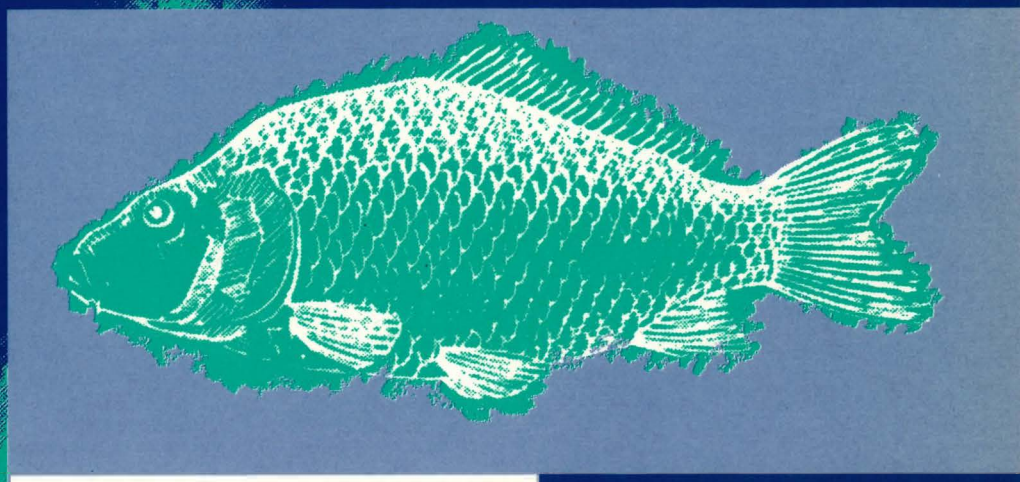
Aquaculture Research



Volume 31, Number 1, January 2000

ISSN 1355-557X

Editors

R W Hardy

S J de Groot

Special issue:

Genetic Issues in Aquaculture
Guest editors: Alan Lymbery, Rob Doupé, Greg Jenkins and Tina Thorne

Blackwell Science

www.blackwell-science.com/are

Aquaculture Research

Aims and scope *Aquaculture Research* is an international Journal which publishes papers on applied or scientific research relevant to freshwater, brackish and marine aquaculture. It covers all aquatic organisms, both floristic and faunistic related directly or indirectly to human consumption.

In addition to publishing papers reporting results of original research, the journal will also include review articles, short communications, technical papers and book reviews. Young scientists are particularly encouraged to submit short communications based on their own research.

Despatch *Aquaculture Research* is despatched within the UK by 2nd class post, within Europe by air mail, and to other continents by various forms of air-speeded delivery: to the US by air freight for forwarding by periodicals class post*, to India by air freight for guaranteed local delivery, and to all other countries by accelerated surface post. Add to the cost of regular subscription £24.00/$36.00 for air mail delivery outside Europe.

Business correspondence All other business correspondence, including orders for offprints and advertising space should be addressed to *Aquaculture Research*, Blackwell Science Ltd, Osney Mead, Oxford OX2 0EL (Tel: +44 1865 206206, Telex 83355 MEDBOK G, Fax: +44 1865 721205.) Information on this journal and other Blackwell Science publications is available on the Blackwell Science Homepage at: http://www.blackwell-science.com

Subscriptions *Aquaculture Research* is published monthly (one volume per annum). Subscription prices for 2000 are: £743.00 (Europe), $1299.00 (USA and Canada) and £817.00 (Rest of the World), in all cases post free. Subscribers in Canada must add 7% to the subscription price, to allow for GST. Subscribers in Europe must quote their VAT registration number or state that they are not registered. Subscribers in the following countries who are not VAT-registered must add VAT at the appropriate rate: Belgium (6% TVA/BTW), France (2.1% TVA), Germany (7% MWST), Spain (4% IVA), The Netherlands (6% BTW). Orders for current subscriptions and back issues should be sent to Blackwell Science Ltd., Journal Subscriptions, PO Box 88, Oxford OX2 0NE, UK.
Tel: +44 1865 206180 or 206038,
Fax: +44 1865 206219,
e-mail:journals.cs@blacksci.co.uk

Paper The Publisher's policy is to use acid-free permanent paper, to the draft standard ISO/DIS/9706, made from sustainable forests using chlorine-free pulp. The paper used in this journal has an ECO-CHECK 4 star rating.

***Periodicals class postage paid at Rahway, NJ. Postmaster send address corrections to *Aquaculture Research*, c/o Mercury Airfreight International Ltd., 365 Blair Road, Avenel, NJ 07001, USA.**

Aquaculture Research, 2000, **31**, 1–2

Genetics in the aquaculture industry

A J Lymbery[1], R G Doupé[2], G Jenkins[3] & T Thorne[4]

[1]Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

[2]Centre for Ecosystem Management, Edith Cowan University, Joondalup, WA 6027, Australia

[3]South Metropolitan College of TAFE, Fremantle, WA 6160, Australia

[4]Fisheries Western Australia, Perth, WA 6000, Australia

Correspondence: A J Lymbery, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

This issue of *Aquaculture Research* contains a series of papers that were presented at a symposium on the application of genetics to the aquaculture industry. While the topics of the symposium have global relevance, many of the examples make specific reference to the Australian situation.

Aquaculture in Australia is a rapidly growing industry. More than 60 aquatic species, including crustaceans, molluscs, finfish, crocodiles and microalgae, are presently cultured in Australia, although less than 10 species support around 80% of the total value of the industry (Brown, Van Landeghem & Schuele 1997). The successful culture of comparatively few species suggests that Australian aquaculture remains species limited. The industry has had a chequered history, and there are numerous examples where apparently suitable candidate species have failed for one reason or another.

