Capturing and maintaining genetic diversity for the establishment of a long-term breeding program for barramundi (*Lates calcarifer*) aquaculture

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Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Statement of Authorship

Chapter 1

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Chapter 2

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Laboratory methods : S.L., J.D.

Statistical analysis : S.L.

Manuscript writing : S.L., N.R.

Chapter 3

Data collection : S.L.

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Manuscript writing : S.L, N.R.

Chapter 6

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Thesis summary

Mass spawning hatchery practices using small broodstock populations, in addition to the cannibalistic nature of some fish species, contribute to a reduction of genetic diversity from parent to offspring and throughout the juvenile grow-out stages. This is of concern when establishing a selective breeding program for such species because the genetic diversity that is captured in the start-up and initial generations of the program is the basic ingredient for future genetic improvement. The aim of this thesis was to examine methods for capturing and conserving genetic diversity in mass spawning barramundi (*Lates calcarifer*), when constructing a base population for a long-term selective breeding program for the species.

Involving 21 males and 12 females, the transfer of genetic diversity from broodstock to offspring in a large commercial mass spawn was investigated in chapter 2. Previous studies had indicated that substantial amounts of genetic diversity were lost using mass spawning techniques, which are normal practice for the commercial barramundi industry. A high participation rate of parents was detected among the large spawning group used in this study (n = 31). Broodstock contributions were skewed and the contribution by individual dams and sires was as high as 48% and 16% respectively at one day post hatch (dph). Barramundi progeny were monitored throughout the juvenile stages to investigate the conservation of genetic diversity, during the periods of larval metamorphosis and size grading (to inhibit cannibalism).

A reduction in allelic richness (A_r) was identified from broodstock to offspring at 1 dph, (A_r was 3.94 among broodstock and 3.52 among offspring sampled). However, no further loss of A_r or genetic diversity was detected in the offspring from

1 to 90 dph, which included the period of metamorphosis, multiple size grading events and losses through size culling, mortalities and the sale of juveniles. The effective population size (N_e) in the broodstock group ranged from 10.1 – 16.7, well below the broodstock census size of 33, whereas the rate of inbreeding was less than 5%. The results from the mass spawn provided reproductive and demographic parameters that could be used to inform the design of a base population for a barramundi selective breeding program.

In chapter 3, 407 mature captive broodstock under current use in eight commercial barramundi hatcheries were pedigree tested using 17 microsatellite markers, to determine their suitability for inclusion into a base population. Levels of genetic diversity within each hatchery and the degree of relatedness between individuals were estimated and compared. Genetic diversity was moderate within each broodstock group (A_r ranged from 2.67 – 3.42) and heterozygosity ranged from 0.453 – 0.537. Relatedness estimates within hatcheries were generally low and ranged from -0.003 to 0.273. Structure analysis revealed that captive Australian broodstock were broadly divided into two genetic stocks and suggested that hatchery individuals were either sourced from the two stocks or represented an admixture between them. From the results, an assessment was made of the genetic suitability of existing domesticated broodstock as contributors to the base population.

Chapter 4 sampled 1205 barramundi individuals from 48 wild sites covering a broad distribution range. Levels of wild genetic diversity were estimated and compared to captive groups from chapter 3. The wild collections were found to cover two broad ranging genetic stocks, an eastern and western stock and a central stock of genetic admixture (F_{ST} = 0.076). The majority of captive individuals were assigned to the eastern stock (59%), followed by the western stock (23%) and central

region of admixture (13%). Levels of genetic diversity, as determined by allelic richness (A_r), were slightly lower in the captive groups (average A_r = 3.15) when compared to the wild populations (average A_r = 3.40). Some genetic variation was unrepresented in the captive groups and it was concluded that the inclusion of wild individuals would enhance overall levels of genetic diversity in a base population for selective breeding.

