

DEVELOPMENT OF A BREEDING STRATEGY FOR HYBRID ABALONE

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SUMMARY

The Australian temperate abalone fishery is based on two main species, the greenlip and the blacklip abalone. Sectors of the emerging abalone aquaculture industry have a commercial preference for the hybrid between these two species and wish to genetically improve the hybrid. To decide on an appropriate hybrid breeding strategy, Great Southern Waters and CSIRO, have initiated a trial over four year classes designed to determine if pure-species breeding values are correlated with hybrid breeding values. Discouragingly, early results from the first year class show a relatively weak and non-significant inter-species family correlation at 21 months of age; suggesting that recurrent selection for weight at 21 months in a blacklip breeding population may not result in the improvement of F1 hybrid growth. However, this weak and non-significant correlation may have been due to a number of extraneous factors and further studies are planned to investigate these.

INTRODUCTION

Abalone aquaculture is a relatively new but expanding industry in Australia. Blacklip (*Haliotis rubra* (Leach)) and greenlip (*H. laevisgata* (Donovan)) abalone, and their interspecific hybrid are the principal species farmed. These species and their hybrid are in the early stages of domestication and, accordingly, there is only a limited understanding of their genetic architecture. Sectors of the industry have a commercial preference for the hybrid as it exhibits favourable growth and behavioural characteristics in culture, as well as desirable product qualities.

Great Southern Waters Pty Ltd (GSW). GSW produces farmed blacklip × greenlip hybrid abalone. The company initiated a family-based selective breeding programme in 2003 with the objective of increasing growth and meat yield while maintaining the distinctive aesthetic characteristics and meat quality of its ‘Jade Tiger™’ product. To date, the company has focused its breeding research and development efforts on (i) establishing a closed, diverse and healthy breeding population, (ii) establishing trials to verify the advantages of hybrid abalone under GSW’s culture system, (iii) developing appropriate data capture and management systems and (iv) establishing progeny trials to estimate genetic parameters required to design, refine and implement a hybrid abalone breeding strategy.

Benefits of hybrids. Hybridisation has been used as a tool for improving the growth, behavioural, flavour, reproductive and processing characteristics of many commercially important plant and animal species (Kerr *et al.* 2004). Possible explanations for hybrid superiority over pure species, include: (i) heterosis; (ii) complementarity (e.g. where the intermediate expression of traits in the hybrid is superior, from an economic or management perspective, to the extreme expression of these traits in the ancestral species); and (iii) exploitation of greater allelic diversity through recombination and selection within backcrossed or advanced-generation (i.e. composite) hybrids (Falconer and Mackay 1996, Potts and Dungey 2004).

Abalone reproductive biology. Although unilateral cross-incompatibility is not complete, the use of blacklip dams and greenlip sires is more successful than the reciprocal cross. Female blacklip

abalone take approximately three years to reach sexual maturity and males can be mature at two years. Most animals live approximately five years in culture. Abalone are highly fecund (0.1 to 8.0×10^6 eggs per female depending on body size), but one of the principal difficulties experienced by abalone breeders is the synchronised induction of spawning (Elliott 2000). However, these problems have to some extent been overcome through the use of domesticated broodstock and the adoption of appropriate spawning methods and infrastructure.

For breeding purposes animals are maintained in individual family tanks until they reach a size suitable for physical tagging (about 15 mm). Difficulties with spawning, limited infrastructure, and limited skilled personnel restrict the number of individual families that can practically be produced in any one spawning season to a maximum of 40 to 50.

Hybrid breeding strategies under consideration. Three hybrid breeding strategies are being considered: (i) pure species selection (ii) reciprocal recurrent selection with forward selection and (iii) composite hybrid breeding (Kerr *et al.* 2004). To decide on a strategy, GSW and CSIRO have initiated a trial over four year classes, designed to determine if pure-species breeding values are correlated with hybrid breeding values (i.e. to establish to what extent recurrent selection in pure species could be used to genetically improve deployed F1 hybrids). This paper outlines the preliminary findings from the first year class which was spawned in the 2006/07 season.