In 1995, a review of world aquaculture resources by the Food and Agriculture Organization (FAO 1995) identified genetic and diversity issues as a major constraint to the future development of aquaculture. There are two areas in which genetics is especially important in aquaculture development: (1) the genetic improvement of important production traits; and (2) genetic implications of the intentional movement (translocation) of organisms for aquaculture or restocking programmes. These two areas provided the foci of the symposium.

Genetic improvement

The power of selective breeding in increasing productivity and efficiency has been amply demonstrated in traditional livestock species. However, aquaculture species, have hardly benefited from modern developments in animal breeding, despite their typically high reproductive capacity and, therefore, high potential for genetic improvement. If genetic improvement is to increase the production efficiency of aquaculture species, the key questions that need to be addressed are:

- Which traits need to be improved (what is the breeding objective)?
- What are the best measures (selection criteria) for these traits?
- What are the best selection methods for improving the traits in the breeding objective?
- What are the research priorities?
- How can we ensure that genetic improvement programmes are implemented by the aquaculture industry?

Translocation

The existence of population genetic structuring has long been recognized in the description of races, stocks and subpopulations of fish and other aquaculture species, and the environmental implications of such structuring have been discussed before (e.g. Ryman & Utter 1987; Billington & Hebert 1991). Despite this, there is still no generally accepted view of the genetic risks posed by translocation, much less a standardized approach to risk assessment. Important questions remain:

- What are the best tools for measuring population genetic structure?
- What levels of gene flow will alter the genetic constitution of local populations?
- Will this have any effects on local adaptation?
- How do we estimate long-term population viability?

The symposium on *Genetics in the Aquaculture Industry* was held in Perth, Western Australia, during September 1998. The symposium was divided between formal presentations and two workshops, involving all delegates. One workshop aimed to develop practical guidelines to implement breeding programmes and to identify research and development priorities for different species. The other workshop sought to identify the scientific information required to assess the genetic risks posed by translocation. The symposium allowed a range of views to be aired, and not all are supported by data. In many cases, data are simply not available. Hopefully, however, the information provided and the exposure of gaps in our knowledge will help to define and prioritize the important issues, and to see old issues from new perspectives.

Acknowledgments

The symposium was sponsored by the Fisheries Research and Development Corporation and the Aquaculture Development Council, and hosted by the Aquaculture Council of Western Australia, Agriculture Western Australia, Edith Cowan University, Fisheries Western Australia and the South Metropolitan College of Technical and Further Education. The organizing committee comprised Simon Bennison, Rob Doupé, Greg Jenkins, Rod Lenanton, Yvette Lourenz, Alan Lymbery and Tina Thorne. Michael Elliot provided enthusiastic facilitation of the workshops. We would like to thank all the invited speakers and the symposium participants for their valuable contributions to the symposium.

References

Billington N. & Hebert P.D.N. (eds) (1991) *International Symposium on the Ecological and Genetic Implications of Fish Introductions (FIN). Canadian Journal of Fisheries and Aquatic Sciences* **48** (Suppl. 1).

Brown D., Van Landeghem K. & Schuele M. (1997) *Australian Aquaculture: Industry Profiles for Selected Species*. ABARE Report 97.3. Australian Bureau of Agricultural and Resource Economics, Canberra.

FAO (1995) *Review of the State of World Fishery Resources: Aquaculture*. Fisheries Circular no. 886. Food and Agriculture Organization of the United Nations, Rome.

Ryman N. & Utter F. (eds) (1987) *Population Genetics and Fishery Management*. University of Washington Press, Seattle.

Aquaculture Research, 2000, **31**, 3–10

Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species

G P Davis & D J S Hetzel
Genetic Solutions Pty. Ltd, PO Box 6082, Fairfield Gardens, Queensland, Australia, 4103

Correspondence: G P Davis, Genetic Solutions Pty. Ltd, PO Box 6082, Fairfield Gardens, Queensland, Australia 4103

Abstract

Genetic improvement of aquaculture species offers a substantial opportunity for increased production efficiency, health, product quality and, ultimately, profitability in aquacultural enterprises. Technologies exist that can be implemented immediately to improve multiple traits that have economic value, while simultaneously accounting for inbreeding effects. Genetic improvement techniques for delivering genetic gain include formal definition of the breeding objective, estimation of genetic parameters that describe populations and their differences, evaluation of additive and non-additive genetic merit of individuals or families and defining the structure of a breeding programme in terms of mating plans. Novel genetic technologies involving the use of DNA-based tools are also under development for a range of aquaculture species. These gene marker technologies can be used for identification and monitoring of lines, families and individuals, monitoring and control of inbreeding, diagnosis of simply inherited traits and genetic improvement through selection for favourable genes and gene combinations. The identification of quantitative trait loci (QTL), and direct or linked markers for them, will facilitate marker-assisted selection in aquaculture species, enabling improvement in economically important traits, particularly those that are difficult to breed for, such as food conversion efficiency and disease resistance.

Introduction

Genetic improvement of farmed aquaculture species has the capacity to deliver cumulative and sustained improvements in production efficiency, product quality and, ultimately, financial profitability of aquaculture enterprises and industries. The potential of these gains has long been recognized as a significant impetus for domestication and controlled breeding for a range of aquaculture species. Gains in profit resulting from genetic improvement have been realized in terrestrial domesticated livestock species, agricultural, horticultural and ornamental plants, forest trees and some aquaculture species, notably salmonids.

