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Improved performance of marron using genetic and pond management strategies

Final FRDC Report – Project No. 2000/215

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Fish for the future

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Non-technical summary

Marron (*Cherax tenuimanus*) are the highest valued freshwater crayfish farmed in Australia. This project addressed the need to increase the profitability of commercial marron farms by improving growth rates and pond management strategies.

The project evaluated progeny produced from wild populations collected from 6 river systems that had not been subjected to the broodstock selection processes on commercial farms. This demonstrated that current management of broodstock, whereby farmers sell the largest crayfish produced and breed from remaining animals, has resulted in slower growth of marron on commercial farms. Marron from all river systems grew faster than industry stocks. The best performing wild river strain, from the Harvey river, grew 82% faster than current industry stocks.

A simple mass selection selective breeding program improved growth rates by 86 -110% in two generations.

Consultation with farmers identified breeding objectives that were applied by researchers to develop a selection index for a more complex pedigree breeding program that permitted simultaneous selection for multiple traits based upon economic merit. This program also permits greater control of inbreeding than mass selection.

Husbandry experiments showed that current refuge densities were suitable for marron production. Paddlewheel aeration practices could be improved by increasing the duration of aeration. Relaying juveniles produced early in the year in northern regions did not improve final production. Size grading of juveniles prior to stocking ponds can, however, increase the average weight of marron harvested by 12 - 58% and decrease the proportion of below market size animals by 54%.

Marron with proportionately shallower abdomens grow faster than those with deeper abdomens. Combined with hide harvesting, this simple condition index can be applied by farmers to evaluate condition of marron in commercial ponds, calculate growth rates and manage feed rates.

Farms based in the more southern, cooler regions, have lower growth rates due to cool water temperatures. In this study the best region from a temperature perspective is Pinjarra, where

lower water temperatures limit growth for only 0.6% of the year, compared to the least favourable region, where temperature limits growth for 33% of the year.

It is essential that commercial marron farms are correctly designed, constructed and professionally managed. Commercial trials involving 147,000 marron reared to market size in 44 commercial ponds over a 5 year period demonstrated the viability of current practices and the increased profit ($33,600/ ha) from farming selectively bred marron developed in this project.

An extension strategy that included open days, research seminars, field trials and open communication with industry was extremely successful. Most key outputs from this project have already been adopted by leading farmers with newer entrants to the industry following their example. As a result the husbandry strategies and software developed by this project have been rapidly adopted by industry and 18,000 elite marron produced from the selective breeding program have been distributed to industry in WA and SA.

The use of marron produced by the FRDC selective breeding program dramatically increases the profitability of farming. For a correctly managed and constructed 50 pond farm replacing industry stock with marron from the selective breeding program increases the IRR from 8.24% to 22%, return on capital from 4% to 40%, yields from 1.5 to 3 t/ha/year and profit from $20,722 to $189,130 /year.

Outcomes achieved to date

This project has provided industry with pond management strategies and improved genetic lines to increase the profitability of commercial marron farms.

The husbandry strategies developed by this project have been rapidly adopted by industry and provide clear evidence that growth and survival is better on correctly designed, constructed and managed marron farms.

The pond data software developed in this project has provided farmers with the ability to efficiently record production and manage broodstock.

The key outcome of the strain evaluation and genetics component of this project was the distribution of 18,000 elite marron from the selective breeding program to juvenile producers in WA and SA. These farmers are mass-producing these animals in order to supply the rest of industry with large numbers of faster growing marron. If correctly managed these stocks should result in supply to industry of 500,000 faster growing marron juveniles by 2008. The outcome from existing farmers rearing these animals for sale is anticipated to be an additional 50 t of production/year compared with farming current genetic stocks. While this is still, insufficient to meet existing global demand for marron, the outcome of this increased production should be increased investment into new marron farms and expansion of marron farming in key regions.

KEYWORDS: Marron, *Cherax tenuimanus*, genetics, husbandry, aquaculture, hides, aeration,

Acknowledgements

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The University of Western Australia provided facilities for strain evaluation and selective breeding programs. Marron farmers in WA and SA provided commercial ponds for field trials.

Additional complimentary projects were funded by the Australian Academy of Technological Sciences and Engineering "Development of an international centre for aquaculture genetics and enhancement of selective breeding program for the production of freshwater crayfish in Australia " and the Aquaculture Development Fund (Western Australia) "Freshwater aquaculture research ponds for collaborative industry, university and government research".

The selective breeding component of this project was facilitated by collaborative research partnerships established with the Danish Institute of Agricultural Science and International Network for Genetics in Aquaculture as a result of funding provided by the Australian Academy of Technological Sciences and Engineering.

Before this report was completed, George Cassells the Senior Technical Officer on the project passed away. The research team gratefully acknowledges his dedication and the considerable experience he brought to this study.

Background

With a farm gate value of $16.00 – $32.00 kg marron (*Cherax tenuimanus*) are the highest valued freshwater crayfish farmed in Australia. In comparison to other species, marron possess the advantage that few reproduce within the 1-2 year production cycle, thereby permitting control of density and implementation of sound production strategies. These advantages were recognised in the early 1970's, with research investment by the Department of Fisheries WA and FRDC resulting in the fundamental breeding, feeding and stocking practices in use by industry today. This pioneering research by Dr. Noel Morrissy, who is now recognised internationally as Australia's leading freshwater crayfish expert, was undertaken at a very low level over 20 years as an adjunct to running the trout hatchery. This limited investment by FRDC ($78,665) and Department of Fisheries WA ($1.2 million over 20 years), has resulted in the existing marron industry that has produced over $7 million worth of marron, over $5 million of which was produced during the last 5 years. Therefore the return on investment into marron research in production alone is $5.5 for every $1 research funds invested (i.e. not including feeds, equipment, jobs etc).

WA is a world leader in live rock lobster export (4,000 t/year from an average annual catch of 11,000 t/year). In comparison, in Europe alone, annual consumption of freshwater crayfish is around 6,300 t, with 2,800 t/year coming from European capture fisheries (Wickens and Lee 2002). The Turkish freshwater crayfish fishery exported to Europe much of the 3,500 t/year shortfall until its collapse in 1986 from 8,000 t/year to 1,000 t/year (Holdich 2002). In comparison current production of marron in Australia is less than 100 t/year (and total production of all freshwater crayfish in Australia is less than 450 t/year) the majority of which is exported live, however clearly this is still well below that required to satisfy global demand. Key research and industry representatives have recognised the advantages to marron export arising from international marketing and transport systems already established for rock lobster. In fact, in contrast to most other aquaculture industries, marketing and transport for marron are

well established, the key issue is how to increase production. After a lengthy embryonic period during the 1970's and 1980's characterised by poor site selection, design and management, marron farming is currently in a phase of unprecedented growth with 212 licensed farmers in Western Australia and 91 in South Australia. This has resulted in a large number of well-designed and constructed marron farms. A conservative estimate of current investment into marron farms in Western Australia is $15 million, indicating the medium term potential for marron production in WA is around 1000 t. Farms are operating successfully from the south coast to Geraldton 750 km north. Around 75% of Australian marron production comes from purpose built farms in Western Australia. The other major producer is South Australia with production coming mainly from purpose built ponds (15%) and wild harvesting (10%). The ratio of farmed to wild marron production in SA has changed rapidly from 30:70 two years ago up to 60:40 at present. While feral marron harvested from streams still form a significant proportion of SA marron production, it is clear that the trend in SA is to follow the WA example of producing marron from semi-intensive purpose built ponds.

Freshwater crayfish industry priorities and directions for research in Australia were identified during a workshop at World Aquaculture Society 1999 (Lawrence 1999). Industry and researchers agree that the two key areas which require research are genetic improvement and pond management strategies to increase growth and decrease size variation.

Farmers wish to use strain evaluation and selective breeding to increase production by increasing growth and reducing the size variation at harvest, so that the majority of animals are above market size. Recent direct monitoring of individual commercial harvests in WA has confirmed that better farmers can produce yields achieved in earlier research trials (2 t/ha/year) (Chatfield and Cassells in prep) and which would sustain attractive returns on capital (Treadwell et al. 1991, and recent Edith Cowan University modelling). However much of the current production is below market size after 12 months and requires another year of growout due to the large size variation at harvest. Selective breeding for improved growth would allow many farmers to move from a 24 month growout pattern to a 12 month production cycle in WA, and in SA, where larger marron are marketed, from 36 to 24 month production cycle.

Previous research has shown generic variation in wild marron which can be used to develop an improved commercial strain for aquaculture (Morrissy et al. 1995, Henryon 1996, Imgrund 1997). In 1999/2000 the Department of Fisheries WA commenced a mass selection program based upon broodstock which represented the fastest growing animals from commercial farms in Western Australia. This FRDC project investigates 4 avenues for genetic improvement of marron 1) Evaluation of wild populations to identify the best performing strain 2) Evaluation of the best performing commercial strains 3) Mass selection of the best performing commercial or wild strain 4) Hybridisation of strains.

While a selective breeding program will increase the quantity of marron produced, farmers in WA and SA also wish to maintain the high quality standards which exist in their industry and have requested husbandry research to further improve the quality of their product. Applied husbandry research has already shown potential for further improving the quality of animals at harvest. In a pilot study to this project, Chatfield and Cassells (in prep) have shown considerable variation in the condition and external quality of marron from different farms. There is strong evidence to suggest that quality of marron is markedly improved in well managed ponds. The effect of management practices on product quality requires further investigation, with a number of relatively simple methods showing potential for improving quality and growth (i.e. aeration, refuge density). Some of the management practices may also reduce size variation.

In addition to increasing quantity and quality of marron produced, farmers require a practical, non-destructive, quantitative, farm-based test to tell if their animals are in good condition. The development and implementation of this test will further refine our definition of crayfish quality and permit farmers to quantify the condition of their marron achieved as a result of improved husbandry. This test will also reduce post-harvest mortalities and lead to increases in profitability by ensuring that only marron in top condition are packaged and sent to market.

In combination, this project seeks to optimise both the quality and quantity of marron produced by working with farmers to genetically improve the animal, identify the optimum system for producing marron and develop a method for farmers to quantify the condition of their marron. The partnership we have established between researchers and industry will ensure rapid adoption by industry of results from the husbandry trials and selection programs in this project.

Need

There is a need for marron aquaculture to follow the example of traditional agriculture and develop domesticated varieties, rather than continuing to rely upon unselected wild stock. In fact, current husbandry techniques on commercial farms, in particular harvesting practices, are likely to result in the selection of slower growing marron for future broodstock (i.e. through early marketing of fastest growing individuals with broodstock chosen from the residual population), as is the case in yabby farming (Lawrence et al. 1998, Lawrence et al. 2006).

Investment in marron farming has grown rapidly in WA and SA with medium term potential of 1,000 t/year. ($20 million) in WA and 250 t/year. ($6.25 million) in SA, based on current investment (conservatively $15 million in WA), anticipated expansion at these farms, performance of better farmers, and site availability (marron are grown from Geraldton to Esperance). As volumes increase the current excellent ex farm prices for marron ($16-32/ kg) may decline and necessitate improved production efficiency. Consultation with industry has identified growth rate and size variation as the main factors affecting profitability of marron farming. Industry's belief in the need for this research is reinforced with significant cash ($9,000 from WA) and in kind contributions.

There is a need to i) compare production of farm stock with that of their ancestral populations to determine the effects of current farm management practices upon marron gene pools, ii) compare different wild stocks to identify the best marron strain for farming, and then iii) genetically improve the best strains.

Just as traditional agriculture has increased growth rates of livestock and poultry by strain evaluation and selective breeding, there is a need to achieve similar gains with marron by developing a genetically improved strain which will result in greatly improved profitability for industry.

In addition, there is considerable debate amongst consultants and farmers as to what are the best methods for producing marron. While experimental trials have shown yields may be increased, demonstration and documentation of the 'best practice' marron farming system has not been undertaken. There is a need for husbandry improvements that can both increase the quantity and improve the quality of farmed marron. The issue of improved quality is vital to farmers, as marron are exported alive but industry currently report mortality rates of up to 11%. Reducing mortality by ensuring marron being sent to market are in top condition will have clear and measurable improvements in profitability. With industry we have developed this

proposal, which combines the needs of industry with the proven freshwater crayfish expertise of Department of Fisheries WA researchers along with facilities and expertise in animal breeding from the University of Western Australia. South Australian farmers and researchers are keen to extend such research activity and results to that state. To facilitate this the project involves WA industry SA industry, SARDI, PIRSA and the University of Adelaide.

Objectives

1. Selection and genetic improvement of stock

1. Identify the fastest growing wild strain of marron.
2. Compare the growth of wild marron strains with a mass selected commercial strain.
3. Determine whether any hybrids have production characteristics that are superior to wild marron strains.
4. Use mass selection to develop a faster growing “domesticated” marron strain or hybrid.
5. Decrease size variation of marron cohorts to increase the proportion of marketable animals.
6. Evaluate performance of the mass selected marron strain on commercial properties.
7. Investigate inbreeding effects by comparing growth of mass selected marron with farm stock.

2. Development of improved husbandry protocols.

1. Compare the effect of aeration upon both production levels and product quality.
2. Determine whether increased hides can alleviate growth reduction due to high density.
3. Evaluate stocking tightly graded juveniles in commercial ponds upon size variation at harvest.
4. Evaluate stocking advanced juveniles into commercial ponds.
5. Trial a non destructive condition index developed for yabbies on marron in commercial farms.
6. Compare the effect of regional variation upon marron growth and production.

3. Extension of results to industry

1. Manual of methods for managing marron ponds to improve husbandry and genetics of farm stock
2. Exchange of information between WA and SA.

Results

Strain evaluation

1.0 Evaluation of marron strains Part I: Strain evaluation

1.1 Growth, sex ratio, breeding success and production characteristics of six river strains and three "domesticated" lines of marron in research ponds

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Introduction

Marron (*Cherax tenuimanus* Smith, 1912) are endemic to isolated freshwater river systems in Western Australia (Lawrence and Jones 2002). These large freshwater crayfish have been the focus of increased aquaculture interest locally, nationally and internationally (Lawrence and Jones 2002). However, the majority of marron stocks for commercial aquaculture originated from the Department of Fisheries, Pemberton fish hatchery. This stock was originally sourced from the Warren River, without prior evaluation of aquaculture production characteristics such as growth or survival (Cassells pers com).

The results from previous studies have shown that there is variation in growth in Australian freshwater crayfish. In a study comparing growth of yabbies collected from allopatric populations throughout Australia (Lawrence et al. 1999, Lawrence et al. in press) showed a 1000% difference in growth among 13 populations of these crayfish. Henryon (1996) showed that there is a 30% difference in growth among 4 populations of marron from different river systems. He also showed a difference in tail yield (10%) and breeding success (11-61%) among the 4 marron populations (Henryon 1996).

More recently polymorphic microsatellite markers have been used to show that there is considerable genetic variation among marron populations from several geographically distinct regions in Western Australia (Imgrund et al. 1997).

The genetic variation reported for marron highlights the potential to identify and develop an improved commercial strain of this species for aquaculture. Increased economic returns can be achieved by selecting the population with the most suitable production characteristics (i.e. growth) and then further gains may be achieved by selective breeding for improved growth, tail and chelae yields (Henryon 1996). Furthermore, selection for faster growth in marron has a greater significance on profit than selection for tail or chelae yields (Henryon 1996). Use of the fastest growing wild marron river strain in the study by Henryon (1996) would increase farm profit by 80%.

In addition, researchers have expressed concerns that the current broodstock management strategies applied by commercial marron farmers may be having a negative affect upon growth of their stocks (Lawrence and Morrissy 2000). This is because most farmers sell the largest marron in their ponds at one year of age and retain the smaller, slower growing animals for another year at which stage they sell the majority of stock and randomly select breeders from these poorer performing crayfish.

The aim of this experiment was to compare the growth, sex ratio, breeding success and production characteristics of marron from a range of isolated river systems, hatchery stocks and a Mass Selected Line with those farmed by industry.

Methods

Collection of broodstock

Broodstock marron for each wild river line were collected from the Harvey River, Margaret River, Donnelly River, Warren River, Shannon River and Kent River between April – June 2000 and acclimated in ponds at the Pemberton Freshwater Research and Aquaculture Centre (PFRAC) in Pemberton (Lat -34.45, Long 116.03) prior to the breeding season (Figure 1).

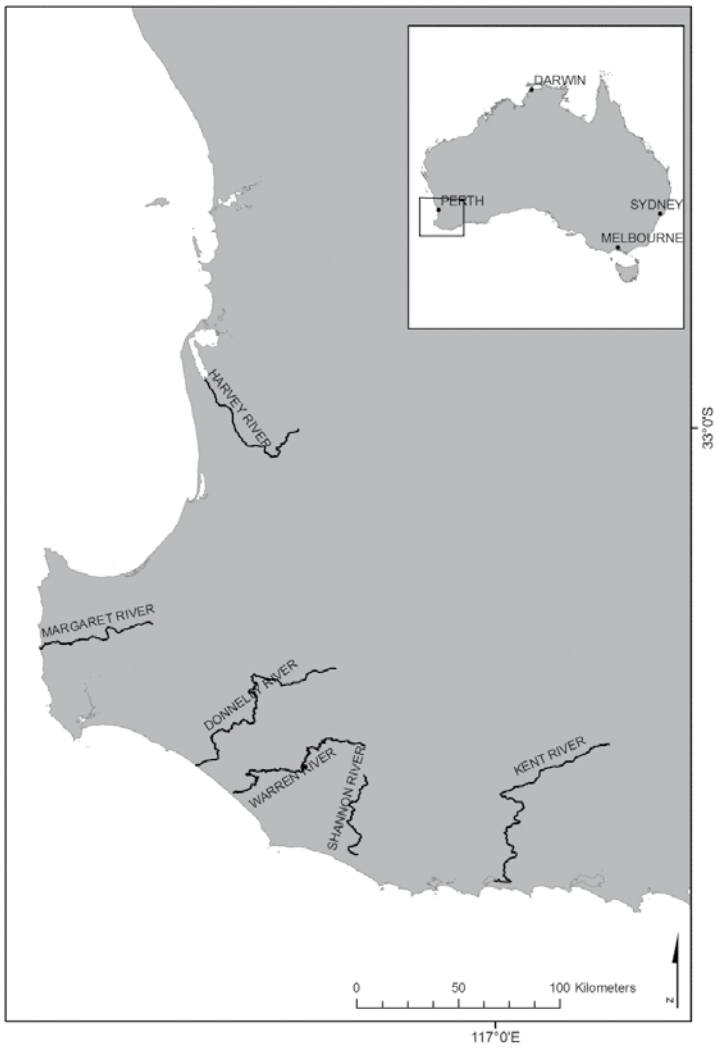

Figure 1. Collection localities for marron strains

Broodstock for the Mass Selected Line was collected from 16 commercial farmers who provided the largest 2-year-old marron (129 male and 148 female) produced from their ponds in June 2000.

Broodstock for the Pemberton Line were randomly selected from 2 year old marron held to the PFRAC hatchery.

Production of juveniles

Mating broodstock from each of the 6 river populations, the Mass Selected Line and the Pemberton Line in 8 m2 concrete ponds at the PFRAC in July 2000 produced juveniles for each strain.

Commercial marron farmers provided juveniles for the Industry Line.

Stocking of experimental tanks

In March 2001, juveniles from all females within each line were pooled and a random sample of 25 juveniles from each strain were weighed prior to stocking tanks.

Eighty juveniles were stocked in each of the 27, 20 m^2 tanks at the UWA Aquaculture Laboratory in Shenton Park (Lat -31.96, Long 115.80) at the rate of 4 juveniles/m^2. Due to the low reproduction of the Harvey Line, each pond for this treatment was stocked with 52 Harvey juveniles and 28 blue juveniles to ensure density was the same for all treatments, whilst still permitting visual identification of the Harvey marron. Each treatment was repeated in triplicate. In total 2,160 juveniles were stocked into 27 tanks for this experiment.

The 27 tanks formed part of a recirculating system with a total volume of over 3,200 m^3 consisting of over 800 m^3 of tanks and aquaria (n = 210) and a 127 m^3 biofilter. Therefore, each tank had the same water chemistry parameters.

Sex ratio and growth of strains

Data on sex ratio and relative growth of each line was collected at 4 monthly intervals commencing in July 2001, prior to this juveniles were too small to sex and weigh without damaging animals.

Every 4 months, over a 10 day period, each of the 27 tanks was drained, marron collected, weighed, sexed and returned to their respective tank. Growth rate data was collected until the marron reached two years of age in November 2002.

Feeding and maintenance of stocks

All marron were fed the WA crayfish reference diet (Morrissy 1990) at similar rates initially. These rates were adjusted according to feed rates derived from Morrissy (1992); adjustments were made daily, according to growth and temperature variations, by visually observing demand feeding.

Water chemistry was recorded 3 times per week (Ammonia, Nitrite, Nitrate) using a WinLab® LF 2400 photometer and pH using a Eutech meter.

Temperature was recorded by a Grant Instruments 1200 series (12-Bit) Squirrel data logger with U type thermistor temperature probes and programmed to record temperature every hour.

Statistical Analyses

Growth data was analysed by ANOVA using the means of replicates within each treatment group. Post hoc multiple pair-wise comparisons were completed using Fishers LSD to test significant differences among treatments.

To test if the sex ratio varied from 1:1 data was analysed using a Chi-square test with 5% level of significance to test the hypotheses Ho: The sex ratio of males to females is 1:1.

Economic analyses were completed using Marron Profit© software (available from the Aquaculture Council of WA, http://www.aquaculturecouncilwa.com) based upon a model 50 pond marron farm designed, constructed and operated according to Department of Fisheries WA recommendations for best practice. The economic effects of growth rates recorded in this experiment were calculated by varying the breeding and husbandry improvement module. Results are presented as change in annual production (t), annual return (profit) ($), break-even price (production cost/ kg) and Internal Rate of Return (IRR) (%).

Results

Water Chemistry

Water chemistry variables (Ammonia range = 0.001 – 0.012 mg/L, Nitrite range = 0.02 – 0.08 mg/L, Nitrate range = 0.05-0.2.5 mg/L, pH range = 7.2-9.1) remained within acceptable limits for marron production for the duration of the experiment.

Water temperature (range 11 - 35°C, mean = 19°C) remained within acceptable limits for marron production for the duration of the experiment (Figure 2).

Figure 2. Average daily water temperature in experimental ponds at UWA Aquaculture Laboratory.

Reproductive success of marron strains

The average percentage of berried females was 53%. The Harvey River Line (13%) had the lowest proportion of berried females and the Warren River Line (84%) had the highest (Table 1).

Table 1. Reproductive success (%) of marron strains 2000/2001 breeding season (n = number of females in breeding pond).

Strain	% Berried females	n
Harvey River	13	90
Margaret River	53	30
Kent River	45	139
Shannon River	56	122
Donnelly River	35	121
Warren River	84	123
Pemberton Line	77	126
Mass Selected Line	64	148

Size of juveniles

The average weight of juvenile marron at stocking was 1.56 g. Margaret River Line (3.10 ± 0.29 g) produced the largest juveniles and Pemberton Line (0.88 ± 0.10 g) the smallest (Table 2).

Table 2. Size of juveniles (g ± se) at stocking (29/3/2001) (n = 25).

Strain	Mean Weight (g)	se
Harvey River	0.93	0.13
Margaret River	3.10	0.29
Kent River	1.07	0.11
Shannon River	2.13	0.18
Donnelly River	1.26	0.15
Warren River	1.35	0.16
Blue Line	2.07	0.18
Pemberton Line	0.88	0.10
Mass Selected Line	1.59	0.18
Industry Line	1.23	0.33

Survival of marron strains

At the conclusion of the experiment survival of the Shannon River Line (27% ± 4.0 se) was lower than that of the other nine strains ($P < 0.017$).

Table 3. Mean survival of marron strains (% ± se) to two years of age (n = 30) (Original stocking density 4/ m^2 = 80 marron/pond in March 2001).

	30-Jul-2001	se	19-Nov-2001	se	13-Mar-2002	se	22-Jul-2002	se	11-Nov-2002	se	17-Mar-2003	se
Harvey River	96	1.1	71	8.0	64	9.7	61	5.3	51	6.1	47	6.5
Margaret River	97	2.1	90	0.7	81	0.8	76	0.7	73	1.1	62	2.9
Kent River	98	1.5	94	1.4	84	0.4	79	2.7	73	2.9	63	6.4
Shannon River	94	4.0	89	4.5	75	4.5	71	5.6	64	4.7	27	4.0
Donnelly River	96	4.2	93	3.2	84	3.3	81	4.4	80	3.0	54	5.8
Warren River	95	4.1	89	3.3	85	3.3	80	3.2	74	2.9	55	3.5
Blue Line	96	2.1	86	4.1	71	6.2	65	7.8	64	5.5	48	8.3
Pemberton Line	98	0.4	95	1.3	86	2.2	80	2.2	73	2.9	61	1.8
Mass Selected Line	94	1.7	91	2.2	77	1.5	67	2.2	62	2.9	47	0.8
Industry Line	85	12.9	82	11.6	78	10.2	72	9.0	70	7.8	50	10.0

Growth rates of marron strains

At the conclusion of the experiment there was a significant difference in the mean weight of marron from different genetic lines ($p < 0.001$) (Table 4). The fastest growing line (Harvey Line) was 82% larger than the Industry Line (Table 4).

Table 4. Mean weight of marron strains (g ± se) (n = 42) (#Pemberton Line consists of two morphotypes "brown" and "blue" at a ratio of 81% Brown : 19% Blue (se 1.52). *during sampling in Nov 2001 two Harvey River morphotypes were observed "brown" and "green" at a ratio of 93% brown: 7% green).

Strain	Initial 29-Mar-2001	se	30-Jul-2001	se	19-Nov-2001	se	13-Mar-2002	se	22-Jul-2002	se	11-Nov-2002	se	Final 17-Mar-2003	se
Harvey River	0.93	0.13	9.30	0.91	24.79	2.77	53.37	2.82	83.47	4.57	106.05	2.46	129.68	1.85
Harvey River (brown)*	-	-	-	-	27.75	0.57	56.91	1.61	88.07	4.16	111.20	4.29	-	-
Harvey River (green)*	-	-	-	-	9.53	3.03	18.24	1.11	28.83	3.57	37.87	4.94	-	-
Margaret River	3.10	0.29	11.31	0.11	22.91	0.06	39.67	0.67	55.91	1.81	68.56	1.69	83.13	2.09
Kent River	1.07	0.11	7.07	0.14	16.82	1.18	33.62	1.90	49.47	0.89	65.44	1.45	79.92	3.52
Shannon River	2.13	0.18	9.96	0.43	20.71	0.92	39.05	1.59	57.26	3.07	74.71	4.37	82.09	1.87
Donnelly River	1.26	0.15	7.07	0.49	16.34	0.73	36.79	1.06	51.04	2.23	63.20	2.77	81.04	3.36
Warren River	1.35	0.16	5.54	0.28	14.23	0.57	31.81	1.05	49.51	1.03	64.81	2.48	77.66	0.35
Blue Line	2.07	0.18	10.09	0.80	24.46	1.52	50.03	4.40	72.07	6.01	93.15	4.89	107.49	6.99
Pemberton Line	0.88	0.10	6.76	0.11	16.44	0.68	35.01	1.12	56.46	2.54	75.97	5.13	94.42	4.08
Pemberon (brown)#	-	-	6.78	0.13	16.67	0.22	35.31	1.35	56.89	2.62	76.12	5.04	94.67	3.77
Pemberton (blue)#	-	-	6.67	0.15	15.88	1.14	33.89	0.35	54.60	2.73	75.00	6.20	91.58	9.13
Mass Selected Line	1.59	0.18	8.78	0.46	20.59	0.81	41.14	1.16	62.66	1.81	79.81	2.86	100.13	2.09
Industry Line	1.23	0.33	3.97	0.59	11.50	1.48	27.57	2.97	41.21	4.41	54.51	6.01	71.42	5.23

The Harvey River (130 g ± 1.85 se), Margaret River (83 g ± 2.09 se), Pemberton Line (94 g ± 4.08 se), Mass Selected Line (100 g ± 2.09 se), and Blue Line (107 g ± 6.99 se) all grew significantly faster than the Industry Line (71 g ± 5.23 se) (Figure 2).

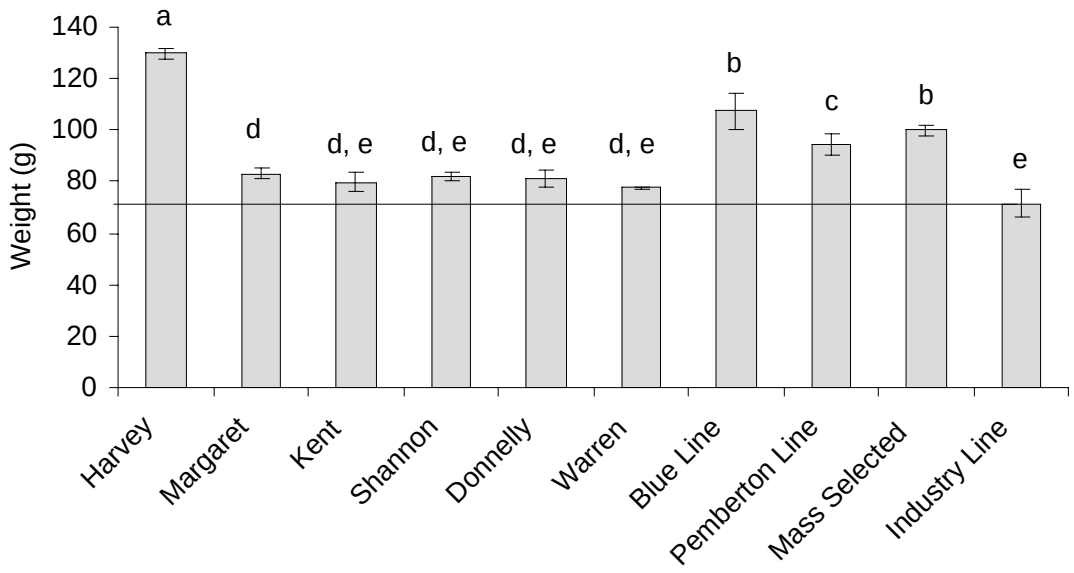

Figure 3. Summary of final mean weight (g ± se) of marron genetic lines after 2 years grow-out (n = 30) (solid line indicates industry mean weight). Means sharing the same superscript are not significantly different P > 0.05.

Size variation of marron strains

The coefficient of variation (CV %) was significantly different among lines (p = 0.001). The Blue Line (35% ± 7.58 se) and Harvey River Line (36% ± 1.56 se) had the lowest coefficient of variation (Table 5).

Table 5. Coefficient of variation (CV % ± se) of marron lines after 2 years (n = 30). Means sharing the same superscript are not significantly different P > 0.05.

Strain	CV	(%)	se
Harvey River	36	a	1.56
Margaret River	45	a,b,c	3.02
Kent River	70	d	0.51
Shannon River	47	a,b,c	7.17
Donnelly River	47	a,b,c	3.61
Warren River	58	c,d	1.93
Blue Line	35	a	7.58
Pemberton Line	51	b,c	4.03
Mass Selected Line	47	a,b,c	6.28
Industry Line	43	a,b	3.84

Sex Ratio of marron strains

All of the strains had an equal sex ratio when stocked as juveniles (Table 6).

Table 6. The sex ratio of females (%) and males (%) (n = 30) in each genetic line at stocking 30/7/2001 (Chi-square test sex ratio 1:1, P > 0.05 indicates that sex ratio is not significantly different from 1:1 at the 0.05 level).

Strain	Female (%)	Male (%)	se	P =
Harvey River	59	41	1.31	0.31
Margaret River	49	51	2.55	0.74
Kent River	50	50	5.30	0.27
Shannon River	47	53	1.66	0.75
Donnelly River	50	50	4.74	0.34
Warren River	52	48	2.01	0.76
Blue Line	40	60	3.28	0.34
Pemberton Line	52	48	1.90	0.77
Mass Selected Line	53	47	4.53	0.30
Industry Line	51	49	4.89	0.32

At the conclusion of the experiment there was no difference in sex ratio, although Shannon River Line showed a tendency towards more females (Table 7).

Table 7. The sex ratio of females (%) and males (%) (n = 30) in each genetic line at the conclusion of the experiment (17/03/2003) after two years grow-out (Chi-square test sex ratio 1:1, P > 0.05 indicates that sex ratio is not significantly different at the 0.05 level).

Strain	Female (%)	Male (%)	se	P =
Harvey River	62	38	2.12	0.35
Margaret River	56	44	5.49	0.26
Kent River	48	52	4.15	0.52
Shannon River	68	32	5.35	0.11
Donnelly River	52	48	5.67	0.39
Warren River	57	43	1.92	0.39
Blue Line	42	58	2.88	0.75
Pemberton Line	46	54	3.44	0.59
Mass Selected Line	52	48	3.44	0.72
Industry Line	54	46	2.76	0.74

The reduced survival of Shannon River marron between November 2002 and March 2003 (Table 4) is most likely due to the reduced survival of males during the summer period (Table 7 & 8).

Table 8. The sex ratio of females (%) and males (%) (n = 30) in each genetic line in November 2002.

Strain	Female (%)	Male (%)	se
Harvey River	65	35	4.42
Margaret River	53	47	3.85
Kent River	49	51	5.50
Shannon River	51	49	4.27
Donnelly River	48	52	0.23
Warren River	51	49	2.76
Blue Line	41	59	4.51
Pemberton Line	47	53	2.44
Mass Selected Line	50	50	1.43
Industry Line	46	54	1.43

Sexual dimorphism

For most genetic lines, males were larger than females before 24 months old (Table 9.) There was no difference in the mean weight of males and females from the Kent, Blue and Pemberton Lines (Table 9).

Table 9. Mean weight of males and females (g ± se) (n = 30) for each genetic line at 23 months old (11/11/02).

Strain	Female Mean wt (g)	se	Male Mean wt (g)	se	P =
Harvey River	98.92	3.79	120.76	10.88	0.031
Margaret River	55.87	3.97	82.70	1.65	0.003
Kent River	57.36	5.50	76.24	8.70	0.140
Shannon River	61.18	4.88	88.61	3.05	0.009
Donnelly River	53.12	0.81	73.35	6.98	0.045
Warren River	54.09	1.72	75.67	6.37	0.031
Blue Line	85.04	7.69	96.73	9.23	0.386
Pemberton Line	67.37	3.81	83.83	6.58	0.096
Mass Selected Line	63.96	3.95	95.67	3.21	0.003
Industry Line	40.52	6.01	66.53	7.08	0.049

Precocious sexual maturity and effect upon growth

The Harvey Line (8.42%) had the lowest percentage of early maturing females (Table 10). For all genetic lines, except Industry Line ($P = 0.29$) , females that did not breed in their first year were larger than those that mated ($P < 0.05$) (Table 10).

Table 10. Percentage of berried females and mean weight of berried and non berried females (g ± se) for each genetic line (n=30).

Strain	% Females berried	se	Mean Weight Non-berried females	se	Mean Weight berried females	se	P =
Harvey River	8.42	5.99	103.62	6.78	54.69	3.92	0.013
Margaret River	51.73	15.94	67.54	2.03	43.84	3.45	0.004
Kent River	40.43	1.50	69.59	4.68	39.32	6.94	0.022
Shannon River	20.97	4.37	66.25	4.98	40.15	5.71	0.026
Donnelly River	39.73	10.62	58.31	1.14	44.10	2.16	0.004
Warren River	42.24	6.36	69.42	5.38	34.79	1.84	0.004
Blue Line	34.72	19.30	102.29	6.50	56.05	1.61	0.012
Pemberton Line	26.94	3.80	75.52	5.55	44.94	1.27	0.006
Mass Selected Line	34.50	2.32	70.94	4.13	50.84	4.66	0.032
Industry Line	58.06	13.68	45.31	7.72	34.82	3.96	0.293

There is a strong relationship between % females berried and the mean weight of females in each genetic line (r = -0.81, P = 0.005) (Figure 3). Consequently, the percentage of females berried also affects the mean weight of each genetic line (r = -0.77, P = 0.005). The % berried females therefore has a large contribution to the final mean weight recorded for each strain and therefore growth.

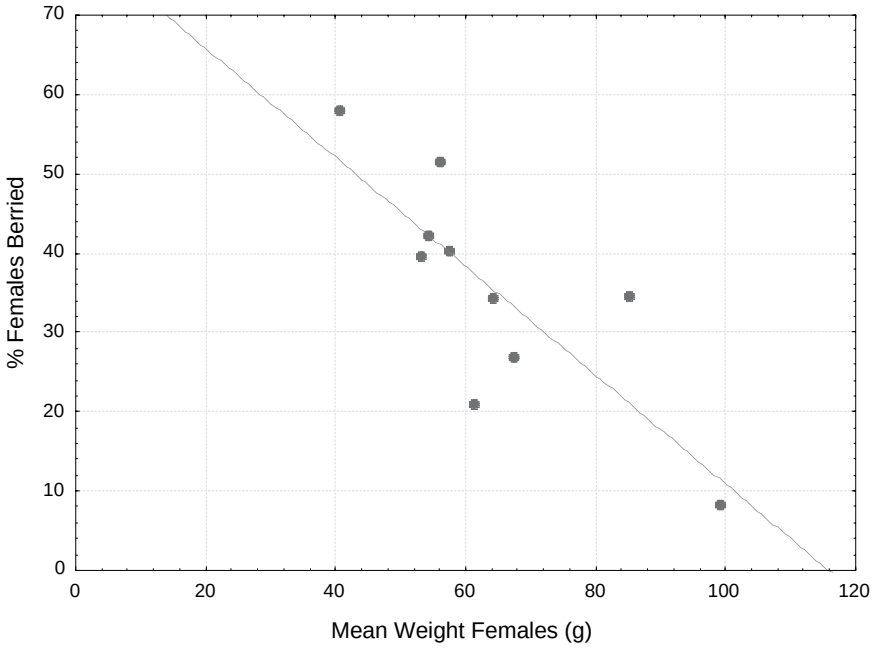

Figure 4. Mean weight females (g) and percentage of females berried in each genetic line (r = -0.81, P = 0.005).

Economic analysis

In comparison to the Industry Line farmed currently, the improved growth rate recorded for the lines in this experiment produced an increase in mean weight of between 9% (Warren River) to 82% (Harvey River) (Table 11).

Table 11. Growth increase of genetic lines compared to Industry Line.

Strain	Final Mean Weight	% Change Compared to Industry Line
Harvey River	130	82
Blue Line	107	51
Mass Selected Line	100	40
Pemberton Line	94	32
Margaret River	83	16
Shannon River	82	15
Donnelly River	81	13
Kent River	80	12
Warren River	78	9
Industry Line	71	0

On a model 50 pond marron farm Marron Profit© software demonstrates that replacing industry stock with the Harvey River Line on an average marron farm (Table 12) or a correctly designed, constructed and professionally managed farm (Table 13) would result in an increase in annual production, annual return (profit) and IRR.

Table 12. Economic evaluation of improved river line on an average marron farm (see Scenario 2, Section 7.1).

	Annual Prodn (t)	Annual gross revenue ($)	Prodn cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	5.7	136,457	25.79	-136,907	-11,936	4.57	0.92	-2.50
Harvey River	10.5	248,352	14.75	1,076,772	93,878	14.59	1.61	19.69

Table 13. Economic evaluation of improved river line on a farm that is correctly designed, constructed and professionally managed (see Scenario 3, Section 7.1).

	Annual Prodn (t)	Annual gross revenue ($)	Prodn cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	6.8	156728	22.08	80408	7010	6.79	1.05	1.47
Harvey River	12.3	285245	12.71	1472286	128361	17.06	1.82	26.92

Therefore the adoption by industry of the best river line of marron evaluated in this study should provide farmers with an increase in production of 82%, increase in profit of 1,730%, a 43% reduction in the cost of production and an increased internal rate of return of between 150-220%.

Discussion

The Harvey River (82%), Blue Line (51%), Mass Selected Line (40%), Pemberton Line (32%) and Margaret River (16%) all grew faster than the Industry Line. The lower growth rates of the Industry Line is most probably due to ongoing selection by farmers of larger animals for sale, with subsequent breeding from smaller slower growing marron. This negative genetic selection is unintentional but has resulted in slower growing animals on commercial farms, when compared with wild populations. The improved performance of the Mass Selected Line demonstrates the ability to rectify this growth disadvantage by selecting broodstock from the largest 2 year old marron.

The best performing genetic line was the Harvey River Line due to fast growth, low CV and fewer early maturing females. In 2003 the Department of Fisheries established a unique recreational fishery management strategy for the Harvey River with a minimum OCL of 90 mm with a bag limit of 5, whereas the OCL for other river systems in Western Australia is 76 mm and a bag limit of 10. This strategy will protect the larger marron in the Harvey River from exploitation and permit these larger animals to breed. This should ensure ongoing neutral or positive selection for growth of the Harvey river marron population.

The commercial value of the Pemberton Line is reduced due to the production of two morphotypes "brown" and "blue" at a ratio of 81% Brown : 19% Blue (se 1.52). Breeding the blue gene out of the Pemberton Line would increase its value for commercial farming. While the colour of the Blue Line limits its value for commercial food production, it does provide a useful well-performing control for evaluating improvement obtained in future genetic selection programs.

The marron strains evaluated in this study had a wider range of breeding success (71%) than those reported previously by Henryon (1996) (53%). Most notable was the difference in % berried females of the Margaret River Line, which was 53% in this study and 11% in Henryon's (1996) experiment, and the Warren River Line, which was 84% in this study and 25% in Henryon's (1996). Conversely, the Donnelly River Line had a breeding success of only 35% in this study, but 61% in Henryon's (1996) experiment. The faster growing Harvey River Line had relatively low breeding success (13%). However, this may be due to the size/age of broodstock collected initially for this project as this strain has delayed sexual maturity in comparison to other lines (Table 10).

There is a strong relationship between early maturing females that mate at only 1 year of age and the mean weight of each genetic line (r = -0.77, P = 0.005). Growth of female marron is reduced when they become sexually mature. Aside from developing a technique to produce only male marron, or females that do not mature, there are two strategies that could improve production. First, farming a late maturing line, such as the Harvey River Line, will reduce the proportion of 1 year old females that reproduce by 50%. Second, a selection program that culls early maturing females and selects for those that do not mature until 2+ years of age is likely to result in larger marron (see section 5.1).

The results of this experiment indicate that the profitability of marron farming can be greatly increased by industry adopting the better performing genetic lines of marron evaluated in this study. Adoption by industry of the best river line of marron evaluated in this study should provide farmers with an increase in production of 82%, increase in profit of 1730%, a 43% reduction in the cost of production and an increased internal rate of return of between 150-220%.

1.2 *Variation in yield of tail flesh among marron strains*

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Introduction

The increase in commercial marron production has led to the development of a breeding program for this species. While growth rate is important to the producer, tail meat yield is significant for the consumer. Growth rate is easily measured, but tail yield cannot be estimated without killing the animals. Therefore an accurate predictor of meat yield in live individuals would be beneficial.

