Family-based selective breeding of New Zealand Abalone, *Haliotis iris*: challenges and opportunities

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Abstract

In 2007 the National Institute of Water and Atmospheric Research in New Zealand initiated a family-based selective breeding programme for the New Zealand Abalone, *Haliotis iris,* with commercial partner OceaNZ Blue Ltd. to investigate the genetics of key performance traits in a cold water and a warm water site and the influence of the early rearing environment on family performance. A total of 101 Abalone families were established using farmed and wild broodstock from multiple locations around New Zealand. Families were reared separately for nine to 14 months prior to tagging at ~15–20 mm shell length and then combined. Families were also pooled during early rearing, up to seven days post-fertilisation and later genotyped. This allowed estimation of individual tank environment effects on family performance. Length, weight and growth rate have been recorded bi-annually plus evaluation of quality traits such as foot darkness and shell colour at a market size of ~80 mm. Relative family performance has generally been consistent across sites, but is moderately influenced by early environment. The growth traits were found to be highly heritable with high genetic correlations among traits indicating good response to selection for increased growth can be expected.

Keywords: abalone; paua, selective breeding; environment; growth; genetic parameters

Introduction

Abalone, *Haliotis iris*, known locally as Paua, are farmed on a small scale in New Zealand where OceaNZ Blue Ltd. are the largest commercial company, producing approximately 100 tonnes per annum (OceaNZ 2009). OceaNZ Blue supply Paua over 75 mm in shell length to international markets and specialise in high quality canned and cryogenically frozen products. *Haliotis iris* is slow growing and can take three to four years to reach market size.

Traits of economic importance in Abalone production include growth to market size, feed conversion, meat yield, shell and foot colour, survival, and meat quality. No quantitative genetic studies have been conducted to date on the species farmed in New Zealand. Evaluation of quantitative genetic parameters such as phenotypic and additive genotypic variation, heritability and correlations are required in order to define a cost effective commercial genetic improvement programme. A family-based genetics programme is required to establish genetic parameters. However, a long term selective breeding programme based on individual family rearing up to tagging requires extensive resources which may not be feasible for a species farmed on a small scale.

The main aims of this study were firstly to establish families and estimate the genetic parameters for key economically valuable traits, secondly to estimate the influence of early rearing effects and environment on family performance and thirdly to assess the feasibility of rearing pooled families versus rearing families separately up to tagging.

Materials and methods

Establishing and rearing families

Broodstock were spawned at the National Institute of Water and Atmospheric Research Mahanga Bay facility according to the method of Moss et al. (1995). Eggs and sperm were mixed from one male and one female to generate an individual cross or "family". Three groups of families were established as the foundation of the selective breeding programme.

Group 1 families. Sixteen families were established on 4 June 2007. Four males (three farmed and one wild broodstock) and four females (two farmed and two wild broodstock) were crossed to each other in a full factorial cross design of four males crossed with four females to generate 16 families.

Group 2 families. Fifty-one families were established between 4 June 2008 and 15 July 2008. A partial factorial cross design was used. The aim was to cross each male to two females and *vice versa*. In total 25

females were crossed to 22 males with the number of crosses per parent ranging from one to four. A mix of wild (n = 42) and farmed (n = 5) broodstock were used.

Group 3 families. Thirty-four families were established on 1 September 2009. A partial factorial cross design was used. Each male (n = 17) was crossed to two females (n =17) and *vice versa*. A mix of wild (n = 32) and farmed (n = 2) broodstock were used.

Overall 92% of the parents produced at least two families. Five parents were reused across the groups to create a genetic linkage between the year classes.

Family rearing. Two rearing strategies were employed for the families. Families were either reared as separate family crosses, one family per tank, until the family individuals were big enough to be tagged and then combined, or pooled with other families of the same group and age during early rearing. The pooled groups enabled the confounding of family and rearing tank to be separated in the statistical analysis. Three pooling times were used to test for differences in pooling strategy:

1. pooled immediately after fertilisation (all Group 3 families),
2. pooled after hatching (24 hours post-fertilisation; all Group 1 and 2 families) and
3. pooled just prior to larval settlement in tanks (7 days post fertilisation; 15 of the 16 Group 1 families).