Genetic improvement techniques that have delivered these gains in terrestrial animal and plant industries include formal definition of the breeding objective, estimation of genetic parameters that describe populations and their differences, evaluation of additive and non-additive genetic merit of individuals or families and defining the structure of a breeding programme in terms of mating plans. For most aquaculture species, these analytical tools are available, but the information needed to implement them successfully is lacking because of difficulty in measurement and/or a lack of domestication or controlled breeding.

Novel genetic technologies involving the use of DNA-based tools are under development for a range of aquaculture species. These gene marker technologies can be used for identification and monitoring of lines, families and individuals, monitoring and control of inbreeding, diagnosis of simply inherited traits and genetic improvement through selection for favourable genes and gene combinations.

This paper discusses the implementation of genetic improvement programmes through their

component technologies. In particular, it focuses on the use of gene markers and their integration into, and enhancement of, genetic improvement programmes based on traditional analytical genetic technologies.

Breeding for profit – defining breeding objectives

The ultimate aim of a genetic improvement programme for an enterprise or industry is to improve profit. Defining objectives for the genetic improvement programme determines the direction the programme will follow and the gains it is expected to achieve. In a formal sense, the breeding objective (H) can be defined as a weighted combination of traits that have economic value.

$$H = trait_i * a_i + trait_j * a_j + \ldots$$

The economic value of a trait can be determined by specifying the production and marketing system being used for the enterprise (Harris & Newman 1994; Newman & Davis 1996). Specification of the system includes feeding and management regimes, age structure of the breeding and growing populations and replacement policies. Specification also includes identifying the sources of income and expenditure from the enterprise and quantifying them. The biological traits that are related to these sources of income and expense are then the components of the breeding objective. For example, income is related to growth rate and survival during the main growth phase for many aquaculture species. Likewise, expenditure is related to traits such as feed efficiency and disease resistance. These traits are the component traits in the breeding objective. In essence, traits in the breeding objective are the traits that make money or that cost money.

The economic value of a trait is the effect on net profit of a marginal increase in the level of that trait (Hazel 1943; Melton, Willham & Healy 1994). This is the partial derivative of the profit equation with respect to each trait in the objective, evaluated at the mean level of all other traits. Not all traits in the breeding objective are expressed at the same time, and some are expressed repeatedly. For example, survival to the post-larval stage is expressed early in the production cycle, whereas final growth rate may be expressed some 2–4 years later, and spawning rates may be expressed repeatedly over a number of cycles or years for some species of shellfish and finfish. This is an issue particularly when you have overlapping generations, such as occurs with salmon and abalone. To account for this, the 'discounted gene flow' method (McClintock & Cunningham 1974) or the 'diffusion coefficient' method (MacArthur & Del-Bosque Gonzales 1990) can be used. These methods account for the frequency and time of expressions and adjust them to present values, so that the value of all traits can be compared appropriately.

Although the traits that are in the breeding objective can be defined, they cannot always be measured. Thus, traits are used as selection criteria that are known to have some genetic relationship with traits in the objective. For example, growth rates and feed conversion efficiency both form part of the breeding objective for many aquaculture species. Growth rate can be measured easily and, thus, it is both part of the objective and a selection criterion. Measuring food conversion efficiency is more difficult, but growth to certain ages may be a good genetic indicator of food conversion efficiency (FCE). Thus, growth at these times may be used as a selection criterion for FCE.

Traits that are chosen as selection criteria are those that will be used to make the genetic change towards the breeding objective. They therefore need to be heritable, inexpensive to measure and, most importantly, genetically correlated with the traits in the breeding objective. Weighting these selection criteria depends on the genetic and phenotypic parameters for these traits and between them and the traits in the objective. The weights can be derived from $b = P^{-1}Ga$, where b is the vector of weightings, P is a matrix of phenotypic variances and covariances among the selection criteria, G is a matrix of genetic variances and covariances between the selection criteria and the traits in the breeding objective, and a is the vector of economic values for traits in the breeding objective. Thus, a selection index is defined and can be calculated for each individual as the sum of the index weightings multiplied by the value for each of these traits. For families or individuals, an index can be developed that weights all of the traits that are selection criteria appropriately to provide a single value for selection, measured in dollars, which will move the breeding programme in the direction of the breeding objective.

The definition of breeding objectives in a formal sense is uncommon in aquaculture species, although Henryon, Purvis & Berg (1999) reported economic values for marron *Cherax tenuimanus* in

Western Australia. Their analysis was comprehensive, including traits of growth, fecundity, survival, tail yield, chelae yield and food consumption in the breeding objective.

How much improvement is possible – defining genetic variation

The extent to which genetic improvement can be made in any component trait or the entire index is dependent on the extent of genetic variation that exists for the trait. Genetic variation can be described at a variety of levels – between species and populations, within populations and within traits.

Populations of wild stocks are often analysed to define population substructure by determining the extent of genetic variation within and between populations. In the past, many of these studies used allozyme loci and, more recently, microsatellites have been used. This switch towards microsatellites has been driven because of the low level of informativeness of allozyme loci relative to microsatellites and the fact that microsatellites have significantly greater utility in the laboratory, allowing a degree of semi-automation and greatly assisting the use of these systems in routine genotyping. Thus, microsatellites are being used increasingly for the study of genetic variation between populations, for wild fisheries management and population genetics applications (Cross, Dillane & Galvin 1997).

While DNA level variation identified by genetic markers has utility in determining the structure of wild populations, it is of academic interest only with respect to aquaculture, where the focus is on productive traits of commercial relevance. In this realm, it is the variation between species and between populations within species (or strains) in economically relevant traits that is important for aquaculture. Species comparisons are not relevant here, as the choice of species is often not a genetic one but more related to economic, management, environmental and legal issues.