MATERIALS AND METHODS

In this study, the 'half-sib' hybrid and pure-species progeny of 21 wild-caught blacklip dams were compared. Each of these dams was crossed with one of seven groups of blacklip sires and one of seven groups of greenlip sires. These crosses were made over 3 spawning runs (Table 1).

Pair-wise fertilisations were undertaken separately and, after fertilisation, eggs from a single dam were mixed to create a pure species and a hybrid maternal family from each dam. Animals were then maintained in single-species (blacklip and hybrid) family tanks for approximately 10 months before 540 individuals from each family were tagged. Total live weight from a random sample of 130 animals per family were taken at tagging and, at approximately 21 months of age, an independent random sample of 60 animals per family were weighed in the same manner. Not all animals weighed at 21 months were weighed at 10 months. Preliminary univariate restricted maximum likelihood (REML) mixed-model analyses, for each species and measurement age, were undertaken by fitting the following model in ASReml (Gilmour *et al.* 2006):

$$\text{WEIGHT} = \text{MEAN} + \text{SPAWN_RUN} + \text{SIRE_GROUP} + \text{FAMILY} + \text{RESIDUAL}$$

where WEIGHT is a vector of log-transformed weight observations, MEAN is the mean weight, SPAWN_RUN are the spawn run (i.e. spawning day confounded with the ratio of wild and farm males in greenlip sire groups; Table 1) effects fitted as a fixed factor, SIRE_GROUP are the sire group effects fitted as a random factor, FAMILY are the maternal family within sire group effects fitted as a random factor and RESIDUAL is a vector of residuals. A multivariate (two species by two measurement ages) model, which extended the univariate model and allowed for covariation between family effects and within-species residuals, was then fitted to estimate variance components and correlations. One- and two-tailed likelihood ratio tests were used to test the significance from zero of family variances and correlations, respectively (Gilmour *et al.* 2006).

For each species and assessment age, the narrow-sense heritability (h^2) and coefficient of additive genetic variation (CV_a) were estimated assuming family variance was explained entirely by additive genetic variation among dams (Falconer and Mackay 1996):

$$h^2 = \frac{4 \times \sigma_{fam}^2}{(\sigma_{fam}^2 + \sigma_e^2)} \qquad CV_a = \frac{\sqrt{4 \times \sigma_{fam}^2}}{\bar{x}}$$

where σ_{fam}^2 is the family within sire group variance; σ_e^2 is the residual variance and \bar{x} is the log-transformed trait mean.

Table 1. Crossing design for the 2006-07 year class (grey = farm sire, black = wild sire).

Blacklip dam	Spawning day	Blacklip sire group ID	Greenlip sire group ID	Blacklip sire groups (four to nine individuals per group)	Greenlip sires groups (four individuals per group)
A	0	1	8	■	■
B	0	1	8	■	■
C	0	1	8	■	■
D	0	1	8	■	■
E	0	2	9	■	■
F	0	2	9	■	■
J	0	3	10	■	■
K	0	3	10	■	■
L	12	4	11	■	■
M	12	4	11	■	■
N	12	4	11	■	■
O	12	4	11	■	■
P	12	5	12	■	■
Q	12	5	12	■	■
R	12	5	12	■	■
S	22	6	13	■	■
T	22	6	13	■	■
U	22	6	13	■	■
V	22	7	14	■	■
W	22	7	14	■	■
X	22	7	14	■	■

RESULTS AND DISCUSSION

Hybrid progeny grew more rapidly than blacklip progeny (Table 2). First-generation hybrids are typically found to give superior performance to pure species in the farmed situation. However, in this study the different genetic background of blacklip and greenlip sires may also have been a factor, since all blacklip sires were wild-caught and most greenlip sires were domesticated farm stock selected according to their size (Table 1).

