Journal of Shellfish Research, Vol. 26, No. 3, 821–824, 2007.

SELECTIVE BREEDING GREENLIP ABALONE (*HALIOTIS LAEVIGATA*): PRELIMINARY RESULTS AND ISSUES

P. D. KUBE,* S. A. APPLEYARD AND N. G. ELLIOTT
CSIRO Food Futures Flagship, CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania, Australia, 7001

ABSTRACT Greenlip abalone (*Haliotis laevigata*) (Donovan 1808) is a preferred aquaculture species in temperate Australia and selective breeding programs are being developed for this species. This study presents the results of a genetic parameter study for a small population grown on a farm in Tasmania, Australia. A total of 21 families were produced from 14 parents, with all parents except one being used in at least two families. Length and total weight were measured at four periods during the grow-out (10, 21, 27, and 38 mo after spawning) and at the final assessment meat and shell weights were also assessed. Because of issues with tag durability, only 17 of the original 21 families were recovered at final assessment. Genetic variation was low and, at best, the age 3 y heritabilities for total weight, meat weight, and length were 0.10, 0.10, and 0.04 respectively. Despite this low genetic variation, economically important gains appear possible in this small population, with a 5% gain in total weight being predicted. Prior to age 2.5 y, the genetic variation for length and weight appeared to be masked by maternal, larval, and settlement effects. The main factors limiting genetic gains in this study were difficulties in raising large numbers of pedigreed families in separate larval and settlement tanks, the effects of variability in the stages up to and including settlement and difficulties in tagging animals. DNA pedigree assignment is seen as a way to overcome these limitations.

KEY WORDS: abalone, *Haliotis laevigata*, selective breeding, genetic parameters

INTRODUCTION

Abalone aquaculture in Australia is a new and expanding industry. In 2006, the production from Australian farms is expected to be 500 tons and by 2010 this is expected to increase to 1,000 tons per annum (S. McLinden, Australian Abalone Growers Association, unpublished data). Abalone farms are located in the temperate regions of Australia and the main species grown is *Haliotis laevigata* (Donovan 1808) (greenlip abalone), which comprises approximately 70% of total production.

One of the main research priorities for the Australian abalone aquaculture industry is to develop selective breeding programs to improve the productivity of farm populations. An essential first step in selective breeding is to measure the genetic variation in commercial traits and a standard measure of the proportion of genetic variation in a population is the heritability. Knowledge of the genetic variation allows rates of genetic gain to be predicted, and the identification of optimal breeding strategies (Tave 1993, Falconer & Mackay 1996, Gjedrem 2005).

There is very little information in the literature about the genetic parameters of cultured abalone. In the only large-scale abalone study, Jónasson et al. (1999) estimated the genetic variation in survival and size of red abalone (*H. rufescens*) in Iceland. A total of 100 research families were produced by mating 29 males and 88 females, and the larvae from each family were grown separately until tagged at 10 mo of age. Heritability estimates for shell length at 8, 10, 18, and 24 mo of age were 0.08, 0.06, 0.27, and 0.34 respectively with a low and negative genetic correlation between survival to four months and shell length. In another study, Li et al. (2005) estimated the heritability of length and weight in 14 full sib blacklip abalone (*H. rubra*) families at four time points. The genetic variation was low and heritabilities for length ranged from 0.07 (at one year) to 0.02 (at 4 y) and for weight they ranged from 0.09 (at one year) to 0.01 (at 4 y).

This study reports the genetic parameters for growth traits of greenlip abalone (*H. laevigata*) from a small farm population in Tasmania, Australia. The aims were to estimate potential genetic gains and identify issues associated with selective breeding for this species. This was a preliminary study done prior to commencing a commercial program and was based upon a small and genetically restricted population. Therefore the results need to be treated with some caution and most probably reflect a conservative estimate of the genetic variation for this species.

MATERIALS AND METHODS

Trial Design

The trial included 21 families from 8 mothers and 6 fathers (in a nested full-sib, half-sib mating design). All parents except one were involved in at least two crosses, and some were involved in up to five crosses. Table 1 shows the crossing design.