Exploiting strain variation involves evaluating the differences between strains for commercial production traits under a commercial environment. An example of a comprehensive strain comparison trial is that for Nile tilapia *Oreochromis niloticus* destined for Asia, where a variety of stocks were compared under a variety of environments as part of the GIFT (genetically improved farmed tilapia) programme (Eknath, Dey, Rye, Gjerde, Abella, Sevilleja, Tayamen, Reyes & Bentsen 1998).

Crosses between strains also need to be evaluated, as heterosis between strains may provide a significant improvement in production efficiency. The GIFT programme evaluated a complete diallel cross design of the nine strains and found an average heterosis level of 4.3% for growth rate, with the best strain combination having an 11% improvement over the best pure strain (Eknath *et al.* 1998). Mating designs can be developed to exploit this type of variation through structured crossing systems and construction of composites.

Genetic variation within populations

Heritability and genetic correlations are the parameters that define the extent of genetic variation within and genetic covariation between traits within a population. Estimation of these parameters for commercially important traits is crucial to the development of successful genetic improvement programmes. Many aquaculture species already have published parameters for key traits such as growth (e.g. salmon *Salmo salar* L. and rainbow trout *Oncorhynchus mykiss*, Gjedrem (1983); Gjerde & Gjedrem (1984); rohu *Labeo rohita*, Gjerde, Reddy, Rye, Jana, Mahapatra, Gupta, Saha, Sahoo, Lenka, Govindaswamy, Tripathi & Gjedrem (1997); shrimp *Penaeus* spp., reviewed by Benzie 1998; and tilapia, Eknath *et al.* 1998). However, for many aquaculture species, there have been no parameters published. This is generally because family and/or individual identification systems are still under development or because appropriate trials have not been conducted to measure these traits where suitable matings have been made. In addition, many of the established industries only have parameters for growth and occasionally survival. A comprehensive set of genetic parameters will include heritabilities and genetic and phenotypic correlations for all traits of commercial importance, i.e. those traits in the breeding objective and those used as selection criteria. These will often include disease resistance, food conversion efficiency, growth and survival under a variety of commercial grow-out conditions and may include traits related to processing and/or consumer acceptance, such as flesh quality and colour.

Genetic parameter estimation has been delayed in many species because of the lack of a suitable

breeding system in which pedigree can be identified unambiguously (Gjedrem 1998). This arises where multiple males are mated to single or multiple females, thereby leading to unknown parentage on the male half of the pedigree. Knowledge of the second half of the pedigree (female) is maintained through spawning and/or growing out families separately. This leads to a confounding of family, maternal and tank effects, and leads to inaccurate genetic evaluation and estimates of genetic parameters. DNA fingerprinting can be used to retrieve pedigree information after animals have been measured or phenotyped. In many species, the first substantial application of gene markers to genetic improvement is through a microsatellite-based identification system that is used to trace pedigrees in tanks or ponds of mixed families. Applications of microsatellite markers include minimizing inbreeding in rainbow trout (Fishback, Danzmann & Ferguson 1997) and retrieving sire parentage information for a selection programme in Kuruma prawns *Peneaus japonicus* (Moore, Whan, Davis, Byrne, Hetzel & Preston 1999a).

Deciding who is best – genetic evaluation

Genetic evaluation is the process of identifying the best individuals, families or lines to breed the next generation. Genetic evaluation procedures estimate breeding values of individuals or families. Observed phenotype is the simplest form of breeding value, but most genetic improvement programmes will benefit from more sophisticated estimates of breeding values (EBVs).

Selection on family means has been used to very good effect to improve salmon (Gjedrem 1998). This approach has significant merit for many aquaculture species in which families can be created in a hierarchical design. Hierarchical designs involve a top tier of sires mated to a number of dams, with each dam producing a variety of progeny, and all progeny are reared in the same environment for evaluation. Thus, full-sib and half-sib family means can be estimated and regressed appropriately by full- or half-sib heritabilities (Falconer 1989) to obtain estimates of family genetic value (full-sibs) or breeding value (half-sibs).

Increasing complexity of genetic relationships, overlapping generations, multiple genetic and non-genetic effects on traits and the need to select for multiple traits means that the analytical approach of choice for genetic evaluation in terrestrial livestock is best linear unbiased prediction (BLUP; Henderson 1973). Many of the same issues are present in aquaculture species and, thus, it is recommended that BLUP be used for calculating EBVs. In general, the model used for BLUP evaluation is $Y = XB + Zu + e$, where Y is a matrix of observations, B is systematic sources of non-genetic variation, u is genetic effects and e is random error. X and Z are incidence matrices relating fixed and random effects, respectively, to observations. Solution of the set of equations this model represents produces estimates of u, which are EBVs.

The model described above is the simplest one, known as a single-trait animal model. However, the model can be varied to analyse different traits and to fit different genetic and environmental effects. One common variation is to model maternal genetic and maternal environmental effects and the correlation between them. Another variation is to fit permanent environmental effects. Importantly, BLUP models can be used that include multiple traits and account for the genetic covariation between traits, even when one trait is not observed on a particular group in the analysis. Further examples can be found in Van Vleck (1994).

The important feature of BLUP models is that they incorporate the numerator relationship matrix (Henderson 1973). This matrix describes the genetic relationship between individuals in a pedigree and allows the estimation of breeding values for all animals in a pedigree, even if the trait of interest is not observed on them. The classic example of this is the estimation of breeding values for bulls that are selected for milk production on the basis of the performance of their daughters. In the case of aquaculture, this means that families and/or individuals can be selected for traits that have not been observed on them through observation of the trait on their relatives. This would include traits such as disease resistance, cold/heat tolerance and harvest traits, such as eating quality.