Finally, a computer simulation model was developed in chapter 5 and used to compare different options for sourcing genetic variation for inclusion into the base population. It was assumed that the primary goal when establishing the base population would be to maximise genetic diversity. Candidates for inclusion into the synthetic base populations were selected according to levels of genetic diversity and relatedness. A range of options were tested, which included the use of candidates from both wild and captive populations. There was a significant reduction in the level of A_r between broodstock and offspring ($P < 0.05$) for many of the options. The best options for retaining genetic diversity were from the base populations constructed from an even representation of wild samples from genetic stocks (WSA_r, broodstock and offspring A_r was 5.21 and 4.75 respectively) and to select captive broodstock according to the lowest mean kinship levels (Cmk_r, broodstock and offspring A_r was 5.05 and 4.69 respectively). Five alternate base population sizes (N_c) were tested to estimate the effective population size (N_e) based on the variance of parental contribution and unequal sex ratio. N_e was 76, 85, 98, 105 and 115 from an N_c of 150, 180, 200, 230 and 250 respectively, and the rate of inbreeding (ΔF) ranged from 0.4 – 0.7%. Under the model presented in this study, an N_c of more than 213 broodstock individuals is required to achieve $N_e > 100$ and $\Delta F < 0.5\%$. The results suggested that a mixture of both wild and captive barramundi should be

included in the base population at the commencement of a selective breeding program for barramundi.

This thesis investigated the effects of hatchery practices, such as mass spawning and size grading on the conservation of genetic diversity. In addition, options for selecting candidates to compose a founding population were explored, and recommendations made to promote the longevity and impact of a selective breeding program for barramundi. The Australian industry has on hand a large number of mature captive broodstock that would be suitable for inclusion into a base population for barramundi selective breeding. However, it would be beneficial to include a selection of wild individuals from regions of high genetic diversity to strengthen the fitness of a base population at the commencement of a selective breeding program.

Summary of chapters

This thesis is presented as a series of manuscripts. Chapter 2 has been published, chapter 3 is under review and chapters 4 and 5 are manuscripts in preparation for publication.

Chapter 2 publication:

Broodstock contribution after mass spawning and size grading in barramundi (*Lates calcarifer*, Bloch).

Loughnan, S.R., Domingos, J.A., Smith-Keune, C., Forrester, J.P., Jerry, D.R., Beheregaray, L.B., Robinson, N.A. **Aquaculture** 2013, 404–405, 139–149.

Barramundi is naturally a mass spawning species, which can be induced to spawn in captivity under conditions that attempt to replicate the natural environment. Due to the high fecundity of females and the inclusion of numerous adults into a spawning group, the production of large quantities of larvae can be high. Relatively few breeders have the potential to supply a large proportion of the grow-out industry. However, the main complications identified by previous studies involving captive mass spawning barramundi, were the low participation rates for particular broodstock and highly skewed levels of parental contribution across all broodstock. With a limited number of contributors, inbreeding rates can be high and genetic diversity can be lost within offspring cohorts, which can complicate the selection of unrelated broodstock candidates for the next generation of breeders. Typically, small broodstock groups of 1 – 2 females and 3 – 5 males are constructed, not only due to high fecundity but space requirements and the costs of maintaining numerous adult barramundi can be high. In this study, a large mass spawn (12 females and 21 males)

not previously applied on this scale was carried out to investigate the level of parental contribution from a large mass spawning group, and the number of parent pair relationships that could be detected within the offspring. The offspring were sampled at regular intervals during grow-out, which provided the opportunity to investigate the conservation of genetic diversity throughout the period of size grading and culling for the avoidance of cannibalism. Previous studies have reported on a loss of genetic diversity by size grading, however, no study has yet monitored the maintenance of genetic diversity throughout the entire cannibalistic stage of juveniles. The major findings from this chapter include a high participation rate of both male and female broodstock and the subsequent production of a large number of parent pair combinations or families. Despite a high rate of participation, contribution levels were unequal and there was a high variance in family sizes. In addition, there was a slight loss of genetic diversity from broodstock to offspring but throughout the period of size grading and culling, no further loss of genetic diversity was detected. The results suggest that a mass spawning group of at least 30 barramundi individuals is required to achieve a high participation rate of breeders and to limit the loss of genetic variation transferred to the offspring.

Chapter 3 in review:

Genetic diversity and relatedness estimates for captive barramundi (*Lates calcarifer*) broodstock populations, informs efforts to form a base population for selective breeding.

Loughnan, S.R., Smith-Keune, C., Jerry, D.R., Beheregaray, L.B., Robinson, N.A.

