}	Unstable pellets	25	0.58±0.03^{d}
	5.0±0.5^{c,d}	Stable pellets	25	0.68±0.03^{c}
	2.9±0.4^{e}	Unstable pellets, algae	14	-
	2.6±0.4^{e}	3 d starved	25	0.58±0.03^{d}
	2.3±0.4^{e}	4 mo starved	25	0.20±0.02^{e}
	4.1±0.3^{d,e}	3 d starved, 3 d fed	25	0.58±0.03^{d}
Communal tanks				
	9.7±1.2^{a,b}	Stable pellets, algae, Daphnia	5	0.68±0.01^{c}
	10.7±1.0^{a}	Unstable pellets, algae, Daphnia	5	0.59±0.02^{d}
Farm Ponds				
Farm 1	6.5±0.5^{c}	Poultry pellets, barley, pond food items	-	0.94±0.03^{a}
Farm 2	8.5±0.9^{b,c}	Salmon pellets, pond food items	3	0.78±0.02^{b}

The marron THCs showed moderate correlation with dry hepatosomatic indices and hepatopancreas moisture content (Table 14), and a weak correlation with SGR in the groups described in Table 13.

Table 14. Regression and correlation between total hemocyte counts (THC) and SGR, hepatosomatic indices (HI_{dry}), and hepatopancreas moisture (HM) content in marron (*Cherax tenuimanus*) reared in the ICCS or communal tanks.

	Regression	Correlation
SGR	$SGR = 2.12 \times 10^{-8} \times THC + 0.450$; $r^2 = 0.38$	r = 0.5***
HI_{dry}, %	$HI_{dry} = 1.469 \times 10^{-7} \times THC + 0.839$; $r^2 = 0.44$	r = 0.7***
HM, %	$HM = -1.755 \times 10^{-6} \times THC + 79.067$; $r^2 = 0.48$	r = -0.7***

5.7 Hemolymph bacteria

Noble crayfish (*A. astacus*) had a variable number of bacteria in their hemolymph during the experiments 1 and 2 (VI) in the ICCS (Table 15). Water baths, either with formalin and methylene blue (experiment 1) or potassium permanganate (experiment 2), did not have a significant effect on hemolymph bacteria levels. The hemolymph bacteria cfu and especially infection prevalence showed a significant increasing trend during the studies, with the proportion of infected crayfish at least doubling in every treatment group.

Marron (*C. tenuimanus*) reared in the ICCS in Curtin University showed bacterial infections in their hemolymph in 60% of the animals studied (N=20) in June 1996, when water temperature in the system was 12-15°C. The bacteria species were not identified.

Table 15. Crayfish hemolymph total bacteria (HTB, cfu in 100 µl blood sample after 2 d incubation in 20°C) and infection prevalence in intensively reared noble crayfish (*Astacus astacus*).

	Experiment 1				Experiment 2			
	Treatment		Control		Treatment		Control	
	cfu	%	cfu	%	cfu	%	cfu	%
Initial	5±3	28	7±4	38	84±36	56	42±26	40
8 wks	34±13	60	51±16	32	-	-	-	-
16 wks	22±14	83	4±0.5	86	39±9(1	94	49±5(2	97

1 = mass growth (over 500 cfu) in 32% of the samples, not included in the mean
2 = mass growth (over 500 cfu) in 20% of the samples, not included in the mean

A total of 11 different gram-negative bacteria were identified in the hemolymph of noble crayfish during the growth studies (V). The taxa Pseudomononadaceae (6 species), Neissericeae, Vibrionaceae (*Aeromonas hydrophila* or *caviae*) and Pasteurellaceae were identified.

The hemolymph of two dying noble crayfish showed a massive growth of *Pseudomonas* sp. when cultured on a bovine agar plate (V).

6. DISCUSSION

In this section I discuss the results of the studies and compare them to published information on co-species or other freshwater crayfish species, focusing on the physiological responses in relation to intensive culture. The ICCS as an culture environment is discussed and then the crayfish production is assessed. The latter part of the discussions focuses on the macro level physiological responses in crayfish as they were caused by the intensive rearing.

6.1 The ICCS as a culture environment

6.1.1 Nutrition

6.1.1.1 Pellets and supplemental feeding

In general, artificial diets, as the only sources of nutrition, were suboptimal resulting in decreased growth rates, and changes in exoskeleton pigmentation. On the other hand, improvements in pellet water stability improved crayfish growth and condition.

Marron (*C. tenuimanus*) fed with water stable pelleted diets grew faster and their production was higher than in marron fed with less stable diets both in the ICCS and communal tank rearing (IV). The differences resulted from both shorter intermolt periods and larger weight gain at molts. Furthermore, groups fed with commercial fish pellets grew at similar rate compared to groups fed with commercial unstable marron pellets. These findings indicate that the commercial diets, when provided in an unstable formulation, were suboptimal to marron reared in tanks.

The leaching, as measured in the present studies, included also to some extent the fine particle matter that was lost due to physical breakdown of the pellets. It can be argued that the actual differencies in pellet stability (IV) were less than reported, and part of the loss in the nutritional value of the pellets was inherent in the pellet manufacturing method.

The type of food affected crayfishes exoskeleton pigmentation. Marron reared in the ICCS lost the natural dark brown exoskeleton pigment (IV), with more severe changes in pigmentation in the groups fed with commercial marron diets. This could indicate severe nutritional imbalance in the

diets, namely lack of carotenoids, which may also have adversely affected marron growth rates (D'Abramo and Robinson 1989).

Nutritional variety, natural production in the system accompaning pelleted diets, may have improved marron condition (IV), and caused differences between tank and ICCS reared groups. However, the effects of pellet contents and texture on the marrons' hepatopancreatic indices were complicated and could mostly be seen as trends, rather than significant differences between the groups. Among the ICCS reared marron, only the starved group's hepatosomatic indices indicated a significantly different, poorer, condition compared to other groups (IV). Therefore, the improved pellet quality, while causing faster growth, had only minor affect on marron condition.

The difference in growth between the groups of noble crayfish (*A. astacus*) fed with different diets (VI) could have resulted from the partial substitution of plant material in the feeds with salmon pellets. The differences, although significant, were irrelevant since the growth was slow in both treatment groups.