The parents were selected from F1 farm stock. The mothers originated from Flinders Island (Tasmania) natural stock and the fathers originated from Cape Portland (north-eastern Tasmania) natural stock. The parental cohorts originated from a small genetic base. The Flinders Island stock originated from nine parents (six females and three males) and the Cape Portland stock originated from five parents (two males and three females). Although the F1 progeny are known to be unrelated, there is a reasonably high probability that any two mothers and any two fathers in our study are related as half-sibs ($P = 0.67$ for fathers and $P = 0.44$ for mothers).

Spawnings occurred in January and February 2002 and families were produced in two batches separated by 21 days. Batches 1 and 2 consisted of 9 and 11 families respectively. The number of ova collected and fertilized for each mating was between 100,000 and 150,000.

*Corresponding author. E-mail: peter.kube@csiro.au

TABLE 1.

Crossing design used in the greenlip abalone family trial.

	Dams								Total Crosses
	A	B	C	D	E	F	G	H	
Sires									
1	X	X	X						3
2	X	X	X						3
3	X	X	X						3
4				X	X	X	X	X	5
5				X	X	X	X	X	5
6				X					1
Total crosses	3	3	3	3	2	2	2	2	21

The families were maintained on separate settlement plates until age 10 mo (November 2002). At this time, low larval numbers and low numbers of settled individuals were noted for some families. Juveniles were tagged when they were removed from settlement plates using colored beads, which were glued to the shell of each individual to identify the family. Between 40 and 164 individuals were tagged per family.

Measurements

Measurements on individual abalone were undertaken on four occasions using digital callipers (lengths in mm) and electronic balances (weights in g). The first measurement (T1) was at 10 mo after spawning (November 2002) when the families were removed from the settlement plates. Only length was measured at this occasion. The second measurement (T2) was at 21 mo (October 2003) and length and weight were assessed. The third measurement (T3) was at 27 mo (April 2004) and also assessed length and weight. On this occasion additional tagging was performed with numbered tags, also glued to the shell, to allow identification of specific individuals. The fourth and final measurement (T4) was at 38 mo (May 2005) and length, total weight, meat wet weight, and shell wet weight were assessed.

The summary statistics for each measurement are shown in Table 2. Because of poor tag durability, a reduced number of animals were recovered at each measurement and only 17 of the original 21 families were recovered at final assessment.

TABLE 2.

Summary of data measurements for the greenlip abalone family trial. SD = standard deviation, min = smallest value, max = largest value, and n = sample size.

Variable	Unit	Age (months)	Mean	SD	Min	Max	n
Length T1	mm	10	11.7	3.4	3.6	26.3	1887
Length T2	mm	21	36.3	5.5	21.9	61.5	852
Weight T2	g	21	5.9	2.9	1.4	30.1	851
Length T3	mm	27	53.1	4.6	43.0	67.2	270
Weight T3	g	27	17.8	4.7	9.9	32.9	270
Length T4	mm	38	73.1	4.6	60.8	83.8	204
Weight T4	mm	38	45.7	8.5	24.0	69.1	204
Meat weight T4	g	38	16.6	2.8	10.0	25.0	203
Shell weight T4	g	38	14.2	3.0	5.7	24.7	203

Analysis

Variance components for each of the measured traits were estimated using residual maximum likelihood methods. ASReml (Gilmour et al. 2002) was used to fit the following univariate individual animal mixed model:

$$Y_{ijk} = \mu + B_i + A_k + F_j + \varepsilon_k$$

where Y_{ijk} is the observed value, μ is the overall mean, B_i is the fixed effect of the 1 ith batch (2 batches, which represent spawning time), A_k is the additive genetic effect of the kth animal, F_j is the random effect of the jth full-sib family (21 individual full-sib families), and ε_{ijk} is the residual random effect. The larval and settlement environments for each family were not replicated and, consequently, the term "family" represents a combination of the effects of individual family larval and settlement environments, egg and sperm quality on fertilization and larval growth stages, and the genetic effects of dominance. The earlier mentioned model was also used to calculate breeding values for the parents and progeny. These breeding values were used to estimate potential genetic gains from selection.