Table 2. Back-transformed least squares (LS) means, narrow sense heritabilities (h^2) and coefficients of additive genetic variation (CV_a) for weight by species and assessment age.

Species	Time of assessment	LS mean (95% CI) (g)	h^2 (SE)	CV_a (%)
Blacklip	10 months (tagging)	1.55 (1.44 - 1.67)	0.97 (0.25)***	22.5
Blacklip	21 months	12.64 (11.89 - 13.44)	0.20 (0.09)***	7.2
Hybrid	10 months (tagging)	1.75 (1.57 - 1.93)	1.29 (0.30)***	29.7
Hybrid	21 months	17.08 (15.03 - 19.41)	0.28 (0.12)***	7.7

*** P<0.001

The extreme heritabilities and high coefficients of additive genetic variation reported for 10

months of age indicated that family variation observed at this early measure was not entirely explained by additive genetic variation but was, in part at least, explained by nursery effects (e.g. maternal, fertilisation, early-husbandry and tank effects).

The inter-age hybrid family correlation was strong (Table 3), suggesting that relative family performance did not change substantially between 10 and 21 months of age, possibly due to the persistence of nursery effects. However, the inter-age blacklip family correlation was weak (albeit positive) and not significantly different from zero. This weak and non-significant inter-age correlation could have been due to genotype-by-age interaction in growth, but was also consistent with a reduction in the influence of nursery effects over time (Kube *et al.* 2007).

Table 3. Family correlations for weight between assessment ages within species and between species within assessment ages.

Comparison	Category	Additive genetic correlation (SE)
Inter-age	Blacklip	0.37 (0.25) ^{ns}
Inter-age	Hybrid	0.72 (0.15)**
Inter-species	10 months (tagging)	0.74 (0.11)***
Inter-species	21 months	0.38 (0.29) ^{ns}

^{ns} not significant, * P<0.05, ** P<0.01, *** P<0.001

The strong and significant inter-species family correlation observed at 10 months, probably reflected nursery effects (excluding tank effects, as they were independent across species) more than additive genetic effects. Discouragingly, the 21-month inter-species family correlation, which is likely to be a more accurate estimate of the additive genetic correlation, was only weakly positive and non-significant. This suggests that recurrent selection for weight within the blacklip population may not result in the genetic improvement of F1 hybrid growth. However, this weak and non-significant correlation may have been due to nursery effects persisting beyond 10 months of age (particularly in the hybrid); biases among families due to variation in the survival of paired crosses after egg mixing; or a lack of statistical power (i.e. Type II error), given the relatively low number of families studied. Further studies are planned to investigate these possible explanations using data from this year class and others but, until these studies are completed, pure-species recurrent selection cannot be pursued with confidence.

Future studies. A sample of animals from each half-sib family will be genotyped to enable pedigree reconstruction, the precise estimation of genetic parameters and the examination of non-additive genetic effects. Assessment of later-age weight and other economically important traits will also be undertaken. Additionally, studies on advanced-generation hybrids and backcrosses will be carried out to determine the suitability and optimal composition of a composite breed.

REFERENECES

- Elliott, N.G. (2000) *Aquac Res* **31**:51-59.
 Falconer, D.S. and Mackay, T.F.C. (1996) "Introduction to quantitative genetics" Longman, Harlow.
 Gilmour, A.R., Gogel, B.J., Cullis, B.R. and Thompson, R. (2006) "ASReml User Guide Release 2.0" VSN International Ltd, Hemel Hempstead, UK.
 Kerr, R.J., Dieters, M.J. and Tier, B. (2004) *Can J For Res* **34**:209.
 Kube, P.D., Appleyard, S.A. and Elliott, N.G. (2007) *J Shellfish Res* **26**:821.
 Potts, B.M. and Dungey, H.S. (2004) *New For* **27**:115.