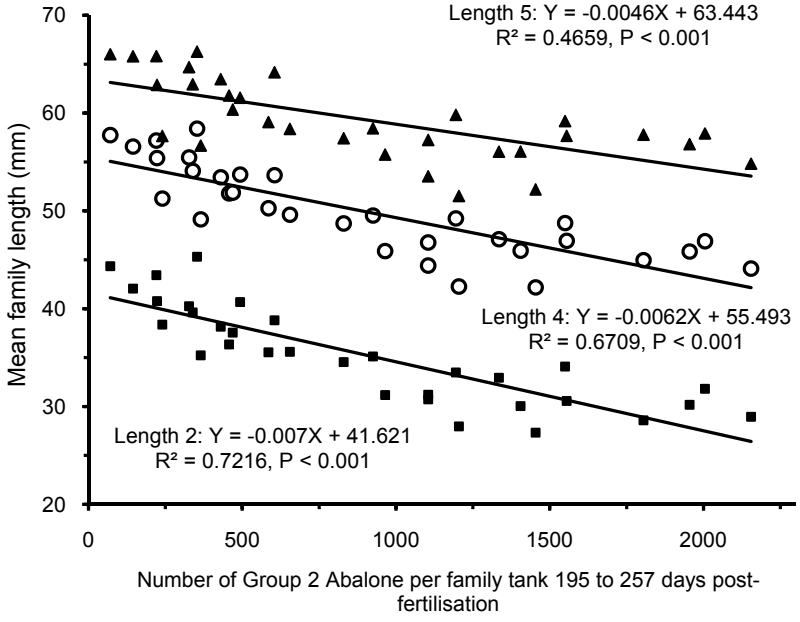
In all pooling strategies, a similar number of individuals per family were randomly collected from each family tank and combined into a single tank for further rearing.

The individual families were reared in separate tanks from fertilisation until tagging with one tank per family. Numbers per tank were standardised where possible. Families and the pooled groups were first tagged after nine to 14 months when they reached a mean shell length of ~15–20 mm using 3 mm spring tags (Mollusc Pty Ltd., Mallacoota, Victoria, Australia). Parentage of the pooled groups was established at a later date using DNA genotyping. Due to differential survival some families had lower numbers during early rearing and not all families survived to the larval settlement stage, seven days post-fertilisation. Families were reared and assessed at a cool water location at the Mahanga Bay facility near Wellington and at a warmer water location at OceaNZ Blue's commercial facility near Whangarei, Northland. Families were transferred from Mahanga Bay to OceaNZ Blue either as pooled larvae for settlement and later tagging, or post-tagging. When surviving numbers allowed, between 120 and 140 individuals per family were tagged from each rearing site. From the pooled groups a random sample was selected from the tank for tagging. All individuals were re-tagged at least once using 4 mm spring tags to ensure that at least one tag was retained.

Genotyping and parentage analysis

For parentage analysis, tissue was obtained from tagged parent broodstock and pooled family individuals by scraping the bottom of the foot and preserved in 100% ethanol. For DNA extraction, the tissue was added to 200µL extraction buffer (5% Chelex 100, 0.1% Tween20, 4 µg proteinase K) and incubated at 60°C overnight before boiling for 10 minutes. The Abalone parentage testing panel was developed from simple sequence repeat (SSR) markers supplied by OceaNZ Blue (7 SSRs) and Canterbury University (2 SSRs). A further marker was selected from microsatellites isolated by genomic (454) sequencing. The ten SSR markers were combined into a single multiplex polymerase chain reaction panel. The optimal polymerase chain reaction conditions for the multiplex were an annealing temperature of 56°C, a $MgCl_2$ concentration of 2.0 mM and primer concentrations ranging from 0.1 µM to 0.4 µM. Using fluorescently labelled primers, the amplification products were run on an ABI3730 genetic analyser (Applied Biosystems, Carlsbad, CA, USA) and allele sizes determined using the GeneScan™ –500 LIZ®

Figure 1 Early rearing effects and the influence of the estimated number of Abalone surviving in each separate family tank prior to tagging on Group 2 mean family length (mm) post tagging (n = 32). Length at Assessment 2 = ■; Length at Assessment 4 = O; Length at Assessment 5 = ▲.