Narrow sense heritability was estimated as the proportion of additive genetic variance to total variance as follows, where the terms are the variance components for the terms in the earlier mentioned model:

$$h^2 = \sigma^2_A/(\sigma^2_F + \sigma^2_A + \sigma^2_\varepsilon).$$

RESULTS

Genetic differences appear to be masked by the influences of the spawning, larval, and settlement environments up until about age 2.5 y. This is seen by the large "batch" and "family" variances and small additive genetic variances at ages 10, 21, and 27 mo (Table 3). The influences of batch and family appeared to steadily decline and by 38 mo had almost disappeared. The batch effects represent a 21-day separation in spawning time. The family effects measured in the early stages of the grow-out are, we believe, most likely to be a measure of the maternal effects and the different larval and settlement environments. Any additive genetic effects appear to be swamped by this noise. Although dominance may be a contributor to family variance, this effect (if present) is probably relatively small given the known variability between different settlement tanks, and the known influences of that variability. Furthermore, the fact that the family effects erode at about the same time as the batch effects suggests this is an environmental and/or a maternal effect and not a true genetic effect.

Additive genetic variation for total weight, meat weight, and shell weight at age 38 mo appeared to be present, but heritability estimates in this small population were low and standard errors high (Table 3). For length at 38 mo, heritability was very low. The high standard errors are probably caused by the small number of parents and low numbers of relationships that contribute to the estimate of additive variance. These terms were shown to be statistically significant when fitting a simpler model with sire and dam as fixed effects. Additive genetic variation could not be detected in earlier measurements regardless of the model fitted presumably because of the over-riding

TABLE 3.

Variance components and heritabilities for traits measured in greenlip abalone families at four time points.

Variable	Batch	Family	Additive Genetic	Residual	h^2
Length T1	5.75 ± 6.06	5.34 ± 1.40	0.00 ± 0.00	5.26 ± 0.17	0.00
Length T2	9.34 ± 13.94	5.95 ± 2.31	0.00 ± 0.00	18.63 ± 0.92	0.00
Weight T2	2.13 ± 3.23	1.36 ± 0.51	0.00 ± 0.00	4.26 ± 0.21	0.00
Length T3	5.86 ± 9.01	2.45 ± 1.61	0.00 ± 1.29	17.09 ± 1.83	0.00
Weight T3	6.01 ± 9.10	2.38 ± 1.51	0.14 ± 2.11	17.82 ± 2.05	0.01 ± 0.05
Length T4	0.00 ± 0.00	0.05 ± 0.88	0.82 ± 2.15	19.49 ± 2.36	0.04 ± 0.10
Weight T4	0.00 ± 0.00	0.26 ± 3.27	6.97 ± 9.42	66.03 ± 8.69	0.10 ± 0.10
Meat wt T4	0.00 ± 0.00	0.13 ± 0.49	0.93 ± 1.33	8.47 ± 1.15	0.10 ± 0.11
Shell wt T4	0.00 ± 0.00	0.00 ± 0.00	1.27 ± 1.15	6.52 ± 0.96	0.16 ± 0.12

influence of variability from maternal, larval, and settlement environments.

The potential genetic gains from this population can be illustrated by examining the parental breeding values for total weight (Table 4). Despite the very small size of this population (only 14 parents) and the low heritability, economically important genetic gains appear possible. Crossing between the best two parents from this population would give a gain of approximately 5% in total weight. Examination of the breeding values of the progeny (not shown here) indicates that similar gains are possible when selecting larger groups of animals from the progeny. For example, when two unrelated groups of the progeny are selected, with 15 animals in each group, gains are also 5%.