The feeding rates were low in all of the marron studies (I, III, IV) compared to some other studies (Mills *et al.* 1994, O'Brien 1994), while several others report similar feeding or consumption rates to those in the present study (McClain 1995a, Seals *et al.* 1997). The food conversion ratios (FCR) were also low, on the average 1.7 (I). Furthermore, low FCRs have been achieved previously on low feeding rates (Kowarsky *et al.* 1985, Lowery 1988), both suggesting a more effective usage of resources. Low feeding rates were used, because pelleted marron feeds dissolved when inserted in water (I, III, IV) limiting pellet acceptance and causing growth of fungal organisms on pellet remains.

The present system of manual feeding, normally once a day, is nutritionally ineffective and ignores the individual differences in crayfishes feeding habits. It was shown that marron readily grazed from the feeding cups above the water level. The cup feeding also resulted in improvements in ICCS hygiene, providing benefits in the system maintenance. There were evidence showing that the differences in pellet processing methods, leading to differences in pellet texture, resulted in different acceptance when administered by feeding cups. This experiment was carried out in southern winter, resulting in slow growth, and the findings should be confirmed under conditions where marron are able to exhibit higher growth rates.

The variations in the feeds studied in these experiments, combinations of pellets, system forage production and supplemental fish and vegetables, did not offer a balanced diet for any of the three

crayfish species (I, III, IV, VI). Commercial marron pellets gave the best results both in growth and survival providing highest production rates (I, III, IV, VI) but caused changes in carapace pigmentation (IV).

6.1.1.2 Food items production

The natural food items production in the ICCS improved crayfish growth and condition. It also enhanced exoskeleton pigmentation.

The higher level of the food items production in the ICCS resulted in faster growth and higher production of marron (*C. tenuimanus*) than in the clean ICCS with similar size compartments (300 cm^2) (III). Furthermore, marron condition was slightly better among groups reared in a dirty system. However, the improvements in growth or condition due to food items production caused problems in survival, and the survival in dirty system in marron was 71-83% (I) and in clean system close to 100% (IV). This was largely because production and decomposion was not controlled in the ICCS. This might have caused problems with dissolved oxygen depletion (Mills *et al.* 1994) at night or when the water temperature rose, but was not measured.

Production of plant forage in the ICCS provided crucial minerals for marron to complete carapace mineralisation and also to obtain nutrients and, finally, to grow faster. It also ensured that marron got additional nutrition over the pellet feeding regime. Morrissy (1984) also concluded that detrital supplementation in the intensive system enhances exoskeleton pigmentation, a finding verified in these studies (IV).

The THCs were similar in the fed marron reared in the dirty system as were in the starved marron in the clean system. The reasons for the decline of the THCs in the fed group in the dirty system remain unclear, but could be related to the low DO or other stressors caused by organic load.

6.1.2 Water quality

Water quality remained optimum for crayfish throughout the experiments, except for short periods when water temperature exceeded the optimum range.

The water temperature remained at the optimum level for crayfish (Holdich and Lowery 1988, Mills *et al.* 1994) in all the growth experiments (I, III, IV, VI) with minor exceptions. The temperatures in the ICCS installed in the Curtin University of Technology field trial area exceeded the high optimum temperature for marron (*C. tenuimanus*) (26°C, Morrissy 1990, 1992b) in a few cases during the summer months (I, III, IV). The mean temperatures in the experiments on Scandinavian species could be regarded as less than optimum for growth (Huner and Lindqvist 1984, Huner *et al.* 1993). Noble crayfish (*A. astacus*) in the present studies experienced increased mortality, and thus stress, when reared at temperatures from 20 to 23°C.

Diurnal temperature variation (III) affected growth of marron and could have caused stress. Present ICCS design with stainless steel tanks maintained at ambient air temperatures with low water volume and long retention time made the ICCS a very efficient heat exchanger. Crayfish, being poikiloterm, are vulnerable to temperature changes and may be experiencing a constant stress in the ICCS due to temperature fluctuations. Diurnal temperature variation in farm ponds is approximately 3°C during summer indicating more favorable conditions (J Jussila, unpublished data). Experiments carried out later showed that simple solutions, i.e. sump tanks were buried in the ground, could minimize temperature variation resulting in diurnal variation being less than 2°C.

Total ammonia, un-ionised ammonia and nitrite levels measured in the ICCS were below the levels that have been found to inhibit growth in cultured fish or crayfish (Ackefors *et al.* 1994). This could indicate that the ICCSs acted as efficient biofilters themselves or that the crayfish densities, up to 25 crayfish m^{-2}, were too low for accumulation of nitrogen compounds. In addition, low nitrogenous compound concentrations were measured in intensive systems where red claw (*Cherax quadricarinatus*) was raised (Du Boulay 1995). These findings could indicate that in the small, experimental systems the nitrogen dynamics are different from the large scale, commercial systems since the removal of metabolites was achieved even without purpose built biofilters.

One of the growth inhibitors in the ICCS, the inborn factors (Morrissy 1984), could be ammonia or hydrogen sulphide (H_2S). The present studies have show no indication of downstream growth inhibition (III) suggesting that the role of metabolites or pheromones in the system is minor. On the

other hand, marron were able to detect density in relation to total water volume in the ICCS (I), which was probably caused by the difference in chemical dilution between the systems.

Dissolved oxygen levels changed immediately with changes in the water temperature and decreased levels were detected especially during extended high temperatures periods. DO saturation levels decreased as low as 60% frequently which could have caused growth inhibition, as has been reported in juvenile spiny lobsters (*Panulirus cygnus*) (Chittleborough 1975), especially if the water temperatures were elevated, or the oxygen consumption was high. Some freshwater crayfish species (*A. astacus*, *A. leptodactylus*, *O. limosus*), on the other hand, have been shown to be able extract oxygen from very low levels (Ackefors and Lindqvist 1994). Short periods of low DO and high temperature can cause delays in molting (Jussila 1995b) and thus decreased growth rates. Prolonged suboptimal conditions increased mortality causing substantial losses especially if heat peaks were repeated.

Differences in pH were minor between the studied systems and the effects of different pH on growth parameters in the ICCS most probably indicated influences of other parameters. Low pH conditions have been reported to inhibit carapace mineralisation and growth in freshwater crayfish (Aiken and Waddy 1992), but the levels obtained in the present studies were well above these inhibitory levels. Other water quality parameters, calcium, total hardness, and conductivity, also indicated optimum conditions.