Size Standard (Applied Biosystems, Carlsbad, CA, USA) and GeneMapper® Software v.3.7 (Applied Biosystems, Carlsbad, CA, USA). Parentage was assigned using a proprietary pedigree analysis programme developed by AgResearch (KG Dodds, Personal communication), comparing DNA profiles of the progeny against all combinations of parents within a group of families. The probabilities and limit of detection were calculated to assist in the parentage analysis. Greater than 95 % of pedigrees were uniquely assigned.

Data collection and trait analysis

Measurements on individual Abalone were conducted biannually from tagging onwards, usually in late autumn and late spring to capture the end of the summer and winter growth periods. Standard assessments were shell length (mm) and total weight (g). Daily growth rates between assessments (μm/d) were also analysed to remove the auto-correlation between length measurements over time. Data in the paper are presented for Assessments 1 (266 to 530 days post-fertilisation), Assessment 2 (447 to 545 days-post-fertilisation), Assessment 4 (580 to 723 days post-fertilisation), Assessment 5 (850 to 926 days post-fertilisation), Assessment 6 (1047 to 1107 days post-fertilisation) and Assessment 8 (1244 to 1293 days post-fertilisation). Interim assessments (Assessments 3 and 7) on smaller numbers of individuals are not included in the results. On one occasion (Assessment 6) foot darkness and shell colour were also assessed for the Group 1 families. This was based on a visual number score ranging from 0.5 (lighter) to 5 (darker) for foot darkness and from 1 (lighter) to 4 (darker) for shell colour.

Family contribution within the pooled groups

Pooling the families reduces early environmental effects but can result in uneven family contributions due to differential survival and competition. To estimate family contribution the 16 Group 1 families were pooled post hatching and just prior to settlement to create 5 different pooled groups:

1. pooled at hatch, reared at Mahanga Bay prior to tagging then at Mahanga Bay;
2. pooled prior to settlement, reared at Mahanga Bay prior to tagging then at Mahanga Bay;
3. pooled at hatch reared at Mahanga Bay prior to tagging then at OceaNZ Blue;
4. pooled prior to settlement, reared at Mahanga Bay prior to tagging then at OceaNZ Blue and
5. pooled at hatch reared at OceaNZ Blue prior to tagging then at OceaNZ Blue.

Data analysis

Data were analysed using linear mixed models. For all traits age at assessment was fitted as a covariate, contemporary group as a fixed effect and animal as a random effect. Contemporary group was defined as the animal's year of fertilisation by settlement site and family for individual family groups, and year by

Figure 2 Contribution of the 16 Group 1 families to the pooled groups based on the parentage assignment. Pooled group 1 was pooled at hatch, reared at Mahanga Bay prior to tagging then at Mahanga Bay; Group 2 was pooled prior to settlement, reared at Mahanga Bay prior to tagging then at Mahanga Bay; Group 3 was pooled at hatch, reared at Mahanga Bay prior to tagging then at OceaNZ Blue; Group 4 was pooled prior to settlement, reared at Mahanga Bay prior to tagging then at OceaNZ Blue; Group 5 was pooled at hatch reared at OceaNZ Blue prior to tagging then at OceaNZ Blue. Between 397 and 474 individuals were genotyped per pooled group. Parentage: Families 1–4 = Female 1; Families 5–8 = Female 2; Families 9–12 = Female 3; Families 13–16 = Female 4.