The aim of this experiment was to determine if the yield of tail meat can be accurately predicted from linear measurements made on live animals. A second aim was to determine the amount of variation in tail flesh yield and body morphology among strains of marron that might be useful to producers in a breeding program.

Industries such as those using cattle, sheep and pig have identified meat yield and flesh composition as very important traits when selecting stock, because that is what the consumer is interested in buying. In general, the tail of each marron is 42% of the total body weight with the flesh in the tail constituting around 31% of the total body weight (Morrissy 1976). Recovery rates such as this compare favourably to marine crayfish species such as the Western Rock Lobster (tail flesh and shell 40%) (Morrissy 1976). The recovery of tail meat from marron also compare very well with other freshwater crayfish such as *Procambarus* spp. (red swamp crayfish and white river crayfish) with 11-25% and *Orconectes* spp. (rusty crayfish and calico crayfish) with 6-16% (Huner 1993). The recovery of tail meat from marron is also much higher than *P. leniusculus* (11-15%) and *Astacus. leptodactylus* (9-13%), the two main freshwater crayfish species in Britain (Harlioglu 2001).

The claws of marron provide approximately 15-20% of bodyweight, and contain around 10% flesh, so they can be an important meat source (Merrick 1991). However, reducing the claw size can be beneficial, to give a smaller claw weight to carapace weight ratio.

Selective breeding requires the identification of animals in the population that are superior in the characteristics included in the breeding objectives. This can be simple for traits such as growth rate, which is estimated from their bodyweight and age. However, others such as tail yield are difficult to measure without slaughtering the animals. Huner et al. (1990) predicted meat yield of the crayfish *Astacus astacus* and *Procambarus clarkia* using regression equations based on measurements of their cephalothorax length. Ryan (2001) used measures of body weight, tail length, width and depth to develop a regression equation to predict meat yield in marron (R^2 = 98.6, MSE = 3.2 for females). The only issue with this kind of regression is it requires taking several measures of distance to predict a volume (tail meat) which means the error component can be high. Standardisation of measurements is therefore necessary for best accuracy. Predictions such as this would be beneficial to the marron industry for both selective breeding and for changing the way animals are priced.

Methods

Predictive equations for tail meat

Several equations for predicting the tail meat yield of marron were developed using measures of the length, width and depth of the tail, carapace width, orbital carapace length (OCL) and bodyweight. The sex and the strain of marron were also taken into account.

Differences in tail flesh as a percentage of bodyweight for male and female marron were compared among strains using 390 animals from 6 different river populations (Table 1) that ranged in size from 23 g to 275 g. The ratio between the tail and OCL for males and females were then used to account for any differences found in tail meat yield among strains.

Table 1. The number of males and females sampled from each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Male	25	32	39	23	25	36
Female	23	36	43	41	23	44
Total	48	68	82	64	48	80

These animals were all fed crayfish pellets at the same rate and were all 2 years of age (see Section 1.1).

Linear Measurements

Each animal was hand-sexed by the presence of penes at the base of the fifth pair of pereiopods for the males and gonopores at the third pair of pereiopods for the females. The bodyweight of each animal was recorded.

Three measures of the tail were taken, in accordance with those by Rhodes and Holdich (1979), Grandjean et al. (1997) and Huner et al. (1990) on the White-clawed crayfish *Austropotamobius pallipes* (Lereboullet) and the Noble crayfish *Astacus astacus* (linne'). The tail length was taken from the edge of the carapace to the anus (Figure 1); the tail width (Figure 1) and depth (Figure 2) were both taken on the second tail segment from the carapace.

The Orbital Carapace Length (OCL) was measured on each animal from where the carapace meets the tail up to the eye socket on the right side of the animal. A measure of carapace width (Figure 3) was taken across the carapace. Chelicera length (Figure 3) and width (Figure 3) measurements were also recorded.

Each animal was assessed for moult stage and any that were found to have moulted recently were discarded from the experiment.

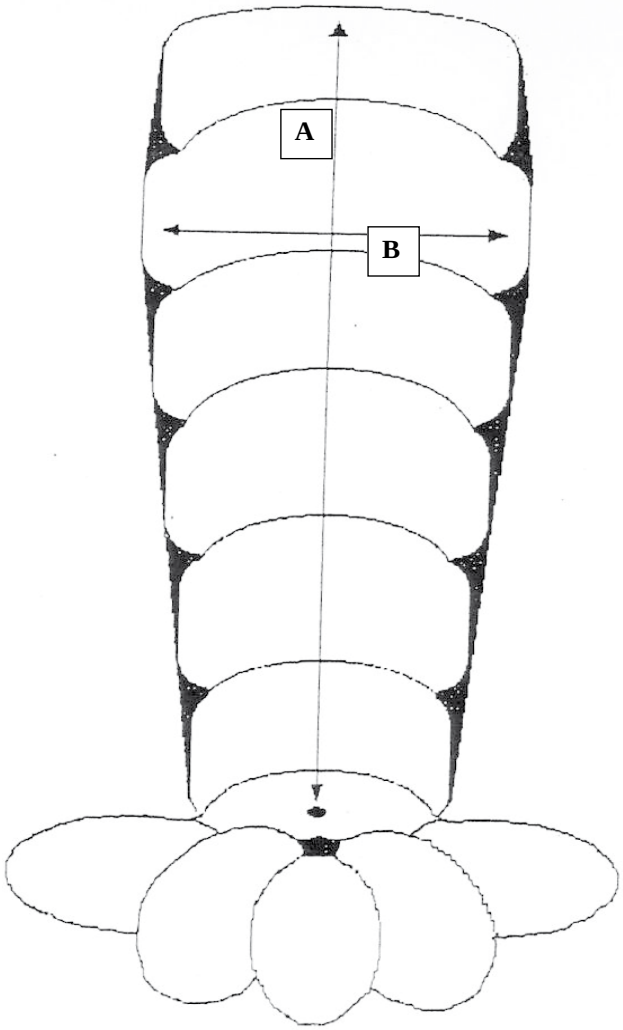

Figure 1. The position that the tail length (A) and tail width (B) measurement was taken on each animal.

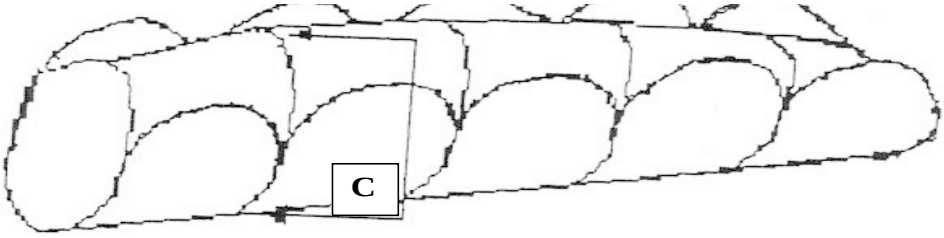

Figure 2. The position that the tail depth (C) measurement was taken on the tail of each animal (measured dorsal-ventrally).

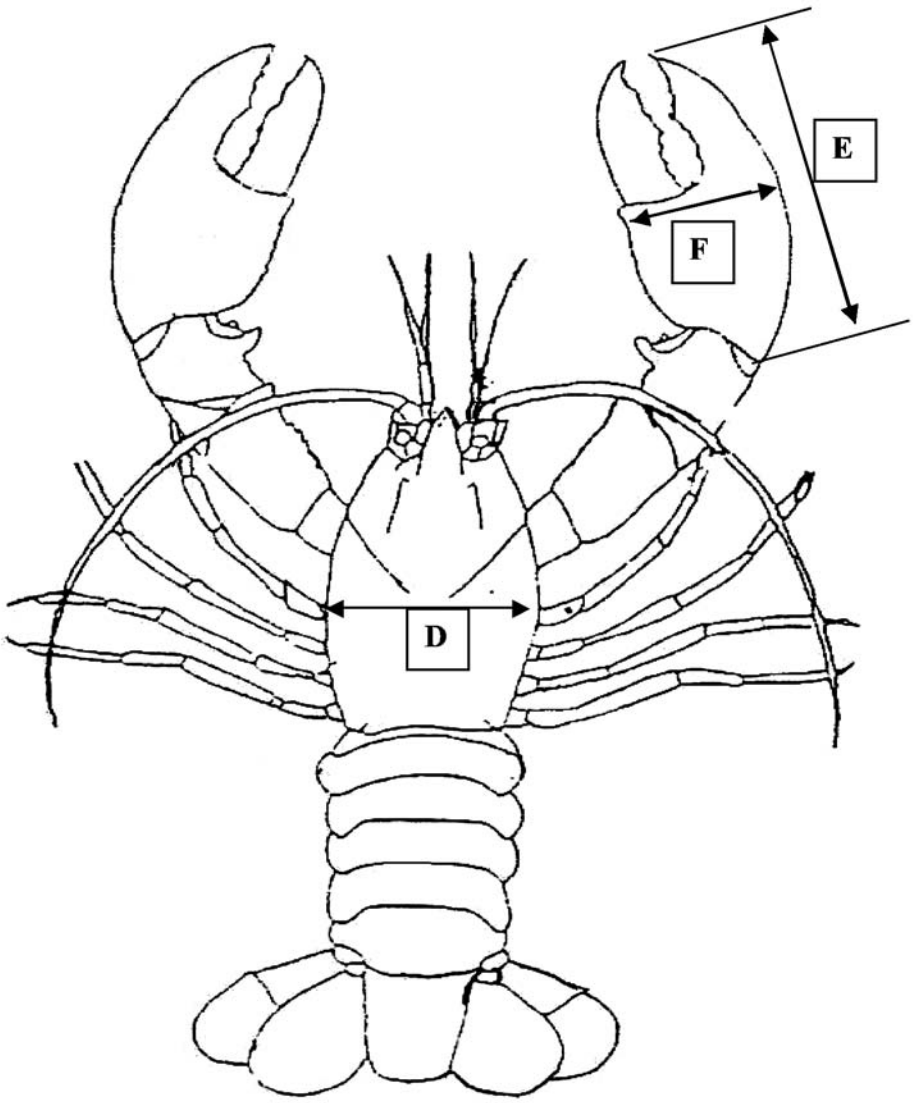

Figure 3. The position of the carapace width (D), chelicera length (E) and chelicera width (F) measurements were taken on each animal.

Experimental Procedure

Animals were sexed, weighed and vernier calipers were used to take all linear measurements. Animals were marked using liquid paper on their carapace to distinguish individuals. Animals were then cooked in 55 liters of boiling water for 4 minutes. The animals were removed and cooled in icy water at approximately 5°C for 3 minutes. The weight of each animal after cooking was measured and recorded. The tail flesh of each animal was removed, weighed and recorded.

Statistical Design

All statistical analyses were performed using Microsoft Excel, and Genstat 6.1. The equations for predicting tail flesh yield were calculated by multiple linear regressions based on objective measures of each animal. The sex of the animal and which strain it was derived from were also included in developing the equations. The accuracy of these equations was tested by an adjusted R squared value and a mean squared error value.

Tail meat yield

The tail meat yield of males and females as a percentage of bodyweight for each strain was recorded. The percentages were transformed by the arc sin2 function to give the data a normal distribution (Quinn and Keough 2002), allowing one-way analysis of variance to be used.

From this transformed data, 2 sets of one-way analysis were done, one between males from each strain and the other between females of each strain. Male and female values were treated separately because the differences in their morphology can affect results when their numbers are not balanced, which is the case in this experiment. All strains were compared to one another using a multiple comparison Bonferroni test, which allowed a comparison between all the strains even though the number of animals was unbalanced (Quinn and Keough 2002). This test is stricter than a general analysis of variance because it adjusts the significant levels based on error rates during the multiple testing (Quinn and Keough 2002). Any differences in meat yield between strains were accounted for by multiple comparison analysis of variance on the average tail length, tail width and tail depth to OCL ratio for males between strains and females between strains.

Meat Yield in Females and Males

The meat yield in females and males was analysed by using the measures of meat yield, transformed as described above, and a t-test within each strain, between the average values for male and female tail meat yield. Another t-test was done for tail meat yield between all males and all females used in the experiment. A t-test between the average ratio of tail length to OCL calculated for pooled values of males and females was used to try and account for any differences in tail flesh yield between males and females.

Meat yield from different river populations

The meat yield from different river populations was analysed by using measures of claw length and width to determine if a particular strain grew disproportional claws. This was done by using a one-way analysis of variance of the average claw ratio for each strain. The proportions were measured by the following claw ratio in equation 1;

$$\frac{\text{Left Claw Length/Left Claw Width}}{\text{Right Claw Length/Right Claw Width}}$$

Equation 1. The equation for testing the proportionality of each animal's claws.

This ratio gives values between 0 and 2, with a value of 1 showing the claws on a particular animal are in perfect proportion.

A one-way analysis of variance was also used to test for differences in claw size as a proportion of overall body size for males and females among the 6 strains. This was done by comparing the average of the ratio of claw width to OCL, shown below in equation 2;

$$\frac{\text{Left Claw Width}}{\text{OCL}} + \frac{\text{Right Claw Width}}{\text{OCL}}$$

Equation 2. The equation for testing the claw size, measured as claw width, as a proportion of body size, measured as OCL.

The analysis of variance was between the average value of this ratio for males from each strain and then another analysis of variance between strains for the females. This is because males are known to have larger claws, which would affect the accuracy of the analysis (Rhodes and Holdich 1979).

Results

Predictive equations for tail meat

All the measures used to predict tail meat yield had very high adjusted R squared values and low mean squared values (Table 2).

Table 2. The predictive regression equations for tail flesh (g) of marron. Sex is represented as male (1) or female (0) and strains represented as 1 when used and 0 when not.

Body measurements	Predictive equation for tail flesh (Y =Tail flesh yield)	Adjusted R2	Mean squared error
Bodyweight (BW)	Y=2.24BW + 2.2	95.1	2.06
Tail length (TL)	Y=1.08TL – 44	85.4	3.55
Tail width (TW)	Y=2.25TW – 40.5	89.4	3.02
Tail depth (TD)	Y=2.99TD – 31.92	86.3	3.44
Tail length, Tail width, Tail depth	Y=0.32(TL) + 0.87 (TW) + 1.17(TD) – 42.95	93.9	2.30
Bodyweight, Sex (S)	Y=0.25BW – 1.88S + 2.2	95.9	1.88
Bodyweight, Sex, Tail width, Strain Donnelly (D) Industry (I) Kent (K) Margaret (M) Pemberton (P) Warren (W)	Y=0.19BW – 0.95S + 0.5TW(D) + 0.49TW(I) + 0.55TW(K) + 0.48TW(M) + 0.51TW(P) + 0.49TW(W) – 7.70	96.8	1.67
Bodyweight, Sex, Tail width, OCL, Tail length, Tail depth, Carapace width (CW)	Y=0.17BW + 0.23CW – 1.18S – 0.22OCL + 0.52TD + 0.09TL + 0.29TW – 11.34	96.9	1.65

The most accurate equation for predicting the tail flesh yield (Y) of male and female marron in grams was calculated using bodyweight (BW), sex (S), orbital carapace length (OCL), tail length (TL), tail width (TW), tail depth (TD) and carapace width (CW) and is shown in equation 3. This equation had an adjusted R square value of 96.9 and the lowest mean squared error value of 1.65.

Y = 0.17BW + 0.23CW - 1.18S -0.22OCL + 0.52TD + 0.09TL + 0.029TW - 11.34

Equation 3. The most accurate equation for predicting tail meat yield in marron.

The most accurate predictive equation when taking the strain of the animals into account used bodyweight, sex, tail width and strain and is shown in equation 4. It has an adjusted R square value of 96.8 and a mean square error of 1.67.

Y = 0.19BW - 0.95S + 0.5TW (D) + 0.49TW (I) + 0.55TW (K) + 0.48TW (M) + 0.51TW (P) + 0.49TW (W)

Where

D – Donnelly strain

I – Industry strain

K – Kent strain

M – Margaret strain

P – Pemberton strain

W – Warren strain

Equation 4. The most accurate equation for predicting tail flesh yield of marron using strain as a factor.

Using only bodyweight and sex as the components for predicting tail flesh yield shown in equation 5, was also quite accurate, with an adjusted R square value of 95.9 and a mean square error value of 1.88.

Y = 0.25BW - 1.88S + 2.2

Equation 5. The simplest predictive equation for tail flesh of marron.

Yield of tail meat between strains

For the females, the difference between the average for the highest (Kent) and lowest (Margaret) yielding strains was 2.2% (Table 3). The analysis of variance between the transformed values for females found significant differences among the strains ($P < 0.001$). To determine which strains were significantly different to one another a multiple comparison Bonferroni test was used (Quinn and Keough 2002). This test showed that the mean yield of tail meat from the Kent river strain was higher than all other strains (t-value > 3.3), except the Industry strain. The Industry strain was shown to be significantly higher than the lowest yielding Margaret river strain.

For the males, the difference between the average for the highest (Kent) and lowest (Industry) yielding strains was 3.1% (Table 3). The analysis of variance for the transformed values of tail meat yield in males also showed there to be significant differences among the strains ($P < 0.001$). As for the females, a multiple comparison Bonferroni test was used to detect differences between each strain. This test revealed that the mean yield of tail meat from the

Kent strain was significantly higher than all other strains (t-value > 3.2), except the Pemberton strain. The Pemberton strain was significantly higher than the lowest yielding strain, Industry.

Table 3. The average yield of tail meat as a percentage of bodyweight for males and females from each strain. These values after the arc $\sin^2$ transformation are also given.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Female (%)	27.1	27.7	29.3	27.7	27.9	28.5
Female adjusted	0.076	0.079	0.088	0.079	0.080	0.083
Male (%)	24.6	24.9	27.2	25.2	26.0	24.1
Male adjusted	0.062	0.064	0.076	0.066	0.069	0.060

Tail length to OCL proportions between strains

The values in Table 4 represent the average length of the tail as a proportion of the animal's body size, measured in this case as OCL. The actual values are not as important as how they differ between strains. A higher value for one strain indicates a longer tail compared to body size than another strain.

Table 4. The average value for the ratio of tail length to OCL for females and males of each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Females	1.31	1.36	1.34	1.28	1.31	1.34
Males	1.27	1.30	1.27	1.24	1.25	1.27

A one-way analysis of variance was used to test for significant differences between strains. Again males and females were kept separate to avoid bias due to sexual dimorphism.

For the females, significant differences were found in the average tail length to OCL ratio among the strains ($P < 0.001$). The multiple comparison tests found that the Industry and Kent river strains had a significantly longer tail than the Donnelly river strain. The Warren river strain had a significantly longer tail than the Pemberton, Donnelly and Margaret river strains. For the males, there were no significant differences between the strains ($P = 0.151$).

Tail width to OCL proportions between strains

A multiple comparison analysis of variance was used to test for significant differences of values in Table 5 between strains. For the females, only the Kent strain was larger than the Donnelly strain ($P = 0.04$). This corresponds to the Kent river strain having a wider tail on average than the Donnelly river strain.

For the males, the Pemberton strain had a wider tail than the Donnelly and Industry strains ($P = 0.001$).

Table 5. The average value for the ratio of tail width to OCL for females and males of each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Female	0.607	0.613	0.613	0.597	0.608	0.606
Male	0.577	0.576	0.576	0.567	0.584	0.567

Tail depth to OCL proportions between strains

A multiple comparison analysis of variance was used to test for significant differences between the strains (Table 6). For the females, the Kent river strain had significantly deeper tails than all other strains, except the Pemberton strain (t-stat > 3.3).

For the males, the tails of the Kent and Pemberton strains were significantly deeper than the Donnelly and Industry strains (t-stat > 3.2).

Table 6. The average value for the ratio of tail depth to OCL for females and males of each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Female	0.375	0.380	0.399	0.368	0.394	0.372
Male	0.384	0.383	0.394	0.371	0.398	0.375

Meat yield of male and females within strains

The values in Table 7 show that there are differences between male and female tail meat yield within every strain tested (t-stat > 1.96). The values in Table 3 show that there is between 1.9% to 4.4% difference in average tail meat yield between males and females in the strains tested.

Table 7. The t-statistic values for the t-test between the average tail meat yield of males and females within each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
t-stat	2.01	2.00	1.99	2.04	2.01	1.99

Overall meat yield of males and females

The average yield of tail meat was 2.8% higher in females than males (Table 8). The t-statistic showed that this is a significant difference in yield (t-stat = 1.96).

Table 8. The average tail meat yield for all males and females used in the experiment.

	Males	Females
Number of animals	180	210
Average Yield (%)	25.4	28.2

The difference in yield of tail meat between males and females over a range of bodyweight is also provided in figure 4. The trendline shows that the yield of tail meat is higher in females than males at the same bodyweight.

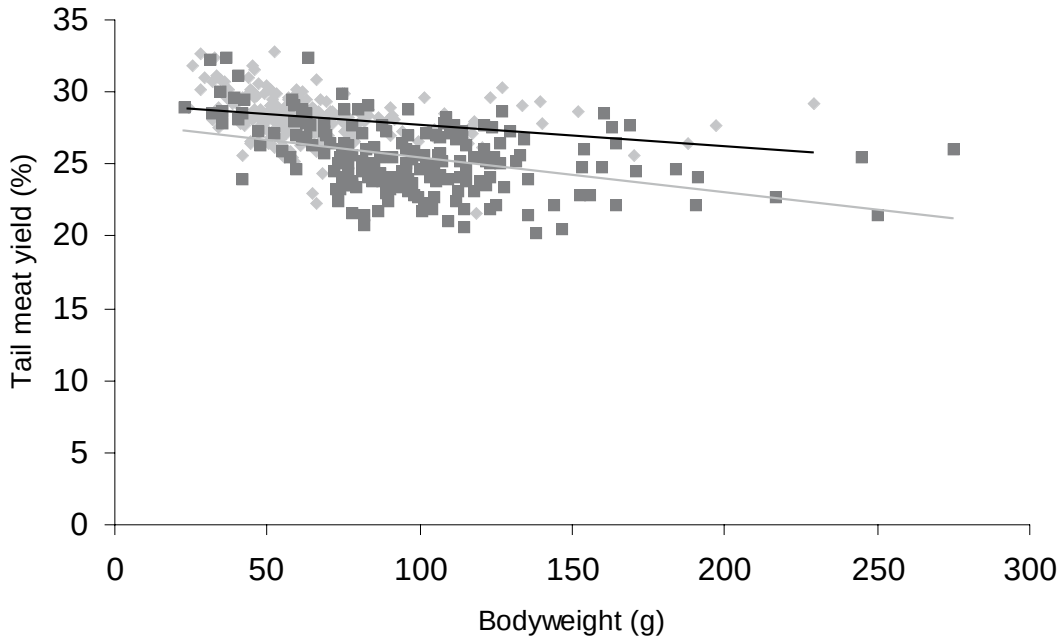

Figure 4. The tail meat yield of males (■) and females (♦) and their respective bodyweights. A trendline is also shown for males (---) and females (---).

Tail length to OCL proportions of male and females

A t-statistic of the values in Table 9 showed that females have a significantly higher tail length to OCL ratio (t-stat < 0.001). This means that female marron have a longer tail than males relative to body size, measured as OCL.

Table 9. The average values for tail length to OCL ratio of all males and females.

	Male	Female
Number of animals	180	210
Tail length to OCL	1.27	1.33

Claw to claw proportions

All strains had a very high degree of symmetry in their claws (Table 10), and the analysis of variance showed no significant difference among the strains (P = 0.822).

Table 10. The value for the proportionality ratio of the claws in each strain. A value of 1 indicates perfect symmetry between claws.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Claw Ratio	1.01	1.01	1.01	0.99	1.01	1.00

Claw to body size proportions

The analysis of variance between the values in Table 11 for males found a significant difference among the strains (P < 0.001). A further multiple comparison Bonferroni test showed that the Kent river strain had significantly smaller claws relative to OCL than all other strains, except the Pemberton strain.

Table 11. Values for the ratio between the average claw width and OCL for males and females of each strain.

	Margaret	Warren	Kent	Donnelly	Pemberton	Industry
Male	0.40	0.39	0.34	0.38	0.36	0.40
Female	0.32	0.32	0.29	0.31	0.30	0.31

For the females, there was also a significant difference among the strains in the average claw width to OCL ratio (P < 0.001). The multiple comparison Bonferroni test again showed that the Kent river strain had significantly smaller claws relative to OCL than all other strains, except the Pemberton strain.

Discussion

Measurements of tail morphology can be used to predict tail meat yield in marron. Several of the equations had an adjusted R square value of over 95.0 and a mean square error value below 2.0. They also take factors such as sex and the strain of the animal into account when predicting tail meat yield, making a single equation applicable to all animals. Ryan (2001) produced an equation for males and another for females, and used a single Pemberton cross Industry strain to develop his equations. The most accurate of these equations developed by Ryan (2001) for predicting yield of tail meat was for males and had a higher adjusted R square of 98.6 than any of those developed in this experiment and a mean square error value of 3.2.

The high level of accuracy of the equations also developed here, though, suggests that the situation should dictate the type of equation used. For example, in the field to quickly monitor the yield of tail meat of stock, the grower may choose to use the equation using just bodyweight

and sex (R^2 = 95.9, MSE = 1.88) because it requires only one measurement for a high level of accuracy. It is not worth the producer taking several more measurements of OCL, carapace width and tail length, width and depth for an equation that is only a slightly better predictor (R^2 = 96.9, MSE = 1.65). But, to price an animal based on its tail flesh yield the predictor that gives the most precise value of flesh yield available should be used. This may require several body measurements but is necessary to ensure the price paid by the consumer and to the producer is justified.

As an example of how much difference these equations can amount to, the values for a 102.9 g female from the Kent river strain was put into each equation. This animal was used because Kent females were found to have the highest tail flesh yield and, as such, are an example of a potential future stock in a common weight range. The regression using only sex and bodyweight as predictors estimated 26.04 g of tail flesh, while the second regression with a higher level of precision using sex, bodyweight, OCL, carapace width and tail length, width and depth measurements estimated 26.64 g of tail flesh.

This shows that by going to the trouble to take the extra measurements, only an extra 0.6 g of flesh was estimated. This appears a waste of time for the breeder if there are many animals to measure, but if their price is determined by the flesh yield, the second regression is predicting roughly an extra 2.3% in tail flesh, which would amount to 2.3% extra revenue. Showing again that when pricing an animal, precision in estimating the yield of tail meat is important.

Yield of tail meat of marron differs among river populations. Male and female tail flesh yield was analysed separately due to their morphology differences (Rhodes and Holdich 1979; Lindqvist and Lahti 1983). However, the Kent river strain was shown to have a significantly higher tail flesh yield as a percentage of bodyweight than all other strains tested, except for Pemberton strain for males and Industry strain for females. This corresponds to Kent having a 3.1% and 2.2% higher yield of tail flesh for males and females than the lowest yielding strains (Industry and Margaret). If prices were determined by the flesh yield, this would correspond to a 3.1% or 2.2% higher amount of revenue to the producer just by stocking the Kent strain instead of Industry or Margaret.

Henryon (1994) found the Warren strain to have the highest yield of tail meat of those he tested. The Kent river strain was not included in Henryon's study but, in this experiment, it had a significantly higher yield of tail flesh than the Warren strain. This indicates that of the strains tested so far, the Kent river strain has the highest tail flesh yield as a proportion of bodyweight.

Measures of tail length, tail width and tail depth from each strain for males and females as a proportion of body size, measured as OCL, were used to try and account for the differences in tail flesh yield among strains. The highest yielding Kent strain was expected to have an overall larger tail compared to body size. However, this failed to be the case. Of the males, there were no differences in tail length between the strains, while the Pemberton strain had a significantly wider tail than the Donnelly and Industry strains. The Kent strain did have a deeper tail than the Donnelly and Industry strains, but it was not significant.

Of the females, the tail length to body size comparison between each strain gave mixed results, with no strain having a clear advantage in tail length compared to the others. The highest yielding Kent river strain had a significantly wider tail than the Donnelly strain and a deeper tail than all other strains, except for the Pemberton strain. This still does not suggest there are clear differences in the relationship between tail size as a proportion of body size and the yield of tail meat among the strains tested, so what makes the Kent have a higher yield of tail flesh?

Overall, the tail size of the higher yielding Kent strain has a tail that is not much bigger compared to the size of the whole animal than any other. But instead provides a higher proportion of weight in a whole body live weight measurement. The Kent river strain may therefore be smaller in other areas of the body not providing tail flesh.

Females have a higher yield of tail meat than males because the tail is larger in proportion to body size. The comparison of tail flesh yield as a proportion of bodyweight between male and females in each strain were all significantly different, with the females having a higher yield in each strain. A comparison of the tail flesh yield of all males with all females also showed the females to average a higher yield than males (2.8%). Overall, females have a significantly longer tail than males, supporting earlier work on morphology differences between male and female freshwater crayfish by Rhodes and Holdich (1979) and Lindqvist and Lahti (1983) that females have a larger abdomen used to care for offspring.

Marron from different river populations vary in their body morphology. Claws in all strains were found to have a high degree of symmetry, but the size of the claws in proportion to the rest of the body varied between strains. For both males and females, the Kent river strain had smaller claws when compared to their body size, measured as OCL, than all other strains except the Pemberton strain. This could help explain why the Kent river strain was earlier found to have a higher yield of tail flesh than most other strains but has a tail that was not clearly larger in proportion to the rest of the body for males and females of any other strain.

The Kent river strain may be growing smaller claws because it spends less energy fighting and competing for resources and so has more for general body growth. Perhaps marron in the future that have been selected for tail size, growth or lower aggression will have claws that are smaller than unselected strains.

2.0 Evaluation of marron strains Part II: Hybrid evaluation

2.1 Growth, sex ratio, fertility and production characteristics of marron hybrids

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Introduction

Hybridisation has traditionally been used to develop improved varieties of crops, livestock and poultry for terrestrial agriculture and, more recently, fish, crustaceans and molluscs for aquaculture (Benzie et al. 2001, Bosworth et al. 1994, Menzel 1987, Purdom 1993, Rahman et al. 1995, Tave 1993).

In freshwater crayfish, hybridisation of *Cherax* species within the yabby group from geographically isolated populations produced progeny that were infertile, female only or male only (Lawrence and Morrissy 2000, Lawrence et al. 2000). The male only hybrids produced from mating *Cherax rotundus* females x *Cherax albidus* males provided a 2 times increase in average size and a 4.6 times increase in crop value in experimental ponds than the commonly farmed *C. albidus* (Lawrence 2004).

In the south-west of Western Australia, a farming industry has developed for the marron (*Cherax tenuimanus*), which is a much larger and more valuable crayfish than the yabby (Lawrence 1998). Genetic variation has been shown to exist among wild marron (*C. tenuimanus*) populations (Imgrund 1998). This variation contributes to growth differences among marron populations in aquaria (Henryon 1996), reared communally in a concrete pond (Henryon 1996), and in combination with environmental factors may also affect wild fisheries. Although improved growth due to heterosis was not found from a diallel cross of 3 marron populations (Donnelly, Warren and Deep) (Henryon 1996), other hybrids from different river systems may have hybrid vigour or other traits that are useful for aquaculture such as skewed sex ratios or infertility.

The aim of this experiment was to compare the production traits of growth rate, sex ratio and fertility of hybrids with those of the 6 parent river strains.

Methods

Collection of stocks

Marron were collected from six wild populations Harvey River, Margaret River, Donnelly River, Warren River, Shannon River and Kent River between April – June 2000 and acclimatised in ponds at the Pemberton Freshwater Research and Aquaculture Centre (PFRAC) in Pemberton (Lat -34.45, Long 116.03) prior to the breeding season (Figure 1).

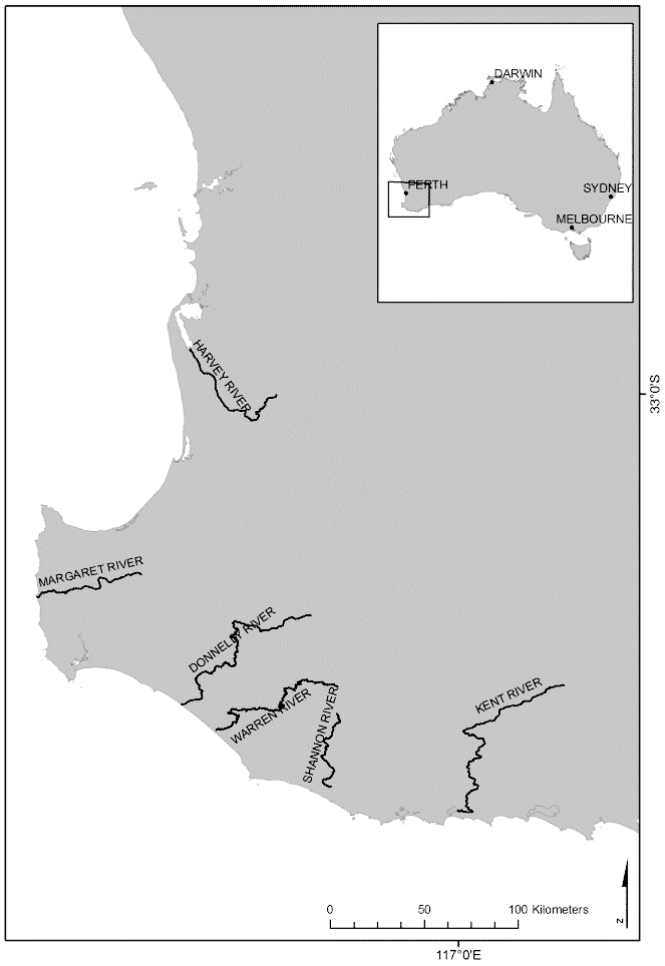

Figure 1. Collection localities for marron strains.

Production of hybrids

Hybrids were produced by establishing reciprocal crosses of these 6 populations in 10 concrete ponds (6 m x 2 m x 0.5 m) each divided into 3 equal sections (2 m x 2 m) by plastic mesh attached to a wooden frame at PFRAC in July 2000. A minimum of five males and five females from each river strain were placed in each section. Crosses established in July 2000 that did not produce hybrid progeny in January 2001 from the 2000/01 breeding season were repeated in July 2001 (in July 2001 the Margaret River female x Shannon male hybrid cross that previously produced progeny during the July 2000 breeding season was repeated to provide both a validation of breeding protocols and a control population between years) and again in July 2002.

Females carrying hybrid eggs were transferred to indoor 120 L glass aquaria at the UWA Aquaculture Laboratory, Perth in December 2000 and 2001. Each aquarium had a gravel substrate. To maintain water quality, each aquarium had a continuous inflow and drained to a communal biological filter, ensuring that environmental differences were minimised. Water chemistry (Ammonia, Nitrite, Nitrate and Ph) was recorded 3 times per week using a WinLab® LF 2400 photometer and pH using a Eutech meter.

Temperature was recorded by a Grant Instruments 1200 series (12-Bit) Squirrel data logger with U type thermistor temperature probes and programmed to record temperature every hour.

Stocking and rearing of hybrids
After hybrid juveniles were released from females in January 2001 and 2002 the adults were removed and mesh shelter for juveniles placed into aquaria.

Hybrid juveniles from all females within each cross were pooled and restocked into aquaria. Each treatment was repeated in triplicate and randomly stocked into 120 L aquaria at 25 juveniles/aquarium. To provide relative growth data, juveniles from each of the 6 wild strains were also stocked at the same density.

The 150 aquaria used in this experiment formed part of a recirculating system with a total volume of over 3200 m^3 consisting of over 800 m^3 of tanks and aquaria (n = 200) and a 127 m^3 biofilter. Therefore, each aquarium had the same water chemistry parameters.

Marron were fed daily to satiation on a rotating diet of marron pellets, prawn pellets, trout pellets, frozen daphnia and frozen bloodworms. Uneaten feed was siphoned out the following day.

Sex ratio and growth of hybrids
Data on sex ratio and relative growth of hybrids, on both wild and domesticated lines, was collected at 4 monthly intervals commencing in July 2001. Prior to this, juveniles were too small to sex and weigh without damaging animals. Growth rate data was collected until the marron reached 20 months of age (July 2002, July 2003).

Fertility of hybrids
At the conclusion of the growth evaluation trials in July 2002 and July 2003 the fertility of two year old hybrids was evaluated by pooling hybrids from each treatment into 22 separate 3 m diameter tanks at PFRC, one for each line, along with three control lines (Pemberton, Kent, Selected Stock). Fertility of hybrids was recorded in November 2003 and 2004 by observation of berried females in ponds and in February 2004 and 2005 by release of juveniles.

Statistical Analyses
Growth data was analysed by ANOVA using the means of replicates within each treatment group. Post hoc multiple pair-wise comparisons were completed using Fishers LSD to test significant differences among treatments.

To test if the sex ratio varied from 1:1 data was analysed using a Chi-square test with 5% level of significance to test the hypotheses H_o: The sex ratio of males to females is 1:1.

Heterosis for growth rate was calculated according to the following formula (Tave 1993):

$$\text{Heterosis} = \left[\frac{\text{average of the reciprocal hybrids - average of the two parental groups}}{\text{average of the two parental groups}}\right] \times 100$$

Results

Water chemistry

Water chemistry variables (Ammonia range = 0.001 – 0.012 mg/L, Nitrite range = 0.02 – 0.08 mg/L, Nitrate range = 0.05-0.2.5 mg/L, pH range = 7.2-9.1) remained within acceptable limits for marron production for the duration of the experiment.

Water temperature (range 11 - 27°C, mean = 18°C) remained within acceptable limits for marron production for the duration of the experiment (Figure 2).

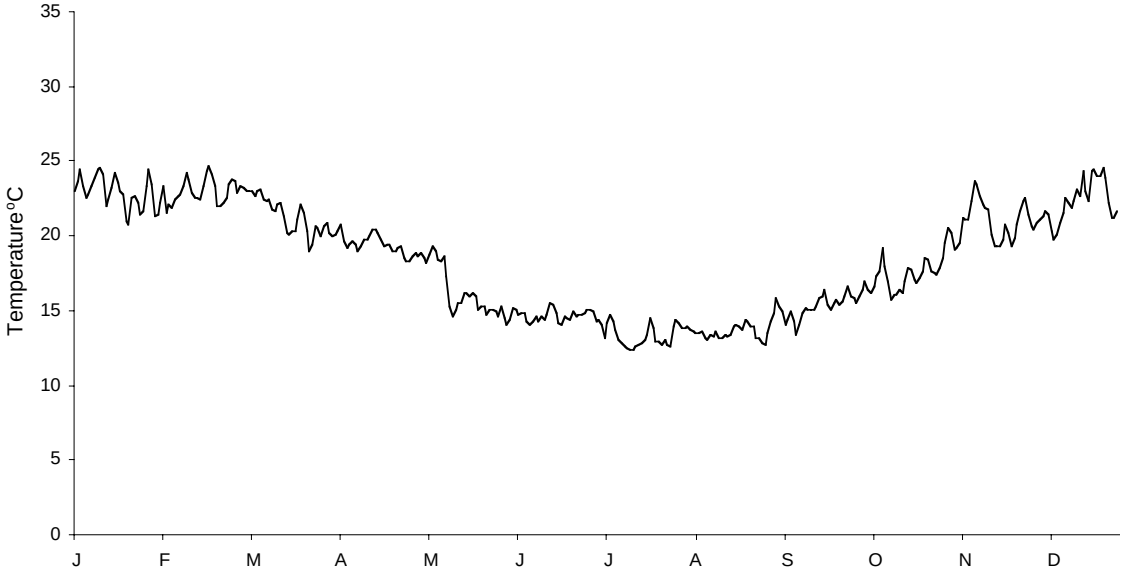

Figure 2. Average daily water temperature in aquaria at UWA Aquaculture Laboratory.

Production of hybrids

In each hybrid cross, one to eight females produced offspring, except for the Margaret River male x Harvey, Donnelly and Kent female mating combinations which failed to produce juveniles (Table 1).

Of 30 possible reciprocal hybrid mating combinations 26 were produced (21 hybrid populations were produced in 2001 and 5 additional populations were produced in 2002) (Table 1).

Table 1. Hybrids produced from marron strains.

(X = juveniles produced 2000/2001 breeding season X = juveniles produced 2001/2002 breeding season, * = no mating success)

		Male					
		Harvey	**Margaret**	**Donnelly**	**Warren**	**Shannon**	**Kent**
Female	**Harvey**	X	*	X	X	*	X
	Margaret	X	X	X	X	X X	X
	Donnelly	X	*	X	X	X	X
	Warren	X	X	X	X	X	X
	Shannon	X	X	X	X	X	X
	Kent	X	*	X	X	X	X

The mating success of hybrid crosses that produced juveniles ranged from 10 – 100% (Table 2).

Table 2. Mating success (% females berried) among river lines (X = juveniles produced 2000/2001 breeding season, X = juveniles produced 2001/2002 breeding season, * = no mating success).

		Male					
		Harvey	**Margaret**	**Donnelly**	**Warren**	**Shannon**	**Kent**
Female	**Harvey**		*	33	20	*	50
	Margaret	66		16	40	50 20	33
	Donnelly	20	*		90	83	60
	Warren	100	50	60		60	40
	Shannon	25	10	33	25		40
	Kent	50	*	37	78	75	

Sex Ratio of hybrids

The sex ratio of marron within each of the hybrid crosses was not significantly different from 1 male :1 female ($P > 0.05$) (Table 3). The Shannon male x Margaret River female produced an intersex juvenile that possessed both male and female external genitalia.

Table 3. The sex ratio of females (%) : males (%) (mean ± se) n=3 in each genetic line (Chi-square test sex ratio 1:1, P > 0.05 indicates that sex ratio is not significantly different from 1:1 at the 0.05 level) * = no mating success.

		Male					
		Harvey	**Margaret**	**Donnelly**	**Warren**	**Shannon**	**Kent**
Female	**Harvey**		*	49:51 ± 5.88 P = 0.73	46:54 ± 1.31 P = 0.47	*	45:55 ± 4.71 P = 0.76
	Margaret	51:49 ± 10.56 P = 0.38		54:46 ±4.46 P = 0.74	50:50 ± 2.98 P = 0.81	45:49:6 ± 5.00 P = 0.60	47:53 ± 2.36 P = 0.94
	Donnelly	37:63 ± 4.62 P = 0.44	*		56:44 ± 7.20 P = 0.51	44:56 ± 4.44 P = 0.77	57:43 ± 4.52 P = 0.49
	Warren	42:58 ± 0.60 P = 0.58	50:50 ± 0.00 P = -	57:43 ± 5.09 P = 0.77		21:79 ± 3.13 P = 0.12	45:55 ± 9.33 P = 0.35
	Shannon	39:61 ± 9.42 P = 0.40	51:49 ± 5.79 P =0.72	44:56 ± 5.92 P = 0.56	48:52 ± 10.39 P = 0.51		45:55 ± 4.41 P = 0.67
	Kent	51:49 ± 4.55 P = 0.88	*	52:48 ± 5.92 P = 0.80	42:58 ± 4.83 P = 0.82	50:50 ± 12.32 P = 0.47	

Growth of Hybrids

Growth of hybrids was comparable for only the first 15 months as thereafter, due to limitations with tank size, survival varied among replicates.