A potential harmfull metabolite, hydrogen sulphide (H_2S), was not analysed in the culture system water. It has been suggested that this compound might have inhibited marron growth in some of the previous studies carried out in the intensive systems (Noel Morrissy, Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, personal communication). The ICCS, as used in most of the present studies (III, IV, V, VI), did not contain substrate in the culture tanks, thus preventing unaerobic conditions necessary for hydrogen sulphide production

6.1.3 Water volume

Marron (*C. tenuimanus*) growth and production were improved by increase in total ICCS water volume.

An increase in total water volume in the system resulted in improvement in both growth and survival of marron (I). Biomass production, being a function of growth and survival, increased 1.5 fold in the large sump tank system compared to the small sump tank system. These findings suggest that the inhibitory effect of high stocking density on growth in the ICCS may be dependent on water volume in addition to the surface area of culture system (I). As the density effect seemed to be volume related, it is in an agreement with the previous findings indicating chemical communication in crayfish (Dunham 1978, Tierney *et al*. 1984, Hazlett 1985, 1989, 1990, 1994, Bechler 1995), and, consequently, that these pheromones or metabolites have to be eliminated from culture system water (Lee and Wickins 1992, Ackefors *et al*. 1994). On the other hand, the larger water volume may have allowed for improved natural food production or dilution of inhibitory metabolites (III).

In fish, several biological and chemical processes are successfully used in intensive, recirculating systems when eliminating metabolites from the water to improve production (Otte and Rosenthal 1979, Sutterlin *et al.* 1984). Furthermore, Carro-Anzalotta and McGinty (1986) have found a volume and surface area related density effect in tilapia (*Tilapia nilotica*), which suggests similar type of combined density inhibition as described in this thesis.

The larger water volume resulted in marron being in better condition than marron reared in the smaller water volume (II). The differences between the two groups were significant with the former showing similar condition as semi-intensively farmed marron, while the latter groups condition resembled that of the wild marron (II). This, together with the difference in the growth rate, suggests that the ICCS rearing conditions could be significantly improved by the culture systems having sumps with large total water volume.

6.1.4 Bacteria in culture environment

Several different bacteria were present in the culture system water but the numbers were below harmful levels.

The number of *Aeromonas* sp. and especially total water bacteria (TWB) in the experimental systems were lower than in natural lakes in Finland during summer (Leena K. Korhonen, National Public Health Institute, Kuopio, Finland, personal communication). The ICCS was, thus, relatively

free of bacteria and levels detected should not have caused an increase in mortality or even stress. However, mortality was high, which indicated increasing stress in crayfish.

Tap water, which was used in these experiments (V), is normally free of human pathogenic bacteria and the number of total bacteria is low (Miettinen *et al.* 1993). So, most of the bacteria in the culture system originate from the bacterial flora of the crayfish, feed, or was introduced by the people who worked with the system. *Aeromonas* sp. and some other bacteria are present in Finnish lakes, so that crayfish from wildstocks could be possible vectors for bacterial contamination.

An increase in the water temperature was the factor stimulating growth in TWB. In other studies, Scott and Thune (1986) have shown that increase in hemolymph total bacteria (HTB) in cultured crayfish was also related to increases in temperature or decreases in dissolved oxygen. Thus, an increase in TWB and an increase in crayfish stress, act synergistically to enhance bacterial disease problems. Growth rates also increase at high temperatures and even though faster growth rates are beneficial, the increasing mortality (Lowery 1988) negates the benefits from growth.

6.1.5 Physical environment

Marron (*C. tenuimanus*) growth was limited due to physical characteristics of the ICCS. The most effective growth inhibitor was crayfish density.

Limited space or surface area, due to holding of the crayfish in individual compartments, compromised growth of the larger marron (III, Jussila 1996b). When marron were taken from communal ponds and placed into individual rearing compartments in the ICCS, the natural hierarchical population structures were broken down with following consequences:

1) Growth rates of small marron were improved even above their growth rates in extensive or semi-intensive communal systems.
2) The limited compartment area inhibited growth when space factor (k) was less than 45 or any compartment dimension was shorter than marron's total length.
3) The mean growth rates decreased.
4) Survival decreased with decreasing compartment size.
5) Production (g cm^{-2}) increased with decreasing compartment size.

In the present studies growth of marron was inhibited when the density factor (k) declined below 45 (III). In previous studies, Du Boulay *et al.* (1995), Ackefors *et al.* (1995), and Goyert and Avault (1978) had similar findings in freshwater crayfish, while Geddes *et al.* (1988) found no inhibition at k values below 50. Lobsters (*H. americanus*) were found to tolerate either higher (van Olst and Carlberg 1978) or lower densities (Aiken 1980) than in the present study. Weight gain at molt was responding to space limitation before the effects could be found in intermolt period. In the latter study, limited space affected both weight gain at molt and intermolt period. Normally, survival was also lower in the groups reared in the smaller compartments.

Marron growth rates in the communal tanks in lower densities were similar to growth rates in the ICCS in higher densities (IV). Higher densities should cause, through density inhibition (Morrissy *et al.* 1995a), slower growth rates, but the density mechanisms seemed to give equal inhibition after the threshold density was exceeded. The reasons for the growth inhibition could have been simple experimental conditions in the communal tanks (IV) that provided only suboptimal conditions, or the marron genome. Furthermore, the breakdown of hierarchical structures, as suggested also by Ackefors *et al.* (1995), might have affected crayfish growth in the ICCS.

Size of the PVC tube hides could also be a factor affecting crayfish growth in the ICCS. In the present experiments only one size hides have been used and crayfish did not have a chance to choose the size of hide suiting their body size. Under natural conditions crayfish choose habitat to suite their physical dimensions, i.e. larger animals occupy habitats with larger physical dimensions (Hogger 1988, Foster 1993).

ICCS tanks, as used in marron studies, were light colored (plain stainless steel), forcing crayfish to adapt to a new environmental color. Thus, the changes in exoskeleton pigmentation (IV) might partly been caused by an adaptation to the environment. The surface of the tanks was smooth and marron could not move as easily as in their natural environment, which might have caused further stress.

Crayfish in all the experiments were reared under conditions where water flow was as low as possible, normally from 2 to 4 L min^{-1}. Kowarsky *et al.* (1985) has shown that marron grow faster when the water flow in the culture tanks in the ICCS is minimized.

6.2 Interpretation of condition and growth indices

Hepatopancreatic indices were shown to give limited but valuable information on crayfish condition. Growth rates, expressed as specific growth rate (SGR) in these studies, should be interpreted with caution.