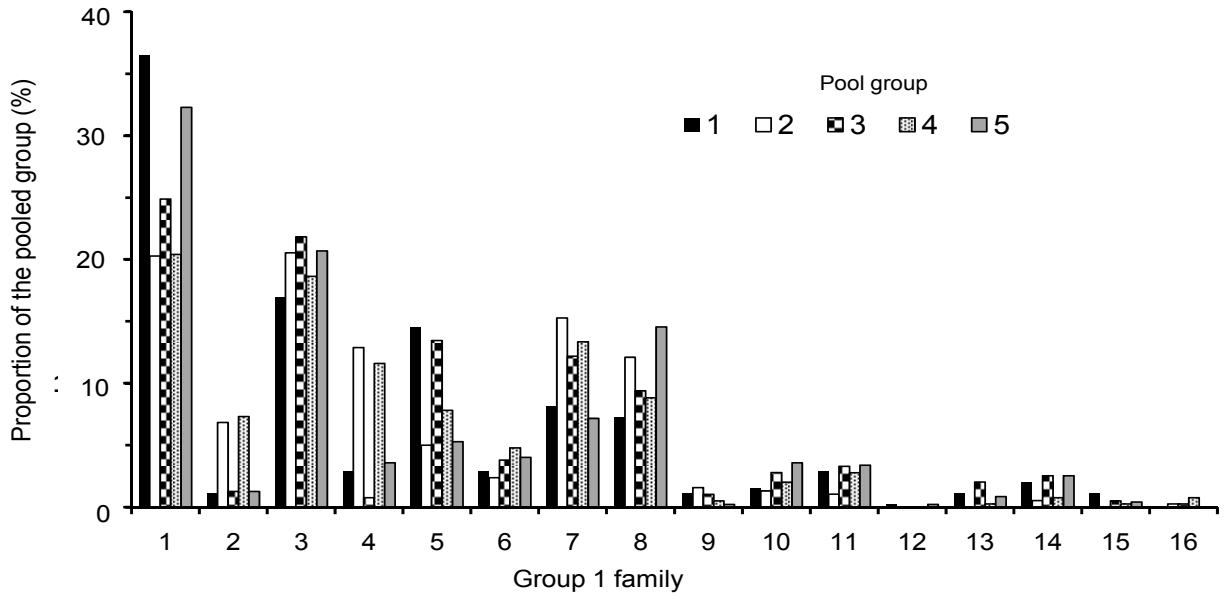


Table 1 Summary statistics for the family dataset including heritability ± standard error and the number of families at each assessment and average age (days post fertilisation). The numeral after each characteristic name indicates the Assessment when the measurement was collected. Number of individuals measured at each assessment were: Assessment 1: 29,064; Assessment 4: 18,997; Assessment 5: 13,658; Assessment 6: 5,739; Assessment 8: 5,115.

Measurement	Heritability	Mean	Phenotypic standard deviation	Coefficient of variation	Number of families	Average age (days)
Length 1 (mm)	0.43 ± 0.11	22.0	5.7	0.26	95	386
Length 4 (mm)	0.40 ± 0.10	43.3	10.2	0.24	95	648
Length 5 (mm)	0.44 ± 0.11	58.2	9.0	0.15	61	897
Length 8 (mm)	0.47 ± 0.11	70.9	8.1	0.11	16	1,263
Weight 1 (g)	0.55 ± 0.12	1.46	1.07	0.73		
Weight 4 (g)	0.50 ± 0.11	11.7	8.7	0.74		
Weight 5 (g)	0.51 ± 0.11	26.7	12.7	0.48		
Weight 8 (g)	0.46 ± 0.21	50.6	17.8	0.35		
Growth rate 1–4 (μm/d)	0.49 ± 0.11	37.3	21.0	0.56		
Growth rate 4–5 (μm/d)	0.56 ± 0.12	58.6	29.3	0.50		
Growth rate 5–8 (μm/d)	0.20 ± 0.11	69.0	22.7	0.33		
Foot darkness score 6	0.32 ± 0.16	1.48	0.59	0.40	16	1,085
Shell colour score 6	0.74 ± 0.27	2.71	0.65	0.24		

Table 2 Genetic parameters for Abalone lengths at Assessments 1, 4, 5 and 8 (L1–L8) and weights at the same assessments (W1–W8). Heritability estimates are shown in bold, with phenotypic correlations above the diagonal and genetic correlations below the diagonal.