There was a significant difference in the growth of hybrids and their parent strains (P = 0.01). The fastest growing hybrid strain was Kent River ♀ x Margaret River ♂ (Figure 3).

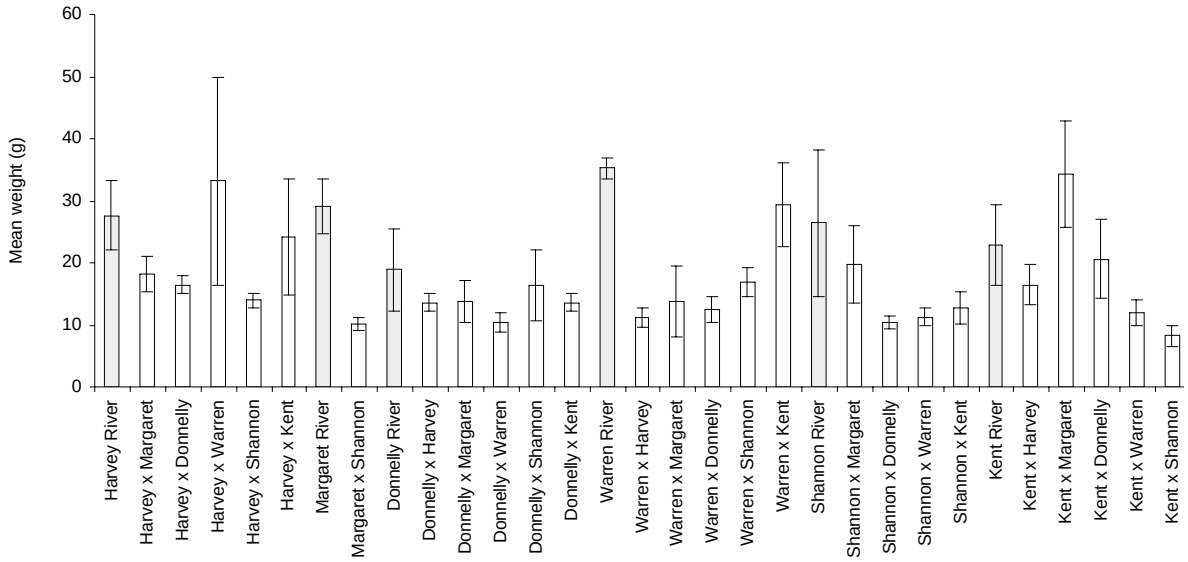

Figure 3. Mean weight (g ± se) of hybrids x (clear bars) and parent strains (shaded bars) (n=31) at 15 months of age in aquaria.

Most of the hybrids in this experiment did not grow faster than the "pure" river strains (Figure 3). Of the 25 hybrids that produced sufficient juveniles for analysis 22 grew slower than the average of the parent stains and 3 grew faster than the average of the parent strains (Table 4).

Table 4. Comparison of growth of 25 hybrid populations with the mean of parental wild strains.

Hybrid	Mean Wt Hybrids (g)	Mean Wt Parents (g)	% Difference
Kent x Margaret	34.17	25.97	32
Harvey x Warren	33.19	31.39	6
Warren x Kent	29.39	29.04	1
Kent x Donnelly	20.64	20.87	-1
Harvey x Kent	24.23	25.21	-4
Donnelly x Shannon	16.37	22.63	-28
Harvey x Donnelly	16.49	23.23	-29
Shannon x Margaret	19.73	27.73	-29
Donnelly x Kent	13.57	20.87	-35
Kent x Harvey	16.43	25.21	-35
Harvey x Margaret	18.14	28.33	-36
Donnelly x Harvey	13.59	23.23	-42
Donnelly x Margaret	13.84	23.99	-42
Warren x Shannon	16.85	30.8	-45
Harvey x Shannon	13.9	26.97	-48
Shannon x Kent	12.78	24.61	-48
Warren x Donnelly	12.49	27.05	-54
Shannon x Donnelly	10.49	22.63	-54
Warren x Margaret	13.8	32.16	-57
Kent x Warren	11.92	29.04	-59
Donnelly x Warren	10.32	27.05	-62
Margaret x Shannon	10.14	27.73	-63
Shannon x Warren	11.27	30.8	-63
Warren x Harvey	11.16	31.39	-64
Kent x Shannon	8.25	24.61	-67

Heterosis

Heterosis can be calculated for hybrids for which the mean weight of both parental groups and reciprocal hybrids (male x female and female x male crosses from parental strains) are known. Of the 25 hybrids produced in this experiment there were 10 sets of reciprocal hybrids (i.e. 20 hybrids) (Table 5). All reciprocal hybrids showed negative heterosis (ranging from 18 – 58%) for mean weight (Table 5).

Table 5. Heterosis (%) for mean weight of hybrids produced from wild river strains for which reciprocal hybrids were produced.

River strain (♀ x ♂ and ♂ x ♀)	Heterosis (%)
Kent River x Donnelly River	-18
Harvey River x Kent River	-19
Harvey River x Warren River	-29
Kent River x Warren River	-29
Harvey River x Donnelly River	-35
Donnelly River x Shannon River	-41
Shannon River x Margaret River	-46
Shannon River x Warren River	-54
Shannon River x Kent River	-57
Donnelly River x Warren River	-58

Fertility of Hybrids

Of the 26 hybrid populations produced 3 populations did not mate, 2 aborted eggs, 3 berried and 15 produced juveniles (Table 6). Three hybrids could not breed because they were lacking females (Table 6).

Table 6. Fertility of hybrids in 2003 and 2004 (B = berried, A = aborted J = juveniles, NB=Not berried, nm = no males, nf = no females, * = no hybrids produced).

		Male					
		Harvey	**Margaret**	**Donnelly**	**Warren**	**Shannon**	**Kent**
Female	**Harvey**		*	J J	nf	*	nf
	Margaret	J		NB	J	A J	A
	Donnelly	NB	*		nm J	J	J J
	Warren	B	nf	B		J J	J J
	Shannon	J	NB	B	J		J
	Kent	A	*	J	J	J	

Male Margaret River marron were not only less likely to hybridise with animals from other river systems, but when they did the hybrids did not breed (Table 6). Conversely, female Margaret River marron hybridised readily with animals from other river systems and most of the hybrids were fertile (Table 6).

Discussion

The majority of hybrids evaluated in this experiment did not grow as well as the pure river strains. There was no evidence of hybrid vigour. Where heterosis for growth could be calculated (from 20 of the 25 hybrid populations produced) in all cases it was negative (-18 - -58%). This indicates that random crossing of river lines, or stock introductions to a marron farm, are unlikely to result in improved growth. Therefore, the crossing of river lines should only be undertaken as part of a carefully designed breeding plan (see section 5.0) to introduce specific traits (i.e. reduced claw size, larger abdomen size) that contribute to breeding objectives.

The Margaret River male x Harvey, Donnelly and Kent female mating combinations failed to produce juveniles. In the two crosses where Margaret River males did mate with females from other rivers (Warren and Shannon) the resulting hybrids did not breed. The reciprocal cross of Margaret River females mated with males from all other river lines in this experiment. Three of the 5 resulting hybrid populations were fertile and produced juveniles.

None of the hybrids had a skewed sex ratio. Therefore, hybridisation of marron does not offer a potential solution for producing monosex populations.

3.0 Evaluation of marron strains Part III: Social interaction among marron strains

3.1 Evaluation of social interaction among five river strains and three "domesticated" lines of marron in research ponds.

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Introduction

It is not known if the fastest growing marron are aggressive, dominant or passive animals. Previous examples from agricultural systems have shown that the most aggressive or dominant animals and plants often grow faster. However, this can result in increased size variation by repressing the growth of other animals or plants in the same plot. Consequently, genetic selection programs generally aim to develop more passive "domesticated" animals that are less aggressive and show decreased size variation in comparison to "wild" stocks.

In a previous study comparing the growth of marron strains from three different rivers (Warren, Deep and Donnelly) they were reared communally in a single pond (Henryon 1996). In contrast, during this FRDC project, growth and production characteristics of marron from 6 different river populations were compared in separate ponds with three replicates per strain (Section1.1). While this showed the relative performance of each strain, it did not investigate how strains perform in the presence of each other.

The aim of this experiment is to determine whether growth of marron from different river populations is affected by stocking populations communally.

Methods

Collection of stocks

Broodstock marron for each wild river line were collected from the Margaret River, Donnelly River, Warren River, Shannon River and Kent River between April – June 2000 and acclimated in ponds at the Pemberton Freshwater Research and Aquaculture Centre (PFRAC) prior to the breeding season (Figure 1).

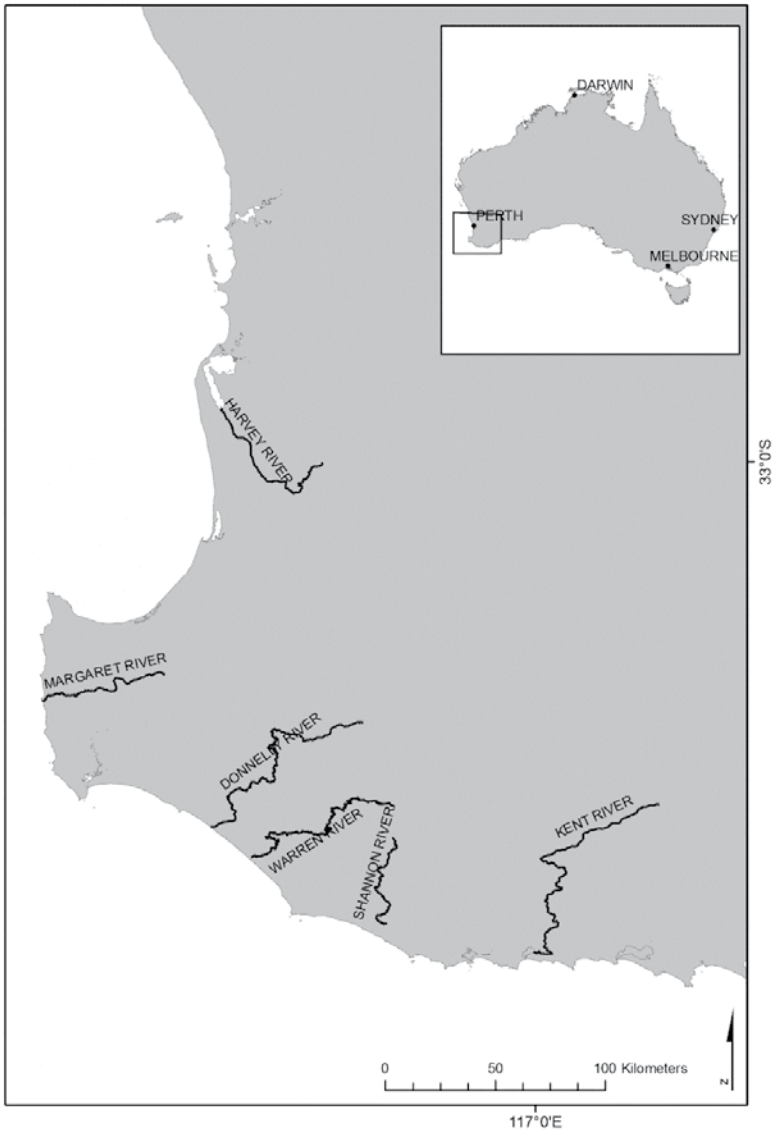

Figure 1. Collection localities for marron strains.

Broodstock for the Mass Selected Line was collected from commercial farmers who provided the largest 2 year old marron produced from their ponds in June 2000.

Broodstock for the Pemberton Line were randomly selected from 2 year old marron held to the PFRAC hatchery.

Production of juveniles

Juveniles for each strain were produced by mating each of the 5 river populations, the Mass Selected Line and the Pemberton Line in 12 m^2 concrete ponds at the PFRAC in July 2000.

A commercial marron farmer provided juveniles for the Industry Line.

Identification of strains

In order to identify marron from each strain they were marked using different coloured Visual Implant Elastomer (VIE) tags (Northwest Marine Technology Inc.) for each strain (Table 1).

Table 1. VIE tag colour codes for marron strains.

Strain	Colour code
Kent River	Orange
Margaret River	Yellow
Shannon River	Pink
Donnelly River	Green
Warren River	Pink and Yellow
Pemberton Line	Pink and Orange
Mass Selected Line	Pink and Green
Industry Line	Yellow and Orange

The VIE is a two part coloured fluorescent elastomer that can be injected into the abdomen of the marron as a liquid that soon cures into a pliable, bio-compatible solid, and after it has set, provides visual identification of each strain.

Juveniles from all females within each cross were pooled prior to tagging and a random sample of 25 juveniles from each strain were then injected with different colour combinations of the elastomer (Table 1). The marron from each strain were then placed into separate aquariums for 2 weeks to confirm tag retention.

Stocking of ponds

Prior to stocking a random sample of 25 juveniles from each strain was weighed. To determine whether some marron strains repress the growth of others we stocked 3 replicate 20 m^2 ponds at the UWA Aquaculture Laboratory with animals from 5 river strains (low breeding success by the 6th strain, Harvey River, prevented including this line) Margaret River, Kent River, Shannon River, Donnelly River and Warren River and three "domesticated lines" Industry Line, Mass Selected Line and Pemberton Line (Total 8 strains). Ten individuals per strain were stocked in each pond.

The three tanks used in this experiment formed part of a recirculating system with a total volume of over 3200 m^3 consisting of over 800 m^3 of tanks and aquaria (n = 200) and a 127 m^3 biofilter. Therefore, each tank had the same water chemistry parameters.

Sex ratio and growth of strains

Data on sex ratio and relative growth of each line was collected at 4 monthly intervals commencing in July 2001, prior to this juveniles were too small to sex and weigh without damaging animals. Growth rate data was collected until the marron had been reared for two years in March 2003.

Feeding and maintenance of stocks

All marron were fed the WA crayfish reference diet (Morrissy 1990) at similar rates initially. These rates were adjusted according to feed rates derived from Morrissy (1992); adjustments were made daily, according to growth and temperature variations, by visually observing demand feeding.

Water chemistry was recorded 3 times per week (Ammonia, Nitrite, Nitrate) using a WinLab®LF 2400 photometer and pH using a Eutech meter.

Temperature was recorded by a Grant Instruments 1200 series (12-Bit) Squirrel meter/logger with U type thermistor temperature probes and set to record temperature every hour.

Validation of Visual Implant Elastomer (VIE) tags
In July 2003, after the conclusion of the experiment, marron that were surplus to the following breeding program were dissected to validate the readability and accuracy of the VIE tagging technique. Thirty-four tagged marron were randomly selected and the VIE tag colour of each animal and time required to determine each tag code was recorded by three staff members. Marron were then dissected and the correct tag colour assigned to each marron.

Statistical Analyses
ANOVA was used to determine the relative performance of strains stocked communally in this experiment. Post hoc multiple pair-wise comparisons were completed using Fishers LSD to test significant differences among strains.

Student's *t*-test was used to compare performance of strains stocked communally in this social interaction experiment with strains in "monoculture" in the related strain evaluation experiment conducted simultaneously in the remaining 27 ponds at the UWA Aquaculture Laboratory (see Section 1.1).

Results

Water Chemistry
Water chemistry variables (Ammonia range = 0.001 – 0.012 mg/L, Nitrite range = 0.02 – 0.08 mg/L, Nitrate range = 0.05-0.2.5 mg/L, pH range = 7.2-9.1) remained within acceptable limits for marron production for the duration of the experiment.

Water temperature (range 12 - 35°C, mean = 19°C) remained within acceptable limits for marron production for the duration of the experiment (Figure 2).

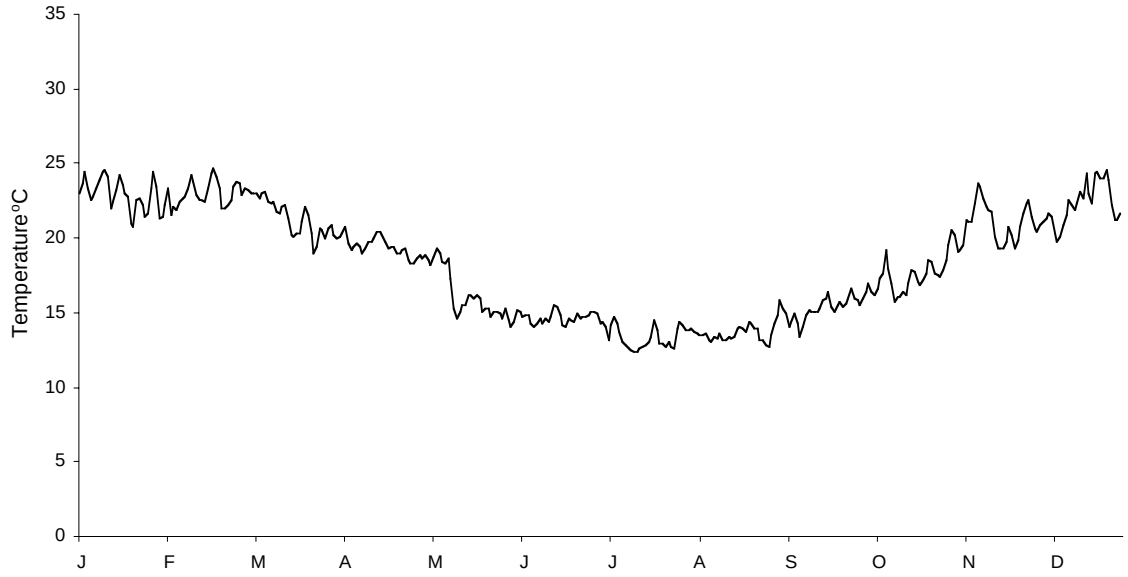

Figure 2. Average daily water temperature in experimental ponds at Shenton Park Aquaculture Laboratory.

Survival of marron strains

Tag readability compromised the accuracy of data collection in this experiment, as shown by survival of 103% for Shannon in July 2001 and an increase in survival for Margaret River and Industry Lines in Nov 2001 and Industry Line in Nov 2002 (Table 2). However these inconsistencies, ranging from 3-6%, represent only 1-2 misread tags. As with the strain evaluation trial (Section 1.1) Shannon showed a decrease in survival between July 2002 and March 2003 (Table 2).

There was a significant difference in survival ($P = 0.03$) (Table 2). Margaret (77%) and Kent River (70%) Lines had significantly higher survival ($P < 0.03$) than Mass Selected (20%) Donnelly (27%) and Industry (33%) Lines (Table 2).

Table 2. Mean survival of marron strains (% ± se) (n = 24). (At the conclusion of the experiment means sharing the same superscript are not significantly different P > 0.05).

Strain	30-Jul-2001	se	19-Nov-2001	se	13-Mar-2002	se	22-Jul-2002	se	11-Nov-2002	se	Final 17-Mar-2003	se
Margaret River	97	6.7	103	12.0	93	3.3	87	8.8	80	17.3	77b	3.3
Kent River	97	3.3	93	6.7	97	8.8	83	12.0	80	15.3	70b	25.2
Shannon River	103	3.3	97	6.7	80	0.0	80	5.8	67	16.7	47ab	6.7
Donnelly River	93	3.3	80	5.8	70	11.5	57	3.3	50	11.5	27a	6.7
Warren River	97	6.7	83	8.8	77	3.3	70	10.0	67	13.3	53ab	8.8
Pemberton Line	97	3.3	97	6.7	90	5.8	73	8.8	53	3.3	43ab	8.8
Mass Selected Line	90	10.0	73	3.3	67	8.8	33	14.5	33	14.5	20a	5.8
Industry Line	93	6.7	97	8.8	70	10.0	57	6.7	63	6.7	33a	3.3

Growth rates of marron strains

There was a significant difference in the final mean weight of marron strains ($P < 0.001$). The Mass Selected Line (106 ± 18 g) ($P = 0.04$) and Pemberton Line (104 ± 10 g) ($P = 0.05$) marron were significantly larger than Industry Line (62 ± 15 g) (Table 3).

Table 3. Mean weight of marron strains (g) (Mean ± se) (n=24) (At the conclusion of the experiment means sharing the same superscript are not significantly different $P > 0.05$.)

Strain	Initial 29-Mar-2001	se	30-Jul-2001	se	19-Nov-2001	se	13-Mar-2002	se	22-Jul-2002	se	11-Nov-2002	se	Final 17-Mar-2003	se
Margaret River	3.10	0.29	13.22	1.30	29.43	2.09	48.14	0.81	72.90	4.58	81.58	8.18	94.65^{ab}	7.72
Kent River	1.07	0.11	5.12	0.77	11.86	1.58	31.78	7.13	41.25	3.55	55.18	9.43	69.91^{ab}	17.25
Shannon River	2.13	0.18	8.63	0.87	19.03	2.59	36.61	1.13	67.35	8.97	91.06	16.54	97.88^{ab}	19.29
Donnelly River	1.26	0.15	5.39	0.66	13.18	1.62	29.28	1.92	45.71	2.64	57.44	5.11	74.93^{ab}	7.55
Warren River	1.35	0.16	5.58	0.85	14.93	2.85	32.38	4.47	50.57	7.03	77.08	8.62	93.19^{ab}	7.19
Pemberton Line	0.88	0.10	6.86	0.87	17.75	2.05	38.27	4.54	60.27	7.55	87.33	17.15	104.07^{a}	10.27
Mass Selected Line	1.59	0.18	7.42	0.69	18.24	1.35	35.77	0.09	72.26	19.51	78.79	3.62	105.93^{a}	18.39
Industry Line	1.23	0.33	3.63	0.96	11.89	2.06	24.78	1.64	33.51	2.34	54.95	9.31	62.41b	14.61

Comparison of growth between social interaction (communal) ponds and mono-strain culture
There was no difference in the growth of the marron strains in social interaction (communal) ponds or mono-strain ponds (P>0.05) (Table 4, Figure 3).

Table 4. Comparison of final mean weight (g ± se) of marron strains in communal ponds (social interaction) or mono-strain ponds (n = 48).

Strain	Mean Weight (g) Social Interaction	se	Mean Weight (g) Mono-strain	se	% Difference	P =
Shannon River	98	19.3	82	1.9	16	0.46
Warren River	93	7.2	78	0.4	16	0.10
Margaret River	95	7.7	83	2.1	12	0.22
Pemberton Line	104	10.3	94	4.1	10	0.43
Mass Selected Line	106	18.4	100	2.1	6	0.77
Donnelly River	75	7.6	81	3.4	-6	0.50
Industry Line	62	14.6	71	5.2	-9	0.59
Kent River	70	17.3	80	3.5	-10	0.60

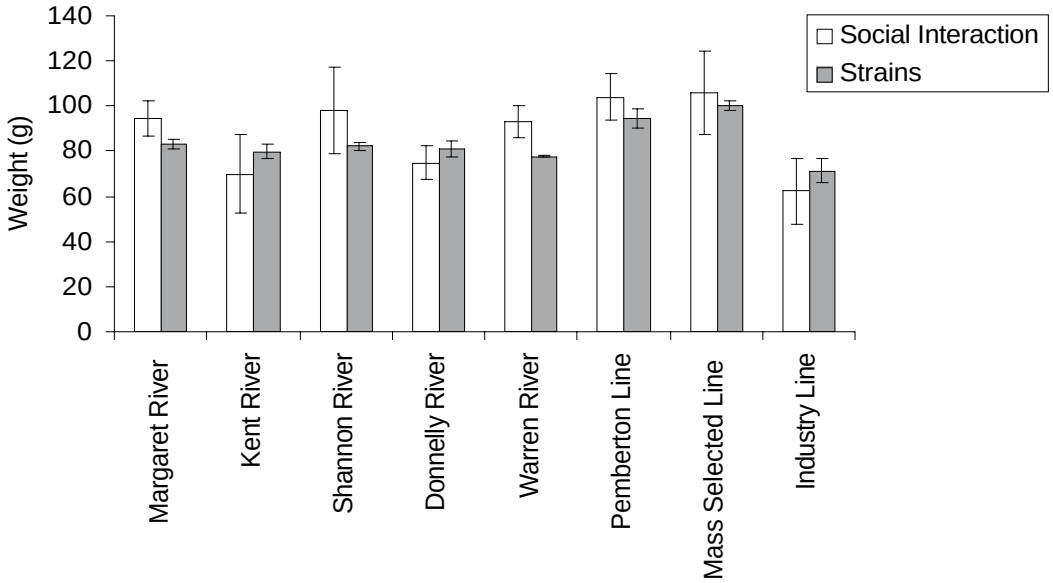

Figure 3. Comparison of final mean weight (g ± se) of marron strains reared in either social interaction (communal) ponds or mono strain ponds (n = 48).

Comparisonofsurvivalbetweensocialinteraction(communal)pondsandmono-strainculture
Survival of the Mass Selected Line (P = 0.02) and Donnelly River Line (P = 0.04) was lower in social interaction ponds than mono-strain ponds (Table 5). Survival of Margaret River marron was higher in social interaction ponds than mono-strain ponds (P = 0.03) (Table 5). However, the survival data for the Margaret River Line must be considered with caution due to the potential to overestimate these animals as shown in the following section.

Table 5: Comparison of final survival (% ± se) of marron strains in communal ponds (social interaction) or mono-strain ponds (n=48).

Strain	Survival (%) Social Interaction	se	Survival (%) Mono-strain	se	% Difference	P =
Shannon River	47	6.7	27	4	20	0.06
Margaret River	77	3.3	62	2.9	15	0.03
Kent River	70	25.2	63	6.4	7	0.62
Warren River	53	8.8	55	3.5	-2	0.9
Industry Line	33	3.3	50	10	-17	0.19
Pemberton Line	43	8.8	61	1.8	-18	0.12
Donnelly River	27	6.7	54	5.8	-27	0.04
Mass Selected Line	20	5.8	47	0.8	-27	0.02

Validation of Visual Implant Elastomer (VIE) tags
At the conclusion of the experiment research staff identified VIE tag colours with an accuracy (mean ± se) of 73 ± 8.7% (range 56% - 85%). The time taken to read VIE tags implanted in the marron averaged 39 ± 4.04 seconds/tag (range 9 - 124 seconds/tag).

Dissection of marron also showed that VIE tags moved not only within the abdomen, but also throughout the body cavity. Consequently, animals that had been tagged with two colours (Warren River, Pemberton Line, Mass Selected Line and Industry Line) had a risk of being misidentified as a line with a single colour tag (Margaret River, Shannon River, Donnelly River or Kent River) because only one colour can be visible in the abdomen when observed externally.

Discussion

There was no difference in the growth of the marron strains in social interaction (communal) ponds or mono-strain ponds.

Survival of Margaret River marron (77%) was higher in social interaction ponds than mono-strain ponds (62%). In contrast, the reduced survival of Mass Selected Line (20%) and Donnelly River Line (27%) indicates that they were more vulnerable to competition in ponds with other marron strains.

The survival and growth data indicates that selection for growth can be undertaken in communal ponds using tagged marron. However, selection for survival of less aggressive marron should be completed in separate ponds for each line.

The use of VIE tags has two constraints; first when selecting bloodstock that had been tagged as juveniles two years previously, the tags could only be read with an accuracy level of 73%. Second, the use of VIE tags adds considerably to the cost of breeding programs as the average time taken to read each tag is 39 seconds. Therefore, for a system capable of holding 3000 pedigree marron such as UWA Aquaculture Laboratory or 1 commercial size marron pond, the time required to identify VIE tagged marron with a 73% degree of accuracy would add 32 hours to data collection each sampling period, therefore despite higher initial capital costs the use of PIT microchip coded tags may be more cost effective in breeding programs.

3.2 Introduced marron displace native marron within the Margaret River by competitive exclusion

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Introduction

Where crayfish species have been introduced beyond their natural range, either accidentally or intentionally, (Vorburger and Ribi 1999), they have often had adverse effects on the existing crayfish fauna, including the local elimination of native species (Bovbjerg 1970, Capelli 1982, Butler and Stein 1985, Söderbäck 1991, Garvey and Stein 1993, Guiasu and Dunham 1999).

In Western Australia there are two types of marron, smooth marron which is common and widespread and hairy marron, which is restricted to a single river, the Margaret River. Twenty years ago, smooth marron were introduced to the Margaret River and as a result has caused the rapid displacement of the native hairy marron which is now listed as critically endangered. Smooth marron and hairy marron have been described as separate species (Austin and Ryan 2002), but the nomenclature of these marron species is currently under review by ICZN (International Commission on Zoological Nomenclature) Case 3267 (Molony et al. 2006).

The causes underlying the displacement of Margaret River marron have not been determined, however other studies on displacement by non-native crayfish have implicated predation susceptibility, disease, reproductive interference and competitive exclusion (Capelli 1982, Butler and Stein 1985, Lodge et al. 1986, Alderman et al. 1990, Söderbäck 1991, Usio et al. 2001).

Crayfish with similar habitat requirements have been reported to compete aggressively with one another for access to valuable resources that are limited in supply. The aim of this experiment was to investigate competitive exclusion as the possible cause for the displacement of native hairy marron by smooth marron in the Margaret River.

Methods

Experimental design

The first experiment recorded the agonistic interactions between interspecific pairs of marron. Pairs were same-sex and of equal and unequal sizes. A pair was placed into an experimental aquarium for one hour and the frequency and direction of offensive and defensive behaviour was used to determine dominant and subordinate marron. The same pairs of marron were used in the second and third experiments. In the second experiment a pair of marron were placed into

an aquarium, separated by a glass divider and each animal was provided with an experimental shelter. Independent shelter use between species was compared by testing whether they differed in preference for the experimental shelters provided. In the third experiment, the glass divider was removed and only one shelter was provided. A pair of marron was put into the aquarium and competition for shelter was examined to determined factors that affect shelter acces.

Experimental Marron

The marron used in the experiment, were the progeny of marron that had been captured from two rivers in the southwest of Western Australia. Progeny with parents from the Warren River were chosen to represent introduced smooth marron, as the marron introduced to the Margaret River are thought to have come from the Warren River. Progeny with parents native to the Margaret River (hairy marron) were used also. All marron used were of the same age (see Section 1.1 for methods used to produce the Margaret River and Warren River genetic lines used in this experiment).

A total of 20 males and 20 females of each of the two species were randomly selected for these experiments. Marron that were missing appendages or had less than whole chelipeds were not selected. Post- moult marron with soft exoskeletons were discarded.

Measurements and Tagging/Identification

One week before experiments began, marron were removed from their holding aquaria, dried and weighed to the nearest gram. The length and width of the ocular carapace and the chelicera were measured with digital calipers (0.01 mm). A small plastic label with a number for identification was glued to the carapace of each marron. Labels were attached with superglue and marron remained out of the water for approximately 5 minutes, to allow the glue to dry.

Treatment groups and pair selection

Marron were separated into groups by species and by sex. Within these four groups, marron were ranked from smallest to largest according to the ocular carapace length, as it was highly correlated ($R^2 = 0.96$) with total body size. Males and females were treated separately and paired in the following size combinations: Treatment 1: Margaret River larger than Warren (MR > W, n = 5). The five largest Margaret River marron were paired with the five smallest Warren marron; Treatment 2: Margaret River smaller than Warren (MR < W, n = 5). The five smallest Margaret River marron were paired with the five largest Warren marron; and Treatment 3: Margaret River equal to Warren (MR = W, n = 5). Five Margaret River marron were paired with five Warren marron of equal size. Ocular carapace length was significantly different ($P < 0.05$) between pairs in treatments 1 and 2 but not between pairs in treatment 3 ($p > 0.05$).

Aquaria - Holding aquaria

A total of 20 holding aquaria measuring 80 cm x 50 cm x 31 cm were filled with water. The bottom of each aquarium was covered with a fine layer of pea-gravel. Two marron of the same sex and species were put into each aquarium. Each aquarium contained one black mesh 'hide', which provided refuge for the marron, as well as 2 short polyvinylchloride (PVC) pipes that could also be used as shelters. Each aquarium was covered with a lid, made from coarse plastic mesh (1 cm mesh diameter), to prevent marron escaping. Aquaria were all connected as part of the UWA Aquaculture Laboratory recirculating system. Water Chemistry parameters (ammonia, nitrite, nitrate) for these aquaria are reported in Section 2.1. Water temperatures were checked every 2 days and ranged between 13°C - 16°C, during the course of the experiment.

Marron were subject to a photoperiod of 10 hours light: 14 hours dark.

Aquaria - Experimental aquaria

The 10 aquaria used for observations were the same, but contained no other features such as shelter. To prevent marron seeking refuge in the corners of an aquarium, a sheet of glass 15 cm x 31cm was placed across each corner held in place with silicon, so each aquarium had an octagonal shape. Water quality was maintained by a filter in each aquaria. As marron could take refuge behind filters they were removed from all aquaria before the commencement of each experiment. Pumps were moved into the corners behind the glass dividers and not accessible to the marron.

Feeding

Marron were fed every 2-3 days to satiation. They were fed commercial marron pellets, also referred to as 'crayfish reference diet' (carbohydrate = 52g/100g; protein = 21g/100g; lipid = 8g/100g; fibre = 4g/100g; moisture = 10g/100g and ash = 4g/100g) (Lawrence 1998). Aquaria were checked daily for pellets that had not been consumed and were siphoned out to prevent bacterial growth.

Experiment 1: Agonistic contests

Each experimental aquarium was divided transversely into two equal halves by a 6mm-thick glass divider. Before the contest, members of a matched pair of marron were removed from their respective holding aquariums and placed on different sides of the divider in the experimental aquarium. They were left to acclimatise for 1 hour before the divider was lifted and they were allowed to interact. All agonistic encounters were recorded for 1 hour. Agonistic behaviours could be categorised as one of four types (Bovbjerbs 1953);

Defensive behaviour

1) **Avoidance**; no threatening behaviour is discernible, yet the subservient crayfish retreats and gives the other crayfish a wide berth in all its movements

Offensive behaviour

2) **Threat**; an approach by a crayfish with its chelae outspread and held in strike position. This can be sufficient to cause the retreat of the other crayfish before the aggressor actually strikes the other.

3) **Strike**; a unilateral aggression, in which the aggressor approaches with outspread chelae, which are suddenly thrust out to hit the other crayfish. The second crayfish retreats without defending itself.

4) **Fight**; a bilateral contact and the most dramatic. Two crayfish meet each striking out at each other, locking chelipeds and occasionally catching other body parts. Infrequently one crayfish attempts to jerk away while still held by the other, which may lead to the loss of a body segment. The fight generally lasts less than a minute and is terminated by a quick backward movement by one of the crayfish, deemed the loser

An individual animal was determined to be dominant if it displayed a high frequency of offensive behaviours and if these offensive behaviours were unidirectional, that is, if that individual predominantly displayed them.

Only head-on tension contacts, (within 45^{o} of either side of the longitudinal axis of each marron) were recorded. Any marron seen walking over the other, retreating from the rear or engaging in side contact and general contact were not recorded as tension contacts (Penn and Fitzpatrick 1963). All observations were made at night between 1900 and 2200 hours, under dim, red light.

Two pairs were watched simultaneously and at the end of the hour, marron were placed back into their holding tanks.

Experiment 2: Preference for shelter

Independent shelter use by each species was compared to determine whether either species differed in their preference for the experimental shelter provided. The same interspecific pairs of marron from Experiment 1 were used. A marron pair was placed simultaneously into the experimental aquarium, on different sides of the glass divider. Individual marron were provided with a shelter made of PVC pipe, measuring 5cm in diameter and 20 cm in length. These pipes were the same as those that marron had been accustomed to using in the holding aquaria.

A pair of marron was placed into an aquarium at 1200 hours and left to acclimatise for 20-hours. Observations began at 0800 hours, individuals from each pair were checked and their relative position (or shelter occupancy) was recorded. A marron was recorded to be "in" the shelter if at least ¾ of its body was inside. The shelter occupancy of each pair was checked every hour from 0800 hours to 1700 hours for two days.

A total of 20 observations of independent shelter occupancy were recorded. For each marron, the number of times shelter was occupied was expressed as a percentage of the total number of observations (Henryon 1989). Marron were placed back into their holding tanks at the end of the two days of recordings.

Experiment 3: Competition for limited shelter

The same interspecific pairs of marron from Experiment 1 were used in Experiment 3 to test if more aggressive and dominant marron could successfully exclude subordinate marron from limited shelter.

Before the experiment the glass dividers were removed from all experimental aquariums. At 1200 hours, a marron pair was placed simultaneously into an aquarium that had only one shelter and left to acclimatise for 20 hours. Observations began at 0800 hours and individuals were checked for their relative position. Shelter occupancy was recorded and the occupant of the shelter was identified. A total of 20 observations of shelter use were made, and for each marron, the number of times the shelter was occupied was expressed as a percentage of the total number of observations.

Statistical analysis

To test for species differences in frequencies within each behaviour type from the three treatment groups a Wilcoxon signed-ranks-test was used. Within each treatment group, the proportion of time that each species displayed a particular behaviour was calculated as follows:

Margaret River (avoid) = frequency of avoidance by MR marron/total frequency of avoidance (MR avoid + W avoid). This was used to determine the direction of offensive and defensive behaviours and species dominance.

Independent t-tests were used to determine differences in size between intraspecific pairs of marron in the holding tanks. These were used to test for the influence of previous fight experience in the interspecific pairs of marron used in the agonistic contests.

Species differences in chelicera at the same body size were tested using a ratio of total chelicera volume to ocular carapace length. The total chelicera volume for individual marron in treatment 3 only was calculated as follows:

TCV= (Left chelicera length x left chelicera width) + (Right chelicera length x right chelicera width)

Independent t-tests were used to determine if species differed in their preference for shelter and (paired) dependent t-tests were used to determine species differences in competition for shelter.

Results

Experiment 1: Agonistic contests

When larger males from the Margaret River were tested against their smaller counterparts from the Warren River (treatment 1), behaviours were unilateral, that is, the marron from Margaret River initiated all the threats, strikes and fights while those from the Warren River always avoided their aggressor (Figure 1). There was a significant difference in the frequencies of avoidance behaviour displayed and threat behaviour displayed by the two species ($P < 0.05$).

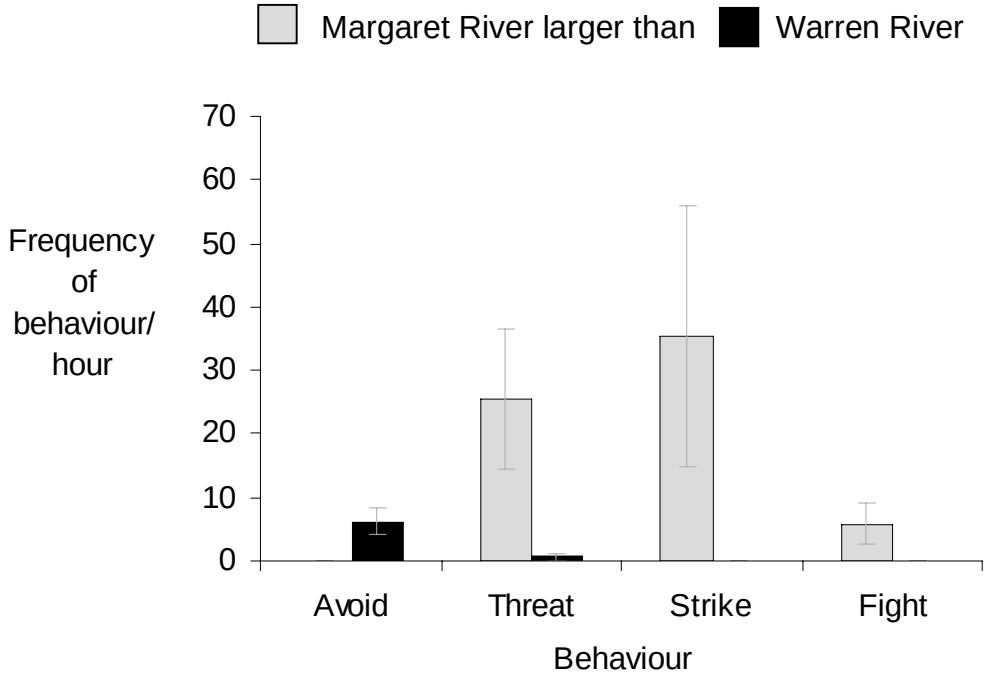

Figure 1. Frequency of the behaviours displayed by male marron in Treatment 1:

When larger males from the Warren River were tested against their smaller counterparts from the Margaret River (treatment 2), behaviours were again unilateral but in the opposite direction. In this treatment, marron from Warren River initiated all the threats, strikes and fights while those from the Margaret River always avoided their aggressor (Figure 2). There was a significant difference in the frequencies of avoidance behaviour displayed and threat behaviour displayed by the two species ($P < 0.05$).

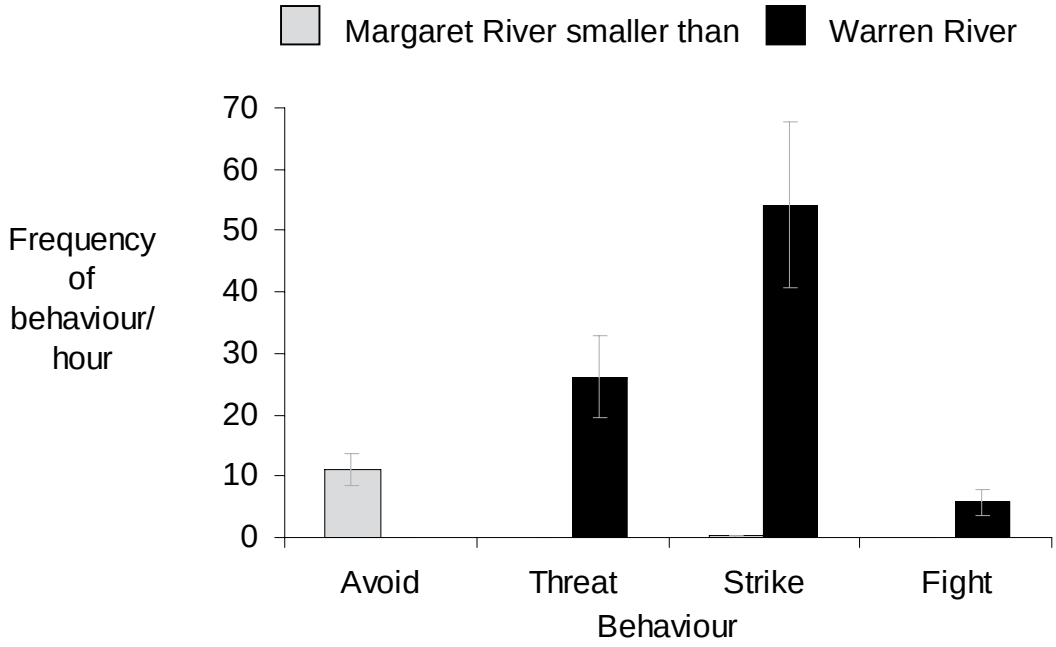

Figure 2. Frequency of the behaviours displayed by male marron in Treatment 2**.**

The frequencies of the strike and avoid behaviour were different for treatments 1 and 2. For example, larger Warren marron displayed the strike behaviour at a higher frequency than larger Margaret River marron. The smaller Margaret River marron displayed avoidance behaviour at a higher frequency than smaller Warren River marron.