Marron (*C. tenuimanus*) growth rates in these experiments (I, III, IV) were strongly influenced by either their genome or selection prior to them being placed in the experimental systems. The marron were from a slow growing, commercially farmed subpopulation, sometimes referred as runts. Since the experimental crayfish were provided by commercial farmers, the experimental stocks could not be of controlled origin or breeding line. This problem has been previously discussed by Celada *et al.* (1989, 1993).

The attempts to estimate the pond period SGRs, as indicated in previous sections, were the only means to standardize the marron (III, IV). In addition, the age of marron could be defined. The noble crayfish (*A. astacus*) growth history could not be estimated, as they were trapped from the wild populations (VI). Signal crayfish (*P. leniusculus*) were reared in an experimental farm, so that their growth rates could be estimated to be SGR 0.45. This was significantly higher than obtained in the same crayfish during ICCS rearing.

The growth rate index, SGR, is based on the assumption that the growth of experimental animals is exponential over the study period (Ricker 1975, Morrissy *et al.* 1986, Hopkins 1992). The strength of exponential trend, especially during short studies, is variable, but the SGR is based on a constant, strong exponential trend defined by the Neper constant (e=2.71849...). SGR, while being a suitable index to describe crustacean growth in long term experiments (Evans and Jussila 1997), might fail to meet the demands of short term experiments, because the stepwise growth pattern is then more influential. It would be beneficial to further develop this index to better describe growth in short experiments and to give more support to comparisons among studies of variable length. Also, when using SGR there were difficulties in comparing growth of crayfish that had significantly different initial weights (J Jussila, unpublished data).

Hepatopancreatic indices, especially wet hepatopancreas ratio or moisture content, can be used as approximate measures to evaluate the condition of crayfish reared under different environmental conditions (Mannonen and Henttonen 1995) or nutrition (IV, VI, McClain 1995a, 1996b), as was also indicated in the literature review in this thesis. The large hepatopancreas size, especially when it is related to low hepatopancreas moisture content, can be taken as an indicator of good condition in crayfish. Several studies have indicated that the hepatopancreatic indices can be useful, if they are

used in a co-operative manner (IV, VI, McClain 1995a, 1995b, Musgrove 1997) where also growth or environmental factors, if possible, are estimated as part of condition index complex. Also, the hepatopancreatic indices can be used as condition indices, if the crayfish have been obtained from the natural environment (Mannonen and Henttonen 1995) or reared in the semi-intensive farms (McClain 1995a, 1995b) and comparisons are made among groups inhabiting similar environments.

It was shown during the present studies that a high lipid diet caused high hepatosomatic indices and low hepatopancreas moisture contents in marron (IV). Traditionally, this could have been taken as an indicator of good condition. However, the growth rates of the high lipid diet group were low, and thus the accumulation of energy and other reserves in hepatopancreas resulted from an unbalanced diet. This suggests, that the combination of growth and condition indices could give a more reliable basis for the overall condition or performance estimates than either of the indices alone. Therefore, high hepatosomatic index and low hepatopancreas moisture content in crayfish was necessary but not solely a condition for fast growth. Furthermore, good condition in crayfish can be taken as a key marginal prerequisite for fast growth.

Sexual maturation and also the reproductive cycle affects hepatopancreatic indices, which has been clearly shown in noble crayfish (Huner *et al.* 1990). In marron, there were no differences between sexes in hepatopancreatic indices in the present studies (II).

Crayfish condition, when estimated from hepatopancreatic indices, has to be evaluated with caution. Previous studies on the wild populations of the co-species have to be examined, and any differences from what is observed in wild populations should be considered carefully. Also, parameters in the experimental environment, and crayfish itself (growth components, mineralisation, stress indicators, etc.), have to be considered together with the hepatopancreatic indices to achieve widest possible support for the interpretations.

6.3 Physiological responses

6.3.1 Growth and molting

Crayfish growth in the ICCS was slow compared to wild or semi-intensively reared populations. This was reflected as either smaller weight gains at molt or longer intermolt periods or both.

Growth rates of the marron (*C. tenuimanus*) in the present studies (I, III, IV) were lower than those of tank or pond reared marron (IV, Morrissy *et al.* 1990, Mills *et al.* 1994) and comparable to those recorded in previous studies on ICCS reared marron (Kowarsky *et al.* 1985, Tsvetnenko *et al.* 1995). The factors affecting growth in the ICCS (III) were similar to those affecting crayfish growth (Lowery 1988) in semi-intensive farming.

Marron growth rates in some of the present studies were comparable to their estimated growth rates in the semi-intensive farms (III, IV), indicating that they could have been the slow growing subpopulation of the farmed stock. Furthermore, the smaller marron obtained faster growth rates in the ICCS than their larger co-species, and even exceeded their estimated growth rates in the semi-intensive farms (Jussila 1996b). This may have indicated breakdown of hierarchical dominance structures in the ICCS or implyed that larger animals were facing more severe growth inhibition when placed into the ICCS. Also, larger size marron may be strongly territorial or required larger territories than their smaller co-species and thus their growth may have been inhibited in the fixed size compartments in the ICCS.

Production of marron in the ICCS was significantly higher than in semi-intensive farming systems (II, III), and it was similar to the highest production figures obtained in semi-intensive farms over a full year under optimal conditions (Morrissy 1979, 1992a). The average production in semi-intensive farms is usually significantly lower, down to 17% of the present ICCS production (Mills *et al.* 1994). These production estimates were obtained in studies where nutritionally inadequate feeds have been used and marron have shown signs of nutritional deficiencies. Also, the growth of marron was slow, below commercially acceptable growth rates, which would possibly result in close to zero commercial biomass production after the normal growout period (24 mo). This means, that production figures are highly unrealistic.

Morrissy (1984) observed a 52% weight gain at molt for marron weighing from 1g to 120 g. These figures were higher than in the present experiments and partly explains the slower growth in the ICCS compared to semi-intensive pond conditions (III, IV). Intermolt periods (III, IV), on the other hand, were relatively short in fast growing marron groups, and comparable to previous observations (Morrissy 1984).

Noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) showed poor growth rates and, because of that and high mortality rates, also the production was low. Growth rates of noble crayfish were slow or comparable to growth rates obtained under semi-intensive conditions (Gydemo and

Westin 1989, Ackefors *et al.* 1992, Taugbøl and Skurdal 1992), while signal crayfish growth has been reported to be much faster than obtained in the present experiments (Celada *et al.* 1993, Tulonen *et al.* 1995, Westman and Nylund 1984). The weight gain at molt in noble crayfish and signal crayfish (VI) was also less than reported previously (Ackefors *et al.* 1995) and the estimated intermolt period was longer than obtained in the smaller co-species (Henttonen *et al.* 1993, Ackefors *et al.* 1995). These findings could indicate, that the environment or nutrition provided in the ICCS offered suboptimal growth conditions for the Scandinavian species.