There has been some suggestion that there may be no benefits of BLUP selection over simple phenotypic selection for aquaculture species where selection is on a single trait, because of the generally high fecundity (Gjoen & Gjerde 1998). However, most genetic improvement programmes will involve selection on multiple traits and for traits where maternal effects and other influences are likely to be important, and therefore BLUP selection is likely to be of significant benefit.

Managing improvement and inbreeding – mating designs

An appropriate breeding structure with mating decisions based on maximizing expected genetic merit in subsequent generations and minimizing the impact of inbreeding will provide the optimal path for genetic improvement in aquaculture species. The reproductive capacity of many aquacultural species has led to fears that mass selection based on phenotype and without identification will lead to high rates of inbreeding and subsequent reproductive and productive failure. Managing this while maximizing genetic improvement has led to the development of family selection as the most popular mating structure (Gjedrem 1998). The use of BLUP approaches to genetic evaluation will allow inbreeding to be monitored and the effect of inbreeding on genetic variance to be accounted for in predicting breeding values.

Inbreeding can be managed through a structured breeding programme that allows a high degree of relationship to exist between some individuals but prevents them mating, so that inbreeding only accumulates slowly. This issue has been examined in nucleus breeding structures in terrestrial livestock where inbreeding can be high (Woolliams 1998). Inbreeding is a particular concern where inbreeding depression is high for commercially important traits or where the extent of genetic load is so high as to significantly reduce viability. There are few estimates of inbreeding depression in the literature for aquaculture species. Examples such as that of Pante, Gjerde & McMillan (1998) show that inbreeding depression for growth in rainbow trout is similar to that for terrestrial species. If this is a general phenomenon, then inbreeding will be able to be managed adequately in well-designed genetic improvement programmes.

Incorporating gene marker technology into genetic improvement programmes for aquaculture species

Dramatic advances in molecular biology have led to the development of a science for examining the basis of genetic processes known as molecular genetics. Knowledge of the structure and function of animal genomes is leading to a significant improvement in the understanding of the biology of all livestock production species. These technologies are now beginning to be applied to aquacultural species with the aim of improving animal health and production and, ultimately, the profitability of aquaculture enterprises.

The basic tools of genome research are genetic markers, genome maps, which may be genetic or physical in nature, and the association of genetic marker variation with phenotypic or observed variation in traits of economic importance.

Genetic markers

Genetic markers come in a variety of formats in modern molecular biology, although initial marker systems were based on protein polymorphisms and morphological characteristics. There is a wide array of DNA-based molecular marker types (Georges 1998), including restriction fragment length polymorphisms (RFLPs), variable number of tandem repeat (VNTR) markers, single-stranded conformation polymorphisms (SSCPs), amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) markers, also known as microsatellites, and single nucleotide polymorphisms (SNPs).

All these markers are designed to achieve a single objective, to detect differences in DNA sequence between chromosomes. Some detect multiple loci in a single reaction, whereas others detect variation at only a single locus. Some are length-based polymorphisms, while others detect variation at a single nucleotide. Some require large amounts of DNA for analysis, whereas others use the polymerase chain reaction (PCR) to amplify scant DNA to allow analysis. Many of these properties are important, because they affect the cost of analysis and the suitability to automation. The focus here is the application of genetic markers in genetic improvement programmes.

Genome maps

Genome maps have three major functions (Hetzel & Moore 1996): (1) they are repositories for gene mapping information, i.e. the location, order and spacing of genes or genetic markers on chromosomes; (2) they facilitate the mapping of genes and markers of unknown location; (3) they enable mapped genes in one species to be identified with homologous regions in another.

Genetic or linkage maps document the synteny, order and spacing of genes or genetic markers. In essence, they provide flag-posts along the genome

that allow locations to be identified. They are obtained by analysing the co-segregation of allelic forms of different markers within pedigrees. Physical maps assign markers and syntenic groups to specific chromosomes and localize and orient their arrangement on these chromosomes. They provide concrete evidence of the location of genes and markers rather than the inferred evidence from linkage mapping. A wide variety of techniques for physical mapping are available, including *in situ* hybridization and somatic cell hybrids. Most recently, the highly efficient method of radiation hybrids has been used to great effect in many mammalian species.

Genome mapping efforts are under way for a number of major aquacultural species including:

- Salmonids (salmon) SALMAP; Lie, Danzmann, Guyornard, Holm, Hoyheim, Powell, Slettan & Taggart (1997).
- Tilapia; Kocher, Lee, Sobolewska, Penman & McAndrew (1998).
- Channel catfish *Ictalurus punctatus*; Liu (1999).
- Kuruma prawns *Penaeus japonicus*; Moore *et al.* (1999a).
- Black tiger prawns *Penaeus monodon*; Moore, Wilson, Whan, Bierne, Lehnert, Chu, Pongsomboon & Tanssanakajon (1999b).

Most of these efforts are using microsatellite markers, although in some crustacean species, the development of microsatellite markers is difficult, and suitable maps have been generated using AFLP markers (Moore *et al.* 1999a). Development of comprehensive maps with substantial (> 80%) genome coverage remains an involved and lengthy process, because of the difficulty of developing effective marker systems for each new species. However, this obstacle can largely be overcome by using multilocus markers, such as RAPDs and AFLPs, in the large full-sib families that are commonly generated in breeding programmes for most aquaculture species.