When male marron were tested in equal size pairs (treatment 3), both species displayed all four behaviours but at different frequencies. Warren River marron displayed the strike most frequently, followed by the threat behaviour. Margaret River marron also displayed the strike most frequently, followed by the threat behaviour, but these behaviours were not observed as much as those displayed by the Warren River marron. Margaret River marron displayed avoidance more than Warren River marron, and the fight behaviour was displayed almost equally by both (Figure 3). There was no significant species difference in frequency of any behaviour type and neither species could be determined as dominant.

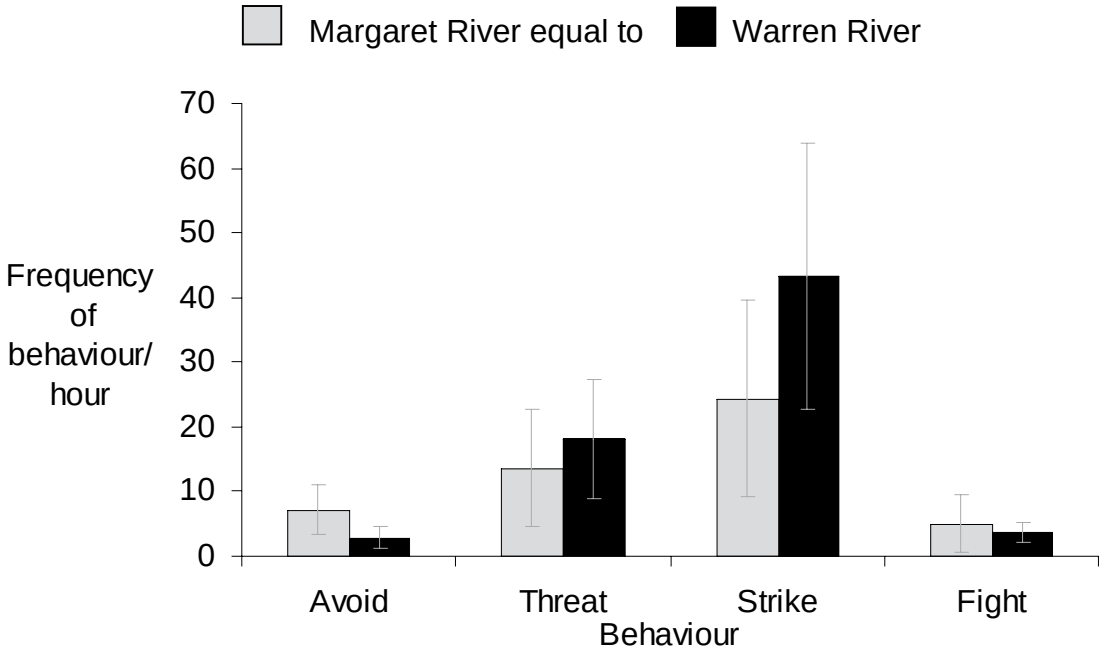

Figure 3. Frequency of the behaviours displayed by male marron in Treatment 3.

When larger females from Margaret River were tested against their smaller counterparts from the Warren River (treatment 1) all four behaviours were displayed by both but at different frequencies. Margaret River marron displayed the strike and threat behaviour the most and at approximately the same frequency. Warren River marron displayed the strike and threat at approximately the same frequency also, but these behaviours were observed less than the larger Margaret River marron. Avoidance behaviour was displayed more frequently by the Warren River marron and Margaret River marron displayed fight behaviour more frequently (Figure 4). There was no significant difference in the frequency of any behaviour type displayed by either species and although Margaret River marron exhibited offensive behaviour slightly more than Warren River marron, interactions were not common and dominance could not be determined.

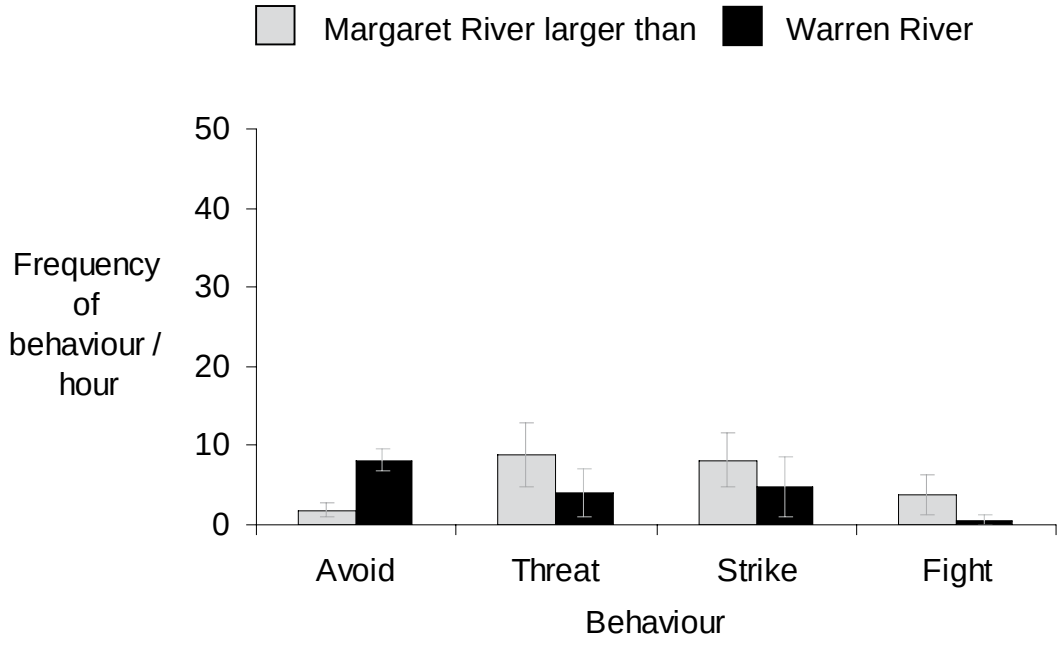

Figure 4. Frequency of the behaviours displayed by female marron in Treatment 1.

When larger females from the Warren River were tested against smaller females from the Margaret River (treatment 2), behaviour was clearly directional with the larger Warren River marron displaying only offensive behaviours, while marron from the Margaret River displayed avoidance quite frequently (Figure 5). There was a significant difference in the frequencies of the avoid, threat and strike behaviours displayed, with the dominant marron being the Warren River marron.

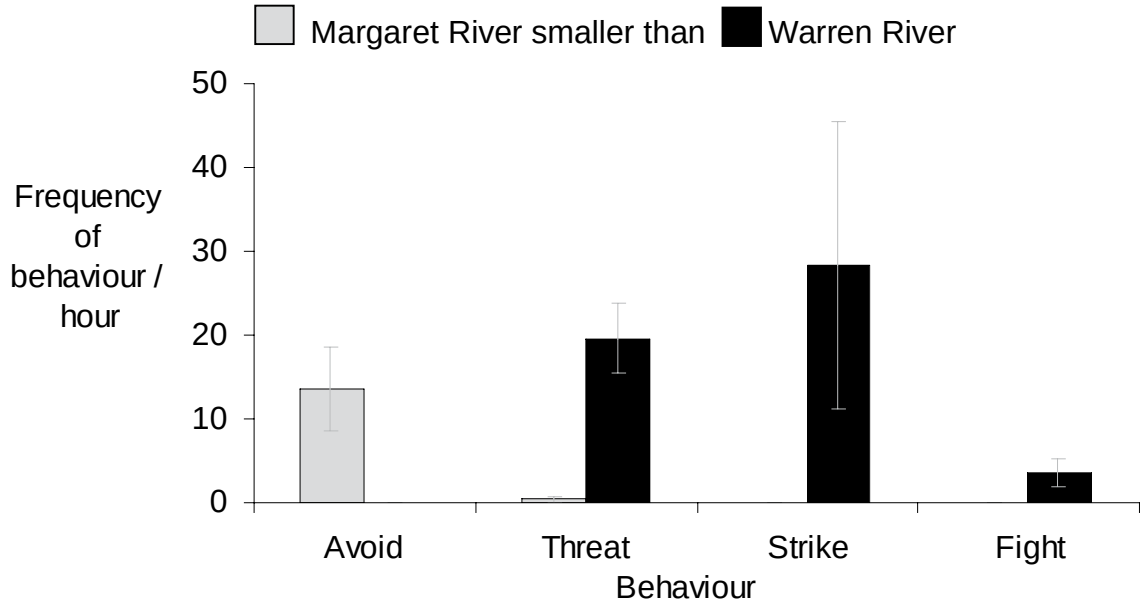

Figure 5. Frequency of the behaviours displayed by female marron in Treatment 2.

A comparison between treatments 1 and 2 for females, shows a large difference in frequency of the threat and strike behaviour in particular and also a difference in the avoid behaviour. Warren River marron displayed the threat behaviour almost double that of Margaret River marron, when they were larger and the strike behaviour almost triple that of Margaret River marron, when they were larger. When Margaret River marron were smaller they displayed the avoid behaviour, over four times as much as Warren River marron did when they were smaller.

In Treatment 3 (MR = W, n = 5), offensive behaviours were displayed by the W marron and again behaviour was clearly directional. The larger W marron displayed predominantly offensive behaviours and it determined that they were the dominant species. They displayed the strike behaviour most, followed by the threat behaviour. MR marron displayed defensive behaviour but it was not high in frequency.

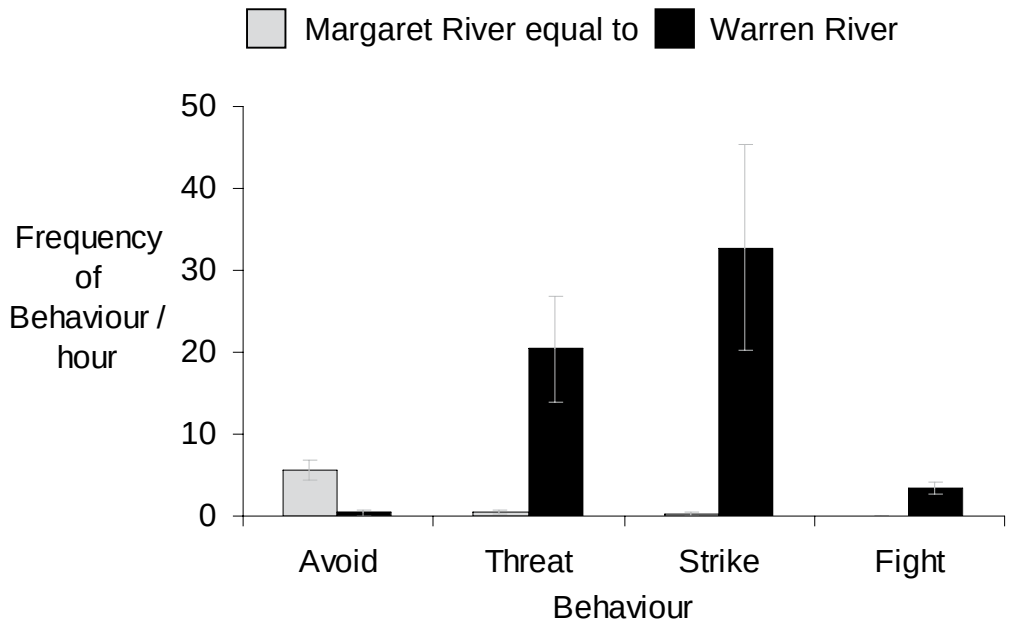

Figure 6. Frequency of the behaviours displayed by female marron in Treatment 3.

A comparison between treatments 2 and 3 shows that the females from the Warren River displayed the strike and threat behaviours at almost the same frequency for both treatments.

At the same body size there was no significant difference in total chelicera volume/ ocular carapace length ratio between species ($p > 0.05$).

Other behaviour during contests

The "chase" behaviour was observed quite often during the agonistic contests, especially by female marron from the Warren River. This behaviour was observed when females were of equal size and of unequal size, in which the female marron from Warren River were larger. Following a retreat by the Margaret River marron after a threat or strike by the Warren River marron, the more aggressive Warren River female chased after the Margaret River female, snapping and waving its chelipeds.

Marron in holding aquaria

Marron in the holding aquaria also engaged in agonistic behaviour. During feeding marron pairs from both species would engage in agonistic interactions as food pellets were dropped into the aquaria.

Marron were observed using the PVC shelters that had been provided

The differences in size between intraspecific pairs of female marron from the Margaret River and pairs of female marron from the Warren River kept in the holding aquaria were not significant.

Differences between treatment groups

The mean difference in ocular carapace length for male marron in treatment 1 was 15.05 mm, while marron in treatment 2 had a mean difference of 7.8 mm. The mean difference in ocular carapace length between female marron in treatment 1 was 5.7 mm, while the mean difference in treatment 2 was 8.0 mm.

Table 2. The mean ocular carapace lengths of Margaret River and Warren River marron, and the mean differences in ocular carapace length between the two species from each treatment group. MR: Margaret River WR: Warren River.

Treatment	Sex	Sample	Mean OCL ± SE (mm)		Mean Difference in OCL length (mm)
		(n)	MR	WR	
Treatment 1: MR>WR	Male	5	59.1 ± 2.10	44.04 ± 1.00	15.05
Treatment 2: MR<WR	Male	5	47.66 ± 0.80	55.46 ± 1.10	7.8
Treatment 3: MR=WR	Male	5	51.95 ± 0.37	50.44 ± 0.65	1.5
Treatment 1: MR>WR	Female	5	45.91 ± 0.50	40.19 ± 0.30	5.7
Treatment 2: MR<WR	Female	5	40.35 ± 0.65	48.38 ± 1.70	8.0
Treatment 3: MR=WR	Female	5	43.41 ± 0.14	43.35 ± 0.37	0.1

Experiments 2 and 3: Shelter preference and shelter competition

When male marron were kept separately, they showed no differential preference for shelter use ($p > 0.05$) and shelter use was minimal by both species. Margaret River marron from treatments 1 and 3 did not use their shelters over the two days. Warren marron did use their shelter, but occupancy was very low (Table 4).

Shelter use was minimal and shelter occupancy did not significantly differ within any Treatment, when only one shelter was provided (Table 4).

Table 4. Shelter occupancy by male marron from the Margaret River (MR) and the Warren River (WR) when shelters were not limited and when they were limited.

Treatment	**MR > WR**		**MR < WR**		**MR = WR**	
Marron	MR	WR	MR	WR	MR	WR
% Shelter occupancy (shelters not limited)	0%	24%	14%	6%	0%	8%
% Shelter occupancy (shelters limited)	28%	0%	19%	0%	0%	21%

When female marron were kept separately, both species were observed using the shelters. In treatment 1, the shelter occupancy for Warren River marron over the two days was 92% and for Margaret River marron it was 65%. In treatment 2, the shelter occupancy for Margaret River marron over the two days was 91%, while for Warren River marron it was only 34%. Shelter occupancy was the same for both species in Treatment 3 at 74% (Table 5). Although shelter occupancy was high for both species in all treatments (with the exception of Warren River marron in treatment 2), differential preference for shelter use was only found to be significant between species in Treatment 2 ($P < 0.05$).

Shelter occupancy was significantly different in treatment 1 ($p < 0.05$) and treatment 2 ($p < 0.05$), when only one shelter was provided. Larger marron from treatment 1 and 2, were observed to be occupying the shelters more when they were limited, while shelter occupancy of the smaller marron from both treatments was zero or close to (Table 5).

Table 5. Shelter occupancy by female marron from Margaret River (MR) and Warren River (WR) when shelters were not limited and when they were limited.

Treatment	MR > WR		MR < WR		MR = WR	
Marron	MR	WR	MR	WR	MR	WR
% Shelter occupancy (shelters not limited)	65%	92%	91%	34%	74%	74%
% Shelter occupancy (shelters limited)	74%	0%	2%	65%	57%	29%

Discussion

This study showed that size and species influenced agonistic interactions and dominance, and agonistic behaviour varied within males and females. Introduced marron can outcompete native marron in agonistic contests.

Female marron from the Warren River were clearly more aggressive than those from the Margaret River of equal size. This finding contradicts the theoretical model of animal conflict put forward by Maynard Smith and Parker (1976) not once, but twice. First, the model of animal conflict predicts that in contests between individuals with similar potential to secure a resource, such as those with the same body size, fights should escalate and aggressive interactions should be more severe, with either individual having the chance to become dominant. However, aggressive behaviour was clearly unidirectional and was most obvious in female marron from the Warren River. Second, the model of animal conflict predicts that when there are no detectable differences in resource holding potential between individuals and the contest still produces a winner, it can often be attributed to the differences in the internal state or motivation of the winner, such as increased hunger or burrow ownership. This second prediction makes sense in a natural environment where animals must compete for access to resources such as food and shelter. However, as these experiments were conducted in a controlled environment, all marron were fed to satiation and placed into experimental aquaria void of shelter or refuge for only one hour, it is likely that aggressive differences between Margaret River and Warren River female marron was a characteristic more likely to be attributed to species differences.

Differences in agonistic behaviour were not apparent between male Margaret River and Warren River marron of equal sizes. Offensive behaviours were not unidirectional and dominance could not be determined. This finding supports the prediction that individuals with similar resource holding potential will fight more aggressively and for longer before one animal withdraws, because each opponent has an equal chance of 'winning' a contest. (Maynard Smith and Parker 1976).

Differences in agonistic behaviour were apparent in male and female marron of unequal sizes. Aggressive behaviour was more frequent when marron were larger, regardless of species, indicating that size was an important factor in the outcome of contests. This result is consistent with other reports where crayfish size has influenced the outcome of a contest (Bovbjerb 1956, Lowe 1956, Rabini 1985, Vorburger and Ribi 1999). The difference in frequencies in avoidance and threat behaviour displayed by male marron in unequal size pairs, were significant and supports the theoretical model of animal conflict proposed by Maynard Smith and Parker

(1976). The authors suggest that when there are differences in the resource holding potential of two individuals, the individual with the lower resource holding potential may chose to engage in the contest only briefly or not at all, as the costs of involvement are high. This was clearly the case with the smaller male marron, as they chose to avoid the larger marron rather than fight, irrespective of species.

Although larger female marron were more aggressive in the unequal size pairs, there was a difference in the frequency of aggressive behaviours, when Margaret River marron were larger and when Warren River marron were larger. Aggressive behaviour was displayed far more by female marron from the Warren River when they were larger and differences in frequencies in avoidance, threat and strike behaviour were significant. Differences in frequencies in behaviour were not significant when female marron from the Margaret River were larger. This result supports the animal conflict model and, supports a species difference in aggression between the females.

Overall the male marron were more aggressive than females. This higher level of aggression between males could be a function of sexual selection. Emlen and Oring (1977) found evidence that females tend to select males with a superior ability to acquire resources that are needed for reproduction, or males who can dominate other males through overt fighting, as mates.

Males and females differed in their preference for shelter and shelter use. Males from both species rarely used shelters nor was there a trend in the use of shelters between the size classes. In the first experiment, the larger male marron dominated the smaller male marron in the contests, but the results were not as clear in the experiments involving shelter, that is, the dominant individuals did not access the shelters.

In their natural environment, shelters are one of the most important resources for crayfish, as they serve to minimise predation pressures. Hazlett et al. (1992) argued that a sheltered area would be occupied more in the presence of danger because of the protection it afforded, regardless of whether the source of potential danger or death to a crayfish was a competitor or a predator. The lack of use of shelters by the males in experimental aquaria indicates that neither marron from a pair felt that it was threatened.

Female marron pairs exhibited a high preference for shelter and there were definite trends in shelter use in the size classes. When both members of a pair were provided with shelter, the smaller marron from the unequal size classes occupied the shelter more frequently than did the larger marron, irrespective of species. It is possible that the smaller marron felt threatened in the presence of the larger marron, supporting the finding made by Hazlett et al. (1992) and stated above.

When shelter was limited, size was the factor that influenced access with the larger female marron from both species accessing the shelter. This is consistent with other experiments involving fiddler crabs, lobsters and crayfish, that have demonstrated that the establishment of dominance is a key factor involved in the acquisition and maintenance of shelter (Hyatt and Salmon 1978, O'Neill and Cobb 1971, Garvey and Stein 1993).

However, in pairs of equal size female marron, dominance did not translate into a shelter advantage. Guiasu and Dunham (1999) stated that usually the more aggressive and dominant crayfish species can competitively exclude subordinate crayfish species from access to preferred shelter and feeding areas. The more aggressive Warren River marron were seen in the shelter much less than the subordinate Margaret River marron, when only one shelter was provided. Again the explanation may be that the Warren River marron did not feel threatened by the presence of Margaret River marron.

The higher preference for shelter by female marron could be a reflection of reproduction strategies. Bovbjerb (1970) found that when females are carrying eggs and young, they must seek a retreat and a study by Figler et al. (1995) demonstrated that ovigerous and brooding *P.clarkii* females win aggressive contests against conspecific male intruders in shelter defence.

In order to conserve the threatened native marron from the Margaret River, it is vital to determine the cause behind the displacement by the introduced marron. Females from the Warren River were clearly more aggressive than females from the Margaret River. This difference in species aggression that was detected provides support that competitive exclusion mediated through direct aggressive interactions is likely to be at work within the Margaret River.

If these differences detected in the laboratory are also evident in the wild, then this finding could have direct implications on the future survival of the native marron. Agonistic behaviour and interspecific competition among crayfish have the ability to regulate the density of the species and influence the crayfish community assemblage (Garvey and Stein 1993). If one sex of a species is somehow competitively disadvantaged in agonistic contests, the continued survival of the species as a whole then becomes threatened.

4.0 Selective breeding I: Mass selection

4.1 Performance of two generations of mass selected marron on commercial farms and in research facilities.

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Introduction

Mass selection of farm stock can be used to improve growth. Mass selection in a breeding programme is where selection is based on individual merit and by truncation. An individual whose phenotypic value (i.e. growth rate) is equal to or exceeds the truncation point is used for breeding, while those with lower values (weights) are culled or sold. Family relationships are ignored in mass selection programmes.

In its simplest form a mass selection breeding program for one trait (i.e. growth) selects the largest males and females from a pond, or series of ponds, for broodstock, while the smaller, slower growing animals are culled or sold. The key advantages of this type of selection are that; 1) farmers can implement mass selection on their own properties if they are prepared to invest the time and resources required to collect measurements, keep records and familiarise themselves with the basic quantitative genetics theory and equations required to select broodstock, 2) fewer ponds are required, 3) costs are lower, 4) the formulas, statistics and computer programs are simpler and easier to use than those for a pedigree breeding program, 5) there is no requirement to individually tag marron, 6) large numbers of juveniles can be produced with limited resources, and 7) more broodstock can be used.

However, there are also a number of disadvantages: in particular 1) gains are reduced over the long term, 2) there is little or no control over the effective breeding number because there is no control over mate selection, and 3) there is little control of inbreeding. In fact, a mass selection program can, if poorly designed, result in quite high levels of inbreeding because if a farmer merely selects broodstock from the largest marron produced on their own farm, or even worse from only one pond, they are likely to be from the same parents.

The performance of freshwater crayfish can be affected by regional variation in environmental parameters (i.e. temperature) and the type of system their performance is being measured in: tanks in a research facility compared to commercial farms. Generally, freshwater crayfish growth in research tank systems is lower than that in earthern ponds (Lawrence et al. 2002). A comparison of the performance of a control strain between the two types of growing systems provides validation and, if necessary, calibration of the results from the research tanks to ensure that accurate predictions of industry outcomes from using genetic improvement programmes can be made. Therefore, when embarking on a research programme to identify and improve a genetically superior strain of crayfish it is essential to establish two factors: 1) is the performance

trait being measured in the research facility representative of what occurs in a commercial system and 2) does the "best" strain perform best in all regions/environments or are different strains required for different regions.

The aim of this experiment was to 1) determine if mass selection of the largest 2-year-old marron from industry stock could improve growth, 2) compare the performance of mass selected and industry marron in commercial ponds 3) compare performance of two lines of marron (Pemberton and Mass Selected) in two different regions and 4) compare the performance of Pemberton, Mass Selected and Industry Lines in two different systems; commercial farms and research tanks.

Methods

Experimental design

A Mass Selected Line was developed from the largest 2 year old marron from 16 farmers in WA. Three different lines; Mass Selected – Generation 1, Pemberton and an Industry Line were grown on commercial farms in two different regions of Western Australia (Pinjarra (north) (Lat -32.63, Long 115.87) and Denmark (south) (Lat -34.96, Long 117.35) and in tanks at the UWA Shenton Park Aquaculture Research Laboratories in Perth. In the second experiment the Mass Selected Line-Generation 2 was compared with industry lines in Western Australia at Mt Barker (Lat -34.63, Long 117.66) and South Australia at Kangaroo Island (Lat -35.72, Long 137.93). The growth of each line was measured and compared between regions and between research and commercial farm systems. The growth of each line was measured over an 2 year period for experiment 1 (generation 1) and a 1 year period for experiment 2 (generation 2).

Experiment 1 (Generation 1: Mass Selected Line)

Production of juveniles

In June-July 2000, prior to the breeding season, a Mass Selected Line was developed by obtaining the largest 2 year old marron from 16 farmers (129 male and 148 female). Marron from all farms were pooled and stocked into a 150 m^2 breeding pond at PFRC at Pemberton (Lat -34.45, Long 116.03). In August 2000 the Mass Selected and Pemberton lines were spawned in the breeding ponds at PFRC Pemberton. Juveniles for the Industry Line were produced by commercial marron farmers.

Stocking of farms

In Autumn 2001 two commercial farms located in the northern (Pinjarra Lat -32.63, Long 115.87) and southern (Denmark Lat -34.96, Long 117.35) WA marron growing regions were stocked with marron to compare the Pemberton, Mass Selected and Industry Lines (Figure 1).

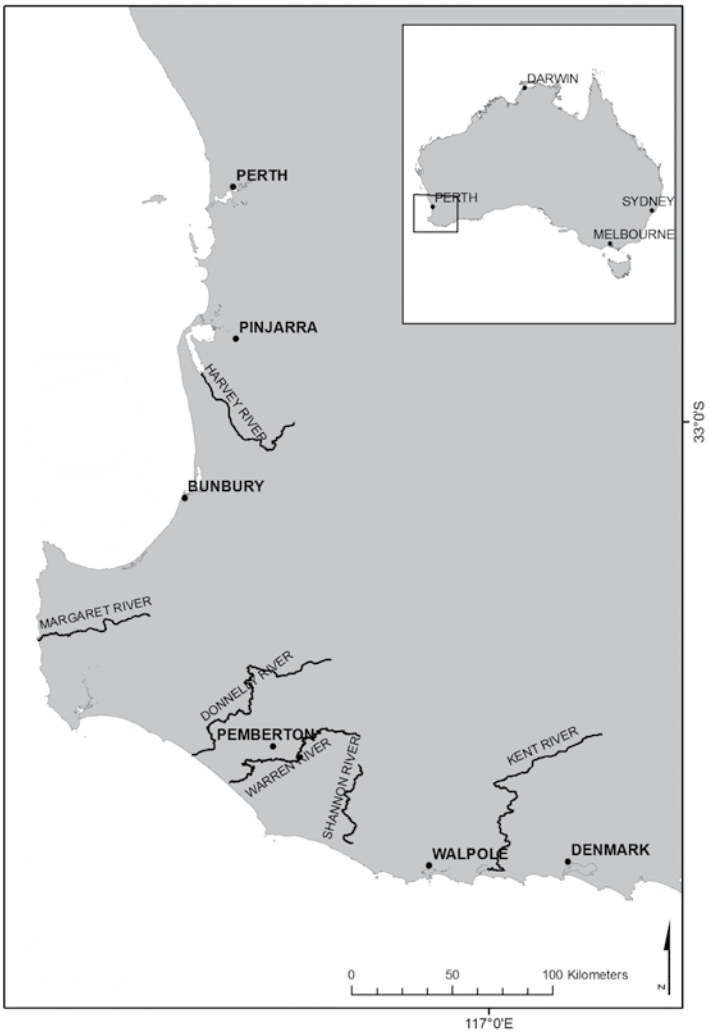


Figure 1. Location of commercial farms Pinjarra and Denmark, PFRC hatchery in Pemberton and UWA Aquaculture Laboratory Shenton Park, Perth.

At the northern (Pinjarra) and southern (Denmark) farms, 6 ponds on each farm were stocked in duplicate with the three strains (Pemberton, Mass Selected and Industry Line) at the rate of 3/m^2 (Pinjarra, 3,364 - 4,139/pond – as pond sizes varied; Denmark, 2,136/pond).

To provide relative growth data of the performance of genetic lines in tanks and commercial ponds, juveniles from each of the 3 Lines (Pemberton, Mass Selected and Industry) were also stocked in triplicate in 20 m^2 tanks at a density of 4/m^2 at the UWA Aquaculture Laboratory in Shenton Park (Lat -31.96, Long 115.80) (Figure 1) (see section 1.1 for detailed methods).

Pond Management

Pond water temperatures were recorded using Tiny Tag Data Loggers® and downloaded using Gemini Logger Management® (GLM) software (see Section 6.7 Regional variation for detailed water quality data).

The ponds were managed and fed according to standard marron industry techniques (Lawrence and Jones 2001). Ponds were aerated by paddlewheels for 20 minutes three times per day (0600, 1800, 2400). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed the WA crayfish reference diet

(Morrissy 1990). All marron were fed the WA crayfish reference diet (Morrissy 1990) at similar rates initially. These rates were adjusted according to feed rates derived from Morrissy (1992); adjustments were made daily, according to growth and temperature variations, by visually observing demand feeding.

At 6-weekly intervals a random sample of marron were collected from each pond by sampling the hides to monitor stock condition and adjust feed rates if necessary. These marron were weighed individually and returned to the ponds.

Marron at the UWA Aquaculture Laboratory were managed according to the methods described in Section 1.1.

Data collection

At the end of year 1, in April/May 2002 growth and survival data were collected from the whole population by draining ponds. Marron were removed, sexed and weighed individually. The southern farm (Denmark) and UWA Aquaculture Laboratory marron were restocked to continue the experiment until the industry standard 2 year grow-out period was completed. High mortalities at the northern (Pinjarra) farm diminished the future value of this component of the experiment, therefore only two ponds of the Mass Selected Line were restocked (ponds 1 n = 1,492 & 2 n = 1,022) and the Industry Line was pooled into 1 pond (Pond 6, n = 1,736).

In April/May 2003, at the conclusion of the two-year experiment, ponds and tanks were drained and marron were removed, sexed and weighed individually.

Data analysis

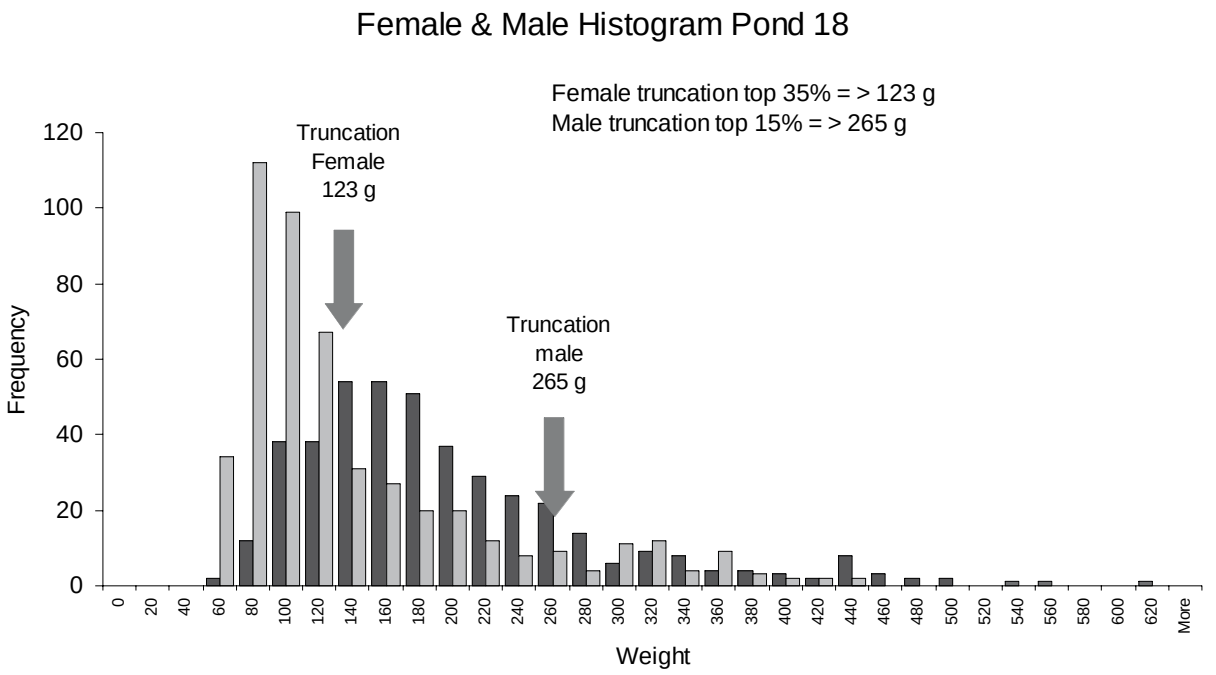
Data for mean weight were analysed by analysis of variance. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance ($\alpha = 0.05$). Post hoc multiple pair-wise comparisons were completed using Fishers LSD to test significant differences among treatment means.

The gross value of production was calculated at the conclusion of the experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade using Marron Profit© software (available from the Aquaculture Council of WA, http://www.aquaculturecouncilwa.com) for a model 50 pond marron farm.

Experiment 2 (Generation 2: Mass Selected Line)

In July 2003, after the completion of Experiment 1, the largest 20% of males (n=10, mean = 175 g, range = 246 - 366 g) and 40% females (n = 44, mean = 175 g, range = 97 – 265 g) of the Mass Selected Line from the northern farm were transferred to 150 m^2 breeding ponds at PFRC for producing the Generation 2 of the Mass Selected Line.

From the southern farm, the largest 15% of male and 35% of female 2-year-old marron from the Mass Selected Line were selected for Generation 2 broodstock (Figure 2). From pond 18, 165 females greater than 123 g (mean = 218 g, range 123 – 426 g) and 71 males greater than 265 g (mean = 362 g, range 265 – 605 g) were selected (Figure 2). From pond 19,216 females greater than 125 g (mean = 209 g, range 125 – 477 g) and 41 males greater than 249 g (mean 344 g, range 249 – 520 g) were selected (Figure 2). These broodstock were transferred to five 150 m^2 breeding ponds (1 male : 4 females) at PFRC Pemberton for producing the Generation 2 of the Mass Selected Line.



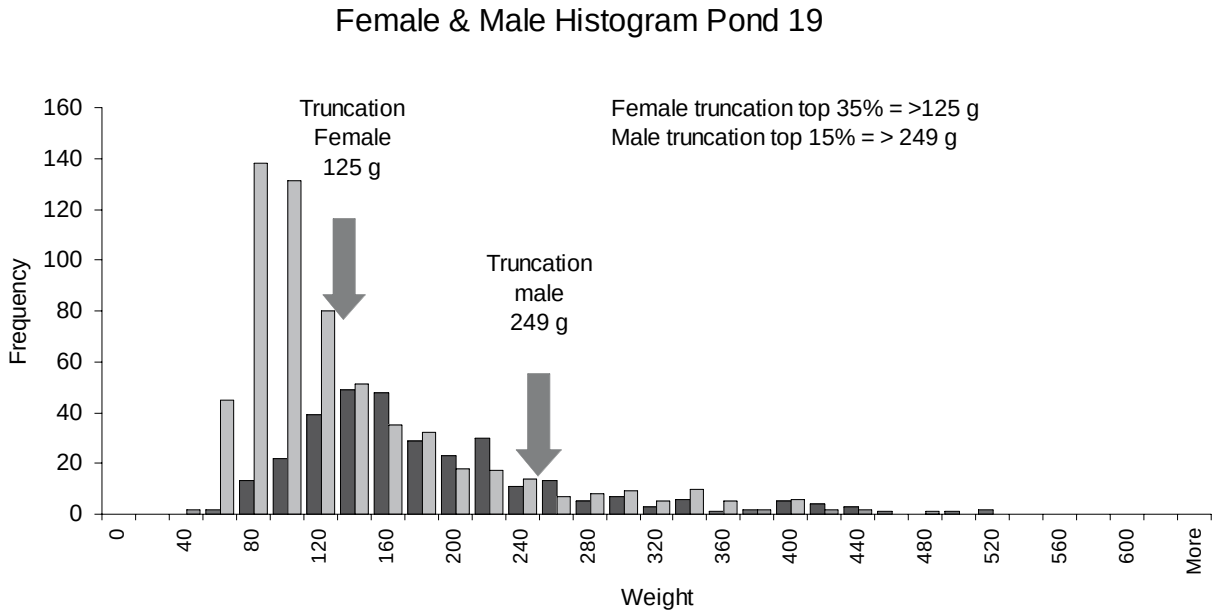


Figure 2. Size histograms of male and female Mass Selected marron from southern farm (ponds 18 and 19). (arrows indicate truncation point for broodstock selection). Dark Grey = males and Light Grey = females.

Progeny from the first generation of mass selected broodstock (Generation 2: Mass Selected Line) were collected from the 5 breeding ponds at PFRC in May 2004. They were pooled and then stocked into commercial ponds at Mt Barker (Lat -34.63, Long 117.66) (2 ponds, stocked at 2.8/m^2, pond 7 = 2,419, pond 14 = 2,268) and Kangaroo Island (Lat -35.72, Long 137.93) (1 pond, stocked at 3/m^2 = 3,000). (See section 5.2 for related experiment) (Figure 4). To evaluate the performance of the Mass Selected Line on each farm, control ponds were stocked with Industry Line (Mt Barker 2 ponds, Kangaroo Island 1 pond).

The mean weight of Generation 2: Mass Selected Line at stocking was 6.93 ± 3.28 g in May 2004.

Figure 3. Locations of commercial farms Mt Barker and Kangaroo Island stocked with Generation 2: Mass Selected Line.

The commercial ponds were drained in May/June 2005. Each marron was sexed and weighed individually to record growth. Survival as recorded in each location.

Data analysis

Data for mean weight and yield were analysed by analysis of variance. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance (α = 0.05). Post hoc multiple pair-wise comparisons were completed using Fishers LSD to test significant differences among treatments.

The gross value of production was calculated at the conclusion of the experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade using Marron Profit© software for a model 50 pond marron farm.

Results

Experiment 1 (Generation 1: Mass Selected Line)

Year 1: 2001-2002

Survival

At the southern farm (Denmark) the Industry Line had higher survival than the Mass Selected (P = 0.02) and Pemberton (P = 0.01) lines (P = 0.02) (Table 1).

Failure of automatic timers operating the paddlewheel aerators at the northern farm resulted in dissolved oxygen levels of < 2mg/L in ponds. As a result there was poor survival (range 0-39%) (Table 1.). There was no difference in survival among lines at the northern farm (P = 0.47).

There was no difference in survival among Industry, Mass Selected and Pemberton Lines at the UWA Aquaculture Laboratory in Shenton Park (P = 0.32).

Table 1. Marron survival after 1 year (n=21 ponds).

	Industry (%)	se	Pemberton (%)	se	Mass Selected (%)	se
Northern (Pinjarra)	19	19	6	5	33	7
Southern (Denmark)	91	2	72	4	69	1
UWA (Shenton Park)	72	9	80	2	67	2

Growth

At all three locations the Mass Selected Line grew faster than the Industry Line (Table 2).

At the southern farm (Denmark), the Pemberton (68 ± 0.67 g) (P < 0.001) and Mass Selected (64 ± 0.62 g) (P < 0.05) lines both grew faster than the Industry Line (57 ± 1.72 g) (P = 0.015) (Table 2).

The poor survival of animals at the northern farm resulted in no statistical difference in growth among the Mass Selected Line (52 ± 3.94 g), Industry (31 ± 0.0 g) and Pemberton Lines (36 ± 4.19 g) (P = 0.14) (Table 2).

The Mass Selected (63 ± 1.8 g (P = 0.003) and Pemberton lines (56 ± 2.5 g) (P = 0.01) both grew faster than the Industry Line at the UWA Aquaculture Laboratory in Shenton Park (41 ± 4.4 g) (P = 0.007) (Table 2).

Table 2. Marron weight (g ± se) after 1 year (n=21 ponds).

	Industry (g)	se	Pemberton (g)	se	Mass Selected (g)	se
Northern (Pinjarra)	31	0.0	36	4.2	52	3.9
Southern (Denmark)	57	1.7	68	0.7	64	0.6
UWA (Shenton Park)	41	4.4	56	2.5	63	1.8

Year 2: 2002 - 2003

Survival

Survival of Mass Selected and Pemberton Lines was lower than the Industry Line at the southern farm (P = 0.005) (Table 3).

There was no difference in survival among Industry, Mass Selected and Pemberton Lines at the UWA Aquaculture Laboratory in Shenton Park (P = 0.34) (Table 3).

Table 3. Marron survival after 2 years (n=18 ponds).

	Industry (%)	se	Pemberton (%)	se	Mass Selected (%)	se
Northern (Pinjarra)	0.5	-	-	-	12	3.6
Southern (Denmark)	75	1	52	3	44	1
UWA (Shenton Park)	50	10	61	2	47	1

Growth

The Mass Selected Line grew faster than the Industry Line at all three locations (Table 4). The Pemberton and Mass Selected lines both grew faster than the Industry Line in Denmark and at the UWA Aquaculture Laboratory in Shenton Park (Table 4).

Table 4. Marron weight (g ± se) after 2 years (n=18 ponds).

	Industry (g)	se	Pemberton (g)	se	Mass Selected (g)	se
Northern (Pinjarra)	94	-	-	-	128	0.5
Southern (Denmark)	116	5.2	94	4.1	100	2.1
UWA (Shenton Park)	71	5.2	94	4.1	100	2.1

Size variation of marron strains

There was no difference in the coefficient of variation (CV %) among genetic lines (P = 0.28) (Table 5)

Table 5. Coefficient of variation (CV %) of marron lines after 2 years (n=18 ponds).

	Industry (%)	se	Pemberton (%)	se	Mass Selected (%)	se
Northern (Pinjarra)	39	-			54	3.8
Southern (Denmark)	53	3.3	56	0.1	58	0.4
UWA (Shenton Park)	43	3.8	51	4.0	47	6.3

Economic analysis

In comparison to the marron farmed currently (Industry Line), the improved growth rate recorded for the Mass Selected Line in this experiment produced an increase in mean weight of between 30 – 41%. The Pemberton Line also grew faster (26 - 32%) than the Industry Line.