The hidden growth potential, an outcome of the stunting effect in some Finnish noble crayfish populations (Huner and Lindqvist 1986, 1988, Huner *et al.* 1995), did not materialize in these experiments (VI), even though the growth was faster in the ICCS compared to wild populations (Huner and Lindqvist 1988). In another study, noble crayfish growth was improved when the rearing conditions were dramatically improved (Laurent *et al.* 1995), indicating that the growth in noble crayfish in Scandinavia might not be limited by their genome.

6.3.2 Sexual maturation in the ICCS reared marron

Marron (*C. tenuimanus*) reached sexual maturity in the ICCS, even when reared only on artificial pelleted diets.

Marron reached sexual maturity under ICCS conditions slightly earlier, and at a significantly smaller size, than reported in the wildstock (Morrissy 1974). The smaller size at maturity at similar age compared to wild marron indicated that the sexual maturity could be related to certain number of molts, as was suggested also by Henttonen *et al.* (1993) and Huner and Lindqvist (1995) or, alternatively, a certain period of immaturity, instead of size. Marron in the ICCS have been reported to gain less weight at molt than marron in the semi-intensive rearing (I, III, IV, Morrissy 1984), and so their size at maturity could be smaller if the maturity is dependent on the number of molts or time spent in the immature life stage.

Reaching sexual maturity requires both adequate resources (Tyler and Calow 1988) and suitable environmental conditions (Morrissy 1974). Both clean and dirty ICCS (III) allowed marron to mature, and the age and the size of maturity for both sexes in both systems were similar. This indicated that the present pelleted commercial diets, even though they resulted in slow growth and changes in carapace pigmentation (IV), provided at least the minimum amount of nutrients and energy for marron to reach sexual maturity. This happened despite the finding, that the compartment size caused an inhibition in growth (III), most probably because of a density stress related symptom.

The marron in the ICCS may have allocated the limited resources available into reproduction under conditions that did not favor growth or which resulted in decreased growth rate. Under such conditions, the ability to gain advantage over co-species by growing faster was limited and the individuals might have been more fit when putting effort to reproduction (Tytler and Calow 1985).

Female ovaries were in most cases developed to stage II as described by Morrissy (1974), and since the mating itself was not possible in the ICCS, there were no evidence of female marron being capable of producing offspring. There is some evidence showing that red claw (*C. quadricarinatus*) do not mate successfully in a tank culture situation (Anon 1995), and it was also my experience (J Jussila, unpublished data) that even though marron were held communally in tanks they failed to mate. There is evidence from other experiments showing that marron can succesfully reproduce under tank conditions (Louis H. Evans, Aquatic Science Research Unit, Curtin University, Western

Australia, personal communication; Noel Morrissy, Bernard Bowen Fisheries Research Institute, Western Australian Marine Research Laboratories, personal communication).

6.3.3 Exoskeleton mineralisation and pigmentation

Exoskeleton mineralisation was decreased in the ICCS, to greater extent in Nordic species. The exoskeleton pigmentation, on the other hand, was more severely changed in marron (*C. tenuimanus*).

The levels observed for marron, noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*) carapace calcium, magnesium or total mineral concentrations (II, VI) were low compared to the concentrations observed in wild co-species (Welinder 1974, 1975, Huner *et al.* 1976, Huner and Lindqvist 1985, France 1987, Lahti 1988, Jussila *et al.* 1995). Mills and Lake (1970), on the other hand, obtained significantly lower calcification in *Parastacoides tasmanicus* and *Astacopsis fluviatilis* than in the present studies.

Carapace calcium concentrations obtained in the present studies were similar to those reported for freshwater crayfish inhabiting acidic waters and having difficulties in calcium metabolism (Malley 1980, France 1983). This might indicate that when pH or calcium in ambient water is not a limiting factor in calcium metabolism, as was the case in these experiments (II, VI), the availability of calcium in nutrition is still of utmost importance. Furthermore, the characteristics of pristine waters in Western Australia (Morrissy 1988) might have an effect on calcium, and also other minerals, metabolism in marron. Malley (1980) also noted that as a result of calcium deficiency, the crayfish remained longer in intermolt from stage B on, suggesting that interferences with calcium metabolism, in addition to inhibiting carapace mineralisation, also inhibited crayfish growth. This could partly explain slow growth rates in noble crayfish and signal crayfish (VI).

Similar mineral concentrations in the ICCS and pond reared marron (II) indicate that the ICCS could provide sufficient conditions, i.e. availability of minerals in water or feeds, for exoskeleton mineralisation. On the other hand, growth rates of ICCS reared marron were lower than in pond reared marron (Morrissy 1979, 1980, 1990, 1992a), which should have resulted in ICCS reared marron having higher concentration of minerals in the exoskeleton (Wheatly and Ayers 1995). Thus,

the calcium concentrations, while indicating successful mineralisation, may have been slightly low, relative to the growth rate, in the ICCS reared marron.

The calcium content in the culture system water should have been high enough to ensure calcification in noble crayfish or signal crayfish (Lowery 1988, Järvenpää *et al.* 1996), but the feeds used in the system might have had insufficient calcium. This is the opposite to arguments concerning intensively reared marron (II), where a combination of pellet feeding and natural food items production may have provided sufficient amount of minerals to complete carapace mineralisation.

Marron showed changes in exoskeleton pigmentation when fed only with commercial marron pellets (IV), indicating that this diet did not provide sufficient carotenoid level for pigmentation. This could also have compromised marron growth (D'Abramo and Robinson 1989, Sommer *et al.* 1991), even though Tsvetnenko *et al.* (1995) did not find growth inhibition in marron fed with carotenoid poor diets. Henttonen *et al.* (1993) observed a severe change in noble crayfish exoskeleton pigmentation after crayfish had been reared individually on diets either supplemented or without plant material. The conclusion was that detritus and cannibalism enhances exoskeleton pigmentation and that under intensive holding conditions, when crayfish are reared individually, the diets have to be enriched with carotenoids. Celada *et al.* (1993) observed no changes in signal crayfish pigmentation after 80 d experiments on artificial diets, even though the laboratory reared groups were pale colored. Lochmann *et al.* (1992), on the other hand, observed growth enhancement by astaxanthin enriched diets, while no changes in exoskeleton pigmentation were recorded in red swamp crayfish (*Procambarus clarkii*).