Associating gene markers with traits

The stated aim of many of these mapping efforts is to enable the mapping of genes affecting commercially important traits. These genes may be identified as single genes inherited in a Mendelian fashion that essentially control an observed phenotype. Alternatively, they may be regions of the genome identified as accounting for a significant proportion of the variation in a trait that is quantitative in nature. This latter type, also known as quantitative trait loci (QTL), are considered particularly useful in genetic improvement programmes as complementary to breeding value estimates of genetic merit.

Simply inherited traits

Simply inherited characteristics can be mapped by producing families informative for the characteristic and analysing them for co-segregation of the characteristic and markers via linkage analysis (Georges 1998). Informative families are those in which at least one of the parents is heterozygous for the characteristic. Screening both parents and progeny with markers will allow the localization of the gene and perhaps the development of a diagnostic test.

A single gene may control some disease resistance traits, particularly those that represent metabolic or other disorders, where the genetic lesion in a particular gene is obviously responsible. Gross morphological changes, such as spots, stripes and colours, can be the result of a defect in a single gene. This allows the opportunity to select for or against a particular morphological type, enabling the development of strains that are branded with an observable genetic marker in the form of a morphological trait.

Detection of quantitative trait loci (QTLs)

Many traits of economic importance have a continuous distribution on the observed or underlying (liability) scale. These traits are generally modelled as being controlled by many genes of small additive effects (Falconer 1989). Leading studies in a variety of organisms have shown that relatively large genetic effects can be identified and localized to a particular region of the genome (Andersson, Haley, Ellergren, Knott, Johansson, Andersson, Andersson-Eklund, Edfors-Lija Fredholm, Hansson, Hakansson & Lundstrom 1994, Georges, Nielsen, Mackinnon, Mishra, Okimoto, Pasquino, Sargeant, Sorenson, Steele, Zhao, Womack & Hoeschele 1995). These effects are known as quantitative trait loci (QTLs), and they are detected by analysing phenotypes with linked marker maps. The identification of markers linked to QTLs for these traits is a significant exercise but has the capacity to deliver benefits for specific traits. In particular, QTL detection has the capacity to deliver significant gains for traits that are: (1) difficult or expensive to measure, such as eating quality or feed conversion efficiency; (2) can only be measured on one sex or class of animal, such as fecundity of females; (3) can

only be measured after the normal date of selection, such as lifetime growth or reproductive characteristics; (4) can only be measured by destroying potential breeding stock, such as flesh quality traits; (5) should only be measured on animals environmentally separate but related to breeding animals in the genetic improvement programme; this is the case with most disease resistance traits, where there is a desire to determine genetic value for resistance without necessarily exposing valuable animals to the pathogen or parasite.

The identification of QTLs and specification of the markers closely linked to them is a major undertaking requiring an appropriate design, resources to phenotype and genotype the population adequately and an adequate analysis of its results (Bovenhuis & Meuwissen 1996). There are a variety of methods used for the analysis of QTL detection experiments. However, in essence, they all attempt to estimate the association between a genotype and a QTL. The key issues associated with QTL detection analyses are the design, assumptions about the genetic model, the statistical model used to represent the genetic model, whether a direct or indirect mapping approach will be used and the analytical approach that is used to fit the statistical model to the data.

QTL detection studies are being conducted in a wide range of aquaculture species, and reports are now appearing in the literature. Examples include QTL for temperature tolerance in rainbow trout (Danzmann, Jackson & Ferguson 1999), QTL for growth in Kuruma prawns (Davis, Moore, Whan, Crocos, Coman, Keys & Preston 1998) and IHN resistance in rainbow trout (Rodriguez, LaPatra, Williams & May 1999).

Marker-assisted selection (MAS)

Marker-assisted selection (MAS) is the use of gene markers linked to QTLs in genetic improvement programmes. Ideally, it is integrated with a comprehensive performance recording and genetic improvement programme. MAS will have most application for traits that are difficult and expensive to measure. Several studies have indicated that MAS can increase selection response (Davis & DeNise 1998). In particular, for traits that can only be measured after selection decisions are made, the rate of genetic improvement can be increased substantially (Meuwissen & Goddard 1996).

A variety of techniques exist for incorporating marker information into genetic evaluation systems. However, the majority of work has focused on the markers as fixed effects in a normal genetic evaluation analysis that produces BLUP EBVs for the residual breeding value and an EBV for the QTL effect (reviewed by Davis & Denise 1998).

Ultimately. the genetic improvement of aquaculture species will be best served through the judicious application of existing approaches modified to the species, production system and traits of interest. A well-designed programme will incorporate components of breeding objectives, genetic evaluation and mating designs that will use gene marker information optimally as it is developed.

References

Andersson L., Haley C.S., Ellergren H., Knott S.A., Johansson M., Andersson K., Andersson-Eklund L., Edfors-Lija I., Fredholm M., Hansson I., Hakansson J. & Lundstrom K. (1994) Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* **263,** 1771–1774.

Benzie J.A.H. (1998) Genetic improvement of prawns. In: *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production* **27,** 103–110.

Bovenhuis H. & Meuwissen T. (1996) *Detection and Mapping of Quantitative Trait Loci.* Animal Genetics and Breeding Unit, University of New England, Armidale.

Cross T., Dillane E. & Galvin P. (1997) An evaluation of currently available molecular markers for different applications in aquaculture genetics (abstract). *Aquaculture* **173,** 6.

Danzmann R.G., Jackson T.R. & Ferguson M.M. (1999) Epistasis in allelic expression at upper temperature tolerance QTL in rainbow trout. *Aquaculture* **173,** 45–58.