On a model 50 pond marron farm Marron Profit© software demonstrates that replacing industry stock with the Mass Selected - Generation 1 Line on an average marron farm (Table 6) or a correctly designed, constructed and managed farm (Table 7) would result in an increase in annual production, annual return (profit) and IRR.

Table 6. Economic evaluation of Mass Selected – Generation 1 Line (30 and 41% improvement) on an average marron farm (see Scenario 2, section 7.1).

	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	5.7	136,457	25.79	-136,907	-11,936	4.57	0.92	-2.50
Mass Selected 30%	7.5	177,395	20.14	307,122	26,776	8.84	1.18	5.62
Mass Selected 41%	8.1	192,405	18.67	469,932	40,971	10.2	1.27	8.59

Table 7. Economic evaluation of Mass Selected – Generation 1 Line (30 and 41% improvement) on a farm that is correctly designed, constructed and professionally managed (see Scenario 3, Section 7.1).

	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	6.8	156,728	22.08	80,408	7,010	6.79	1.05	1.47
Mass Selected 30%	8.8	203,746	17.28	589,632	51,407	11.13	1.34	10.78
Mass Selected 41%	9.6	220,986	16.04	776,347	67,685	12.52	1.44	14.19

Therefore the adoption by industry of the Mass Selected Line – generation 1 produced by this study should provide farmers with an increase in production of 30 – 40% increase in profit of 860%, a 22 - 28% reduction in the cost of production and an increased internal rate of return of between 64 – 123%.

Experiment 2 (Generation 2: Mass Selected Line)

Breeding success

The percentage of berried females recorded in November 2003 from the 5 breeding ponds was 57 ± 2.7% (mean ± se) (n = 354 females).

Year 1: 2004-2005

Survival

Although an algal bloom reduced the survival of Mass Selected stock at Mt Barker, there was no difference in survival between Mass Selected and Industry Lines at Mt Barker and Kangaroo Island (P = 0.051) (Table 8).

Table 8. Marron survival after 1 year (n=6 ponds).

	Industry (%)	se	Mass Selected (%)	se
Mt Barker	78	1	31	11
Kangaroo Island	108	-	86	-

Growth

The Mass Selected Line grew faster than the Industry Line at both locations. At Mt Barker, the Mass Selected Line (111 ± 6.2 g) grew 110% faster than the Industry Line (53 ± 0.4 g) (P = 0.01) (Table 9). There was a difference in growth between the two farms (P = 0.03 but the interaction of genetic line by farm was not significant (P = 0.13).

Table 9. Marron weight (g ± se) after 1 year (n=6 ponds).

	Industry (g)	se	Mass Selected (g)	se	P
Mt Barker	53	0.4	111	6.2	0.01
Kangaroo Island	36	-	67	-	

In comparison to experiment 1, generation 2 of the Mass Selected Line at 1 year of age was 5 – 133% larger than the first generation of this line (Table 2 and Table 9).

Size variation of marron strains

The coefficient of variation (CV %) was lower in the Industry Line than the Mass Selected Line at Mt Barker (P = 0.02) (Table 10). There was no difference in the CV between farms (P = 0.30), or between lines (P = 0.10). However, there was a significant interaction effect (P = 0.03) of genetic lines between the two farms.

Table 10. Coefficient of variation (CV %) of marron lines after 1 year (n=6 ponds).

Site	Industry (%)	se	Mass Selected (%)	se
Mt Barker	43	0.01	61	3.11
Kangaroo Island	65		54	

Economic analysis

In comparison to the currently farmed Industry Line the improved growth rate recorded for the second generation of the Mass Selected Line in this experiment produced an increase in mean weight of between 86 – 109% at 1 year of age.

On a model 50 pond marron farm Marron Profit© software demonstrates that replacing industry stock with the Mass Selected - Generation 2 Line on an average marron farm (Table 11) or a correctly designed, constructed and managed farm (Table 12) would result in an increase in annual production, annual return (profit) and IRR.

Table 11. Economic evaluation of Mass Selected – Generation 2 Line (86 and 110% improvement) on an average marron farm (see Scenario 2, section 7.1).

	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	5.7	136,457	25.79	-136,907	-11,936	4.57	0.92	-2.50
Mass Selected 86%	10.7	253,811	14.46	1,135,976	99,040	14.97	1.64	20.77
Mass Selected 109%	12.0	285,196	13.01	1,476,398	128,719	17.09	1.82	26.99

Table 12. Economic evaluation of Mass Selected – Generation 2 Line (86 and 110% improvement) on a farm that is correctly designed, constructed and professionally managed (see Scenario 3, Section 7.1).

	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
Industry	6.8	156,728	22.08	80,408	7010	6.79	1.05	1.47
Mass Selected 86%	12.6	291,514	12.47	1,540,183	134,280	17.46	1.85	28.16
Mass Selected 109%	14.2	327,561	11.24	1,930,587	168,317	19.67	2.06	35.3

Therefore the adoption by industry of the Mass Selected Line – Generation 2 produced by this study should provide farmers with an increase in production of 86 - 109% increase in profit of

1,800 - 2,300%, a 44 – 50% reduction in the cost of production and an increased internal rate of return of between 157 – 274%.

Discussion

The results from this study show that selective breeding of industry stocks may provide rapid improvements in performance.

The results from Experiment 1 showed that the Industry Line, which was originally distributed from the PFRC, grew slower than the Pemberton Line. The Pemberton Line has not been selected for faster or slower growth, while broodstock management of the Industry Line has involved selling the largest 1 year old marron and breeding from the remaining marron at 2 years of age. This has resulted in a 25 - 41% decrease in growth of Industry stock at two years of age compared with the Pemberton Line. In this experiment, mass selection of the slower growing Industry Line was able to rapidly address this negative genetic selection and provided a gain of 30 – 41% in average weight at harvest in only one generation.

The results from Experiment 2 showed that growth of the Industry Line could be further improved by a second generation of mass selection. The second generation of Mass Selected marron grew 86 – 109% faster than the Industry Line.

The increase in mean weight of mass selected marron compared with industry marron, indicates that selective breeding could have a significant impact upon marron farming. In particular, further improvement of growth by selective breeding offers the potential to shift marron production from a 2 year to a 1 year harvesting cycle.

This experiment has also shown that the mass selected marron produced from this breeding program grow faster than current industry stocks from different farm lines and in different regions.

In the short term, this program is capable of producing a large number of faster growing juveniles, while utilising relatively few resources. However, it does run the risk that in the longer-term higher levels of inbreeding will occur. One effective strategy to control inbreeding is by implementing a pedigree breeding program (see sections 5.1 and 5.2).

5.0 Selective breeding II: Pedigree selection

5.1 Breeding objectives and selection index for a marron pedigree breeding program

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Introduction

A pedigree breeding program uses not only the traits of individuals and families, but also a family tree on which to base selection and mating decisions. This requires mapping the genetic history of a particular individual or family to identify the best broodstock.

This is a much more sophisticated and complex breeding program than mass selection, as broodstock are selected not only on their individual merit, but also on the performance (merit) of other members of their family. Simply put, while a marron may not be the largest in a pond, if we know it is closely related to other very large animals it is probable that it also carries similar genes and will pass these on to its progeny.

The key advantages of a pedigree mating design are that; 1) inbreeding and effective breeding number can be controlled, 2) selection for several traits is made simultaneously, 4) larger genetic improvements can be made over the longer term.

The key costs of this mating design are that; 1) a large number of holding ponds or tanks are required to maintain separate family lines, 2) the formulas, statistics and computer programs are complex, 3) marron must be individually tagged or held separately, 4) fewer marron are produced each generation due to the large amount of resources required.

The aim of this component of the project was to 1). Identify breeding objectives for use in a pedigree selective breeding program for marron 2). Develop a strain selection index based upon these breeding objectives 3) Develop a selection index to determine breeding values for individuals, based on the breeding objectives and economic merit for each marron in our pedigree.

Methods

Researchers held a workshop consisting of representatives from industry, experts in developing breeding programs from DIAS (Danish Institute of Agricultural Sciences), genetics researchers from UWA and aquaculture experts from Department of Fisheries.

Breeding objectives were identified and prioritised in this workshop. Researchers then attributed economic merit to the breeding objectives and developed an equation that could be used to rank strains and individuals within strains according to their EBV's (Estimated Breeding Values).

Results

Breeding Objectives

The key factors affecting economic return from marron production identified by industry were growth, size variation, survival, age at sexual maturity and colour (Table 1).

Table1. Breeding objectives for marron and desired selection criteria.

Breeding Objective	Desired criteria	Undesirable criteria
Colour	Black	Blue
Claw size	Small	Large
Carapace area	Small	Large
Tail volume	Large	Small
Age of sexual maturity	≥ 2 years	< 2 years
Size variation	Low	High
Survival	High	Low
Proportion of large marron in cohort	High	Low

Mass selection is suitable of improving a single trait such as growth (Figure 1). However, it is not suitable for improving multiple traits simultaneously. While mass selection for growth can produce larger marron (Section 4.1), in the long term it may have detrimental affects upon the form of marron. This is because marron selected for broodstock may be heavier due to larger claws or a larger carapace, but have a reduced meat yield due to a proportionately smaller abdomen.

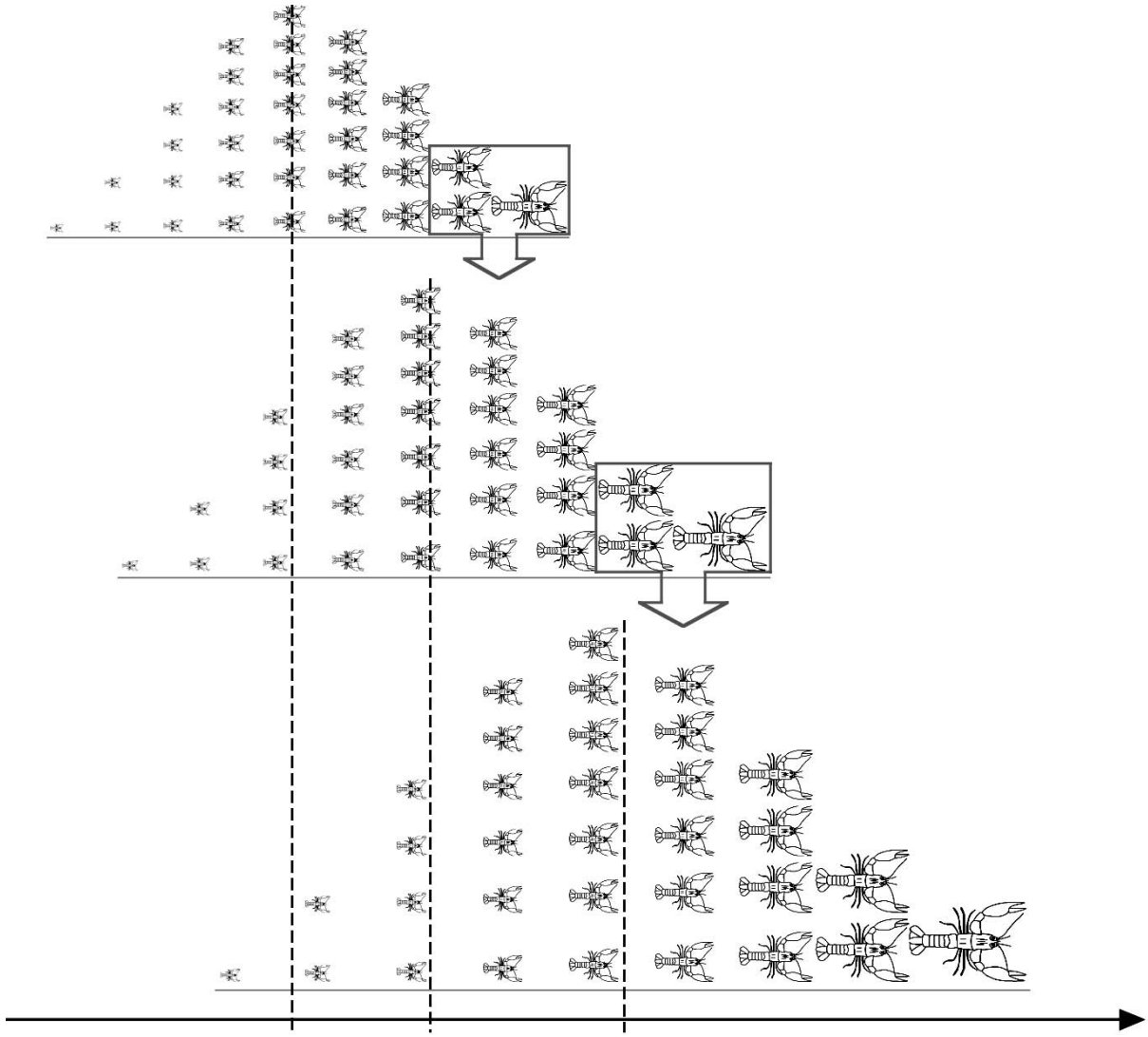

Figure 1. Mass selection for growth improves a single trait (i.e. mean weight).

Pedigree selection, based upon a selection index, permits traits identified in the breeding objectives to be prioritised according to economic merit. This enables family lines to be established that initially combine the best traits of wild river lines then selection to further improve these traits (Figure 2). The advantage of a pedigree selection program is that by selecting for multiple traits the meat yield as a proportion of body weight should increase over subsequent generations.

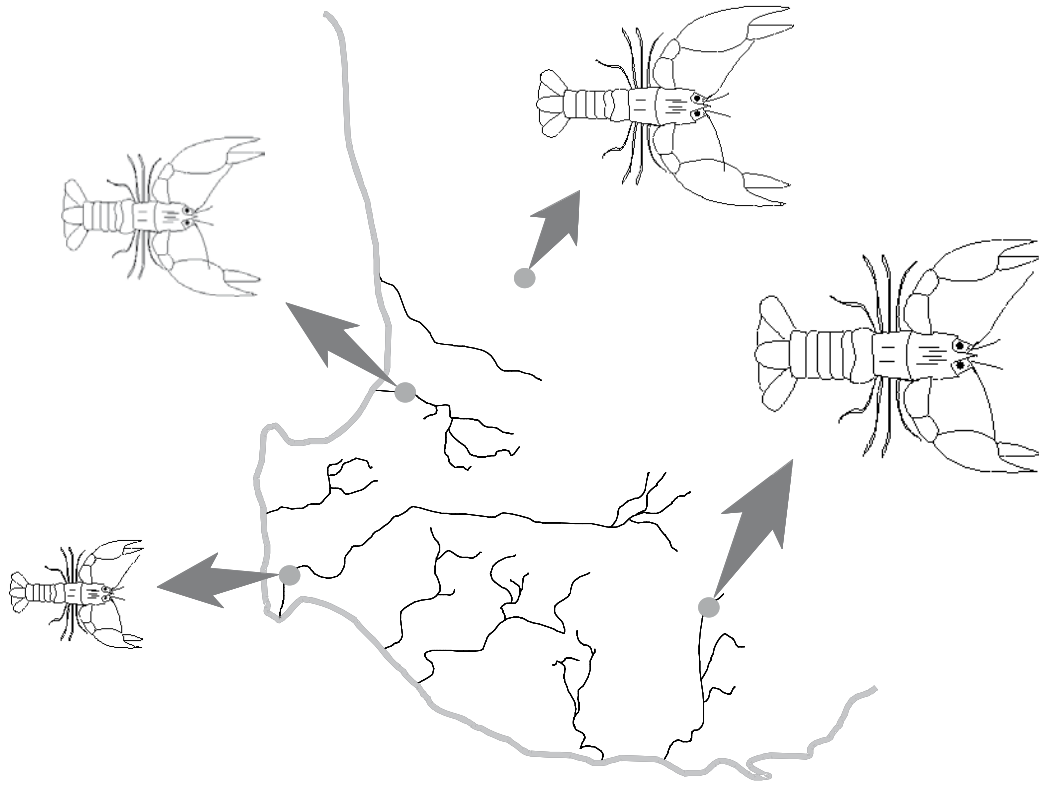

Figure 2. Pedigree breeding programs combine the best traits of wild populations then use a selection index to improve the genetic lines.

Selection index

Based upon the breeding objectives, the following selection index was developed for identifying the best genetic lines to use in the pedigree breeding program:

$$I = NS_{m_i} \cdot \begin{bmatrix} \overline{WT}_{m_i} \cdot \begin{bmatrix} (prop_{<40g} \cdot p_{<40g}) + (prop_{40-70g} \cdot p_{\$40-70g}) + (prop_{71-100g} \cdot p_{\$71-100g}) + (prop_{101-150g} \cdot p_{\$101-150g}) + (prop_{151-200g} \cdot p_{\$151-200g}) \\ + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{151-200g} \cdot p_{\$151-200g}) + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{251-300g} \cdot p_{\$251-300g}) \end{bmatrix} \\ + [\text{Male Tail Volume}_{i\bullet} - \text{Male Tail Volume}_{\bullet\bullet}] \cdot p_{\text{tail}} \\ - [\text{Male Claw Area}_{i\bullet} - \text{Male Claw Area}_{\bullet\bullet}] \cdot p_{\text{claw}} \\ - [\text{Male Carapace Area}_{i\bullet}] - [\text{Male Carapace area}_{\bullet\bullet}] \cdot p_{\text{carapace}} \end{bmatrix}$$

+

$$N_{fi} \cdot S_{fi} \cdot \begin{bmatrix} \overline{WT}_{fi} \cdot \begin{bmatrix} (prop_{<40g} \cdot p_{<40g}) + (prop_{40-70g} \cdot p_{\$40-70g}) + (prop_{71-100g} \cdot p_{\$71-100g}) + (prop_{101-150g} \cdot p_{\$101-150g}) + (prop_{151-200g} \cdot p_{\$151-200g}) \\ + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{151-200g} \cdot p_{\$151-200g}) + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{251-300g} \cdot p_{\$251-300g}) \end{bmatrix} \\ + [\text{Female Tail Volume}_{i\bullet} - \text{Female Tail Volume}_{\bullet\bullet}] \cdot p_{\text{tail}} \\ - [\text{Female Claw Area}_{i\bullet} - \text{Female Claw Area}_{\bullet\bullet}] \cdot p_{\text{claw}} \\ - [\text{Female Carapace Area}_{i\bullet}] - [\text{Female Carapace area}_{\bullet\bullet}] \cdot p_{\text{carapace}} \end{bmatrix}$$

Where

N = Number of marron stocked originally (n)

S = Survival (%)

WT_m = Mean weight of males (g)

WT_f = Mean weight of females (g)

Prop = Proportion with the size grade

P = Price received for animals in the size grade ($)

P_{tail} = Value of tail ($)

P_{claw} = Value of claws ($)

$P_{carapace}$ = Value of carapace ($)

Applying this selection index to the strains evaluated in this project (Section 1.1) identified the Harvey (112.17) as the best line (Table 2).

Table 2. Ranking of marron lines according to the selection index calculated as an average for each line.

Genetic Line	Selection Index
Harvey	112.17
Pemberton	85.38
Mass Selected	83.61
Shannon	71.66
Warren	69.34
Margaret	68.65
Donnelly	66.42
Kent	49.05
Industry	43.78
Blue	0

Although both the Blue and Pemberton Lines performed well, they were both excluded from pedigree selection program to prevent the introduction of the blue colour mutation into any of the Pedigree Lines.

Discussion

Using the results from the strain evaluation experiment (Section 1.1) we used both between-strain and within-strain selection to establish the base population for our pedigree-based breeding program. First, the best broodstock were identified using between-strain selection to identify the families with the best genes for the traits we wished to improve. Secondly within-strain selection was used to select the best individual marron from within each of the best strains.

Pedigree breeding has much better longer term potential than mass selection, provided the industry can support a long term sophisticated breeding program. Whereas mass selection can be used to improve performance in the shorter term by producing a large number of marron that are better than existing industry stocks, but ultimately not as good as those produced by

a pedigree breeding program. On this basis, both are worth pursuing to provide industry with better animals for farming in both the short and long term and this is the strategy we are adopting in the marron genetic improvement project.

Needless to say, unlike mass selection, it would be very difficult for individual farmers to attempt a pedigree-based breeding program on their own properties. However the strains identified by the selection index that have the highest ranking Harvey, Mass Selected, Shannon, Warren and Margaret show the best characteristics for marron farming and may form the basis of future farm based mass selection programs.

5.2 Performance of marron produced from a pedigree breeding program in research ponds

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Introduction

While a pedigree selection program has the advantage that selection for multiple traits can be made simultaneously and inbreeding can be controlled, it is anticipated that in the short term improvements in growth would be less than that achieved from a mass selection breeding program based upon only one trait (i.e. growth).

The aim of this experiment was to compare the growth of marron from a pedigree selection breeding program with the genetic lines that showed the fastest growth in the strain evaluation experiment (Mass Selected, Harvey and Blue marron).

Methods

Using the results from the strain evaluation experiment (Section 1.1) we used both between-strain and within-strain selection to establish the base population for our pedigree-based breeding program based upon the selection index developed in Section 5.1. First, the best broodstock were identified using between-strain selection to identify the families with the best genes for the traits we wished to improve. Secondly within-strain selection was used to select the best individual marron from within each of the best strains.

$$I = NS_{m_i} \cdot \begin{bmatrix} \overline{WT}_{m_i} \cdot \begin{bmatrix} (prop_{<40g} \cdot p_{<40g}) + (prop_{40-70g} \cdot p_{\$40-70g}) + (prop_{71-100g} \cdot p_{\$71-100g}) + (prop_{101-150g} \cdot p_{\$101-150g}) + (prop_{151-200g} \cdot p_{\$151-200g}) \\ + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{151-200g} \cdot p_{\$151-200g}) + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{251-300g} \cdot p_{\$251-300g}) \end{bmatrix} \\ + [\text{ale ail l me}_{i\bullet} - \text{ale ail l me}_{\bullet\bullet}] \cdot p_{\text{tail}} \\ - [\text{ale Claw Area}_{i\bullet} - \text{ale Claw Area}_{\bullet\bullet}] \cdot p_{\text{claw}} \\ - [\text{ale Carapace Area}_{i\bullet}] - [\text{ale Carapace area}_{\bullet\bullet}] \cdot p_{\text{carapace}} \end{bmatrix}$$

+

$$N_{fi} \cdot S_{fi} \cdot \begin{bmatrix} \overline{WT}_{fi} \cdot \begin{bmatrix} (prop_{<40g} \cdot p_{<40g}) + (prop_{40-70g} \cdot p_{\$40-70g}) + (prop_{71-100g} \cdot p_{\$71-100g}) + (prop_{101-150g} \cdot p_{\$101-150g}) + (prop_{151-200g} \cdot p_{\$151-200g}) \\ + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{151-200g} \cdot p_{\$151-200g}) + (prop_{201-250g} \cdot p_{\$201-250g}) + (prop_{251-300g} \cdot p_{\$251-300g}) \end{bmatrix} \\ + [\text{Female ail l me}_{i\bullet} - \text{Female ail l me}_{\bullet\bullet}] \cdot p_{\text{tail}} \\ - [\text{Female Claw Area}_{i\bullet} - \text{Female Claw Area}_{\bullet\bullet}] \cdot p_{\text{claw}} \\ - [\text{Female Carapace Area}_{i\bullet}] - [\text{Female Carapace area}_{\bullet\bullet}] \cdot p_{\text{carapace}} \end{bmatrix}$$

Where

N = Number of marron stocked originally (n)

S = Survival (%)

WT_m = Mean weight of males (g)

WT_f = Mean weight of females (g)

Prop = Proportion with the size grade

P = Price received for animals in the size grade ($)

P_{tail} = Value of tail ($)

P_{claw} = Value of claws ($)

$P_{carapace}$ = Value of carapace ($)

Twenty-two pedigree Lines were produced. In May 2004 these genetic lines were each stocked into 22 tanks at the rate of 60 Pedigree marron : 20 Blue marron/pond. Six additional ponds were stocked with a Mass Selected Line, a Harvey Line, and two High Selected and Low Selected lines (Harvey x Warren mean = 3.52 g, mean High Line = 6.56 g, mean Low Line = 2.25 g; and Warren x Shannon mean = 4.96 g, mean High Line = 5.37 g, mean Low Line = 2.13 g).

The 28 tanks each 20 m^2 that were used in this experiment formed part of a recirculating system with a total volume of over 3200 m^3 consisting of over 800 m^3 of tanks and aquaria (n = 200) and a 127 m^3 biofilter. Each tank therefore had the same water chemistry parameters.

Sex ratio and growth of strains

Data on sex ratio and relative growth of each line was collected at 4 monthly intervals commencing in May 2004, prior to this juveniles were too small to sex and weigh without damaging animals.

Every 4 months, over a 10 day period, each of the 28 tanks was drained, marron collected, weighed, sexed and returned to their respective tank. Growth rate data was collected until the conclusion of the project in July 2005.

Feeding and maintenance of stocks

All marron were fed the WA crayfish reference diet (Morrissy 1990) at similar rates initially. These rates were adjusted according to feed rates derived from Morrissy (1992); adjustments

were made daily, according to growth and temperature variations, by visually observing demand feeding.

Water chemistry was recorded 3 times per week (Ammonia, Nitrite, Nitrate) using a WinLab® LF 2400 photometer and pH using a Eutech meter.

Temperature was recorded by a Cambell Scientific CR10X logger U type thermistor temperature probes and programmed to record temperature every minute.

Results

The Harvey x Warren Line showed the best growth (Table 1). Although selected for multiple traits, 7 of the Pedigree Lines still grew faster than the Mass Selected Line (Table 1).

f the two Pedigree Lines that were graded as juveniles prior to stocking into High or Low lines, one (War x Sha) grew faster (H17 mean = 80 g) and slower (L17 mean = 52 g) than the average for this line (75 g) (Table 1). While the other graded line Har x War, the High Line (H10 mean = 87 g) and the Low Line (L10 mean = 66 g), both grew slower than the Ungraded Line (mean = 90 g) (Table 1).

Table 1. Mean weight (g ± sd) and rank of Pedigree genetic lines at the conclusion of the experiment.

Line	Mean wt (g)	SD	Rank	n
HAR x WAR	90	41.00	1	28
HAR x HAR	90	37.66	2	27
HAR x SEL	89	45.00	3	32
HAR x WAR (H10)	87	30.02	4	26
SEL x SEL	85	50.12	5	41
HAR x WAR	82	32.01	6	29
SEL x WAR	82	44.46	7	36
SEL x MAR	82	30.10	8	36
Mass Selected	81	26.06	9	33
WAR x SHA (H17)	80	23.08	10	37
HAR x MAR	80	30.02	11	24
HAR x MAR	76	41.82	12	38
WAR x SHA	75	31.44	13	38
SHA x MAR	73	40.99	14	41
SHA x SEL	73	38.59	15	30
SEL x SEL	71	43.93	16	42
HAR x WAR (L10)	66	24.32	17	29
SEL x SEL	66	36.42	18	44
SEL x SEL	66	29.21	19	34
SHA x MAR	64	45.10	20	37
SHA x WAR	64	54.63	21	19
SHA x WAR	57	43.10	22	34
WAR x SEL	56	36.05	23	47
SHA x MAR	54	40.32	24	39
SHA x SEL	53	24.63	25	49
WAR x SHA (L17)	52	22.64	26	41
WAR x WAR	46	33.01	27	39
WAR x WAR	34	6.76	28	44

In comparison to the Blue Control Line stocked in each pond the majority of Pedigree Lines (n = 18) grew faster (Table 2). The Harvey (79%) and Mass Selected (93%) Lines also grew faster than the Blue Control Line (Table 2). Therefore 20 of the 24 genetic lines in this experiment grew faster than the Blue Line, which was the best line in the previous strain evaluation experiment (Table 2).

Table 2. Growth comparison of Pedigree Lines with Blue Line.

Line	% Growth Compared to Blue Line
HAR x WAR (H10)	142
HAR x WAR	110
SEL x SEL	105
SEL x MAR	99
SHA x MAR	97
Mass Selected	93
HAR x WAR	82
HAR x MAR	80
HAR x HAR	79
SEL x SEL	68
WAR x SHA	68
HAR x SEL	58
HAR x MAR	53
WAR x SHA (H17)	53
SEL x SEL	42
SEL x SEL	41
SHA x SEL	41
SEL x WAR	39
HAR x WAR (L10)	21
WAR x SHA (L17)	19
SHA x SEL	11
SHA x MAR	11
SHA x MAR	4
SHA x WAR	1
WAR x SEL	-8
SHA x WAR	-13
WAR x WAR	-24
WAR x WAR	-55

The coefficient of variation (%) ranged from 20 – 86% (Table 3). The Warren x Warren Line (20%) had the lowest CV (Table 3).

Table 3. Coefficient of variation (CV%) of Genetic Lines.

Line	CV (%)
WAR x WAR	20
WAR x SHA (H17)	29
Mass Selected	32
HAR x WAR (H10)	35
SEL x MAR	37
HAR x WAR (L10)	37
HAR x MAR	38
HAR x WAR	39
WAR x SHA	42
HAR x HAR	42
SEL x SEL	44
WAR x SHA (L17)	44
HAR x WAR	45
SHA x SEL	47
HAR x SEL	50
SHA x SEL	53
SEL x WAR	54
HAR x MAR	55
SEL x SEL	55
SHA x MAR	56
SEL x SEL	59
SEL x SEL	62
WAR x SEL	64
SHA x MAR	71
WAR x WAR	72
SHA x WAR	75
SHA x MAR	75
SHA x WAR	86

The Shannon x Selected Line had the highest survival (Table 4).

Table 4. Survival (%) of genetic lines.

Line	Survival (%)	n
SHA x SEL	82	49
WAR x SEL	78	47
WAR x WAR	73	44
SEL x SEL	73	44
SEL x SEL	70	42
SEL x SEL	68	41
SHA x MAR	68	41
WAR x SHA (L17)	68	41
SHA x MAR	65	39
WAR x WAR	65	39
HAR x MAR	63	38
WAR x SHA	63	38
SHA x MAR	62	37
WAR x SHA (H17)	62	37
SEL x WAR	60	36
SEL x MAR	60	36
SHA x WAR	57	34
SEL x SEL	57	34
Mass Selected	55	33
HAR x SEL	53	32
SHA x SEL	50	30
HAR x WAR	48	29
HAR x WAR (L10)	48	29
HAR x WAR	47	28
HAR x HAR	45	27
HAR x WAR (H10)	43	26
HAR x MAR	40	24
SHA x WAR	32	19

Selection index

There was a significant relationship between the weight of the dam (g) and the mean weight of the juveniles at 18 months of age (r = 0.64, P = 0.0013; y = 24.6836*exp(0.0172*x)) (Figure 1).

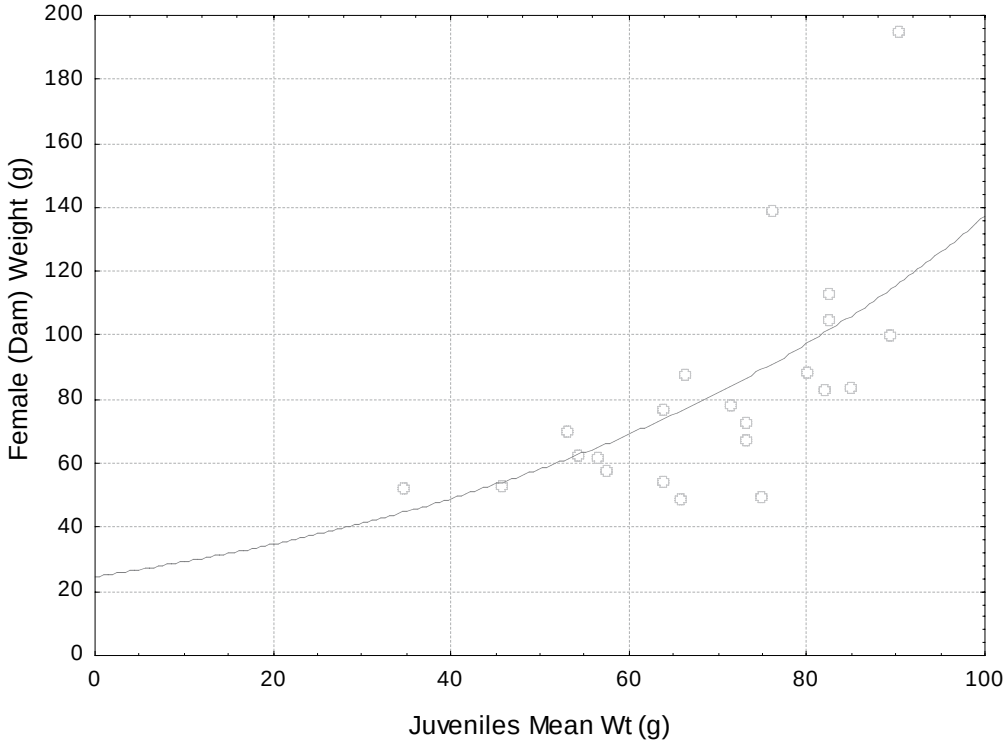

Figure 1. Relationship between weight of the female (dam) and mean weight of juveniles (progeny) at 18 months of age.

There was a significant relationship between the selection index score of the female (dam) and the mean weight of the juveniles at 18 months of age (r = 0.64, P = 0.0013; y = 20.2255*exp(0.0198*x)) (Figure 2).

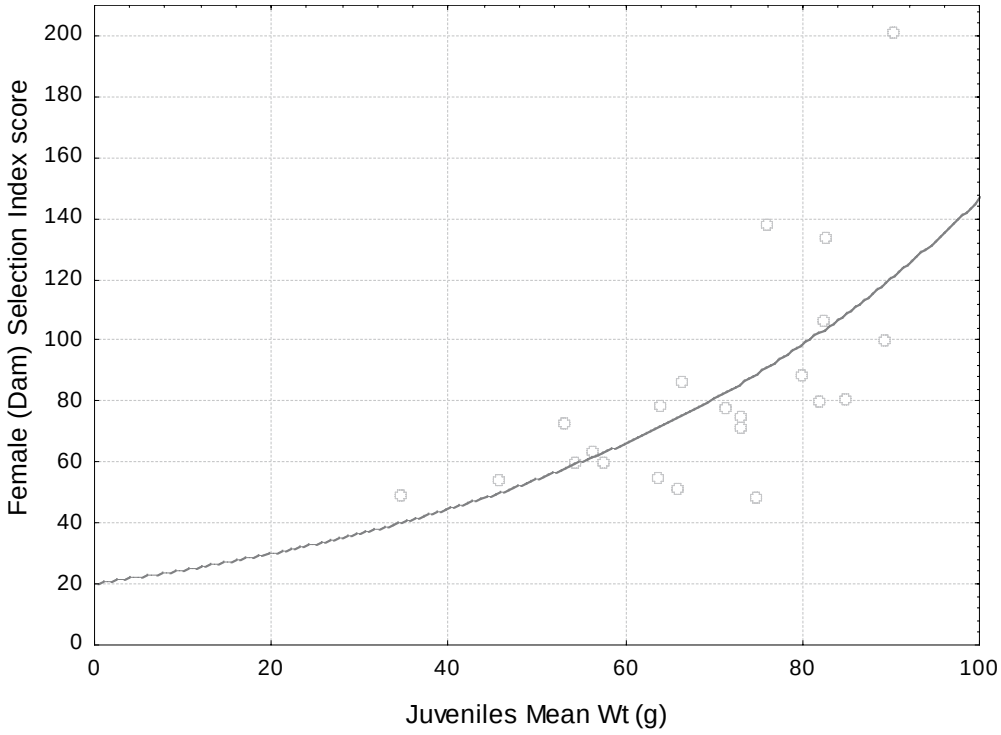

Figure 2. Relationship between selection index score the female (dam) and mean weight of juveniles (progeny) at 18 months of age.

There was no relationship between the weight of the sire and the mean weight of the juveniles at 18 months of age ($r^2 = 0.001$; r = 0.032, P = 0.889), or the selection index score of the sire and the mean weight of the juveniles at 18 months of age ($r^2 = 0.0003$; r = 0.017, P = 0.942).

This data also indicates that under the same, or similar, environmental conditions and management strategies, there is potential for predicting the mean weight of progeny at 18 months of age based upon the weight of females (dams) (Mean wt = -56.3711+66.4583*log10(x)) or the selection index score of females (dams) (Mean wt = -53.577+64.7925*log10(x)) and may therefore complement the previous work on predictive equations by Morrissy et al., 1995).

Discussion

In this experiment we added another layer of complexity to the breeding program by developing a selection index to determine breeding values for individuals, based on the economic merit for each marron in our pedigree (Section 5.1). This selection index while quite complex, enables us to identify and select broodstock based not only on weight (i.e. growth), but also a number of other factors that are of economic importance such as age at sexual maturity, colour, size of claws, size of carapace and size of abdomen. These individual marron were selected and individually tagged,and their progeny reared separately so that the family line of each individual could be determined and used to estimate breeding values (EBV's) for each individual marron to aid the selection of broodstock in the future.

Using EBV's for individual marron, the selection index and sophisticated computer software, means our research team will be identifying the best broodstock based not only weight, but also several other commercially important traits. Incorporating the pedigree information will improve the accuracy of our EBV's and provide us with far greater control over inbreeding and potentially greater returns for farmers over the longer term.

6.0 Husbandry and Pond management

6.1 The effect of size grading of juveniles upon marron production in commercial ponds

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Introduction

Growth and size variation are a major concern of industry because at harvest a significant proportion of the crop is below market size (> 70 g).

One approach to decrease size variation is to tightly grade juveniles prior to stocking growout ponds. Size grading of juvenile freshwater prawns, to remove the smaller 50% of animals prior to stocking ponds resulted in animals that were 19% larger at harvest (Tidwell et al. 2004). Furthermore, the variation of individual sizes at harvest, evaluated by comparing the coefficients of variation (CV) of the upper grade fraction compared to ungraded controls, was significantly lower in ponds stocked with graded juveniles (Tidwell et al. 2004).

However, size grading to stock the largest 50% of juvenile prawns has only been effective when conducted on young juveniles (Tidwell et al. 2002). When size grading was undertaken upon older animals, it did not increase production due to an increased proportion of earlier maturing individuals (Tidwell et al. 2002). In a similar study, a population of older marron (10 – 160 g) that were size graded and restocked into ponds did not grow faster than ungraded animals (Qin et al. 2001).

The aim of this study is to examine the effects of size grading of juvenile marron prior to stocking upon growth, size variation, survival and economic return of marron on commercial farms.

Methods

Experiment 1: The effect of size grading of juveniles upon marron production in commercial ponds (Pemberton)

Six ponds (595 ± 57 m^2 mean ± se) on a commercial marron farm in Pemberton, Western Australia (Lat -34.45, Long 116.03) were stocked with a total of 10,710 juvenile marron (Pemberton Line) at a density of 3 marron/m^2 in June 2001.

Juveniles from 3 nursery ponds were pooled prior to stocking the growout ponds. The pooled juvenile population was randomly divided into 2 groups. From the first group, ungraded juveniles (mean weight = 2.63 ± 0.06 g) were stocked into 3 ponds. From the second group, juveniles for the remaining three ponds were graded using a Apparatebau Gunther Kronawitter (AGK) mechanical bar grader (slot width 7.5 mm). The smallest 50% of these graded juveniles were discarded and the remaining largest 50% of graded juveniles (mean weight = 5.15 ± 0.45 g) were stocked into three ponds. There was no significant difference in the size variation of either graded (CV = 65 ± 14.32%) or ungraded (CV = 56 ± 4.65%) (P = 0.56) juvenile marron stocked in ponds (Figure 1).

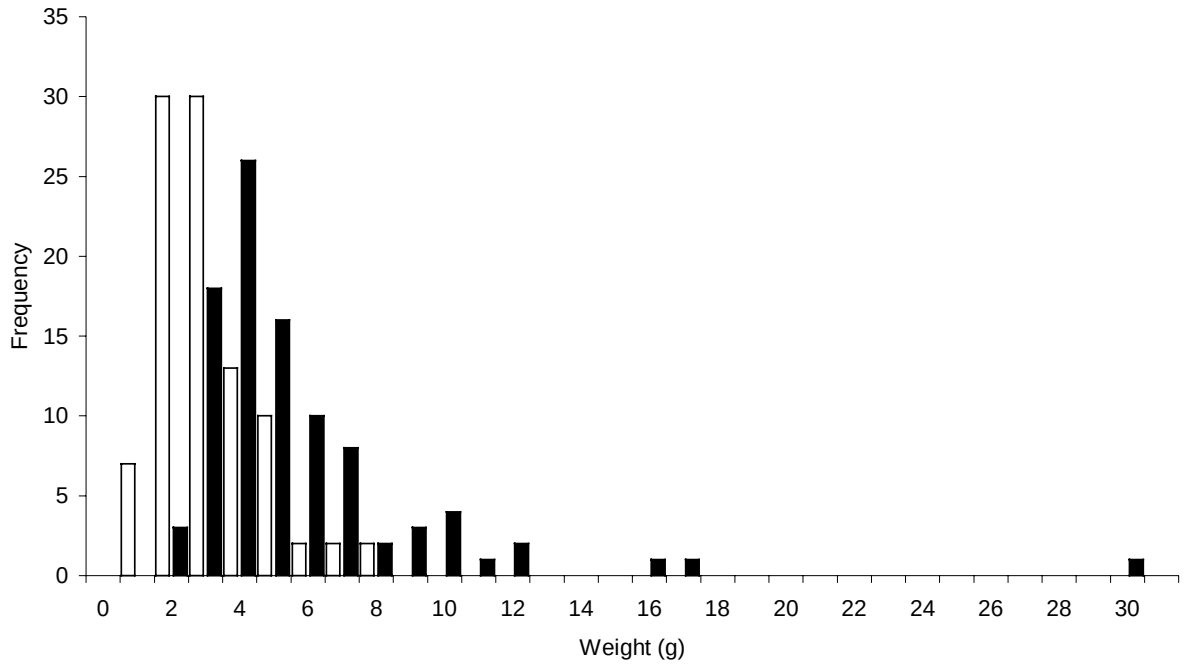

Figure 1. Size distribution of ungraded (clear bars) and graded (shaded bars) juvenile marron prior to stocking in Experiment 1.

Pond water temperatures were recorded using Tiny Tag Data Loggers® and downloaded using Gemini Logger Management® (GLM) software. The ponds were managed and fed according to standard marron industry techniques (Lawrence and Jones 2001). Ponds were aerated by paddlewheels for 20 minutes three times per day (1800, 2400, 0600). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed WA crayfish reference diet (Morrissy 1990). Both groups received similar initial feed rates but this was adjusted according to feed rates derived from Morrissy (1992), with daily adjustment for pond specific feed requirements according to growth and temperature variations by visually observing demand feeding.