The changes in exoskeleton pigmentation could have been caused by the pale colors on the tank walls and the amount or quality of light in the ICCS environment. Similar changes in exoskeleton pigments have been recorded also in other studies with marron and yabbies (*Cherax destructor*), with different changes occurring under differently colored environments (Robin Fowler, Aquaculture Research Unit, RMIT University, Melbourne, Australia, internet communication).

6.3.4 Crayfish condition

6.3.4.1 Hepatopancreatic indices

The hepatopancreas showed inferior condition in intensively reared crayfish compared to semi-intensively reared crayfish. Some of the results showed that hepatopancreatic indices were not sensitive enough to show differences between treatment groups in nutritional studies.

In general, the ICCS reared marron (*C. tenuimanus*) were in better condition than wildstock but worse than tank or pond reared marron (II). The larger total water volume resulted in marron having higher total energy in their hepatopancreases which was also higher in moisture. The difference in condition, as well as in growth rates, between groups from large or small total water volume (I), could have been caused by the difference in the total water volume in the system, i.e. the apparent density. The density causes growth inhibition in marron (Morrissy *et al*. 1995a), which might be stress related. Also, the differences in food items produced in the ICCS (III) could have partly caused the differences in marron condition.

The type and quality of feeds did not have a significant effect on hepatopancreatic indices in marron reared in the ICCS (IV). Instead, the trends indicating than fast growing groups were in better condition, could be seen in hepatopancreatic indices. The intensively reared, starved group showed significantly poorer condition, similarly to previous studies in freshwater crayfish (Whyte *et al.* 1986, Schirf *et al*. 1987, Evans *et al* 1992b).

Intensively reared noble crayfish (*A. astacus*) appeared to be in slightly poorer condition than their wild co-species with smaller size hepatopancreases (VI, Lindqvist and Louekari 1975, Huner *et al.* 1985), while signal crayfish (*P. leniusculus*) condition was significantly poorer than in the wild co-species (VI). The poorer condition in intensively reared crayfish could have resulted from poorer nutrition (Evans *et al.* 1992b, McClain 1995a, 1995b), or more severe environmental stress (Mannonen and Henttonen 1995). These findings, together with the slow growth rates, suggested that noble crayfish were reared under suboptimal conditions. However, signal crayfishes hepatopancreases moisture content indicated poorer condition than in wildstock (VI). Noble crayfish were fed with a combination of vegetables, fish and salmon pellets, while signal crayfish were reared solely on commercial marron pellets (VI). The nutrition might have caused the difference between cultured and wild populations of signal crayfish.

The growth rates and hepatosomatic indices correlated in marron only if the data consisted of several treatment groups, and there were significant differences in growth among the groups (VI), indicating that the hepatosomatic indices were insensitive to individual differences within a treatment group. The relationship between hepatopancreatic indices and SGR was reverse in marron

compared to signal crayfish, which might indicate differences in physiology between these species. On the other hand, the suboptimal conditions resulting in slow growth of signal crayfish could have interfered with the physiological responses.

Trends between SGR and several condition indices were observed in the present studies. These findings were obtained from experiments designed for other purposes, suggesting that the relationships between these indices should be further studied. Correlations between hepatopancreatic indices and growth have also been reported in spiny lobsters (Cockcroft 1997, Musgrove 1997), suggesting the possibility of using hepatopancreatic indices to evaluate the growth potential of crustacean populations.

6.3.4.2 Hemolymph bacteria

Noble crayfish (*A. astacus*) were infected with several bacteria species when reared intensively. This might indicate increasing stress in these crayfish.

Eleven gram negative bacteria were identified in the hemolymph of otherwise healthy noble crayfish and infestation rate of intensively reared crayfish increased in time in the present studies (V). Bang (1970) states that the hemolymph of healthy crustaceans should be free of bacteria. However, several genera of bacteria (*Acinetobacter*, *Aeromonas*, *Arthrobacter*, *Vibrio*, *Bacillus*, *Corynebacterium*, *Pseudomonas*) have been isolated from the hemolymph of crustaceans previously (Cornick and Stewart 1966, Amborski and Amborski 1974, Haskell *et al.* 1975, Scott and Thune 1986).

Aeromonas sp. is one of the most common aquatic bacteria (Rhodes and Kator 1994) and its presence in hemolymph can be expected. Normally, *Aeromonas* is not pathogenic to crayfish, but mass infections may cause mortality (Amborski *et al.* 1974).

Pseudomonad colonies were numerous in a hemolymph sample from a dying crayfish which indicated its likely role as a pathogen. Amborski *et al.* (1974) reported that Pseudomands are present in dying crayfish and it is believed that abundant *Pseudomonas* sp. could cause crayfish deaths (Krieg and Holt 1984, Alderman and Bolglase 1988). Pseudomonads are part of the normal gut flora of noble crayfish (Mickeniene 1983) and can colonise the hemolymph if the immune resistance in the crayfish decreases.

Culture conditions may have an impact on bacterial flora in crayfish hemolymph, due to different infection routes: crayfish stock and eggs, water supply, fodder fish, equipment and supplies, visitors, and wild animals (Nylund and Westman 1992). In the tested indoor crayfish culture system, there were two main sources of bacterial contamination: the crayfish and their diet. Crayfish could transport bacteria from natural waters to indoor culture systems on their cuticle and gills. Furthermore, the potatoes, vegetables and fish used as food are not sterile and therefore potential sources of infections.

Marron (*C. tenuimanus*), reared in the ICCS, showed bacterial growth in their hemolymph, with an infection prevalence of 60% in samples taken during the low temperature season. The slow growth of marron in the ICCS could partly be explained by the bacterial infections, even though bacteria were common in the hemolymph of semi-intensively reared, fast growing marron during the summer season (J Jussila, unpublished data). Therefore, bacteria might be a normal, but not preferred, part of marron life cycle, appearing when the water temperature rises.

These findings indicate that crayfish may have been stressed under the intensive conditions and as a result their immune competence was compromised, allowing growth of several bacteria in their hemolymph. On the other hand, the role of bacteria in crayfish hemolymph is unclear, and low levels of bacteria might be harmless for the host crayfish.