Trait	L1	L4	L5	L8	W1	W4	W5	W8
L1	**0.43**	0.82	0.72	0.65	0.97	0.83	0.73	0.65
L4	0.86	**0.40**	0.79	0.78	0.62	0.96	0.88	0.78
L5	0.83	0.95	**0.44**	0.89	0.68	0.84	0.95	0.87
L8	0.79	0.89	0.96	**0.47**	0.58	0.75	0.86	0.96
W1	0.99	0.85	0.81	0.72	**0.55**	0.82	0.71	0.60
W4	0.88	0.99	0.91	0.89	0.86	**0.50**	0.89	0.77
W5	0.84	0.93	0.99	0.95	0.83	0.94	**0.51**	0.88
W8	0.81	0.90	0.98	0.98	0.75	0.90	0.97	**0.46**

settlement site and pooled group number for the pooled groups. Parameter estimates for all traits were estimated on an age constant basis.

Variance components were estimated using restricted maximum likelihood (REML) procedures fitting an animal model in ASReml (Gilmour et al. 2006). Univariate analyses were used to estimate heritabilities for individual traits. Bivariate analyses were used to estimate the phenotypic and genetic correlations between the various traits. The variance/covariance matrices were bent where appropriate using a weighted bending method (Jorjani et al. 2002; Jorjani et al. 2003) to ensure the matrices were positive definite.

Results

Overall 95 families survived to tagging. However, eight families comprised less than five surviving individuals. The 87 families with over five individuals represent 83 parents, 39 males and 44 females, sufficient numbers to sustain a future breeding programme. Seven of the eight families with poor survival were from Group 2 which had the least consistent family survival during early rearing. The resulting highly variable number of individuals being reared in the separate family tanks up to tagging impacted on density and continued to have a significant influence on the mean length of the families post tagging (Fig. 1); the lower the density the better the early growth.

All families were reared at both sites post tagging. Despite the difference in seawater temperatures and rearing environments the families performed similarly in both environments. A comparison of family mean length at both sites for the families reared individually up to tagging revealed they were significantly correlated ($R^2 = 0.94$, $P < 0.001$, $n = 47$; $R^2 = 0.85$, $P < 0.001$, $n = 47$; $R^2 = 0.68$, $P < 0.001$, $n = 15$) for mean lengths at Assessments 2, 4 and 8 respectively. A similar comparison was also conducted for the 15 families

from the Group 1 pooled families at both sites: ($R^2 = 0.17$, $P > 0.05$; $R^2 = 0.62$, $P < 0.005$; $R^2 = 0.68$, $P < 0.001$) for mean lengths at Assessments 2, 4 and 8 respectively. Initially the family mean lengths were significantly different at the two sites, but by Assessment 8 the family mean lengths were similar. The performance of pooled and individual families reared at OceaNZ Blue was also compared and revealed they were significantly correlated ($R^2 = 0.30$, $P < 0.001$, $n = 41$; $R^2 = 0.31$, $P < 0.001$, $n = 40$) for lengths at Assessments 2 and 4 respectively.

The genotyping of the five groups of pooled families from Group 1 showed there were very unequal contributions from the 16 families within each group (Fig. 2). One family produced 20 to 37% of the individuals in each pool whereas eight of the families each contributed only 0 to 4% of the individuals within each pooled group. The contribution per family was mostly consistent across the pooling strategies. The proportion of each family within the pooled groups was mainly influenced by the female parent, as Families 1–4 (Female 1) were over represented compared to the families produced by Female 3 (Families 9–12) and Female 4 (Families 13–16). By comparison, sufficient numbers survived from 15 of the 16 families reared separately to meet all tagging requirements of the programme.