At 6-weekly intervals a random sample of marron were collected from each pond by hide sampling to monitor stock condition and adjust feed rates if necessary. These marron were individually weighed and returned to the ponds. At the end of year 1, in June 2002, growth and survival data were collected from the whole population by drain harvesting ponds. Marron were removed, sexed and individually weighed. At this stage of the experiment, predation due to water rats (Hydromys chrysogaster) was observed to have compromised survival in the graded ponds. Consequently, while marron from control ponds were returned to their ponds, those from the three graded ponds were restocked into two ponds to maintain the same density as control ponds. In June 2003, at the conclusion of the two year experiment, ponds were again drained and marron were removed, sexed and individually weighed.

Experiment 2: The effect of size grading of juveniles upon marron production in commercial ponds (Kangaroo Island SA)

Six ponds (1066 ± 15 m^2 mean ± se) on a commercial marron farm in Kangaroo Island, South Australia (Lat -35.72, Long 137.93) were stocked with a total of 26,154 juvenile marron at an average density of 4 marron/m^2 in June 2003.

Juveniles from nursery ponds were pooled prior to stocking the growout ponds. The pooled juvenile population was randomly divided into two groups. From the first group, ungraded juveniles (mean weight = 2.36 ± 0.11 g) were stocked into three ponds. From the second group, juveniles for the remaining three ponds were graded. The smallest 75% of these graded juveniles were discarded and the remaining largest 25% of graded juveniles were stocked into three ponds.

Ponds were fed and managed in the same manner as for Experiment 1.

Data analysis

The gross value of production was calculated at the conclusion of each experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade (Table 3).

Data for mean weight and yield were analysed by analysis of variance. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance ($\alpha = 0.05$).

Results

Experiment 1: The effect of size grading of juveniles upon marron production in commercial ponds (Pemberton)

Water temperatures in ponds at Pemberton ranged from 6.5 – 27.4°C, mean 17.4°C (Figure 2).

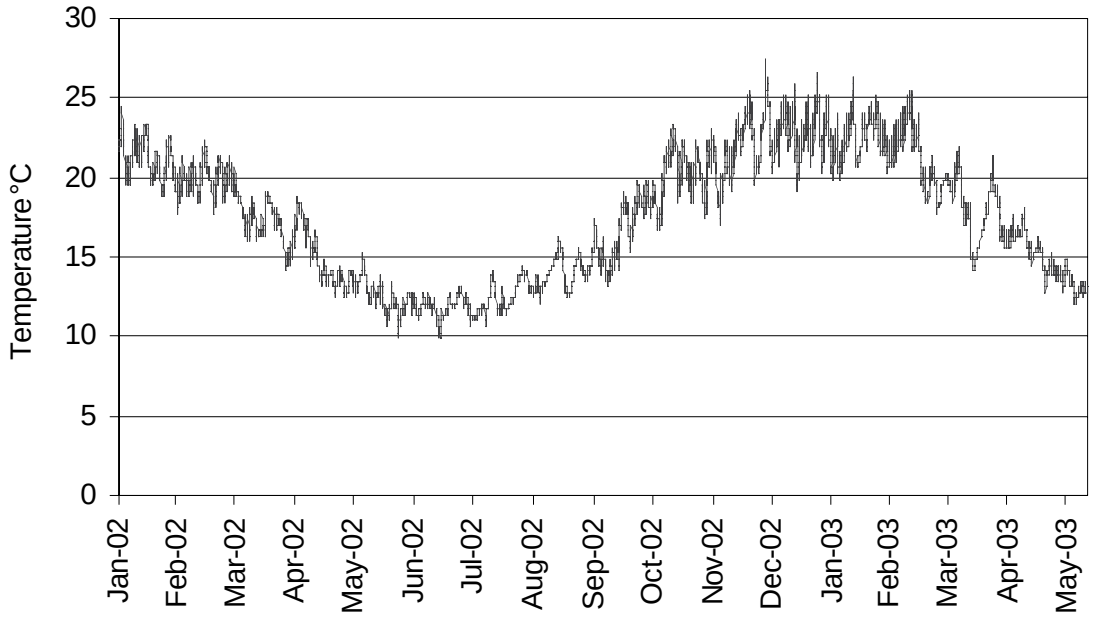

Figure 2. Water temperatures in ponds at Pemberton.

Year 1

At 1+ years of age the mean weight of marron in the three ponds stocked with graded juveniles (105 ± 9.64 g) was not significantly different from the three ponds stocked with ungraded juveniles (81 ± 1.90 g) (P = 0.07) (Table 1). There was no difference in the size variation of marron harvested from ponds stocked with either graded juveniles (CV = 54 ± 6.43%) or ungraded juveniles (CV = 69 ± 7.48%) (P = 0.20) (Table 1).

Table 1. Mean weight, size range and CV of marron from six commercial ponds at Pemberton stocked with either ungraded or graded juveniles after 1 year (n = 6).

	Ungraded	se	Graded	se	P =
Mean Weight (g)	81	1.90	105	9.64	0.07
Size range (g)	0.6-462		4.6-437		
CV Weight (%)	69	7.48	54	6.43	0.20
N (number harvested)	3,822		2,005		

Survival data for year 1 are not presented in Table 1 because of confounding by predation. While predation reduced survival, and therefore remaining density of marron at 1 year of age in ponds stocked with graded juveniles (1.19 ± 0.31/m^2) and ungraded juveniles (1.84 ± 0.24/m^2), this was not significantly different between treatments (P = 0.17).

Year 2

After two years the mean weight of marron harvested from the two ponds stocked with graded juveniles (171 ± 1.43 g) was greater than that of marron from three ponds stocked with ungraded juveniles (152 ± 2.05 g) (P =0.007) (Table 2). There was no difference in the size variation of marron harvested from ponds stocked with either graded juveniles (CV = 50 ± 1.10 %) or ungraded juveniles (CV = 54 ± 3.19%) (P = 0.42) (Table 2).

Table 2. Mean weight, size range, CV, survival, yield and proportion of marron above market size (> 70 g) from five commercial ponds at Pemberton stocked with either ungraded or graded juveniles after 2 years (n = 5).

	Ungraded	se	Graded	se	P =
Mean Weight (g)	152	2.05	171	1.43	0.007
Size range (g)	24 - 575		25 - 527		
CV Weight (%)	54	3.19	50	1.10	0.42
Survival Year1-Year 2 (%)	77	4.33	74	1.64	0.65
Yield (t/ha)	2.23	0.39	2.16	0.00	0.89
% Marketable (>70g)	89	1.97	95	0.65	0.07
N (number harvested)	2,974 (3 ponds)		1,498 (2 ponds)		

Survival of marron between year 1 and year 2 ranged from 70 – 85 % (mean = 76%). There was no difference in survival (P =0.65) or yield (t/ha) (P = 0.89) between marron in ponds stocked with ungraded or graded juveniles (Table 2).

Production ranged from 1.5 – 2.8 t/ha (mean = 2.2 t/ha), providing a gross income of $A36,485 - $A69,397 /ha (mean = $A55,378 /ha) and there was no significant difference between the treatments (P = 0.95). The proportion of the population above market size (> 70 g) was not significantly different in ponds stocked with ungraded or graded juveniles (P = 0.07) (Table 2). However, ponds stocked with graded juveniles had a greater proportion of marron in the 201-250 g size grade (Table 3). While there was a trend for ponds stocked with ungraded juveniles to have a greater proportion of the population in the smaller size grades (0-70 and 70-100) and fewer in the highest size grade (401+), this was not significant (Table 3).

Table 3. Proportion (mean ± se) of marron in size grades after 2 years in Experiment 1 (n = 5 ponds).

Size grade	Ungraded (%)	se	Graded (%)	se	n	P =	Value ($A/ kg)
0-70	11	1.97	5	0.65	5	0.07	0
71-100	19	0.96	13	1.65	5	0.05	15
101-150	31	1.04	32	0.50	5	0.63	20
151-200	18	2.02	24	0.50	5	0.11	27
201-250	9	0.21	11	0.17	5	0.01	28
251-300	6	0.19	6	1.09	5	0.49	31
301-400	6	0.18	5	3.03	5	0.51	32
401+	1	0.42	5	1.78	5	0.08	33

Experiment 2. : The effect of size grading of juveniles upon marron production in commercial ponds (Kangaroo Island SA).

Year 1

At 1+ years of age the mean weight of marron in ponds stocked with graded juveniles (45.82 ± 1.36 g) were larger than ponds stocked with ungraded juveniles (26.42 ± 4.28 g) (P = 0.012) (Table 4). There was no difference in the size variation of marron harvested from ponds stocked with either graded juveniles (CV = 72 ± 1.62%) or ungraded juveniles (CV = 105 ± 15.52%) (P = 0.096) (Table 4). There was no difference in survival (P = 0.12) between marron in ponds stocked with ungraded or graded juveniles (Table 4).

Table 4. Mean weight (mean ± se), size range, CV and survival of marron from six commercial ponds at Kangaroo Island stocked with either ungraded or graded juveniles after 1 year (n=6).

	Ungraded	se	Graded	se	P
Mean Weight (g)	26	4.28	46	1.36	0.012
Size range (g)	1.4 - 373		2.6 - 436		
CV Weight (%)	105	15.52	72	1.62	0.096
Survival (%)	74	1.25	58	7.95	0.124

Year 2

After two years the mean weight of marron harvested from ponds stocked with graded juveniles (136 ± 8.29 g) was greater than that of marron from ponds stocked with ungraded juveniles (86 ± 11.66 g) (P = 0.05) (Table 5). There was no difference in the size variation of marron harvested from ponds stocked with either graded juveniles (CV = 54 ± 3.38%) or ungraded juveniles (CV = 68 ± 4.53%) (P = 0.10) (Table 5).). There was no difference in survival (P = 0.46) between marron in ponds stocked with ungraded or graded juveniles (Table 5).

Table 5. Mean weight (mean ± se), size range, CV, survival, yield and proportion of marron above market size (> 70 g) from commercial ponds at Kangaroo Island stocked with either ungraded or graded juveniles after 2 years (n = 6 ponds).

	Ungraded	se	Graded	se	P
Mean Weight (g)	86	11.66	136	8.29	0.05
Size range (g)	10.9 - 481		17.5 - 456		
CV Weight (%)	68	4.53	54	3.38	0.101
Survival (%)	53	3.56	44	14.50	0.46
Yield (t/ha)	2.15	0.41	2.07	0.34	0.91
% Marketable (>70g)	48	9.23	82	5.43	0.10
Value/ha ($)	40,875	10,459	49,056	6,897	0.61

Production ranged from 1.3 – 2.7 t/ha (mean = 2.1 t/ha), providing a gross income of $A20,195 - $A55,953 /ha (mean = $A44,147 /ha) and there was no significant difference between the treatments (P = 0.61). The proportion of the population above market size (> 70 g) was not significantly different in ponds stocked with ungraded or graded juveniles (P = 0.10) (Table 5). However, ponds stocked with graded juveniles had a greater proportion of marron in the 251-400 g size grades (Table 6). While there was a trend for ponds stocked with ungraded juveniles to have a greater proportion of the population in the smaller size grades (0-70 and 70-100) and fewer in the highest size grade (401+), this was not significant (Table 6).

Table 6. Proportion (mean ± se) of marron in size grades after 2 years in Experiment 2 (n = 6 ponds).

Size grade	Ungraded (%)	se	Graded (%)	se	n	P	Value ($A/ kg)
0-70	28	7.08	8	2.61	6	0.07	0
71-100	17	0.71	13	1.61	6	0.11	15
101-150	23	1.25	22	1.23	6	0.46	20
151-200	16	2.15	21	1.71	6	0.20	27
201-250	10	1.73	17	0.72	6	0.08	28
251-300	4	1.14	11	0.53	6	0.04	31
301-400	2	0.74	8	0.62	6	0.03	32
401+	0	0.25	1	0.46	6	0.51	33

Discussion

In experiment 1 size grading of juveniles prior to stocking ponds increased the average weight of marron harvested by 12.5% and decreased the proportion of below market size animals by 54%, although the latter was not significant. Similarly in experiment 2 size grading of juveniles prior to stocking ponds increased the average weight of marron harvested by 58% and decreased the proportion of below market size animals by 65%, although the latter was not significant (P = 0,07). This simple, low cost technique may be applied by farmers to increase the average weight of marron harvested from commercial ponds.

However, in experiment 1 it involved rejecting 3570 small juveniles with a market value of $A357 – $A1,785 for three ponds. In addition, it is probable that over the longer term, size grading of juveniles without correct management of broodstock, could result in growth depression due to inbreeding. This is because it is likely that a selection strategy that stocks larger juvenile marron will be biased towards siblings from either females that release juveniles earlier or produce larger juveniles, due to maternal, nutritional or genetic factors.

In experiment 1, although graded juveniles were twice the size of ungraded juveniles when stocked, they were only 30% larger at 1+ year of age and 12.5% larger at 2+ years of age. Therefore the growth advantage shown by some juveniles in nursery ponds is not maintained throughout growout. This indicates that juvenile size from non synchronized reproduction is not a trait that can be used for genetic selection to improve the growth rate of marron to market size at two years of age.

6.2 The effect of refuge density upon marron production in commercial ponds

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Introduction

Growth and size variation are a major concern of industry because at harvest a significant proportion of the crop is below market size (> 70 g). One aspect of husbandry that warrants further investigation is the density of refuges, commonly referred to as hides, in marron ponds. Hides are used in freshwater crayfish and prawn farming for a variety of reasons including: reducing interaction among animals; minimising aggressive encounters; providing shelter for recently moulted animals; increasing available substrate in the water column; improving FCR's by increasing periphyton production; increasing production; and reducing stress (Figler et al. 1999, Geddes et al. 1993, Jones and Ruscoe 2001, Tidwell et al. 1999, Tidwell et al. 2000).

A variety of artificial shelter types have been evaluated in freshwater crayfish ponds including plastic pipes (Geddes et al. 1993), mesh bundles, pipe stacks, car tires, elevated cement/fibre-board sheets and cement/fibre-board sheets laid flat on the substrate (Jones and Ruscoe 2001), tanikalon fibre and onion bag mesh (Fellows 1995). In evaluating a number of hide materials, Jones and Ruscoe (2001), showed that the mesh bundles, similar to those used on marron farms were the most effective type of shelter for redclaw (*Cherax quadricarinatus*).

Plastic pipe hides have increased survival of yabbies (*C. destructor*) in ponds stocked at high densities (10-20/m^2) but not growth (Geddes et al. 1993). Similarly, while mesh bundles, tires

and pipe stacks increased survival of *C. quadricarinatus*, they did not improve growth (Jones and Ruscoe 2001). While the addition of hides did not increase growth of either yabbies (*Cherax destructor*) or redclaw (*C. quadricarinatus*), they did increase pond biomass primarily through improving survival (Geddes et al. 1993, Jones and Ruscoe 2001). It is therefore possible that marron production can also be improved by increasing the density of hides as marron utilise a range of artificial refuges in the wild (Molony and Bird 2005).

The aim of this study is to examine the effect of increasing the density of hides upon growth, size variation, survival and economic return of marron on a commercial farm.

Methods

Six ponds (mean 874 ± 35 m^2) on a commercial marron farm in Mt Barker Western Australia (Lat -34.63, Long 117.66) were stocked with 15,724 ungraded juvenile marron (mean weight 2.67 ± 0.25 g) at a density of 3 marron/m^2 in August 2001.

The 1,114 hides required for this experiment were each made from a 7 m length of marron hide mesh (5 mm mesh size) 0.75 m wide with 0.5 m length of twine passed through the centre of the folded mesh and a float and weight at opposite ends according to the methods of Fellows (1995). Three control ponds contained hides at the standard density for commercial marron ponds of 0.15 hides/m^2 (Lawrence and Jones 2001), while the 3 treatment ponds contained twice the number of hides (0.30 hides/m2). The cost of each hide was estimated at $A4.00 each.

Pond water temperatures were recorded using Tiny Tag Data Loggers® and downloaded using Gemini Logger Management® (GLM) software. The ponds were managed and fed according to standard marron industry techniques (Lawrence and Jones 2001). Ponds were aerated by paddlewheels for 20 minutes three times per day (1800, 2400, 0600). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed WA crayfish reference diet (Morrissy 1990). Both groups received similar initial feed rates but this was adjusted according to feed rates derived from Morrissy (1992), with daily adjustment for pond specific feed requirements according to growth and temperature variations by visually observing demand feeding.

At 6-weekly intervals a random sample of marron were collected from each pond by hide sampling to monitor stock condition and adjust feed rates if necessary. These marron were individually weighed and returned to the ponds

At the end of year 1, in August 2002, growth and survival data were collected from the whole population by drain harvesting ponds. Marron were removed, sexed and individually weighed, then returned to the ponds. In August 2003, at the conclusion of the two year experiment, ponds were drained and marron were removed, sexed and individually weighed.

The gross value of production was calculated at the conclusion of the experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade (Table 2).

Data for mean weight and yield were analysed by analysis of variance. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance ($\alpha = 0.05$).

Results

Water temperatures in ponds at Mt Barker ranged from 6.2 – 24.5°C, mean 15.8°C (Figure 1).

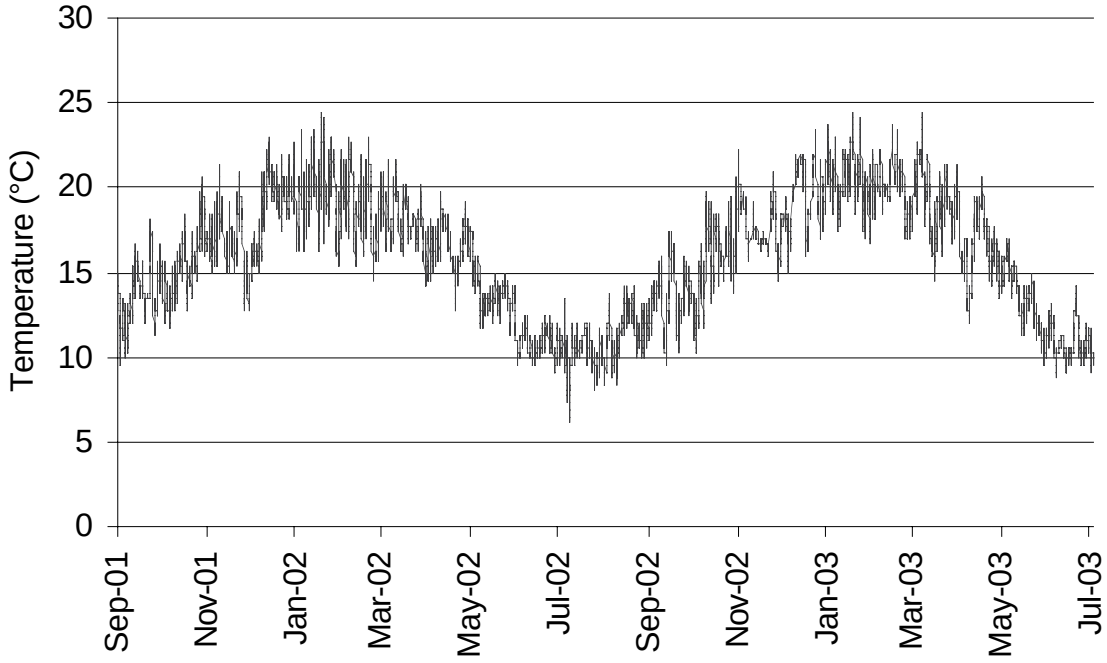

Figure 1. Water temperatures in ponds at Mt Barker.

There was no difference in the mean weight of marron from ponds containing hides at the standard density (0.15/m^2) (116 ± 5.29 g) or double the number of hides (0.30 hides/m^2) (124 ± 4.31 g) (P = 0.29) (Table 1). Size variation of marron from ponds with the standard hide density (0.15/m^2) (CV = 47 ± 0.54%) was not different to those from ponds containing double the number of hides (0.30/m^2) (CV = 45 ± 0.82%) (P = 0.13) (Table 1).

Table 1. Mean weight (mean ± se), size range, CV, survival, yield and proportion of marron above market size (> 70 g) from six commercial ponds at Mt Barker after two years with either hides at 0.15/m^2 or 0.30/m^2 (n = 6 ponds).

	Hides	se	Double hides	se	P =
Mean Weight (g)	116	5.29	124	4.31	0.29
Size range (g)	28 - 485		36 - 503		
CV Weight (%)	47	0.54	45	0.82	0.13
Survival (%)	84	3.41	78	4.14	0.33
Yield (t/ha)	2.88	0.21	2.91	0.24	0.94
% Marketable (>70 g)	79	2.41	86	2.08	0.10
N (number harvested)	6,945		5,706		

Survival of marron to 2 years of age ranged from 70 – 91% (mean = 81%). There was no difference in survival (P = 0.33) or yield (P = 0.94) between marron in ponds with standard hide density (0.15/m^2) or the number of double hides (0.30/m^2) (Table 1).

Production ranged from 2.5 – 3.3 t/ha (mean = 2.9 t/ha), providing a gross income of $A52,296 - $A75,152 /ha (mean = $A62,514 /ha) and there was no significant difference between the treatments (P = 0.77). There was no difference in the proportion of the population above market size (> 70 g) in ponds with double hides (0.30/m^2) compared with standard hide density ponds (0.15/m^2) (P = 0.10) (Table 1, Table 2). While the slight increase in the proportion of animals in the larger size grades (Table 2) did provide an increase in gross return to the farmer of over $A2,700 /ha, the net return from doubling hides, (due to the cost of additional hides $A6,000 /ha) is only economically viable if they are reused for 6 years (i.e. 3 growout cycles).

Table 2. Proportion (mean ± se) of marron in size grades after 2 years in ponds with standard hide density (0.15/m^2) or the number of double hides (0.30/m^2) (n = 6 ponds).

Size grade	Hides (%)	se	Double Hides (%)	se	n	P	Value ($A/ kg)
0-70	21	2.46	14	2.08	6	0.10	0
71-100	26	0.98	27	1.23	6	0.71	15
101-150	30	1.15	32	0.40	6	0.18	20
151-200	15	2.23	18	1.01	6	0.42	27
201-250	5	1.45	7	1.51	6	0.41	28
251-300	2	0.39	2	0.35	6	0.60	31
301-400	1	0.10	1	0.05	6	0.72	32
400+	0	0.03	0	0.03	6	0.10	33

Discussion

There was no significant difference between the mean weight of marron reared in ponds that contained hides at the standard density (0.15 hides/m^2) (116 ± 5.29 g), compared with those from ponds that contained twice the number of hides (0.30 hides/m^2) (124 ± 4.31 g) (P = 0.29). The increased shelter provided from doubling the number of hides did not increase survival. Similarly, the increased provision of substrate for periphyton production did not significantly increase pond carrying capacity expressed as total pond yield (t/ha). Consequently, this experiment has shown that there is no justification for increasing the number of hides in commercial marron ponds above 0.15 hides/m^2.

This husbandry experiment has shown that the current farming system for marron is capable of achieving high yields (2.91 t/ha) and up to 95% of marron above minimum market size at two years of age, with little change to standard production techniques recommended by the Department of Fisheries Western Australia.

6.3 The effect of increased aeration upon marron production in commercial ponds

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Introduction

For stocks to survive in aquaculture ponds dissolved oxygen is the most critical water quality variable (Boyd 1990). In addition to improving survival, studies with prawns and catfish have shown that aeration can double production biomass (i.e.carrying capacity) per pond (Boyd 1990). However, among marron farmers in Australia there is still considerable debate on the value of aeration for improving production.

Previous studies have shown that the most economical method of aerating ponds is using paddlewheels (Boyd 1990). In a comparison of 5 types or aeration (Paddlewheel, aspirator, vertical pump, pump sprayer and diffused air) Paddlewheel aerators were the most efficient (Boyd 1990).

In comparison to the value of a pond of marron ($3,000 - 6,000), the cost of running paddlewheels is low. Bird and Cassells (1996) reported operating costs (as at 10/11/2000) of only 21cents/hr or at current industry aeration regimes only $86/year.

However, there are no precise guidelines on the length of aeration required (Boyd 1990). While most catfish and prawn farms use paddlewheel aerators for 6 – 12 hours every night (Boyd 1990) and high density ponds run aerators 24 hours per day, in contrast marron farmers use very little aeration. The average aeration regime consisting of only 30 min three times per day at late afternoon, midnight and dawn.

The aim of this experiment was to determine if an increase in aeration time from 30 min – 1 hour three times per day 365 days/year is justified by the increase in value of marron harvested for the increased cost of production from $86 /pond to $230 /pond or in real terms around 7 kg of marron.

Methods

In May 2003 on a commercial farm on Kangaroo Island (Lat -35.72, Long 137.93) in South Australia 6 ponds were stocked with juvenile marron (mean = 2.36, sd = 0.55 g) at a density of 5 animals/m^2 (average pond water surface area was 1,000 m^2) and fed the standard industry diet.

Three ponds were aerated for 30 min three times per day at late afternoon (1600), midnight (2400) and dawn (0600). The other three ponds were aerated for 60 min per day at late afternoon, midnight and dawn. (Table 1).

The ponds were managed and fed according to standard marron industry techniques (Lawrence and Jones 2001). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed WA crayfish reference diet (Morrissy 1990). Both groups received similar initial feed rates but this was adjusted according to feed rates derived from Morrissy (1992), with daily adjustment for pond specific feed requirements according to growth and temperature variations by visually observing demand feeding.

At 12-weekly intervals a random sample of marron were collected from each pond by hide sampling to monitor stock condition and adjust feed rates if necessary. These marron were individually weighed and returned to the ponds

At the end of year 1, in May 2004, growth and survival data were collected from the whole population by draining ponds. Marron were removed, sexed and individually weighed, then returned to the ponds. In May 2005, at the conclusion of the two year experiment, ponds were drained and marron were removed, sexed and individually weighed.

The gross value of production was calculated at the conclusion of the experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade.

In year 1 data for mean weight and yield were analysed by analysis of variance. In year 2, Levene's test demonstrated unequal variances for mean weight data ($P = 0.04$), consequently this data was analysed using the Mann-Whitney *U* Test. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance ($\alpha = 0.05$).

Results

Year 1

At 1+ years of age there was no difference in the mean weight ($P = 0.21$), CV ($P = 0.17$) or survival ($P = 0.22$) of marron from ponds with either standard or increased aeration (Table 1).

Table 1. Mean weight, size range, CV and survival of marron from six commercial ponds at Kangaroo Island stocked with increased aeration or standard aeration after 1 year (n = 6).

	Standard Aeration	se	Increased Aeration	se	P
Mean Weight (g)	26	4.28	33	0.36	0.21
Size range (g)	1.4 - 373		0.9 - 434		
CV Weight (%)	105	15.52	76	8.38	0.17
Survival (%)	74	1.25	66	6.15	0.22

Year 2

After two years the mean weight of marron from ponds with increased aeration was larger than those from ponds with standard aeration ($P < 0.05$). There was no difference in survival ($P = 0.75$) or percentage above market size (70 g) ($P = 0.17$) of marron harvested from ponds with increased aeration or standard aeration (Table 2).

Table 2. Mean weight, size range, CV, survival, yield and proportion of marron above market size (> 70 g) from commercial ponds at Kangaroo Island with increased aeration or standard aeration after 2 years (n=6).

	Standard Aeration	se	Increased Aeration	se	P
Mean Weight (g)	86	11.66	112	2.59	0.049
Size range (g)	10.9 - 481		8.9 - 508		
CV Weight (%)	68	4.53	63	2.88	0.37
Survival (%)	53	3.56	51	4.68	0.75
Yield (t/ha)	2.15	0.41	2.61	0.18	0.34
% Marketable (>70g)	48	9.23	65	4.05	0.17
Value/ha ($)	40,875	10,459	56,940	3,756	0.22

Production ranged from 1.3 – 3.0 t/ha (mean = 2.4 t/ha), providing a gross income of $A20,195 - $A64,395 /ha (mean = $A48,908 /ha). There was no significant difference in production ($P = 0.34$) or value/ha ($P = 0.22$) between ponds with increased aeration or standard aeration (Table 2).

Discussion

Marron from ponds with increased aeration (mean weight = 112 ± 2.59 g) were larger than those from ponds with standard aeration (mean weight = 86 ± 11.66 g) and there was less variation among ponds with increased aeration (se = 2.59). This resulted in a 39% increase in gross return to the farmer for an increase in production costs of only 6%. At current market values this equates to an increased gross return of $A1,600 /pond. In comparison to the minor increase in production costs due to additional aeration ($A86 /year), this resulted in an increased net return of over $A1,400 /pond.

Yields of over 2 t/ha were obtained from both treatments. Therefore at current industry standard stocking densities and feed rates aeration is not a limiting factor in achieving carrying capacities of 2 t/ha. However, the data from this experiment indicates that for a relatively minor increase in aeration costs there is potential to further increase pond biomass and feeding rates in commercial ponds.

6.4 The effect of relaying upon marron production in commercial ponds

Project team: C. Lawrence, G. Cassells, S. How and C. Bird

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Introduction

Relaying usually involves transferring juveniles from ponds containing stunted populations to under stocked water bodies where food and space do not limit growth (McClain et al.1993, McClain and Romaire 1995). In general relaying is used to increase production in other species of freshwater crayfish, to overcome reduced growth due to overpopulation and food shortages in systems where crayfish reproduction cannot be controlled (McClain et al.1993).

While density and food have a major effect upon marron production, growth is also dependant upon temperature (Morrissy 1990, Morrissy 1992). Growth of marron is limited by high summer temperatures in the northern marron farming region and by cool winter temperatures in the southern marron farming regions (Morrissy 1990). Therefore in Western Australia it may be possible to improve marron growth by relaying crayfish between northern and southern farms to maximise the period spent in optimum temperature ranges. Furthermore, in Western Australia, it has been reported that due to warmer temperatures the peak spawning period for northerly marron populations (Hutt River) is thought to be in July (Beatty et al. 2005), compared with that of the more southern (Warren River) populations in September/October (Morrissy 1970, Morrissy 1975). Consequently, if this is correct juvenile marron would be released almost 4 months earlier in the north of the state (September) compared with the south (December) (Burton 1995, Beatty et al. 2005, Morrissy 1970, Morrissy 1975). It is not known if early released juveniles from the north have a growth advantage compared with those released later in the south.

However, relaying increases the risk of spreading disease, particularly *Thelohania*, as was experienced by the Western Australian yabby industry transferring juveniles among farm dams (Jones and Lawrence 2001)

The aim of this experiment was to compare the growth of marron produced in the northern marron farming region that were relayed as juveniles onto a farm in the southern region.

Methods

Six ponds (800 ± 0 m^2) on a commercial marron farm in Denmark in the south of Western Australia (Lat -34.96, Long 117.35) were stocked with a total of 14,400 juvenile marron at a density of 3 marron/m^2 in May 2003. Prior to stocking a random sample of 90 juveniles/pond were individually weighed.

Three of the ponds were stocked with juveniles from a northern farm (Geraldton Lat -28.77, Long 114.61), where they had been released in early December 2002. The remaining three

ponds were stocked with juveniles produced on the southern farm (PFRC Pemberton Lat -34.45, Long 116.03), where they were released in late December 2002.

Pond water temperatures were recorded using Tiny Tag Data Loggers® and downloaded using Gemini Logger Management® (GLM) software. The ponds were managed and fed according to standard marron industry techniques (Lawrence and Jones 2001). Ponds were aerated by paddlewheels for 20 minutes three times per day (1800, 2400, 0600). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed WA crayfish reference diet (Morrissy 1990). Both groups received similar initial feed rates but this was adjusted according to feed rates derived from Morrissy (1992), with daily adjustment for pond specific feed requirements according to growth and temperature variations by visually observing demand feeding.

At 6-weekly intervals a random sample of marron were collected from each pond by hide sampling to monitor stock condition and adjust feed rates if necessary. These marron were individually weighed and returned to the ponds

At the end of year 1, in May 2004, growth and survival data were collected from the whole population by drain harvesting ponds. Marron were removed, sexed and individually weighed, then returned to the ponds. In May 2005, at the conclusion of the two year experiment, ponds were again drained and marron were removed, sexed and individually weighed.

The gross value of production was calculated at the conclusion of the experiment by dividing marron into standard industry size grades and applying current farm gate values for each size grade.

In year 1 data for mean weight and yield were analysed by analysis of variance. In year 2, Levene's test demonstrated unequal variances for mean weight data ($P = 0.03$), consequently this data was analysed using the Mann-Whitney U Test. The data for proportions (CV, % survival, proportion of marron above market size (> 70 g) and proportion of marron in size grades) were transformed with the arcsine square root function to satisfy the assumption of normality, prior to use of analysis of variance ($\alpha = 0.05$).

Results

Five months after release juveniles produced in the northern region (Geraldton) (3.04 ± 0.18 g) were smaller than those produced in the southern region (Pemberton) (4.18 ± 0.27) ($P = 0.02$) (Table 1). Juveniles produced in the northern region (Geraldton) (50 ± 1.79 g) had a lower CV than those from the southern region (Pemberton) (67 ± 3.76) ($P = 0.02$) (Table 1).

Table 1. Mean weight, size range and CV at stocking (May 2003) of juveniles produced in the southern region (Pemberton) and northern region (Geraldton) (n = 6).

	Southern juveniles (Pemberton)	se	Northern juveniles (Geraldton)	se	P =
Mean Weight (g)	4.18	0.27	3.04	0.18	0.02
Size range (g)	0.6 – 15.8		0.7 – 7.8		
CV Weight (%)	67	3.76	50	1.79	0.02

The reduced growth of juveniles in the northern region between release in December and stocking in May resulted in a greater proportion of these juveniles in the smaller size categories, in particular between 2 – 3 g (Figure 1).

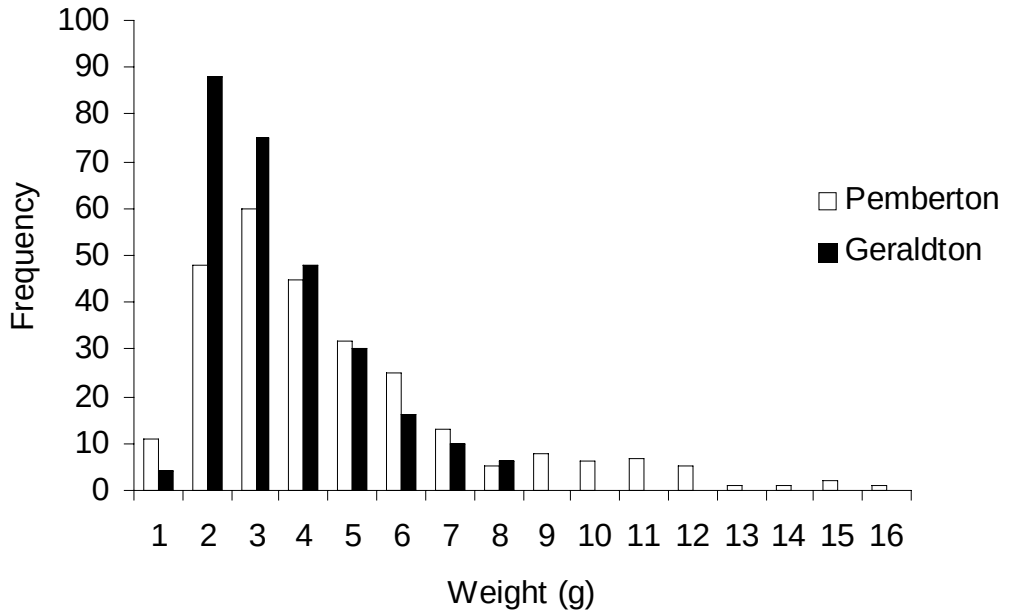

Figure 1. Size distribution at stocking in May 2003 of juveniles produced in the northern region (Geraldton, n = 277) and southern region (Pemberton, n = 270).

Year 1

There was no difference in mean weight (P = 0.40) or survival (P = 0.30) of juveniles produced on either the southern or northern farm (Table 2). The CV of juveniles relayed from the northern farm (54 ± 0.85) was lower than that of juveniles produced on the southern farm (65 ± 2.64) (P = 0.02) (Table 2).

Table 2. Mean weight, size range, CV and survival of marron from six commercial ponds at Denmark stocked with northern relayed juveniles and southern juveniles after 1 year (n=6).

	Southern juveniles	se	Northern relayed juveniles	se	P
Mean Weight (g)	81	10.18	74	2.69	0.40
Size range (g)	12.6 - 408		15.7 - 318		
CV Weight (%)	65	2.64	54	0.85	0.02
Survival (%)	35	8.29	46	3.25	0.30

Year 2

After two years the mean weight of marron from ponds stocked with juveniles produced in the southern region was 30% larger than those from ponds with juveniles relayed from the northern region ($P < 0.05$). There was no difference CV or survival (Table 3).

Remediation of low pH by liming addressed the poor survival recorded in year 1 for both treatments (Southern juveniles = 35 ± 8.29% and Northern relayed juveniles = 46 ± 3.25%) (Table 2), with much higher survival recorded between year 1 to year 2 (Southern juveniles = 80 ± 5.16% and Northern relayed juveniles = 81 ± 1.35%) (Table 3).

Table 3. Mean weight, size range, CV, survival, yield and proportion of marron above market size (> 70 g) from commercial ponds at Denmark stocked with northern relayed juveniles and southern juveniles after 2 years (n=6).

	Southern juveniles	se	Northern relayed juveniles	se	P =
Mean Weight (g)	170	19.35	131	3.77	0.049
Size range (g)	24.9 - 596		27.6 - 489		
CV Weight (%)	54	4.25	49	1.17	0.32
Survival (%)	28	7.57	37	2.89	0.32
Survival (%) Yr 1 – Yr 2	80	5.16	81	1.35	0.74
Yield (t/ha)	1.35	0.27	1.47	0.09	0.69
% Marketable (>70g)	89	4.91	87	2.79	0.71
Value/ha ($)	34,451	5,923	33,543	1,925	0.89

Production ranged from 0.8 – 1.7 t/ha (mean = 1.4 t/ha), providing a gross income of $A29,922 – $A41,822 /ha (mean = $A33,997 /ha) and there was no significant difference between the treatments ($P = 0.89$). The proportion of the population above market size (> 70 g) was not

significantly different in ponds stocked with northern relayed juveniles or southern juveniles (P = 0.71) (Table 3).

Discussion

Although juvenile marron have been reported to be released around 4 months earlier in the northern marron farming region (Beatty et al. 2005), by May when juvenile ponds are drained and marron are restocked into grow out ponds they were 27% smaller than juveniles produced in the south. This is most probably due to juvenile release not occurring until later than that reported by Beatty et al. (2005). It is also possible that higher water temperatures limit juvenile growth between December-March in the northern regions (Morrissy 1990).

After 2 years growth in commercial ponds the marron produced in the southern region were 30% larger than those relayed as juveniles from the northern region. These results corroborate those from the experiments in this project evaluating size grading of juveniles, where the effects of genetic selection for growth by culling the smaller juveniles from breeding ponds prior to stocking were examined (see Section 6.1). Early growth differences among juveniles, due to genetic factors, release date or environmental variation between farms have a significant influence upon final harvest size. As a result farmers should only stock commercial ponds with larger juveniles from each cohort, as small juveniles due to genetic and/or environmental factors fail to achieve the same growth rates and result in smaller marron at harvest.

In addition, the relaying technique is labour intensive and farmers may be transferring genetically inferior "runts" into their commercial ponds, which could exacerbate the "stunting" problem in future generations.

Therefore, although relaying is used in the USA where both crawfish and rice are cultured in neighbouring ponds and in Western Australia, where farmers transfer small yabbies from overpopulated dams into under populated dams, it does not offer benefits for marron production in commercial ponds.

6.5 The use of morphology to determine condition of marron in commercial ponds

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Introduction

Farmers currently have no simple method for quantifying the condition of marron in commercial ponds. While previous studies have shown a relationship between hepatopancreas indices and condition, this technique is destructive and therefore not appropriate for a high value animal such as marron on commercial farms.

Other non destructive techniques for assessing condition such as BIA and TOBEC require equipment that is expensive and therefore are not currently appropriate for commercial marron farms.

This study investigates simple morphological characteristics to determine if growth rate in marron is related to abdomen meat reserves and in particular the variation in abdomen depth observed in marron harvested from commercial ponds.

Methods

Marron that had been reared for 2 years in 11 commercial ponds from 4 different farms (Pinjarra Lat -32.63, Long 115.87, Pemberton Lat -34.45, Long 116.03, Mt Barker Lat -34.63, Long 117.66 and Denmark Lat -34.96, Long 117.35) were randomly sampled (n=20/pond, 10males:10 females) and weighed. Measurements were recorded from each animal for Abdomen Length (AL), Abdomen Width (AW), Abdomen Depth (AD), Carapace Length (CL) and Carapace Width (CW). Growth rate (g/day) was calculated from the mean weight of the population at stocking.

Results

There is a strong negative relationship ($r^2 = 0.68$, $r = -0.82$, $P < 0.001$) between growth rate and Abdomen depth /Abdomen area (Abdomen Width x Abdomen Length) (Figure 1). Marron with proportionately deeper abdomens grow slower than those with shallower abdomens (Figure 1).

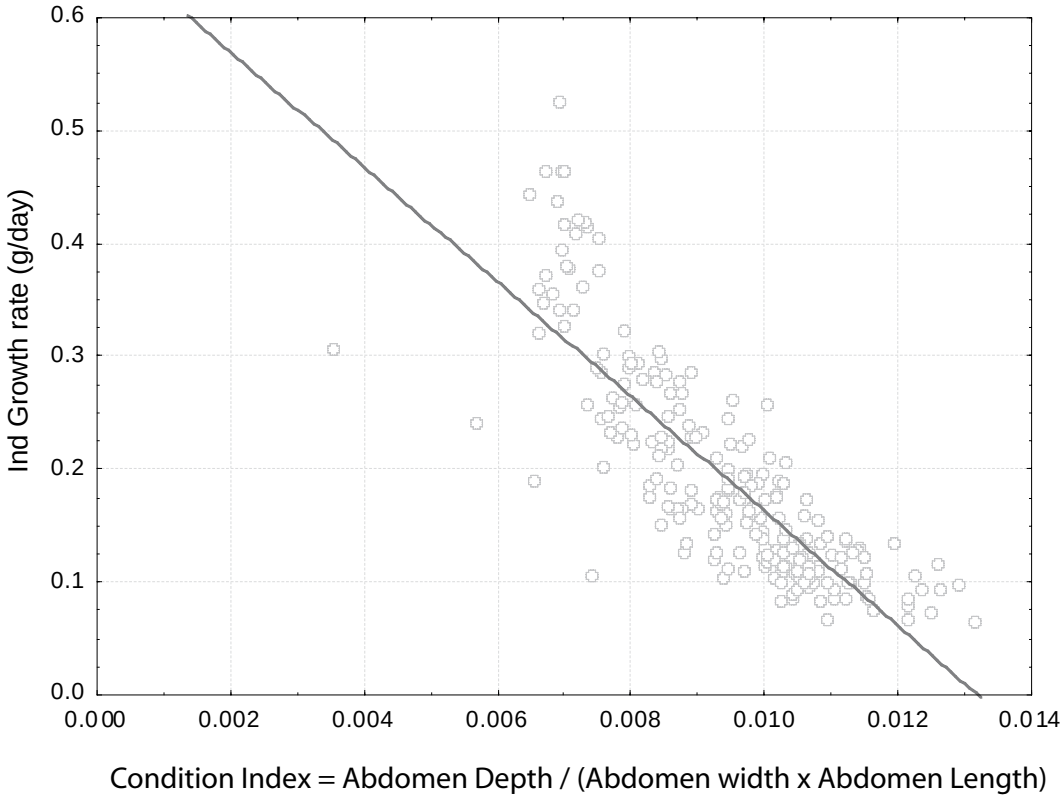

Figure 1. Relationship between growth rate (g/day) and Abdomen Depth/ Abdomen area (Abdomen width x Abdomen Length).