6.3.4.3 Total hemocyte counts (THCs)

Total hemocyte counts (THCs) showed differences among different groups of marron (*C. tenuimanus*) as a result of either rearing condition or nutrition or both.

These studies showed, that THCs changed in response to differences in marron rearing conditions. Low THCs can be taken as indicators of stress in crustaceans (Bauchau 1981), and the decreasing THCs in this study gave indications of poorer nutritional status of the crayfish.

The lower THCs in the ICCS reared marron, compared to the communally reared ones, indicated that the ICCS reared marron might be stressed, caused either by inferior nutrition or suboptimal holding conditions. The reasons for the low THCs in the dirty ICCS reared marron could have been environmental stress caused by the breakdown of organic matter but remains to be investigated. It was also shown that short term starvation could decrease the THCs, and that the

THCs increased to the normal level in three days after feeding was recommenced. Since there was no attempt to isolate or investigate the effects of different stressors (nutrition, water quality, high density, etc.), it is impossible to say, what caused the differences in the THCs between the groups reared in different systems.

The THCs showed moderate correlation with hepatopancreases moisture content and dry hepatosomatic indices in the studied populations. This could indicate that the THCs can be used as a condition indices, which are sensitive to the changes in nutritional status and crayfish condition. Further studies are needed to confirm this relationship.

A decrease in the THCs has been shown to indicate lowered health status in western rock lobster, *Panulirus cygnus*, (Jussila *et al.* 1997) and an increase in the marron THCs was suggesting initiation of the handling and transportation stress in another study (L H Evans and J Jussila, unpublished data). These studies all suggested the usefulness of the THCs as a condition or stress index.

6.4 Culture potential of the studied species

Marron (*C. tenuimanus*) showed highest potential for intensive culture, while their growth rates were lower than those obtained under semi-intensive rearing. Production rates were high in marron and close to nil in noble crayfish (*A. astacus*) or signal crayfish (*P. leniusculus*).

The growth rates of the studied freshwater crayfish species were slow compared to the growth of the semi-intensively farmed or wild populations (I, III, IV, VI). In the latter experiments carried out with marron (IV) individual SGRs were similar or slightly lower than the SGRs obtained for the same animals held in semi-intensive farm ponds before they were taken into the ICCS.

Experimental marron in the present studies were provided by commercial farmers, and the analyses of their growth rates in farm ponds (III, IV) revealed that they could be considered as slow growing. Yet, marron showed the highest potential for intensive culture of the studied species, even with growth rates slower than under semi-intensive experimental or commercial conditions (IV, Morrissy 1974, 1980, 1992a, Semple *et al.* 1995). Villarreal (1991) concluded similarly that marron have high potential for farming because of their high efficiency in converting intake energy into tissue growth, that gives marron superiority over most freshwater crayfish.

The production of marron in the ICCS was considerably higher (I), or comparable or higher (III) than the production in the semi-intensive farms (Morrissy *et al.* 1995a, 1995b). However, because of the slow growth rate, the production figures were highly theoretical and a poor indication of the commercial potential of the ICCS or marron in the intensive rearing. The survival of the marron in the ICCS was comparable, 60-80% (I; dirty system), or higher, 90-100% (III, IV; clean system), to those in commercial farms (Lee and Wickins 1992), which has partly resulted, in addition to high densities (25 ind. m^{-2}), in high production figures.

Noble crayfish showed low growth rates under the tested conditions compared to previous studies (Ackefors *et al.* 1995) and the mortality was high. The assessment of the results was complicated since there is only few reports on growth of this species in intensive culture (Lee and Wickins 1992, Gydemo and Westin 1993, Henttonen *et al.* 1993). Furthermore, noble crayfish from stunted wildstock, possibly genetic runts, failed to show hidden growth potential (VI, Huner and Lindqvist 1986, 1988) in the ICCS. Reasons for the slow growth could have been in the poor system design and unsuitable water quality (Kuopio communal tap water) or either alone. In an unpublished study, farmed juvenile noble crayfish (0+ y.o., farmed in the Finnish Game and Fisheries Research Institute's Research Station in Evo, Finland), that could have had growth potential, ceased to grow when they were transferred into the ICCS (J Jussila, unpublished data).

Signal crayfish showed a similar low potential as noble crayfish (VI), although the growth rates of signal crayfish have been reported to be higher than those of noble crayfish (Tulonen *et al.* 1995), while Gydemo (1989) has questioned the differences in growth rates between these two species. The high survival rate in signal crayfish indicated a possible suitability of the species for high density rearing, or that the farmed stocks might be suitable for ICCS type conditions.

The production of the three studied species in the ICCS, estimated as their growth rate, is still far from being commercially viable.

7. CONCLUSIONS

The general conclusion in the present studies on the freshwater crayfishes physiological responses to intensive rearing was that the particular ICCS modification used (O'Sullivan 1990) provided suboptimal conditions for marron (*C. tenuimanus*), noble crayfish (*A. astacus*) and signal crayfish (*P. leniusculus*). The studies indicated that the growth was slower, exoskeleton mineralisation similar or poorer, and crayfish condition similar or worse than in wild or semi-intensively reared co-species. The THCs and hemolymph bacteria also indicated poorer condition in intensively reared crayfish. The age at sexual maturation indicated that the ICCS provided adequate resources for the process and it was comparable to that in the wild populations. All studied crayfish species showed low commercial potential under intensive rearing.

The nutrition seemed to cause severe inhibition in growth of all the studied species. Increased density inhibited marron growth, which has been shown also previously (Morrissy 1992a, Morrissy *et al.* 1995a), but the slow growing strain of marron (runts) used in these studies partially masked both of these effects. The density effect in noble crayfish or signal crayfish was not studied.

7.1 Growth and molting

Growth of marron (*C. tenuimanus*) was slower in the ICCS than expected in a commercial system. The growth rate was inversely related to total water volume in the ICCS. The nutrition provided in the commercial marron pellets was suboptimal, while pellet water stability resulted in improved growth rate. The ICCS also proved to be susceptible to air temperature fluctuations resulting in high water temperature and low dissolved oxygen levels.

Noble crayfish and signal crayfish grew slower than marron, as could be expected of the Scandinavian species. Noble crayfish from stunted populations did not show significantly faster growth in the ICCS compared to wild populations of the same origin.

The weight gain at molt was lower in both marron and noble crayfish compared to results obtained previously in semi-intensive or intensive rearing systems. Also the intermolt period was longer than previously reported for all of the three studied species.