DISCUSSION

Issues for Selective Breeding

A key factor limiting progress in selective breeding for this species are difficulties in raising large numbers of pedigreed families. The main limitation is the space and facilities required to keep families separate during the larval and settlement stages. More families are needed for two reasons. The first is to be able to understand the nature of genetic variation. It is known that small populations give imprecise estimates of additive genetic variance and heritability, and the high standard errors on variances and heritabilities in this study illustrate this point. We believe 40 families should be a minimum requirement, and ideally more—especially when heritabilities are low. The second reason for having more families is too achieve greater gains. From the small population used in this study, gains in total weight of 5% have been predicted. However, bigger gains are possible with larger breeding populations and, using the standard genetic gain prediction equations (e.g., Falconer & Mackay 1996), it can be shown that gains could be doubled if populations of approximately 100 families were produced.

TABLE 4.

Parental breeding values for total weight calculated at 38 mo of age.

Parental ID Code#	Sex	Breeding Value for Total Weight (g)*	% Gain Over Mean
4	sire	2.8	6%
C	dam	1.8	4%
F	dam	1.1	2%
3	sire	0.9	2%
H	dam	0.4	1%
D	dam	0.3	1%
2	sire	0.1	0%
6	sire	0.1	0%
A	dam	–0.2	–1%
E	dam	–0.9	–2%
G	dam	–0.9	–2%
1	sire	–1.0	–2%
B	dam	–1.6	–3%
5	sire	–2.9	–6%

Parental code as in Table 1.

* Breeding values are expressed as a deviation from the overall mean (mean = 45.7 g).

Another factor limiting progress was loss of tags on the animals. This occurred because of animals rubbing against the tank structure and each other, unreliable adhesives, and poor durability of tags. Only 11% of animals originally tagged at 10 mo (when they were 12 mm in length) were recovered at 38 mo (see Table 2). Most losses occurred when the animals were small. For example, 55% of tag loss occurred between the beginning of grow-out and age 21 mo, 39% between 21 mo and 27 mo, and only 3% between 27 mo and 38 mo. Clearly, the need for effective individual tagging for small animals is an important issue.

The high environmental variation introduced in the early stages of the life cycle is another issue limiting progress. Each family was raised in a separate tank through to settlement and therefore maternal, larval, and settlement effects are confounded with family effects. At the completion of the settlement phase there were large size differences between families that were, most probably, because of environmental differences in the settlement tanks and not the true genetic affects. These differences appeared to mask genetic differences for at least 2.5 y. Furthermore, the effect of spawning different families at different times appears to influence family performance. In this study, there was a 21-day difference between the spawning of batches 1 and 2, and these effects persisted for at least 27 mo after spawning. Presumably larger time differences between batches would have effects that last even longer. Ideally, genetic links are needed between batches so the effects of spawning time can be reliably accounted for. A key challenge therefore is to develop protocols that remove the noise (that is, the nongenetic effects) from the grow-out period. Such protocols would

increase the gains from genetic selection by improving the precision of breeding value estimates and increasing the expression of genetic variation (i.e., increasing the heritability).

Implications for Breeding Programs in H. laevigata

The data from this study suggests genetic variation for growth rate is present in *H. laevigata* and that genetic gains of approximately 5% per generation can be achieved with a small population such as that used in this study. Gain predictions from theoretical genetics (e.g., Falconer & Mackay 1996, Table 13.4) can be used to show that genetic gains could be twice this size with a breeding population of approximately 100 families. The estimates of additive genetic variance and heritability from this study are not precise because of the small numbers of families and parents in this trial. However, they are likely to be conservative because of the small number of parents used and the relatively narrow genetic base from which they originated. Therefore selective breeding programs for this species can, and should, proceed with the confidence that commercially important gains can be achieved.

This study also provides some warnings to those establishing commercial selective breeding programs. Firstly, we have identified three key limitations (difficulties in raising large numbers of pedigreed families, high variability in the stages up to and including settlement, and difficulties in tagging) and selective breeding cannot proceed to a fully commercial activity without addressing these. Secondly, the age at which selections are made needs to be considered carefully. In this study no genetic variation was found before age 2.5 y and therefore early selection is unlikely to deliver good genetic gains. Consideration needs to be given to identifying the optimal selection age. And thirdly, we believe that when planning selective breeding programs the premise should be that genetic variation will not be high. Genetic progress can still be made, but well planned and scientifically based selective breeding programs will be needed that are based on diverse breeding populations and selection methodologies that can exploit all possible pedigree information. Simple mass selection programs are not likely to deliver good genetic gains. An example of the potential of a relatively small program to deliver gains when heritabilities are low is *Eucalyptus globulus* tree breeding in which volume gains of 15% have been made after 2 generations (McRae 2004) despite a heritability of approximately 0.11 (Potts et al. 2004).