The regression equation is:

Growth rate (g/day) = 0.6709-50.8015 x (Abdomen Depth/Abdomen area)

Where Abdomen area = Abdomen width x Abdomen Length

For application on commercial farms this condition index can be summarised according to Table 1.

Table 1. Growth (g/day) for marron in commercial ponds as indicted by condition index Abdomen depth/ Abdomen area (where Abdomen area = Abdomen width x Abdomen Length).

	Growth rate (g/day)	Condition index
Poor growth	<0.15	>0.01
Average growth	0.15-0.25	0.01-0.008
Rapid growth	>0.30	<0.008

Discussion

Marron populations with proportionately shallower abdomens are growing faster than those with deeper abdomens. This simple condition index can be applied by farmers to evaluate the condition of marron populations in commercial ponds.

While it is difficult to randomly sample a population of marron from a commercial pond, as trapping is biased towards large males, this simple condition index shows that by recording three simple measurements Abdomen depth, Abdomen width and Abdomen Length from a relatively small sample of marron (20 /pond) it is possible to calculate if the population of animals are growing slowly (CI > 0.01), average (CI = 0.01-0.008) or rapidly (CI = < 0.008).

While this simple technique has been developed for the management of marron populations in commercial ponds, it could also be applied to farm dam and wild river populations. In these cases, where the age of stock is unknown, it would facilitate management practices by providing a better understanding of the growth rate of "wild" marron populations. For example 40 marron were sampled from two different sites (20 per site) on the Harvey river. Marron from the first site had average condition indices of 0.010, while those from the second site averaged 0.009 (C. Lawrence, unpublished data). If we apply the equation developed in this study to calculate growth rate from condition index, (Growth rate (g/day) = 0.6709-50.8015 x (Abdomen Depth/ Abdomen area)), it indicates that marron in the first sampling site were growing slower (0.19 ± 0.02 g/day) than those at the second sample site (0.22 ± 0.02 g/day).

6.6 The use of hide harvesting as an indicator of marron growth and condition in commercial ponds

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Introduction

Farmers currently have no simple method for determining the size and growth rate of marron in commercial ponds. Unlike fish, the size of marron cannot be observed readily as they do not come to the surface to feed. Similarly, while a sample of fish can be obtained and weighed by seine or scoop netting, this is not appropriate for freshwater crayfish as they become entangled in the mesh and this can result in loss of limbs.

While some marron farmers use trapping to sample their stock, this method provides an overestimate of average weight, as traps are biased towards catching large crayfish (Lawrence et al., in press).

The lack of a suitable technique for obtaining a random sample of marron from a commercial pond, aside from draining the pond, is a major limitation for effectively managing marron farms. A simple technique that can be applied on commercial farms is required so that managers can calculate feed rates, growth rates and plan marketing.

Refuges, commonly referred to as hides, are used to provide shelter for marron in commercial ponds. However, it is not known if marron residing in the hides are representative of the population or are biased towards small or large animals.

This study investigates hide harvesting to determine if it can provide a reliable technique for estimating the average size of marron in commercial ponds.

Methods

Sixteen marron ponds on 3 farms that had been stocked with juveniles 1 – 2 years previously were hide sampled (see section 6.2 for detailed description of hides). Hides were sampled by scooping a hide sampler under the hide and lifting it to the surface. The hide sampler consisted of an 1.0 cm diameter cuboid aluminium frame (40 cm deep x 40 cm wide x 60 cm high). The frame was covered with plastic mesh (2 mm gap width) on all sides except the top. When scooped under the hide the hide sampler retained the hide along with marron that had been living in the hide, whilst permitting water to drain through the mesh back into the pond.

Marron collected by hide sampling were weighed individually and returned to the pond. The number of marron sampled in each pond ranged from 20 – 113 (mean = 51/pond)

Each pond was then drained and all marron collected. From the population a random sample (mean = 702 marron/pond) were weighed individually.

Data was analysed using the regression package in Statistica.

Results

There is a very strong relationship ($r^2 = 0.97$, $r = 0.99$, $P < 0.0001$) between the mean weight of marron collected by hide harvesting and marron collected by draining the pond (Figure 1).

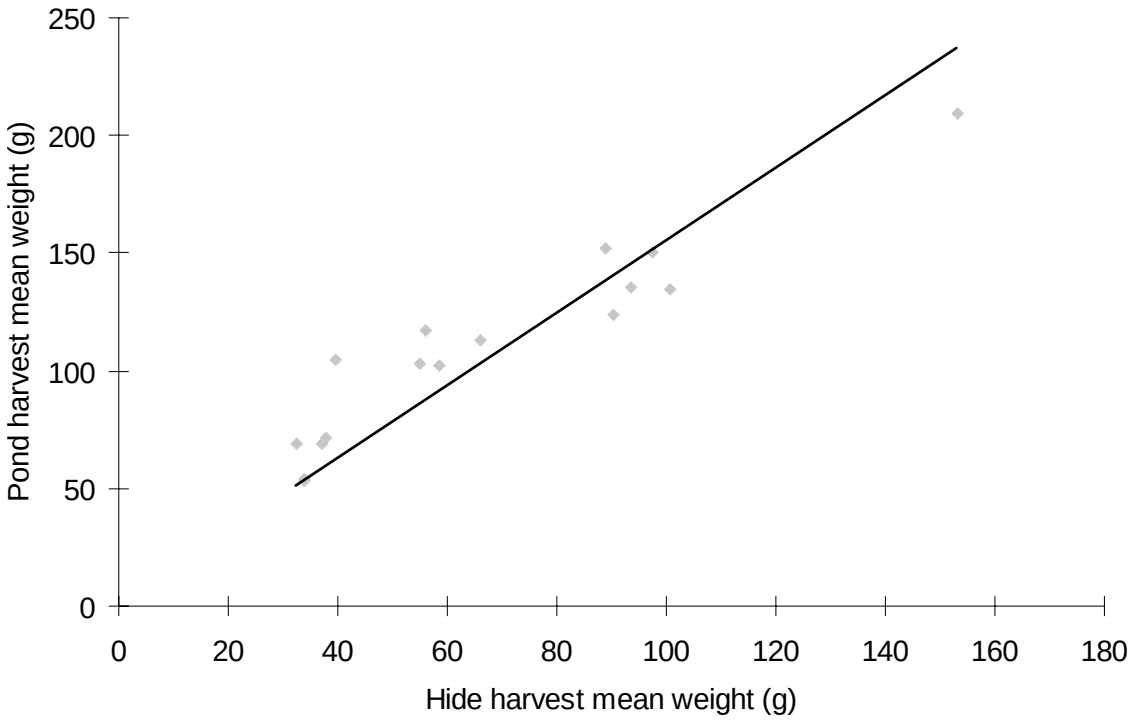

Figure 1. Relationship between pond harvest mean weight (g) and hide harvest mean weight (g).

The regression equation for estimating the mean weight of marron in a commercial pond is:

Mean weight (g) = 1.546 x (Hide harvest mean weight (g))

Discussion

Hide harvesting is a simple, reliable technique that can be used by farmers to determine the average weight of marron in a pond. The equation for calculating pond mean weight from hide harvest mean weight is not entirely suitable for small marron below 30 g, however above this size the relationship is robust. (Figure 1, Table 1).

This sampling technique and equation will permit farmers to calculate growth rates, feed rates and condition of marron (see section 6.5).

For example, by adapting the predictive equations for marron developed by Morrissy (1992), hide harvesting can be used to calculate the feeding rate (Table 1). This information can also be applied to determine if the marron are growing faster or slower than predicted and adapt feeding strategies accordingly (Table 1).

Table 1. Feeding rate for marron in Pemberton initially stocked at 3 juveniles/m^2 according to hide harvesting data (recalculated according to predictive equations by Morrissy 1992).

		Aug	Nov	Feb	May	Aug
	Water Temp ºC	**12.2**	**19.2**	**22.5**	**13.4**	**12.2**
0+ - 1+	Feed as % BW/Day	4.9	3.6	1.8	0.7	0.4
	Feed as g/m²/week	6.8	14.7	20.1	12	8.1
	Hide harvest mean weight (g)	3	8	23	38	46
	Marron mean weight (g)	5	13	35	58	71
1+ - 2+	Feed as % BW/Day	0.4	1.5	1.1	0.6	0.5
	Feed as g/m²/week	9.9	29.7	30.1	20.3	16
	Hide harvest mean weight (g)	52	61	82	109	113
	Pond marron mean weight (g)	81	95	126	169	175

6.7 The effect of regional variation upon marron production in commercial ponds

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Introduction

Marron (*Cherax tenuimanus*) are native to the main permanent rivers in the forested, high rainfall areas in the south west of Western Australia. Marron farming has extended the original distribution of marron from the southwest corner of Western Australia east to Esperance, west to the wheatbelt region and north to Geraldton (Figure 1).

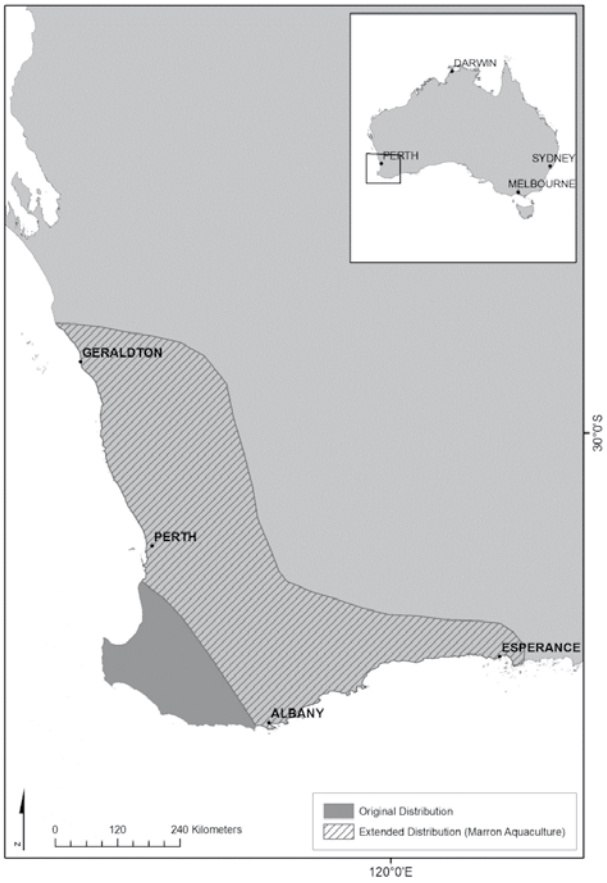

Figure 1. Original and extended distribution of marron for aquaculture in Western Australia.

In addition marron have been introduced to South Australia and New South Wales for aquaculture (Figure 2).

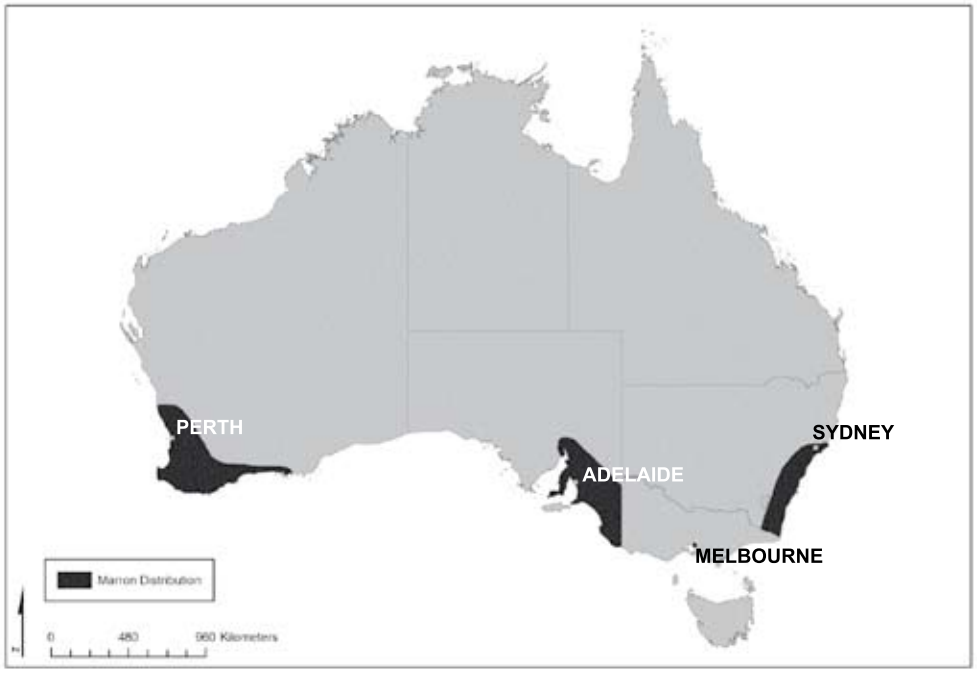

Figure 2. Marron farming regions in Australia.

International interest in marron farming has led to the species being introduced into South America, South Africa, Zimbabwe, Japan, USA, China and the Caribbean.

While it is recognised that variation in environmental parameters (i.e. temperature, water chemistry) can affect growth and survival of freshwater crayfish, it is not known if marron farms outside the original distribution of this species achieve higher or lower growth and production.

The aim of this experiment was to compare key environmental variables and production of marron from commercial farms in Western Australia and South Australia.

Methods

Production (yield t/ha, survival, growth rate) over the industry standard 2 year growout period was recorded from 20 commercial ponds on 4 marron farms in Western Australia (Pinjarra Lat -32.63, Long 115.87, Pemberton Lat -34.45, Long 116.03, Mt Barker Lat -34.63, Long 117.66 and Denmark Lat -34.96, Long 117.35) and one in South Australia (Kangaroo Island Lat -35.72, Long 137.93) (Figure 3). In addition for comparison 3 research ponds were stocked with mass selected juveniles at the Department of Fisheries Pemberton Freshwater Research Centre (PFRC).

Figure 3. Locations of commercial farms.

To address potential differences in growth among genetic stocks from different farms, ponds were stocked with juveniles from cohorts of either Mass selected Generation 1 stock (4 ponds), Mass selected Generation 2 stock (3 commercial ponds and 3 research ponds) or Pemberton stock (13 ponds) that had been produced at PFRC. This also provided the opportunity to compare performance of selected and unselected stock in different regions.

Environmental variables (Water temperature and water chemistry parameters) were recorded from ponds.

Pond water temperatures were recorded using Tiny Tag Data Loggers® and downloaded using Gemini Logger Management® (GLM) software.

Water samples were collected from ponds and analysed by the Australian Government Analytical Laboratories for the following parameters, Alkalinity, CO_3, Ca, Cl, Cu total, Conductivity, Fe, HCO_3, Hardness, K, Mg, Ammonia, Nitrite, Nitrate, Na, P-SR, SO_4 S, Zn total, pH.

All ponds were managed and fed in the same manner, according to standard marron industry techniques (Lawrence and Jones 2001). Ponds were aerated by paddlewheels for 20 minutes three times per day (0600, 1800, 2400). Fifty percent of each pond's water volume was replaced each year to compensate for losses due to evaporation and seepage. Marron were fed WA crayfish reference diet (Morrissy 1990). Feed rates were derived from Morrissy (1992), with daily adjustment for pond specific feed requirements according to growth and temperature variations by visually observing demand feeding.

Results

Environmental parameters

Temperature
Water temperatures at all farms remained below 30°C the upper limit for growth and survival (Figure 4).

Water temperatures at the Pinjarra region were more favourable as they remained above 12.5°C, the lower temperature limit for growth, throughout the year (Figure 4).

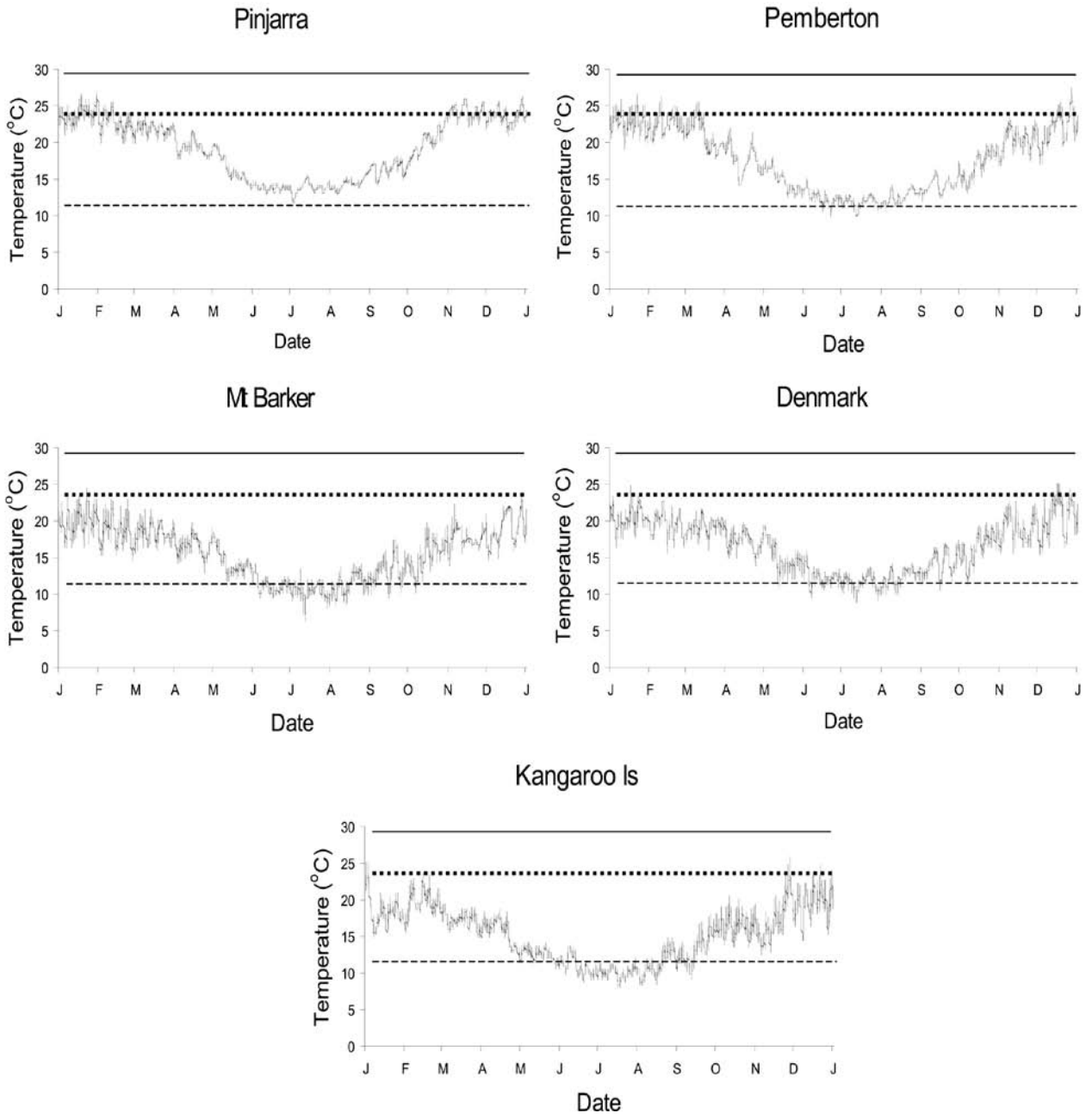

Figure 4. Annual temperature variation at 5 farms Pinjarra, Pemberton, Mt Barker, Denmark and Kangaroo Island (SA) (solid line = 30°C upper limit for survival and growth, dotted line = 24°C optimum temperature for growth, dashed line = 12.5°C lower temperature limit for growth).

Pinjarra had the most favourable temperature profile for growth. In this region for 35% of the year water temperature was within ± 2°C of the optimum temperature for growth. (Table 1). In comparison to Pinjarra and Pemberton, Mt Barker, Denmark and Kangaroo island regions each had a greatly reduced proportion of the year when temperature was within ± 2°C of the optimum temperature for growth (Table 1). At Kangaroo Island in particular, cool water temperatures limited growth for 33% of the year (Table 1).

Table 1. Annual water temperature mean ,minimum, maximum and range from 5 farms in different regions of Australia and proportion of year that temperatures are within ranges that affect growth and survival.

	Pinjarra	Pemberton	Mt Barker	Denmark	Kangaroo Is
Mean	19.2	17.4	15.4	16.2	15.0
Minimum	11.7	9.9	6.2	8.8	8
Maximum	27	27.4	24.5	25.2	25.9
Range	15.3	17.5	18.3	16.4	17.9
Proportion of year (%) <12.5°C (lower temperature limit for growth)	0.6	17.9	27.9	21.0	33.2
Proportion year (%) > 30°C (upper limit for survival and growth)	0.0	0.0	0.0	0.0	0.0
Proportion of year (%) = 24°C (optimum temperature for growth)	8.3	3.8	0.1	0.2	0.3
Proportion of year (%) = 22-26°C (optimum temperature for growth 24 ± 2°C)	35.3	20.6	1.9	4.1	3.4

Water Chemistry

Water chemistry of all farms was within the ranges acceptable for marron production (Table 2). The Mt Barker region had considerably higher Alkalinity, bicarbonate, carbonate, calcium and hardness (Table 2). The increased intensity of the farming practices at the Mt Barker farm are reflected in the higher Ammonia, ortho-Phosphate and sulfate levels (Table 2). The levels of iron at Kangaroo Island (12 mg/L) and Denmark (8.4 mg/L) could limit growth and survival, particularly at low pH (Table 2).

Table 2. Water chemistry from 5 farms in different regions of Australia.

		Pinjarra	Denmark	Kangaroo Island	Pemberton	Mt Barker	Min	Max	Mean
Alkalinity	mg/L	210	31	13	25	100	13	210	76
Ammonia	mg/L	0.14	0.04	0.02	0.07	0.27	0.02	0.27	0.11
Bicarbonate	mg/L	200	31	13	25	96	13	200	73
Calcium	mg/L	45	11	5	9	51	5	51	24
Carbonate	mg/L	5	<1	<1	<1	4	<1	5	<1
Chloride	mg/L	1,680	60	290	193	1,086	60	1,680	662
Conductivity (25C)	mS/m	5,670	220	970	73	353	73	5,670	1,457
Copper - Total	mg/L	<0.005	0.026	0.022	<0.005	<0.005	<0.005	0.026	<0.005
Hardness	mg/L	580	36	70	72	428	70	580	237
Iron - Total	mg/L	0.25	8.4	12.0	2.3	1.1	0.25	12	4.8
Magnesium	mg/L	114	2	14	12	72	2	114	43
ortho-Phosphate	mg/L	0.006	0.009	0.005	0.003	0.029	0.003	0.029	0.010
pH		8.4	7.1	6.7	6.5	9.2	6.5	9.2	7.6
Potassium	mg/L	7	1	3	4	10	1	10	5
Sodium	mg/L	1,010	20	160	90	523	20	1010	361
Sulfate	mg/L	8.1	16	19	22	99	16	99	33
Zinc - Total	mg/L	<0.005	0.020	0.048	0.007	<0.005	<0.005	0.048	0.02

In comparison to natural river systems that contain marron, commercial farms had higher mean levels of Ammonia (63%), salinity (68%), iron (85%), orthophosphate (57%) and potassium (50%) and lower sodium levels (-60%) (Table 2 & 3).

Table 3. Water chemistry from 6 river systems containing marron populations in Western Australia.

		Margaret River	Warren River	Donnelly River	Harvey River	Shannon River	Kent River	Min	Max	Mean
Alkalinity	mg/L	10	70	23	8	6	50	6	70	28
Ammonia	mg/L	0.054	0.052	0.027	0.031	0.027	0.03	0.027	0.054	0.037
Bicarbonate	mg/L	10	70	23	8	6	50	6	70	28
Calcium	mg/L	1	27	6	2	2	38	1	38	13
Carbonate	mg/L	<1	<1	<1	<1	<1	<1	<1	<1	<1
Chloride	mg/L	70	570	120	50	70	1300	50	1300	363
COD	mg/L	10	15	7	<5	42	90	<5	90	28
Colour	colour	3	36	19	29	130	28	3	130	41
Conductivity (25C)	mS/m	25	205	48	19	28	440	19	440	128
Copper - Total	mg/L	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.004	<0.001
Hardness	mg/L	22	310	60	19	28	620	19	620	177
Iron - Total	mg/L	2.5	0.6	0.3	0.1			0.1	2.5	0.9
Magnesium	mg/L	5	58	11	4	5.7	127	4	127	35
ortho-Phosphate	mg/L	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
pH		6.5	7.7	7.1	6.7	6.3	7.1	6.3	7.7	6.9
Potassium	mg/L	1	3	2	<1	1.6	4.9	1	4.9	2.3
Sodium	mg/L	26	250	55	26	40	1500	26	1500	316
Sulfate	mg/L	<5	40	13	6	6	92	6	92	27
Total Oxidised Nitrogen (TON)	mg/L	<0.01	0.11	0.13	<0.01	0.013	0.016	<0.01	0.016	0.048
Total Phosphorus	mg/L	0.015	0.01	0.006	0.016	0.01	<0.005	<0.005	0.016	0.010
Turbidity	NTU	5.9	1.4	0.8	0.7	1.6	1	1	5.9	1.9
Zinc - Total	mg/L	0.028	0.018	0.016	0.016	0.018	0.028	0.016	0.028	0.021

Growth year 1

Growth rates of cohorts of Pemberton Line and generation 2 Mass selected lines varied between regions (Table 4). In general, regions with higher growth rates had a longer period of optimum temperature each year (Table 1 & 4). Where temperature and water chemistry parameters appeared optimal, but growth was still reduced (i.e. Pinjarra) this is attributed to deviation from standard management practice (i.e. failure to operate paddle wheels at Pinjarra).

Table 4. Growth rate (g/day) of 3 lines of marron over a 1 year growout period in 5 different regions (n = 23).

Region	Growth rate (g/day)					
	Pemberton Line	se	Gen 1 Mass selected	se	Gen 2 Mass selected	se
Pinjarra	0.10	0.01	0.14	0.01		
Pemberton	0.22	0.01			0.29	0.01
Mt Barker	0.14	0.01			0.30	0.02
Denmark	0.21	0.02	0.18	0.00		
Kangaroo Island					0.18	0.00
P =	0.003		0.098		0.028	

Production year 2

Growth rates in year 2 were similar to year 1 (Table 4 & 5). As for year 1, aside from Pinjarra (where aeration failure limited survival and growth. The low survival prevented collection of useful 2 year old growth data from the 2 Pemberton Line ponds at Pinjarra), growth rates followed a similar trend of higher growth where temperatures were more favourable (Table 5). The conclusion of the project in July 2005 meant that final 2-year growth rate data for Generation 2 Mass selected stock from the 3 commercial ponds (Mt Barker – 2 ponds, Kangaroo Island -1 pond) could not be collected. However, 2-year growth rate data for Generation 2 Mass selected stock from the 3 PFRC research ponds was collected in July 2006 and is being prepared for publication.

Table 5. Growth rate (g/day) of 2 lines of marron over a 2 year growout period in 5 different regions (n = 15).

Region	Growth rate (g/day)			
	Pemberton Line	se	Gen 1 Mass selected	se
Pinjarra			0.18	0.00
Pemberton	0.21	0.00		
Mt Barker	0.16	0.01		
Denmark	0.22	0.02	0.21	0.01
P =	0.15		0.07	

Discussion

Farms based in the more southern, cooler regions, have lower growth rates due to cool water temperatures. In this study the best region from a temperature perspective is Pinjarra, where cool water temperatures limit growth for only 0.6% of the year, compared to the least favourable region, Kangaroo Island where marron do not grow for 33.2% of the year due to cold water temperatures.

Water chemistry in most regions was conducive to marron farming. However, levels of iron at Kangaroo Island (12 mg/L) and Denmark (8.4 mg/L) could limit growth and survival, particularly at low pH and these farms must consistently lime ponds to maintain high pH levels. In comparison to natural river systems that contain marron, commercial farms had higher nutrient levels, as would be expected from increased feed availability and higher carrying capacities. Increased salinisation of river systems, particularly the Kent river system, is of concern from a biodiversity perspective.

7.0 An economic evaluation of marron production in commercial ponds

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Introduction

This series of experiments have shown that it is possible to increase marron growth by genetic selection and pond management strategies. However, in addition the project has also resulted in a unique data set of marron production in commercial ponds. The economic viability of marron production is a key factor affecting aquaculture investment decisions and the decision to farm marron or an alternative species.

Investment decisions for most aquaculture species are modelled from anticipated production and returns. This information is usually extrapolated from research or pilot scale data that often represents small scale, or short term experiments. These extrapolations are often compromised by several factors including data that does not represent the entire life history of the species, growth and production data from animals that are not reared to market size, data collected under only the most favourable conditions, inaccurate production costs, estimates of price/ kg for produce based upon estimated values. However, there are few examples where economic viability is assessed using actual commercial production figures. This is because few experiments are 1) conducted where animals are reared to market size, 2) are conducted on commercial farms and 3) most businesses are reluctant to publish data upon production and income from their commercial farms

The aim of this component of the project is to investigate the economic viability of marron farming.

Methods

Between the years 2000 – 2005 production growth, survival and yield data were collected from five commercial farms consisting of 44 commercial ponds from stocking to 1 year of production and 34 commercial ponds from stocking to 2 years of production.

The area of commercial ponds ranged in size from 681 m^2 to 1,550 m^2. To permit comparison of yield and gross return among ponds data was converted to yield (t/ha) and gross return ($/1,000 m^2).

Economic evaluation including Annual gross revenue, Annual return (Profit), Production cost/ kg, Net Present Value (NPV), Internal Rate of Return (IRR), Benefit cost ratio and Return on Capital (%) were calculated using Marron Profit© software (available from the Aquaculture Council of WA, http://www.aquaculturecouncilwa.com). The standard Department of Fisheries Marron Profit model was used for all economic evaluation calculations with input production data obtained from actual commercial farm harvest data recorded in this study between 2000-2005.

The Department of Fisheries Marron Profit model is based upon a 50 pond farm (each pond 1,000 m^2), 7 broodstock ponds (each 500 m^2), 7 juvenile ponds (each 200 m^2), settlement pond (1,200 m^2), reed pond (2,500 m^2), a farm manager and casual labour, purging facility, electricity, pumps and water quality monitoring equipment. But does not include land purchase or water supply as these costs are highly site dependant. The entire farm is constructed on 11.5 ha of land.

Results

Production Year 1

After 1 year in commercial ponds the majority of marron were below market size (Table 1). However, farms with faster growing stock achieved up to 86% of market size marron within 1 year (Table 1). Survival was highly variable (range 11 – 100%) among ponds and farms (Table 1). The main factors contributing to low survival were predation by water rats, poor water quality or inoperative paddlewheels.

Table 1. Marron production recorded from 44 commercial ponds 1 year after stocking.

	Mean	se	Min	Max
Mean weight (g)	60	3.63	18	122
Survival (%)	61	4	11	100
Yield (t/ha)	1.0	0.06	0.1	1.8
Gross return /1000m2 pond ($)	1,472	121	726	3,277
Proportion above market size >70 g (%)	28	3	1	86

Production Year 2

The average production was 2.0 t/ha at 2 years of age (Table 2). The average gross return/1000m^2 pond is over 200% greater that that of 1 year production, due to the increased proportion of maron above market size (Table 1 & 2). This supports the current two year production strategy. The minimum values represent almost entire pond crashes due to water rat predation, poor water quality or inoperative paddlewheels (Table 2.). The maximum values were obtained from ponds that did not experience large scale mortalities (Table 2).

Table 2. Marron production recorded from 34 commercial ponds 2 years after stocking.

	Mean	se	Min	Max
Mean weight (g)	131	4.65	62	209
Survival (%)	49	4	1	91
Yield (t/ha)	2.0	0.14	0.03	3.3
Gross return /1,000 m^2 pond ($)	4,535	319	53	7,569
Proportion above market size >70 g (%)	80	2	29	99

Economic return

Scenario 1: Virtual farm .
This scenario considers actual production from all ponds in this project as one virtual farm. The farm therefore experienced some high producing ponds and several ponds that experienced poor production and large scale mortalities (Table 3).

Scenario 2: Average production

This scenario considers all ponds in this project that did not experience major mortalities due to preventable causes. This would be expected to represent an "average" producing farm (Table 3).

Scenario 3: High production

This scenario represents ponds in the project that are correctly designed, constructed and professionally managed to maximise survival and production (Table 3).

Scenario 4: Top 10% of marron farms

The majority of marron production in WA comes from 10% of farms. This scenario represents the top 10% of marron ponds in this study (Table 3).

The majority of marron farms are marginal, with only the top 10% of farms and well-managed farms achieving reasonable returns (Table 3).

Table 3. Economic returns from four scenarios for a 50 pond farm.

Scenario	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
1	4.6	110,644	31.68	-416,526	-36,315	1.16	0.75	-7.62
2	5.7	136,457	25.79	-136,907	-11,936	4.57	0.92	-2.50
3	6.7	156,728	22.08	80,408	7,010	6.79	1.05	1.47
4	7.4	171,231	20.36	237,680	20,722	8.24	1.14	4.35

The profitability of all 4 farm scenarios is increased by the stocking of marron from the FRDC selective breeding program (Table 4). This data is based upon actual performance of generation 1 Mass Selected stock. Therefore it represents an underestimate of returns that have already been achieved by the second generation of selective breeding and further increases in returns expected from subsequent generations.

Table 4. Economic returns from four scenarios for a 50 pond farm using marron produced by the FRDC selective breeding program (Gen 1 Mass Selected) resulting in a 12 month production cycle.

Scenario	Annual Production (t)	Annual gross revenue ($)	Production cost/ kg ($)	NPV ($)	Annual return (Profit) ($)	IRR (%)	Benefit cost ratio	Return on Capital (%)
1	9.5	226,195	16.18	833,860	72,700	13.53	1.47	15.25
2	11.8	278,968	13.31	1,404,698	122,468	17.63	1.78	25.68
3	13.9	320,408	11.49	1,848,229	161,137	20.48	2.01	33.79
4	15.1	350,057	10.65	2,169,307	189,130	22.40	2.18	39.66

Discussion

The top 10% of marron farmers, that currently represent the majority of production in Western Australia, have yields that are 61% higher than the average marron farm. As a result their cost/ kg is 36% lower than the average farmer. However, the cost of marron production from some of the ponds in this study exceeds the current price/ kg paid for marron. The low IRR and cost of production from these less productive farms indicate that the current price paid by processors for marron, in particular larger marron, while excellent, are below that required for some farmers to profitably grow current unselected industry stocks.

The use of marron produced by the FRDC selective breeding program dramatically increases the profitability of farming and for a correctly managed and constructed farm increases the IRR to 22% and return on capital to almost 40% with yields of 3 t/ha/year.

In addition a key factor affecting the economic viability of the less productive marron farms in this study was pond management, in particular control of predators, adequate aeration, management of water quality and correct feeding regimes.

Even so, the current average return/pond of over $4,500 justifies the expansion of marron farming as construction costs are currently around $2,000 /pond. This means that pond construction costs are paid for by the first marron crop.

From an economic and marketing perspective, until production levels can be increased, it is difficult for the marron industry to enter key international markets. For example, in Europe alone, annual consumption of freshwater crayfish is around 6,300 t, with 2,800 t/year coming from European capture fisheries (Wickens and Lee 2002). The Turkish freshwater crayfish fishery exported to Europe much of the 3,500 t/year shortfall until its collapse in 1986 from 8,000 t/year to 1,000 t/ year (Holdich 2002). In comparison current production of marron in Australia is less than 100 t/year (and total production of all freshwater crayfish in Australia is less than 450 t/year), however clearly this is still well below that required to satisfy global demand or enter markets previously supplied by other key producers such as the Turkish freshwater crayfish fishery.

Therefore, with the recent commercialisation of the faster growing genetic lines of marron developed by this FRDC project along with commercial farm trials that have demonstrated the viability of marron farming, it appears that the major remaining limitation to increased marron production in Australia is the application of these principals on existing farms and investment

into new farms, that are correctly designed, constructed and professionally managed. This should result in increased production levels that will in turn enable Australian processors to gain access to some of the larger existing international markets.

8.0 Benefits and Adoption

Marron farmers in WA and SA will benefit directly from this research through animals that grow faster and improved pond management strategies. These are the same benefits and beneficiaries identified in the original application. In addition, this research has clear applications to crustacean farming in general and to selective breeding programs for other aquaculture species.

The use of marron produced by the FRDC selective breeding program dramatically increases the profitability of farming. For a correctly managed and constructed 50 pond farm replacing industry stock with marron from the selective breeding program increases the IRR from 8.24% to 22%, return on capital from 4% to 40%, yields from 1.5 to 3 t/ha/year and profit from $20,722 to $189,130 /year.

Husbandry and pond management strategies have validated best practice techniques for farming marron and identified strategies for further improving production. In combination, the hide harvesting technique and condition index provides farmers with a method to monitor stock and feeding regimes.

These results, combined with the data that shows that growth is improved in areas with favorable temperature regimes, should encourage the construction of well designed and professionally managed marron farms in key regions.

Commercial marron farmers have rapidly adopted the results of the research project. This is due to three main factors 1) Each year the project team conducted an annual research seminar and open day for licensed marron farmers. This provided the opportunity for farmers to be involved in the research process, informed of the most recent research outcomes, and implement these outcomes on their own farms. 2) Field trials conducted on commercial farms trained key farmers in new techniques and best practice farming methods, provided validation of research results, increased farmer confidence in the reliability of research outcomes and permitted demonstration of techniques at industry field days. 3) The scale of experiments was realistic from an industry perspective, the project recorded growth to two years of age of 147,000 marron from 44 commercial ponds and two large research facilities consisting of 22 ponds (PFRC) and 49 tanks and 110 aquaria (UWA Aquaculture Laboratory) over a 5 year period, 4) The research team worked with industry and research partners to develop a commercialization strategy that provided rapid dissemination of stocks from the selective breeding program to commercial farmers. These stocks were distributed to farmers who are experienced juvenile producers via a competitive tender process within 5 months of the conclusion of the project. These farmers are currently breeding from these “elite” marron from the selective breeding program to produce much larger numbers for supply to industry throughout WA and SA.

9.0 Further Development

The selective breeding expertise developed during this project has direct application to genetic improvement, domestication and commercialisation of genetics programs for other aquaculture species, both nationally and internationally. Additional funding provided by the Australian Academy of Technological Sciences and Engineering for the "Development of an international centre for aquaculture genetics and enhancement of selective breeding program for the production of freshwater crayfish in Australia " has resulted in a core team consisting of Dr Craig Lawrence (Department of Fisheries Western Australia), Dr Phil Vercoe (The University of Western Australia) and Dr Mark Henryon (Danish Institute of Agricultural Science) that has been recognized by the International Network for Genetics in Aquaculture as an Advanced Scientific Institution in aquaculture genetics. Increased communication at a national level amongst project leaders involved in similar research would facilitate both the dissemination of techniques developed by this team and opportunities for international collaboration. Similarly, at both a national and international level there is the opportunity for commercial application of this resource.

10.0 Planned Outcomes

The strain evaluation and selective breeding component of the project improved growth rate by 86 – 110%. This exceeds the aim in the project application of developing an improved marron strain that grows 50% faster. At the conclusion of the project 18,000 marron that had been produced by the selective breeding program were distributed to commercial marron farmers via a competitive tender process. These animals now form the basis of an industry multiplier program that should result in large numbers of juveniles available for stocking in 1-2 years. This should result in an increase in production levels in 3 years as industry converts to the faster growing marron stocks.

In addition to validating current best practice farming methods, the husbandry and pond management component of the project produced a simple condition index and hide harvesting technique. This has provided farmers with a method for monitoring stocks that will reduce post-harvest mortality rates by ensuring marron are adequately nourished and in good condition prior to harvest.

11.0 Conclusion

There is considerable potential for improving marron production from commercial farms. This study addressed the original objectives to:

1. Selection and genetic improvement of stock

1. Identify the fastest growing wild strain of marron.
2. Compare the growth of wild marron strains with a mass selected commercial strain.
3. Determine whether any hybrids have production characteristics that are superior to wild marron strains.
4. Use mass selection to develop a faster growing "domesticated" marron strain or hybrid.
5. Decrease size variation of marron cohorts to increase the proportion of marketable animals.
6. Evaluate performance of the mass selected marron strain on commercial properties.
7. Investigate inbreeding effects by comparing growth of mass selected marron with farm stock.

2. Development of improved husbandry protocols.

1. Compare the effect of aeration upon both production levels and product quality.
2. Determine whether increased hides can alleviate growth reduction due to high density.
3. Evaluate stocking tightly graded juveniles in commercial ponds upon size variation at harvest.
4. Evaluate stocking advanced juveniles into commercial ponds.
5. Trial a non destructive condition index developed for yabbies on marron in commercial farms.
6. Compare the effect of regional variation upon marron growth and production.

3. Extension of results to industry

1. Manual of methods for managing marron ponds to improve husbandry and genetics of farm stock
2. Exchange of information between WA and SA.

1. Selection and genetic improvement of stock

The best performing wild strain of marron was the Harvey River Line due to fast growth, low CV and fewer early maturing females. The Harvey River Line (82%), Blue Line (51%), Mass Selected Line (40%), Pemberton Line (32%) and Margaret River (16%), Shannon (15%), Donnelly (13%), Kent (12%) and Warren (9%) all grew faster than the Industry Line. The lower growth rates of the Industry Line compared with all the river lines evaluated in this study is most probably due to ongoing selection by farmers of larger animals for sale, with subsequent breeding from smaller slower growing marron. This negative genetic selection is unintentional but has resulted in slower growing animals on commercial farms, when compared with wild populations.

The Kent river strain had the highest tail meat yield as a proportion of bodyweight for males (27.2%) and females (29.3%). The Kent river strain also had smaller claws in proportion to their body size.

The majority of hybrids evaluated in this experiment did not grow as well as the pure river strains. There was no evidence of hybrid vigour. None of the hybrids had a skewed sex ratio. Therefore, hybridisation of marron does not offer a potential solution for producing monosex populations.

