MARKER ASSISTED BREEDING PROGRAMS FOR FARMED ABALONE

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SUMMARY
A preliminary evaluation was conducted to determine the effectiveness of marker assisted breeding programs for increasing growth rates and reducing inbreeding in farmed abalone. Computer simulation of a population of abalone was used to investigate alternate strategies. Two strategies were investigated 1. A DNA marker was available which was closely linked to a genetic mutation affecting growth rate, and this information was used in marker assisted selection (MAS), and 2. Information from neutral markers (not linked to genes affecting growth rate) was used to reduce the rate of inbreeding. Key findings were that the rate of genetic gain for growth can be increased by up to 16%, in the first generation of selection, if there is a gene affecting growth rate and closely linked genetic marker is available for this gene, and the rate of inbreeding can be reduced by 30% using marker information. These improvements were achieved by genotyping only a small fraction of the total population – only 20 to 40 animals were genotyped at the DNA markers in all strategies.
Keywords: Abalone, Marker Assisted Selection, Inbreeding

INTRODUCTION
Abalone species (*Haliotis sp.*) have recently been domesticated, and are being farmed in Japan, Iceland, USA and Australia. The success of on-farm spawning programs (Fleming and Hone 1996) allows the possibility of selective breeding, with the most economically important trait being growth rate. Disease resistance is also important. While rapid gains for these traits should be possible from traditional selective breeding programs, the availability of DNA marker information in abalone could be used to further improve growth rates, as well as manage other aspects of breeding program design. There are at least 73 microsatellite tests available for Abalone (GenBANK), and efforts are underway both to increase the number of microsatellite tests, and to identify which of these tests are linked to genes affecting important production traits (Baranski *et al.* 2003). Specifically, the tests could be used as 'markers' of genes or chromosome segments to increase the accuracy of selection for growth rate and other traits using MAS, and to decrease the rate of inbreeding (a significant problem in aquaculture species owing to their high fecundity, Kincaid 1983) (though this also means there is opportunity to select out affected animals). Most strategies to avoid inbreeding rely on the availability of good family or pedigree information, with selections made to minimise genetic relationships based on this information. In abalone, such detailed pedigrees do not exist, as the species has only recently been domesticated. An alternative to using pedigree information is to use DNA markers to infer the proportion of the genome which is identical among selection candidates.

In this paper we describe some preliminary evaluations of marker assisted breeding programs for farmed abalone, using a computer simulation of an abalone aquaculture enterprise. As acquiring molecular marker information is expensive, any strategy which uses marker information must seek to

reduce the amount of genotyping required. The breeding programs aimed to maximise a function that seeks to maximise genetic gain, control inbreeding, with a minimal number of genotypings.

MATERIALS AND METHODS

Simulation of QTL and marker evolution in abalone. Computer simulation was used to evaluate alternate breeding strategies. Full details of the simulation model are described in Hayes *et al.* (2002). The starting point for the breeding strategies was a simulated population of 'wild' abalone, simulated as 1000 generations of natural selection in a population of 1000 abalone. Each abalone had a genome of 31 chromosomes. On each chromosome there were 51 loci, 25 of which were QTL (genes) and 26 of which were markers. Mutation at QTL and marker loci generated new alleles, and new QTL alleles affected growth rate and fitness. Offspring were formed by sampling parental gametes. Environmental deviations for animals were simulated to give phenotypes with a heritability of 0.3 (similar to the heritability that has been observed for growth rate of Californian red abalone, Jonasson *et al.* 1999). In generation 1000, there were approximately 5 alleles per marker locus and marker heterozygosity was 0.65.

Breeding schemes. Four breeding schemes were evaluated over 10 generations of breeding, with one generation being 3 years. In all breeding schemes, five males and five females were used to breed a total of 1000 offspring per generation. We assumed that all males and all females were spawned together in the same tank, that is there was random fertilisation of ova. The proportion of the next generation contributed by each of the five males and five females was randomly assigned to be (for each sex) 25%, 12.5%, 6.25%, 3.75% and 2.5%. Phenotypic measurements for growth rate were available for all 1000 offspring each generation. Four breeding strategies were evaluated. **PHENO** (Mass selection) In each generation, the five males and five males with the largest estimated breeding value (EBV) for growth rate were selected. EBVs were calculated as $EBV_i = h^2(y_i - \overline{y})$, where EBV_i is the breeding value of the i^{th} animal, h^2 is the heritability of growth rate (0.3), and y_i is the phenotypic measurement of growth rate, and $\overline{y}$ is the average growth rate in that generation. **10MAS**. In this strategy, two stages of selection were performed. First, the 10 males and 10 females ranked the highest on phenotype were selected. These animals were genotyped at the QTL with the largest effect on growth rate. The genetic variation at this QTL explained approximately 35% of the total genetic variation. Marker assisted breeding values (MEBVs) were then calculated as $EBV_i = h^2(y_i - \overline{y}) + QTL_i$, where QTL_i was the effect at the known QTL for animal i, assumed known without error. Phenotypes were pre-corrected for the QTL effect. From the 10 males and 10 females from the first stage of selection, the five males and five females with the highest MEBVs were selected to breed the next generation. **20MIN**. In each generation, the 20 males and 20 females with the largest estimated breeding value (EBV) for growth rate were genotyped for a single marker at the midpoint of each chromosome. This information was used to construct a relationship matrix. Five male and five female selection candidates were chosen to maximise average EBV and minimise the average marker relationship, from the marker relationship matrix. **10MIN**. As for 20MIN, but with 10 male and 10 female candidates genotyped each generation.