Family data used for the genetic evaluation included data from individually reared families combined post-tagging and from pooled families following parentage assignment (n = 3,318). Basic summary statistics for the traits analysed are presented in Table 1. Heritabilities were moderate to high for all traits. Genetic correlations between the length and weight traits are shown in Table 2 and were all positive and high indicating that the genes that control early and later growth are similar for length and weight. Genetic correlations between weight and length were highest amongst adjacent assessments, and decreased as the time between the two measurements increased.

Foot darkness and shell colour were measured at Assessment 6. These two quality traits were analysed along with weights at the two nearest assessments (Assessments 5 and 8) as more individuals were assessed at these times. Genetic correlations for foot darkness and the two weight traits varied at -4% and +22%. However, the standard errors for these estimates were high at around 42% due to the smaller numbers of individuals measured. As such, the two genetic correlations were not significantly different from each other, and not different from zero. Genetic correlations between shell colour and the two weight traits were consistent at +33% and +39%, but again standard errors for both comparisons were around 36% meaning they were not significantly different from zero. Finally, the genetic correlation between foot darkness and shell colour was high at +72% and significantly different from zero.

Discussion

This programme successfully produced 95 Abalone families. An initial challenge was the differential family survival that influenced early growth. Individuals from families with the lower numbers were generally larger, most likely due to reduced competition for resources within the tank. This difference in length was still evident in Group 2 families 897 days post fertilisation, although the effect diminished with age. Nevertheless, the ranking of families overall in the pooled and individual groups, and at the two sites, was similar regardless of the different early rearing strategies and environments. The heritabilities were also high for weight traits even at the early assessments when early rearing effects such as tank and density effects, are likely to result in high environmental variance (Kube et al. 1997). However, to avoid biasing family performance it was recommended that the numbers reared per family in separate tanks were standardised as much as possible.

One way to ensure all families are reared in the same environment is to pool them shortly after fertilisation. This also reduces the resources required to rear multiple crosses within a family breeding programme. However, the results from the Group 1 pooling experiment showed that distribution of family sizes was highly skewed after pooling. This was mainly influenced by the female parent and could reflect differences in survival due to egg quality (Fukazawa et al. 2005), rather than a genetic effect. Even when numbers added to the pooled group are standardised, differential survival post-fertilisation and during early larval rearing, are likely for Abalone (Lucas et al. 2006). In the case of Group 1 families pooling at either post-hatch or at pre-settlement, made little difference to the proportion of each family surviving. Therefore pooling should be done as soon as possible to reduce costs and minimise early rearing influences on family performance. Even with the skewed family distribution, pooling could still provide a cost effective alternative to separate family rearing, as increasing genotyping to increase the numbers from underrepresented families could still cost less than the infrastructure and labour required to rear multiple families separately for nine to 14 months.

In general the length and weight traits are highly heritable with high genetic correlations between traits. This indicates that high rates of response to selection for increased weight or length can be expected. Growth rates also proved to be highly heritable and had high genetic correlations between assessment periods, especially at later assessments. This indicates that early and later growth is controlled by similar processes. From Fig. 1 we know that density has an impact on growth which persists for some time. However, the high genetic correlations between early and late assessments shows that the design, which includes pooled rearing and use of the

same males and females to generate multiple crosses within and between years, allows for the removal of these biases in the analysis. In a commercial breeding programme the number of assessments could then be reduced. This would be advantageous from an economic perspective, especially if broodstock can be selected earlier as this significantly reduces costs.

Opportunities for improving Abalone growth through selective breeding, and thus reducing the time to market or increasing market size are good. The next stage of this programme will involve harvest evaluation of these families including meat yield and repeat measures of foot darkness and shell colour. A selection index will be developed for OceaNZ Blue in order to select elite broodstock for commercial production and for use in a genetic improvement programme.

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