The production was relatively high in marron in the ICCS, but the slow growth rates negated the commercial potential. The production of noble crayfish and signal crayfish in the present studies was negligible.

7.2 Sexual maturation

Marron reached sexual maturity at an age (1+ y.o.) similar to but at a smaller size (17 g) than wild or semi-intensively reared marron. This indicates that the ICCS offers enough resources for both growth and sexual maturation, even though the environment partly inhibits marron growth.

7.3 Exoskeleton mineralisation and pigmentation

Low carapace mineralisation in noble crayfish and signal crayfish showed that the ICCS could not provide adequate environment to complete the mineralisation. Since the levels of calcium and magnesium were only slightly lower than in wild co-species, it can be argued that the nutrition in the system was suboptimal for mineralisation.

Marron showed intermediate, or slightly low carapace mineralisation compared to the wild or semi-intensively farmed co-species. Exoskeleton mineralisation was better in marron than in the Scandinavian species compared to their wild co-species.

Marron showed changes in exoskeleton pigmentation, a pearly blue color, as a result of a prolonged rearing in the ICCS on commercial marron pellets. This indicated that the marron were experiencing carotenoid deficiency, which also could have inhibited their growth.

7.4 Crayfish condition

Intensively reared marron were in slightly poorer condition than semi-intensively reared marron but in better condition than their wild co-species. The larger water volume in the ICCS improved marron condition compared to marron reared in the small water volume. Furthermore, the condition of marron reared in the dirty ICCS was slightly better than that of marron reared in the clean system.

Hepatopancreatic indices showed that both noble crayfish and signal crayfish were reared on nutritionally insufficient diet. The poorer condition in noble crayfish was more evident than in signal crayfish compared to wild co-species.

The hepatopancreatic indices showed a potential for predicting the growth rates of marron and signal crayfish. The relationships between hepatopancreatic indices and SGR were reverse in marron compared to signal crayfish.

The total hemocyte counts (THCs), obtained in marron reared in the ICCS or semi-intensively, indicated that the ICCS provided insufficient nutrition and resulted in lower THCs than in semi-intensively reared marron. The THCs, on the other hand, correlated significantly with marron growth and hepatopancreatic indices, which suggests that the THCs could be used as condition indices.

Noble crayfish, reared in the indoor system, were infected with a range of gram-negative bacteria. This might be indicating decreased immune system activity and thus a poorer condition of the crayfish. The bacterial infections have traditionally been indicating suboptimal rearing conditions and could severely affect production in an aquatic system. On the other hand, it has been demonstrated previously, that pond reared crayfish can be infected with bacteria and be otherwise healthy.

7.5 The factors affecting growth in the ICCS

The basic factors affecting growth in the ICCS were similar to those functioning in semi-intensive pond conditions: level of dissolved oxygen, temperature and its diurnal fluctuation, food quantity and quality, and density of the crayfish (expressed as compartment size or total water volume). The intermolt period was normally affected by these factors while the weight gain at molt was affected only by diurnal temperature variations.

8. RESEARCH AND DEVELOPMENT OF FRESHWATER CRAYFISH CULTURE SYSTEMS

The ICCS showed low production potential, and several factors that might be inhibiting growth could be identified. The following aspects were considered as crucial for further development of freshwater crayfish farming either under semi-intensive or intensive conditions:

1) Nutrition: Nutritional requirements of freshwater crayfish, and crustacean, have to be investigated in further detail, focusing on improving availability of carotenoids and the ingredient balances in artificial diets. The major problem is leaching of the water soluble components from the pelleted diets (Goldblatt *et al*. 1980), which has to be solved before the nutritional studies are commenced. In fish, Kraul *et al.* (1993) have shown that docosahexaenoic acid (DHA) has improved stress resistance, which might indicate the possibility to study the effect of nutrition as means to improve stress resistance in crayfish. Furthermore, once the feed quality problems are solved, automatic feeding systems suitable for crayfish should be developed because of the benefits in maintanence and costs.
2) Temperature: The growth and sump tank systems have to be improved to minimize diurnal and seasonal temperature fluctuation in the ICCS. Studies on the optimum rearing temperature of the Scandinavian species are required for further development of indoor culture.
3) Physical environment: The presently used ICCS needs adjustable compartments and hides designed to suit the needs of growing crayfish and to optimize the density-economics. The physical environment needs to be improved to ensure that crayfish (health, molting, survival, etc.) and the culture system (especially water quality) can be monitored and the basic maintenance (feeding, cleaning, etc.) carried out without opening the compartment lids or otherwise disturbing the crayfishes.
4) Bacterial infections: Sources of bacterial infections and their impact on the performance of crayfish in intensive culture should be studied. The ICCS should be tested using an efficient system to remove bacteria from the water (ozonizer).
5) The hepatopancreatic indices: The role of hepatopancreas as an indicator of crayfish condition under intensive rearing and the relationship between growth rate and crayfish condition should be further investigated. The seasonal hepatopancreatic cycle in wild marron needs clarification,

as this is a crucial for better understanding of these indices. A non-sacrificial method to assess crayfish condition and stress should be developed.

6) Total hemocyte counts: Experiments to obtain information of the effects of nutrition and environmental stressors on crayfish THCs should be carried out. A better understanding of crayfish stress, and its causes, is needed to improve both farming and post-harvest handling techniques.
7) The present studies were carried out with crayfish which were provided by commercial farmers or trapped from the wild. There should be an effort to use crayfish of known provenance, breeding history and strain in laboratory type experiments. The effect of genetics on marron growth has to be addressed.
8) All the growth studies included in this thesis were too short to evaluate the production of commercial biomass in the system, which is a crucial factor when the viability of any crayfish farming technique is assessed. After the present problems in nutrition are solved, studies lasting at least 12 mo should be carried out to evaluate the commercial potential of the ICCS.

The priority of the future studies should be on freshwater crayfish nutrition to benefit development of both semi-intensive and intensive farming systems. The problems arising from the usage of artificial feeds in crustacean farming have been addressed before (Goldblatt *et al.* 1980, Castell *et al.* 1989, Morrissy 1989) and similar problems were encountered in the present studies. Also the density effect should be studied in further detail and the possibilities to eliminate density communication in the culture environment should be investigated.

The studied modifications of the ICCS, even though they had some potential for crayfish farming, are far from being commercially viable systems. Based on the results of the present studies and the economic reality and resources in freshwater crayfish farming and research in Finland and Western Australia, the effort required for effective development of intensive farming systems is still huge but achievable.

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