For each strategy, genetic value over 10 generations was calculated. Increase in homozygosity was also measured at all markers, to reflect inbreeding. Results presented are averages of fifty replicates.

RESULTS AND DISCUSSION

Evaluation of MAS in abalone (10MAS).

Genetic gain after the first generation of implementing 10MAS was 16% greater than average genetic merit from implementing PHENO, Table 1. Average genetic gain from 10MAS was 5% to 2% higher than from PHENO in subsequent generations.

Table 1. Average genetic value and inbreeding from PHENO and 10MAS.

Generation	*Average genetic value*			*Inbreeding*	
	PHENO	10MAS	% Improvement from 10MAS	PHENO	10MAS
1	2.63	3.12	15.5	0.41	0.41
2	6.36	6.90	7.9	0.45	0.45
5	15.14	15.86	4.5	0.58	0.58
10	25.27	25.91	2.5	0.71	0.71

Inbreeding (average marker homozygosity at all markers) was nearly identical for 10MAS and PHENO (Table 1), indicating MAS does not substantially affect the rate of inbreeding.

Evaluation of breeding strategies to reduced inbreeding (10MIN and 20MIN).

Average genetic merit over generations from 10MIN and 20MIN were very similar, and similar to PHENO, Figure 1. The rate of increase in inbreeding per generation, or marker homozygosity, was very similar for 10MIN and 20MIN, at approximately 2.37%, Figure 1. This represents a 30% reduction in the rate of increase in inbreeding from PHENO.

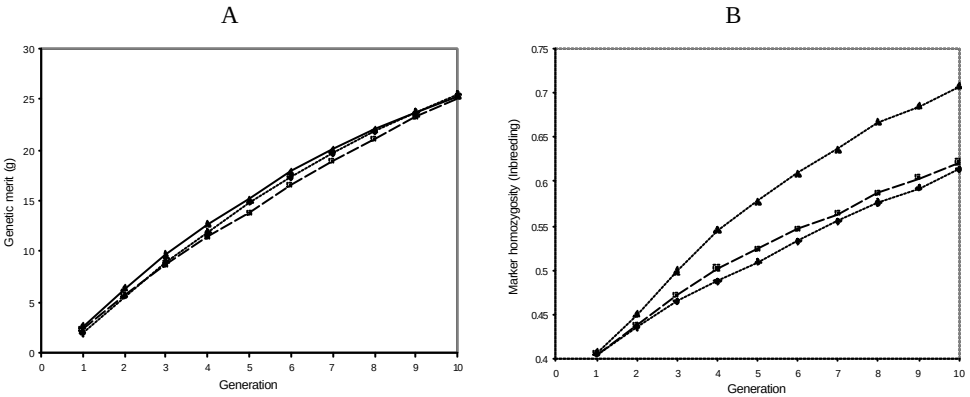

Figure 1. (A) Average genetic value from PHENO (full line, triangle), 10MIN (dotted line, diamond) and 20MIN (dashed line, square) from 10 generations of selection (B) Average progeny marker homozygosity (inbreeding) from 10 generations of selection.

Marker information can be used in at least two ways to increase genetic gains in farmed abalone. If a marker is linked to a QTL which affects an economic trait, this information can be used to increase the accuracy of selection. Secondly our results demonstrate that inbreeding could be significantly reduced by using marker information. Markers for this purpose did not have to be linked to QTL.

In the marker assisted selection strategy (10MAS), we assumed genotypes were available for the QTL with the favourable genetic mutation itself. The first applications of marker assisted selection in abalone will almost certainly be with a DNA marker linked to the QTL, rather than information on the QTL itself. As a result, the genetic gains would be less than predicted here, and more genotyping and trait recording would be required to track linkage phases.

An advantage of the marker assisted breeding strategies proposed is that only a fraction of the population need be genotyped. Essentially the strategies we have proposed use a two stage selection method, with an initial selection round based on phenotype only, followed byfurther selection with marker information. This greatly reduces the cost of using MAS in abalone breeding programs.

Results from investigations of MAS in livestock suggest the greatest gains from using marker information will be for traits which are difficult to select for, such as disease resistance and meat quality, and less for traits such as growth (Meuwissen and Goddard 1996). We have not evaluated gain for these other traits, but our results suggest even for growth using markers linked to genes affecting growth rates leads to quite reasonable gains.

One strategy for improving growth rates of farmed abalone, would be the use of MAS in conjunction with early selection of broodstock. Early growth in Abalone appears to be a poor indicator of how the quickly the abalone grow closer to market sizes (Jonasson *et al.* 1999). In a traditional breeding program, this means broodstock should be selected only when the reach market weights. However by using markers linked to genes with a large effect on growthrate, potential broodstock could be accurately selected at an early age. This would eliminate the need to keep large numbers of abalone through to market size in the breeding program. Alternately, selected broodstock could be spawned early to decrease the generation interval, increasing the rate of genetic gain.

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