Journal **Aquaculture.**

The Australian barramundi industry has on hand a large number of mature broodstock that are currently supplying the grow-out market, however, before selective breeding programs can begin, it is important to assess the levels of genetic diversity and relatedness of current captive broodstock populations. This has not yet been assessed for Australian captive stocks, nor has the application of such information been applied to establishing a base population for selective breeding. Due to the implications of mass spawning investigated in chapter 2, it is also unclear how this has impacted on genetic diversity and relatedness levels across the captive industry. To address these issues, microsatellite DNA markers were utilised to genotype barramundi broodstock from eight major Australian commercial hatcheries. Population structure analysis indicated that captive Australian broodstock were broadly divided into two genetic population groups, genetic diversity levels were moderate and a level of relatedness was detected in each broodstock group. The estimates of genetic diversity and relatedness derived from this study suggest that the Australian barramundi industry has on hand suitable broodstock candidates for the development of a base population for selective breeding from current captive stocks. Although, sourcing additional broodstock from wild regions of high genetic diversity could enhance the fitness of current captive stocks further. The results are discussed

with regard to broodstock management and the development of a base population for selective breeding using existing Australian broodstock.

Chapter 4 to be submitted:
Assignment of captive barramundi (*Lates calcarifer*) broodstock to wild Australian stocks guides captive base population recruitment for selective breeding.
Loughnan, S.R., Smith-Keune, C., Jerry, D.R., Beheregaray, L.B., Robinson, N.A.
Journal **Aquaculture.**

The quality of captive barramundi founder stocks can be enhanced and fitness maintained by including wild individuals from genetically diverse stocks at the commencement of a selective breeding program. Identifying which wild stocks to target can be aided with assignment tests, which can clarify the wild genetic origins of captive individuals and determine the degree of wild genetic diversity not currently represented in captive stocks. In chapter 3, levels of relatedness and genetic diversity were estimated for eight captive broodstock groups under current production, and in this chapter the individuals within each of these groups were assigned to their wild ancestral origins. Levels of genetic diversity and population structure were determined for wild barramundi samples from 48 sites with 16 polymorphic microsatellite loci. Two wild genetic stocks and a region of genetic admixture were detected and levels of genetic diversity were slightly higher in the wild sample collections than the captive groups. Upon developing a base population for the selective breeding of barramundi, wild locations demonstrating high levels of genetic diversity identified in this study should be accessed to gather broodstock candidates. Ideally, an even number of broodstock should be sourced from each of

the three wild genetic stocks, to lower the level of relatedness between individuals and to gather a broad range of genetic diversity for the founding population.

Chapter 5 to be submitted:

Comparison of the use of different source stocks for establishing base populations for selective breeding of barramundi (*Lates calcarifer*).

Loughnan, S.R., Smith-Keune, C., Jerry, D.R., Beheregaray, L.B., Robinson, N.A.

Journal **Aquaculture Research.**

To determine the most appropriate broodstock candidates to use when establishing a base population for barramundi selective breeding, a computer simulation model to predict the maintenance of genetic diversity at 16 microsatellite loci was developed. There are various methods for selecting broodstock candidates for inclusion into a base population, such as selecting according to kinship levels between individuals (mk_r) or choosing individuals from wild regions demonstrating high levels of genetic diversity. Both of these methods were tested in the simulation model. Synthetic base populations were developed from the observed genotypes of captive broodstock from eight hatcheries (accessed from chapter 3) and the genotypes from 48 wild sites were utilised from chapter 4. In addition, chapter 2 provided parental contribution probabilities, which were used to select male and female parents at the commencement of the simulation, to mimic the skewness of parental contribution that can occur in barramundi mass spawning. Overall, this chapter incorporated the findings of the previous studies and utilised the results to recommend the best method for selecting a base population. Under each option there was a loss of genetic diversity from each broodstock group to offspring, although the highest level of genetic diversity was maintained when selecting broodstock

according to low mean kinship values (mk_r). The results suggest that a base population of at least 213 individuals split into five spawning tanks of an equal sex ratio, will provide a N_e of 100 and ΔF of 0.2%. In addition, wild broodstock should be sourced from regions of high genetic diversity and combined with current captive broodstock that have been selected according to the lowest mk_r values. This will help to maintain founder genetic diversity and heterozygosity levels in subsequent generations.