Davis G.P. & DeNise S.K. (1998) The impact of genetic markers on selection. *Journal of Animal Science* **76,** 2331–2339.

Davis G.P., Moore S.S., Whan V., Crocos P.J., Coman G.C., Keys S. & Preston N. (1998) Detection of QTL for growth in the Kuruma prawn (*Penaeus japonicus*) (abstract). *Animal Genetics* (in press).

Eknath A.E., Dey M.M., Rye M., Gjerde B., Abella T.A., Sevilleja R., Tayamen M.M., Reyes R.A. & Bentsen H.B. (1998) Selective breeding of Nile tilapia for Asia. In: *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production* **27,** 89–96.

Falconer D.S. (1989) *Introduction to Quantitative Genetics,* 3rd edn. Longman, Essex.

Fishback A.G., Danzmann R.G. & Ferguson M.M. (1997) Broodstock development at a commercial rainbow trout farm using a semi-automated microsatellite genotyping system (abstract). *Aquaculture* **173,** 5.

Georges M. (1998) Mapping genes underlying production traits in livestock. In: *Animal Breeding Technology for the*

21st Century (ed. by A.J. Clark), pp. 77–101. Harwood Academic Publishers, Amsterdam.

Georges M., Nielsen D., Mackinnon M., Mishra A., Okimoto R., Pasquino A.T., Sargeant L.S., Sorensen A., Steele M.R., Zhao X., Womack J.E. & Hoeschele I. (1995) Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny-testing. *Genetics* **139,** 907–920.

Gjedrem T. (1983) Genetic variation in quantitative traits and selective breeding in fish and shellfish. *Aquaculture* **33,** 51–72.

Gjedrem T. (1998) Developments in fish breeding and genetics. *Acta Agriculture Scandinavia, Section A , Animal Science* **Suppl**. **28,** 19–26.

Gjerde B. & Gjedrem T. (1984) Estimates of phenotypic and genetic parameters for some carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* **36,** 97–110.

Gjerde B., Reddy P.V.G.K., Rye M., Jana R.K., Mahapatra K.D., Gupta S.D., Saha J.N., Sahoo M., Lenka S., Govindaswamy S., Tripathi S.D. & Gjedrem T. (1997) Genetic variation in growth rate of rohu (*Labeo rohita*) in mono and polyculture systems (abstract) *Aquaculture* **173,** 9.

Gjoen H.M. & Gjerde B. (1998) Comparing breeding schemes using individual phenotypic values and BLUP breeding values as selection criteria. In: *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production* **27,** 111–114.

Harris D.L. & Newman S. (1994) Breeding for profit. Synergism between genetic improvement and livestock production (review). *Journal of Animal Science* **72,** 2178–2200.

Hazel L.N. (1943) The genetic basis for construction of selection indexes. *Genetics* **28,** 476.

Henderson C.R. (1973) Sire evaluation and genetic trends. In: *Proceedings of the Animal Breeding and Genetics Symposium in Honor of Dr Jay L Lush, Champaign, IL,* pp. 10–41. American Society of Animal Science, Champaign, IL.

Henryon M., Purvis I.W. & Berg P. (1999) Definition of a breeding objective for commercial production of the fresh water crayfish, marron (*Cherax tenuimanus*). *Aquaculture* **173,** 179–196.

Hetzel D.J.S. & Moore S.S. (1996) Applications of molecular genetic technologies in livestock improvement. In: *Exploring Approaches to Research in the Animal Sciences in Vietnam. ACIAR Proceedings No.* **68,** 115–119. ACIAR, Canberra.

Kocher T.D., Lee W.J., Sobolewska H., Penman D. & McAndrew B. (1998) A genetic linkage map of a cichlid fish, the tilapia (*Oreochronis niloticus*). *Genetics* (in press).

Lie O., Danzmann R., Guyornard R., Holm L.E., Hoyheim B., Powell R., Slettan A. & Taggart J. (1997) Constructing a genetic linkage map of salmonid fishes, SALMAP (abstract). In: *Proceedings of the 6th International Symposium in Genetics in Aquaculture*, Institute of Aquaculture, Stirling, UK.

Liu Z. (1999) The catfish genetic linkage mapping, its current status and future perspectives (abstract). *Plant and Animal Genome* **VII,** 34.

MacArthur A.T.G. & Del Bosque Gonzalez A.A. (1990) Adjustment of annual economic values for time. In: *Proceedings of the 8th Conference of the Australian Association of Animal Breeding and Genetics* **8,** 103–106.

McClintock A.E. & Cunningham E.P. (1974) Selection in dual purpose cattle populations. Defining the breeding objective. *Animal Production* **18,** 237–247.

Melton B.E., Willham R.L. & Heady E.O. (1994) A note on the estimation of economic values for selection indices, a response. *Animal Production* **59,** 455–459.

Meuwissen T.H.E. & Goddard M.E. (1996) The use of marker haplotypes in animal breeding schemes. *Genetics, Selection and Evolution* **28,** 161–176.

Moore S.S., Whan V., Davis G.P., Byrne K., Hetzel D.J.S. & Preston N. (1999a) The development and application of genetic markers for the Kuruma prawn *Penaeus japonicus*. *Aquaculture* **173,** 19–32.

Moore S.S., Wilson K.J., Whan V., Bierne N., Lehnert S.A., Chu K.H., Pongsomboon S. & Tanssanakajon A. (1999b) Molecular tools for mapping the Black tiger shrimp (*P. monodon*) genome (abstract). *Plant and Animal Genome* **VII,** 34.