Selective breeding of industry stocks may provide rapid improvements in performance. Mass selection of the Industry Line provided a gain of 30 – 41% in average weight at harvest in only one generation. The second generation of Mass Selected marron grew 86 – 109% faster than the Industry Line. However, selection for growth may result in heavier marron due to large claws or carapace, but a smaller abdomen. It may also lead to inbreeding by selecting marron siblings that are fast growing for broodstock. To address this a pedigree selection program was developed. This used a selection index to determine breeding values for individuals, based on the economic merit for each marron in our pedigree. It enables simultaneous selection for multiple traits (i.e.growth, age at sexual maturity, colour, size of claws, size of carapace and size of abdomen) and control of inbreeding.

The increase in mean weight of mass selected marron compared with both industry marron indicates that selective breeding could have a significant impact upon marron farming by shifting production from a 2 year to a 1 year harvesting cycle.

2. Development of improved husbandry protocols

Increasing the duration of paddlewheel aeration from 30 to 60 min three times per day, at late afternoon (1600), midnight (2400) and dawn (0600), produced larger marron (mean weight 112 ± 2.59 g) compared with the standard industry practice of 30 min aeration 3 times per day (mean weight 86 ± 11.66 g). Increased aeration also resulted in reduced size variation among marron ponds.

The current hide density is suitable for marron farming. There was no significant difference between the mean weight of marron reared in ponds that contained hides at the standard density (0.15 hides/m^2) (116 ± 5.29 g), compared with those from ponds that contained twice the number of hides (0.30 hides/m^2) (124 ± 4.31 g) (P = 0.29). The increased shelter provided from doubling the number of hides did not increase survival.

Size grading of juveniles prior to stocking ponds increased the average weight of marron harvested by 12 - 58% and decreased the proportion of below market size animals by 54%.

There was no advantage from relaying juveniles that had been produced in the northern region to ponds in the southern region. In fact, at harvest marron produced in the southern region (170 ± 19.35) were 30% larger than those relayed as juveniles from the northern region (131 ± 3.77 g). These results corroborate those from the above experiments evaluating size grading of juveniles. Early growth differences among juveniles, due to genetic factors, release date or environmental variation between farms have a significant influence upon final harvest size. Consequently, farmers should only stock commercial ponds with larger juveniles from each cohort, as small juveniles due to genetic and/or environmental factors fail to achieve the same growth rates and result in smaller marron at harvest.

Marron with proportionately shallower abdomens are growing faster than those with deeper abdomens. This simple condition index can be applied by farmers to evaluate condition of marron in commercial ponds. By recording three simple measurements Abdomen depth, Abdomen width and Abdomen Length from a relatively small sample of marron (20 /pond) it is possible to calculate if the animals are growing slowly (CI > 0.01), average (CI = 0.01-0.008) or rapidly (CI = < 0.008).

Hide harvesting is a simple, reliable technique that can be used by farmers to determine the average weight of marron in a pond. The equation for calculating the mean weight of marron from hide harvest mean weight (Mean weight (g) = 1.546 x (Hide harvest mean weight (g)) is suitable for marron above 30 g. This sampling technique and equation will permit farmers to calculate growth rates, feed rates and condition of marron.

Farms based in the more southern, cooler regions, have lower growth rates due to cool water temperatures. In this study the best region from a temperature perspective is Pinjarra, where cool water temperatures limit growth for only 0.6% of the year, compared to the least favourable region, Kangaroo Island where marron do not grow for 33.2% of the year due to cold water temperatures.

Water chemistry in most regions was conducive to marron farming. However, levels of iron at Kangaroo Island (12 mg/L) and Denmark (8.4 mg/L) could limit growth and survival.

It is essential that commercial marron farms are correctly designed, constructed and professionally managed. The cost of marron production on some farms exceeds the current price/ kg paid for marron. However, the top 10% of marron farmers, that currently represent the majority of production in Western Australia, have yields that are 61% higher than the average marron farm. A correctly managed and constructed 50 pond farm has an IRR of 8.24%, return on capital of 4%, yields 1.5 t/ha/year and profit of $20,722

The use of marron produced by the FRDC selective breeding program dramatically increases the profitability of farming. For a correctly managed and constructed 50 pond farm replacing industry stock with marron from the selective breeding program increases the IRR from 8.24% to 22%, return on capital from 4% to 40%, yields from 1.5 to 3 t/ha/year and profit from $20,722 to $189,130 /year.

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13.0 Appendices

Appendix 1 Intellectual Property

The intellectual property arising from this research project has been made available in this report. The three main components of IP from this project are 1) Selection index equation developed for Pedigree breeding, 2) Software developed for managing commercial pond data sets and selecting broodstock, and 3) Marron produced by the selective breeding program.

At the conclusion of the project 18,000 marron had been produced by the selective breeding program. These animals have been distributed to commercial marron farmers via a competitive tender process.

Appendix 2 Staff List

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Ivan Lightbody
Workshop Engineer (0.05 FTE)

Carey Nagle
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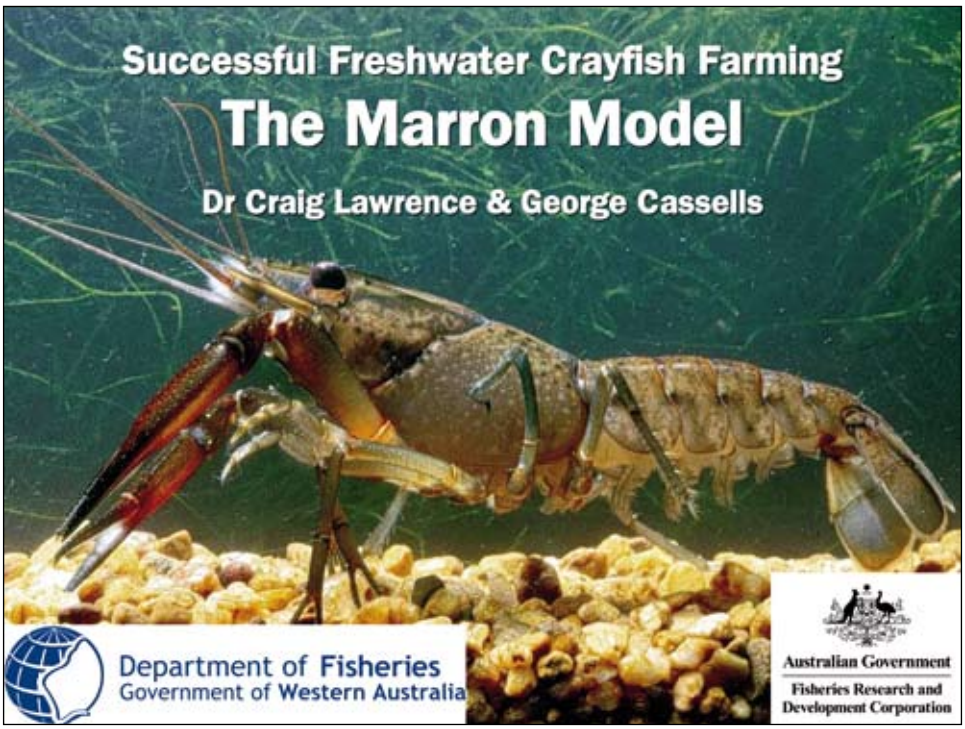
Dr Phil Vercoe
Senior Lecturer (Genetics) (0.1 FTE)

Appendix 3 Successful Freshwater Crayfish Farming – The Marron Model

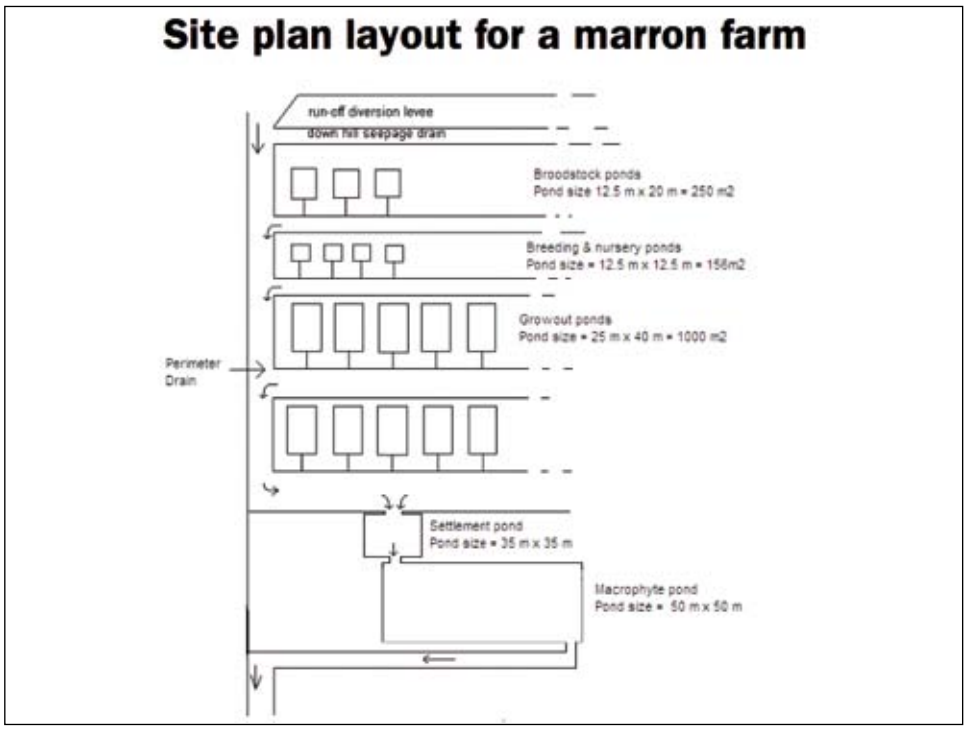
A manual for farmers of methods for managing marron ponds to improve husbandry and genetics of farm stock.

Project team: C. Lawrence, S. How, G. Cassells and C. Bird

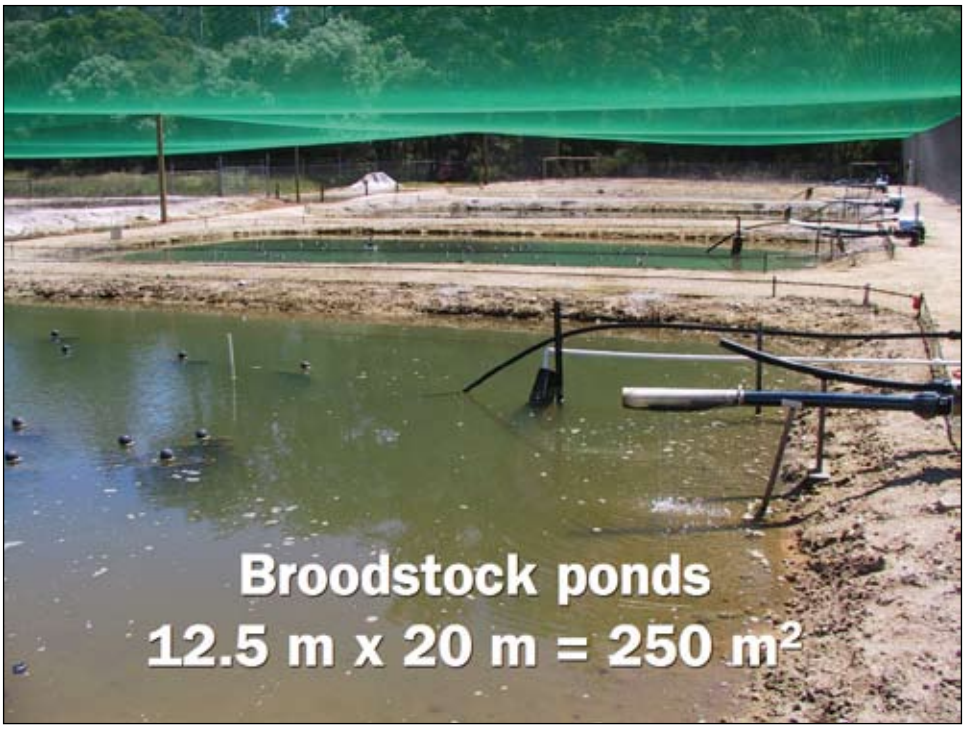


Slide 1. Successful Freshwater Crayfish Farming – The Marron Model. Dr Craig Lawrence and George Cassells.

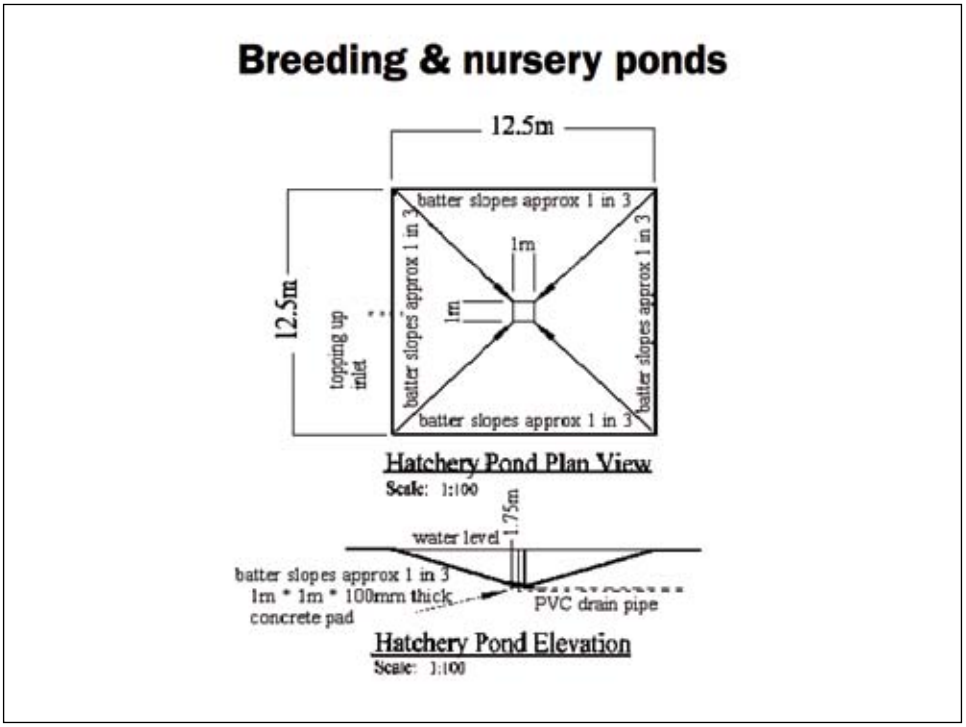
Slide 2. Build and manage your farm correctly.



Slide 3. Site plan layout for a marron farm.

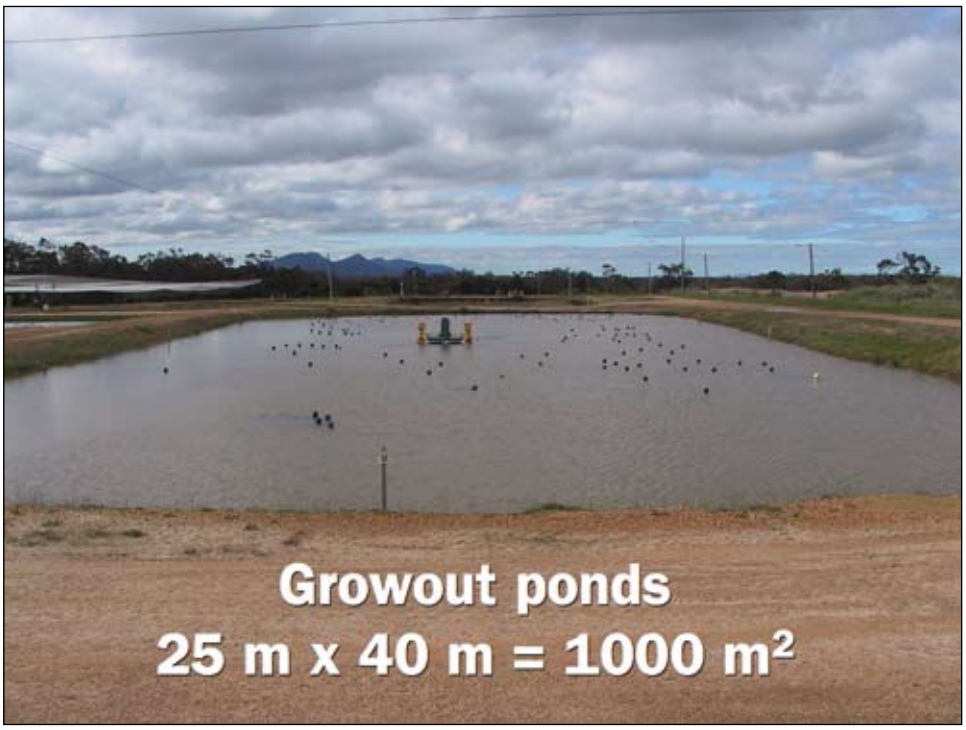


Slide 4. Broodstock ponds 12.5 m x 20 m = 250 $m^{2.}$

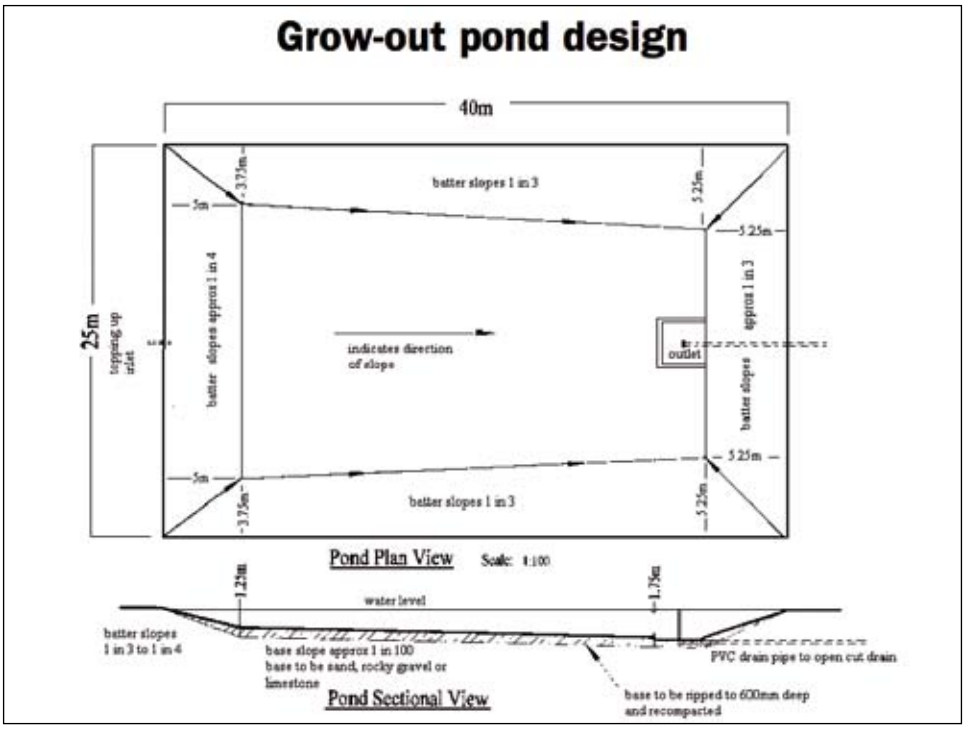


Slide 5. Breeding and nursery ponds.

Slide 7. Use venturis on broodstock, breeding and nursery ponds.



Slide 8. Growout ponds 25 m x 40 m = 1,000 m^2.



Slide 9. Grow-out pond design.

Slide 10. Use paddlewheels on growout ponds. Aerate ponds for 30-60 min three times per day at late afternoon (1600), midnight (2400) and dawn (0600).

Slide 11. Provide a water supply to each pond.

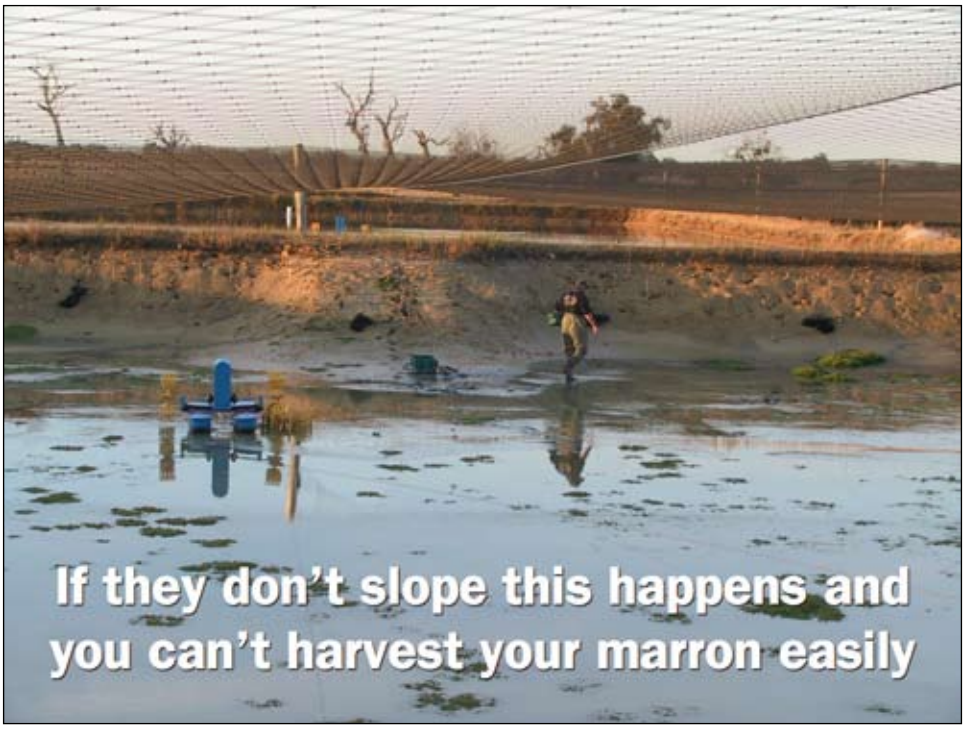
Slide 12. Install drain pipes in each pond.

Slide 13. Install drain pipes in each pond.

Slide 14. Install a concrete harvesting base near the drain.

Slide 15. It will make harvesting much easier.

Slide 16. Make sure your ponds slope towards the drain so they empty correctly.



Slide 17. If they don't slope this happens and you can't harvest your marron easily.

Slide 18. Compact the bottoms and line with gravel – they should be hard enough to drive on.

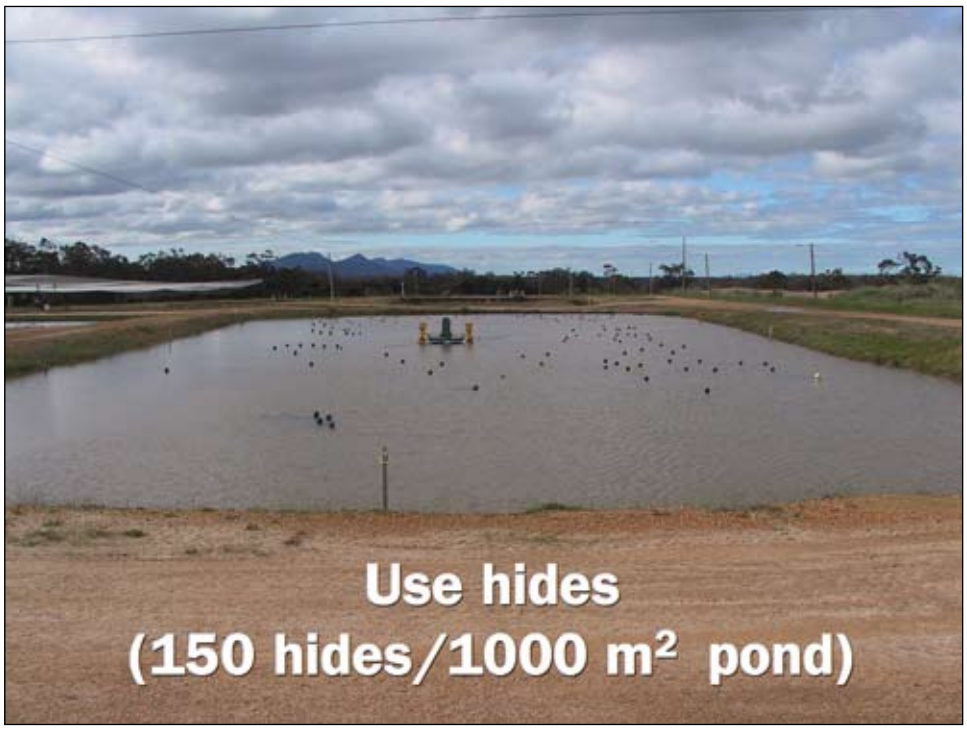
Slide 19. Don’t make you banks too steep. Hard bottom ponds and a bike are essential for easy harvesting.

Slide 20. If you don’t compact bottoms this happens. How can your marron survive in this?

Slide 21. This pond could be improved by hard bottoms – How else can you harvest all your marron?

Slide 22. Install bird netting to prevent predation.

Slide 23. Marron hides are simple to make.



Slide 24. Use hides (150 hides/1000 m^2 pond).

Slide 25. Provide good access to ponds – It certainly makes feeding easier.

Slide 26. Feed marron pellets daily.

Average marron feed rates for Pemberton

		Aug	Nov	Feb	May	Aug
	Water Temp ºC	12.2	19.2	22.5	13.4	12.2
0+ - 1+	Feed as % BW/Day	4.9	3.6	1.8	0.7	0.4
	Feed as g/m?week	6.8	14.7	20.1	12.0	8.1
	Marron Mean Weight g	4.7	13	35	58	71
1+ - 2+	Feed as % BW/Day	0.4	1.5	1.1	0.6	0.5
	Feed as g/m?week	9.9	29.7	30.1	20.3	16.0
	Marron Mean Weight g	81	95	126	169	175

Slide 27. Average marron feed rates for Pemberton.

Slide 28. Marron farming cycle.

Slide 30. Drain ponds in the morning when it is cool.

Slide 31. The day before draining put a syphon in to lower water level overnight.

Slide 32. Syphons are a cheap and simple way to lower water levels.

Slide 33. Overnight the syphons will have lowered the water level to around 1m at the deep end.

Slide 34. Remove hides before completely draining the pond.

Slide 35. When draining ponds let the marron come to you.

Slide 36. They will follow the water down.

Slide 37. Until they all congregate around the screen.

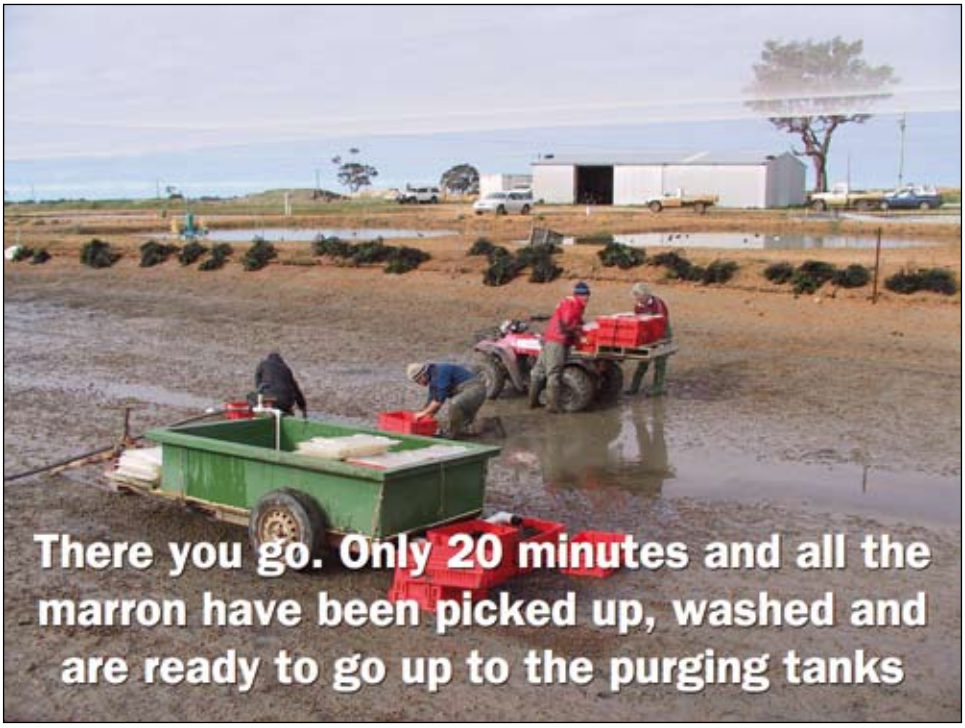
Slide 38. Isn't harvesting easy when you have a drain?

Slide 39. Now it's just a simple matter of picking them up.

Slide 40. Have a trailer and bike ready so you can quickly wash marron in the pond and take them up to your purging tanks.

Slide 41. A trailer with water supply makes washing marron easy.

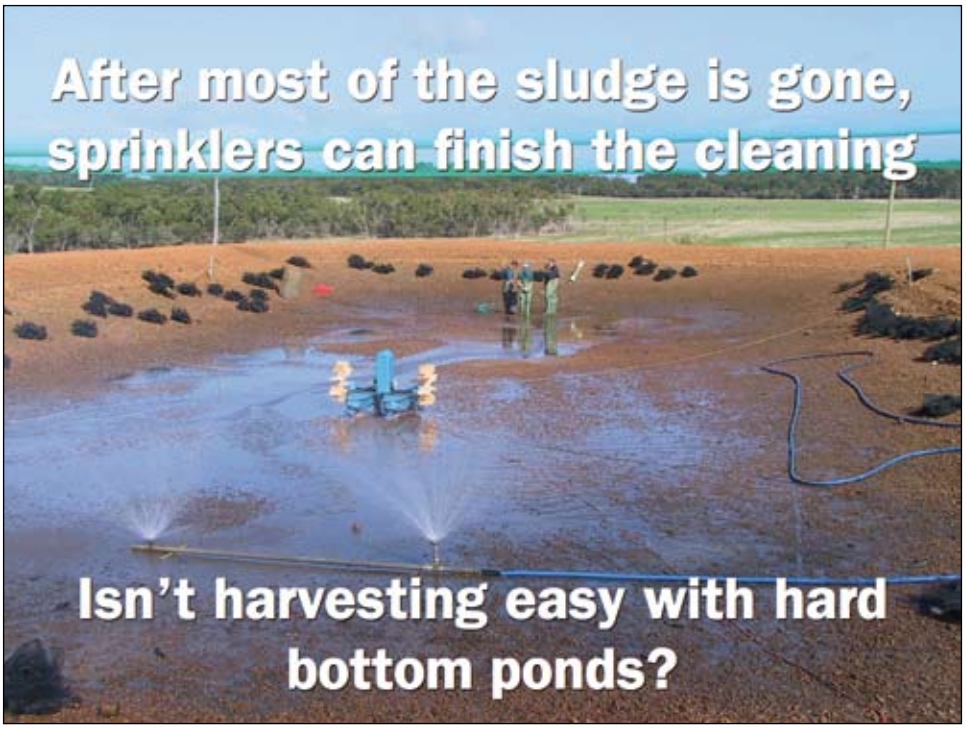
Slide 42. After a quick wash to get the mud off they are ready to go into your purging tanks.



Slide 43. There you go. Only 20 minutes and all the marron have been picked up, washed and are ready to go up to the purging tanks.

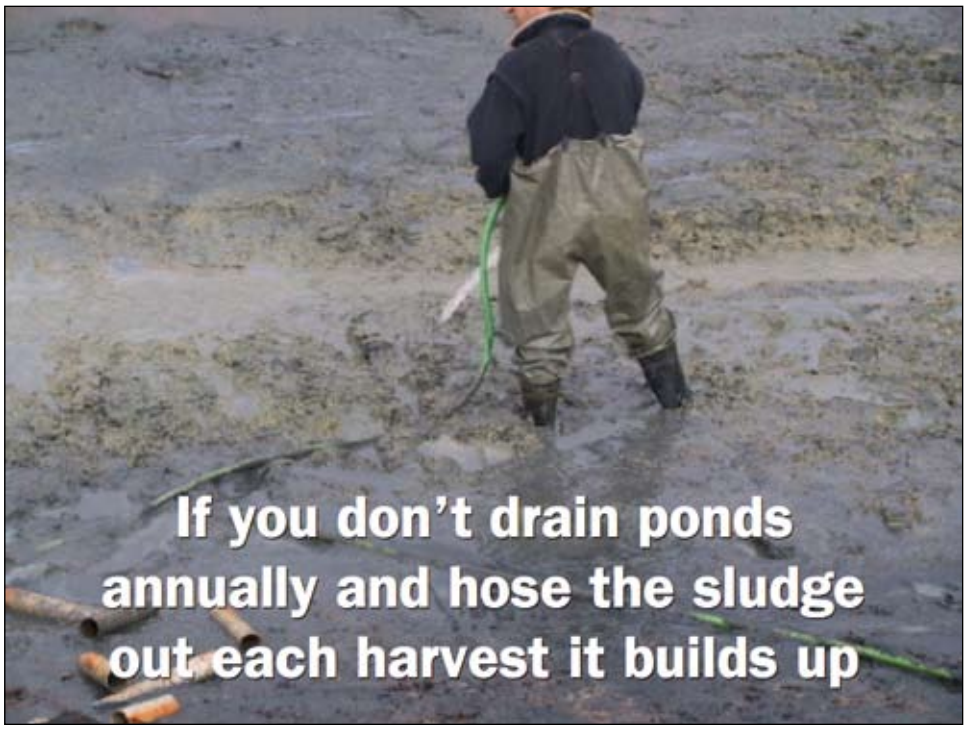
Slide 44. After draining, hose down the sludge from the pond.

Slide 45. Firefighter pumps make short work of hosing out sediment.



Slide 46. After most of the sludge is gone, sprinklers can finish the cleaning. Isn't harvesting easy with hard bottom ponds?

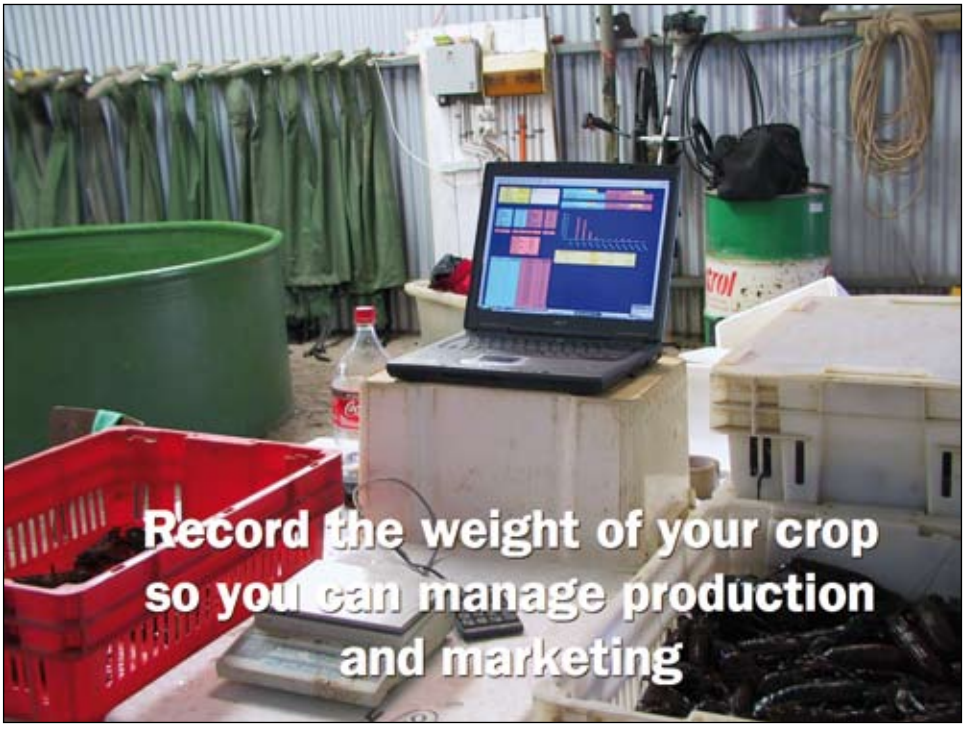
Slide 47. Cleaned and ready to fill for your next crop.



Slide 48. If you don't drain ponds annually and hose the sludge out each harvest it builds up.

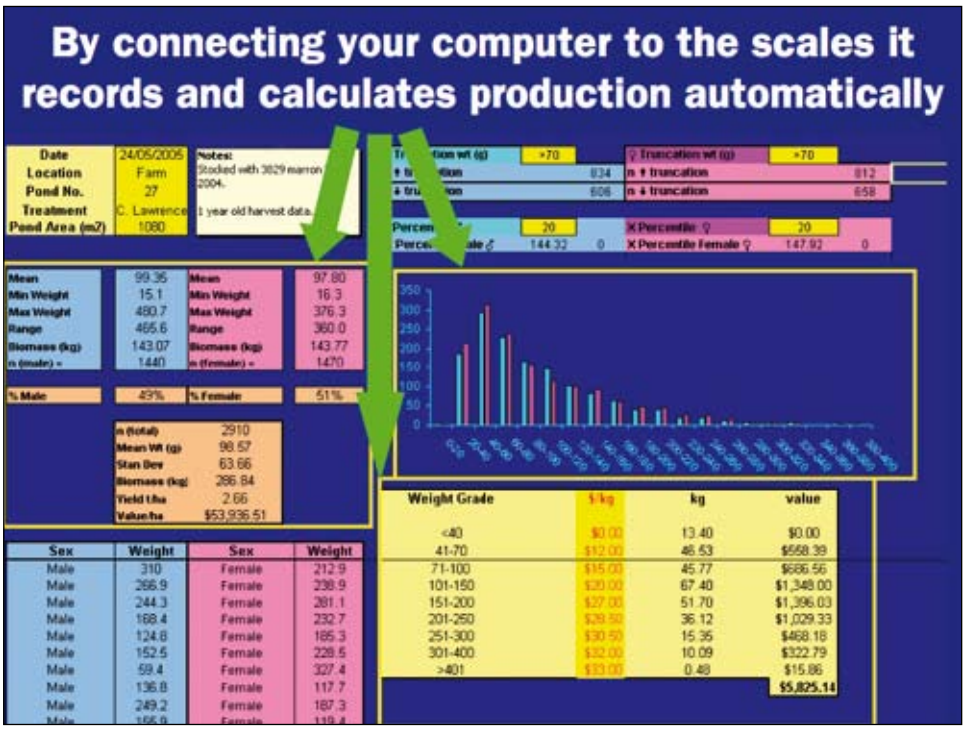
Slide 49. Use purging tanks to gill flush your marron immediately / after harvest.

Slide 50. Make sure your purging tanks are well aerated with a venturi.

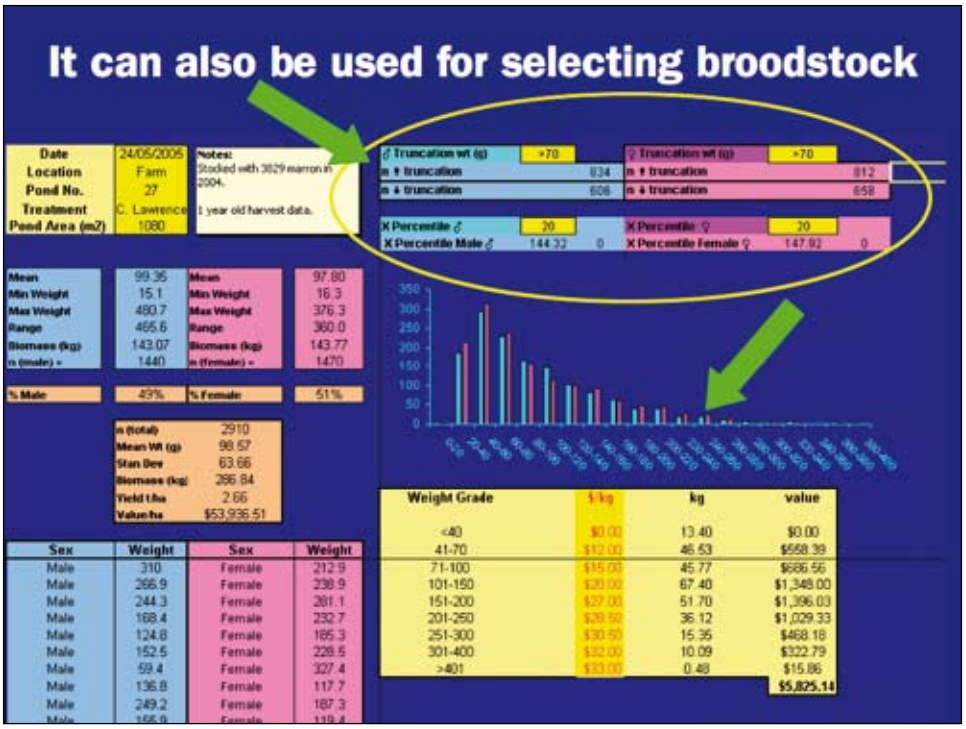


Slide 51. Record the weight of your crop so you can manage production and marketing.

Slide 52. We developed computer software to make data recording easy on commercial farms.



Slide 53. By connecting your computer to the scales it records and calculates production automatically.



Slide 54. It can also be used for selecting broodstock.

Slide 55. Now just pack and grade your crayfish for market.

Slide 56. George, Sandy, Carey, Chris B., Craig, Chris C.

Slide 57. Acknowledgements: We wish to acknowledge the generous assistance of the marron farmers who worked with us on this project.

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2 Towards an assessment of natural and human use impacts on the marine environment of the Abrolhos Islands Phase 1: Data consolidation and scoping, Final Report for FRDC Project 2000/166. Chubb, C.F. and Nardi, K. (2003).

3 Early life history of abalone (*Haliotis rubra, H. laevigata*): settlement, survival and early growth, Final Report for FRDC Project 1998/306. Daume, S. (2003).

4 Determining waste excretion parameters from barramundi aquaculture, Final Report for Aquaculture Development Fund of WA. Glencross, B., Rutherford, N. and Hawkins, W. (2003).

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6 Assessment of the nutritional variability of lupins as an aquaculture feed ingredient, Final Report for the Grains Research Committee of WA Project. Glencross, B., Curnow, J. and Hawkins, W. (2003).

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8 The nutritional management of barramundi. Glencross, B. (2004).

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10 Mother-of-pearl shell (*Pinctada maxima*): Stock evaluation for management and future harvesting in Western Australia, FRDC Project 1998/153. Hart, A.M., Friedman, K.J. (2004).

11 Yabby hybrid growout experiment FRDC Project No. 97/319.02 Aquaculture Development Fund of Western Australia, Project No. 41

12 Mitigation of the negative impacts on biodiversity and fisheries values of the refurbishment of Waroona Dam, south-western Australia, Final report for the Water Corporation of Western Australia

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14 Development of a national translocation policy using abalone and prawns as templates for other aquatic species, Aquatic Animal Health Subprogram: FRDC Project No. 2004/080. Jones, B. and Stephens, F. (2006)

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16 Improvement and evaluation of greenlip abalone hatchery and nursery production, Final FRDC Report – Project 2003/203. Daume, S. (2007).

17 Improved performance of marron using genetic and pond management strategies, Final FRDC Report – Project 2000/215. Lawrence, C. (2007)