Future Directions

To establish a more effective selective breeding program in greenlip abalone, we see the development of DNA markers for pedigree assignment as a means of overcoming the limitations outlined above. DNA markers will allow the production of greater numbers of families free of the limitations of separate larval and settlement tanks, and not subject to the problems of tag loss on small animals. We envisage that families would be fertilized separately, but combined immediately after fertilization. Pedigrees will be assigned after taking a biopsy sample, running a suite of microsatellite markers, and matching the markers of the progeny to the markers of the known parents.

The numbers of families that can be produced in a single cohort would be limited only by the number of parents that can be induced to spawn. Such a system would avoid the noise introduced from separate settlement tanks. This would allow the accurate detection of genetic dominance, and may allow the detection of genetic variation at an earlier age.

CONCLUSION

The precision of estimates of genetic variation are limited because of the small scale of this study however, the results suggest genetic variation for growth rate is present in *H. laevigata*. If the results from this study are confirmed in a larger study, then a well-planned breeding program with a diverse genetic base should be able to deliver gains for growth rate in the order of 10% per generation. This study identified three key limitations to an operational selective breeding program. These were difficulties in raising large numbers of pedigreed families in separate larval and settlement tanks, the effects of variability in the stages up to and including settlement, and difficulties in tagging animals.

ACKNOWLEDGMENTS

This research was made possible with the financial and in-kind support of Miles Cropp and his staff at Abalone Farms Australia. The authors gratefully acknowledge this contribution. They also thank Bronwyn Holmes, Mina Brock and Graeme Dunstan from CSIRO Marine and Atmospheric Research for their help with the abalone tagging and measurements, and John Henshall and Sonja Dominik from CSIRO Livestock Industries for comments on an earlier draft of this paper.

LITERATURE CITED

Falconer, D. S. & T. F. C. Mackay. 1996. Introduction to quantitative genetics, 4th edition. England: Longman. 464 pp.

Gjedrem, T. 2005. Selection and breeding programs in aquaculture. Dordrecht: Springer. 364 pp.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham & R. Thompson. 2002. ASReml User Guide release 1.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Jónasson, J., S. E. Stefansson, A. Gudnason & A. Steinarsson. 1999. Genetic variation for survival and shell length of cultured red abalone (*Haliotis rufescens*) in Iceland. *J. Shellfish Res.* 18:621–625.

Li, X., R. Ponsoni, B. Brien, N. H. Nguyen & A. Butterworth. 2005. Selective breeding of farmed abalone in Australia: preliminary genetic analysis of the data from blacklip abalone families established in Victoria in the summer of 2000/2001. In: A. E. Fleming, editor. Proceedings of the 12th annual abalone aquaculture workshop. August 1–3, 2005. McLaren Vale, Australia. Abalone Aquaculture Subprogram, Fisheries Research and Development Corporation, Canberra, Australia.

McRae, T. 2004. Growing trees with the best genetics. In: Integrating forestry into farms, communities and catchments. Proc. Australian Forest Growers Biennial Conference, Ballarat, Victoria. May 3–5, 2004.

Potts, B. M., R. E. Vaillancourt, G. Jordan, G. Dutkowski, J. Costa e Silva, G. McKinnon, D. Steane, P. Volker, G. Lopez, L. Apiolaza, Y. Li, C. Marques & N. Borrelho. 2004. Exploration of the *Eucalyptus globulus* gene pool. In: N. Borrelho. Eucalyptus in a changing world. Proc. IUFRO Conf., Aveiro, Portugal. October 11–15, 2004.

Tave, D. 1993. Genetics for fish hatchery managers, 2nd edition. USA: Van Nostrand Reinhold. 350 pp.