Newman S. & Davis G.P. (1996) Design of structured livestock breeding programs. In: *Exploring Approaches to Research in the Animal Sciences in Vietnam. ACIAR Proceedings No.* **68,** 127–131. ACIAR, Canberra.

Pante M.J., Gjerde B. & McMillan I. (1998) Inbreeding levels and inbreeding depression in a farmed population of rainbow trout (*Onchorhynchus mykiss*). In: *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production* **27,** 119–122.

Rodriguez F., LaPatra S.E., Williams S.R. & May B. (1999) Identification of molecular genetic markers for infectious hematopoietic necrosis resistance in rainbow trout (abstract). *Plant and Animal Genome* **VII,** 109.

Van Vleck L.D. (1994) *Selection Index and Introduction to Mixed Model Methods*. CRC Press, Boca Raton.

Woolliams J.A. (1998) A recipe for the design of breeding schemes. In: *Proceedings of the 6th World Congress of Genetics Applied to Livestock Production* **25,** 427–430.

Aquaculture Research, 2000, **31**, 11–23

Genetic improvement of marine fish – which method for industry?

W Knibb
Israel Oceanographic and Limnological Research, National Center for Mariculture, PO Box 1212, Eilat, 88112, Israel

Correspondence: W Knibb, Israel Oceanographic and Limnological Research, National Center for Mariculture, PO Box 1212, Eilat, 88112, Israel

Abstract

Aquaculture industries and research organizations usually fail to exploit genetics as a tool for enhancing productivity and competitiveness. Yet, there is now some awareness among aquaculturists of the benefits from genetics. There remains little consensus about the types of genetic approaches that are most beneficial for the aquaculture industry. Discord arises because genetics comprises many subdisciplines, each with advocates promoting one field or another as the best method to develop commercially valuable strains. Here, I examine retrospectively the various genetic approaches in aquaculture to assess their commercial benefit.

Why invest in genetics?

Genetic programmes have the power to shape the animal to suit a given purpose or environment – as is evident from the variety of dog breeds, and the foods we consume. Genetic programmes can bring various benefits, most of them related to economic return:

Increased productivity and reliability, yielding benefit to consumer and stability to industry

The ongoing genetic improvement of breeds is often the primary method of increasing productivity in terrestrial agriculture. For example, by comparing the performance of broiler chickens maintained with and without genetic selection since 1957, Havenstein, Ferket, Scheideler & Rives (1994) reported that nearly all the 300–400% growth improvement of modern chicken strains was the result of selection, and only 14% was caused by improved diets. Faster growth translates into greater production with existing facilities, often improved food conversion efficiency and, thus, increased economic gain (see Knibb, Gorshkov & Gorshkova 1998a). Thus, for chickens, genetic gains were made over moderate time scales, and the gain per year (about 10%) compares favourably with other approaches to increasing productivity. Largely because of genetic improvement, chickens are inexpensive, of uniform quality and appearance, and supply is reliable; the reverse is typical of marine fish in the market place. Marine finfish are analogous to chickens before domestication and genetic improvement; fish are genetically wild, adapted for survival in natural environments and are not improved for growth, survival, disease resistance, appearance and carcass composition in captive culture. By analogy with chickens, genetic improvement of marine fish will enhance their competitiveness relative to other food items in the supermarket, which, in turn, can expand and stabilize mariculture industries.

Increased competitiveness leads to profit or survival for companies

Long-term profit margins for primary production aquaculture companies are usually narrow, and only competitive and efficient companies tend to survive (Sweetman 1993). Narrow profit margins apply to most primary (commodity) production agribusiness and should be anticipated for all new

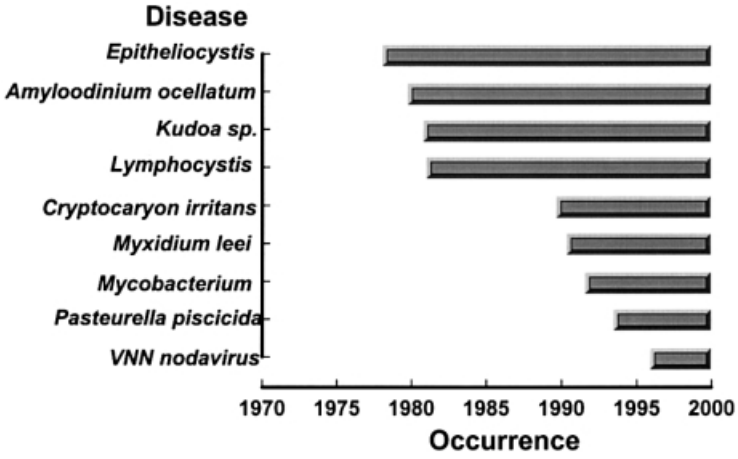


Figure 1 Emergence of diseases in Israeli mariculture (data from A Diamant, pers. commun.).

species under consideration for aquaculture. Use of genetically improved strains is one option for increasing competitiveness. For marginal companies (≈ 5% profit on costs), even modest genetic gains (≈ 10%) can double profits. Where profitability is constrained by environmental regulations and limits on numbers of sea cages, genetically improved strains can increase production with existing infrastructure.

Solutions for the ongoing emergence of pathogens

Losses from existing diseases, as well as the incidence of new economically important pathogens, has increased over the last 20 years in farmed Atlantic salmon *Salmo salar* L. (Salmonidae) (Tilseth, Hansen & Moller 1991). A similar pattern is evident over the last 10 years for mariculture of Mediterranean gilthead seabream *Sparus aurata* L. (Sparidae) and European seabass *Dicentrarchus labrax* L. (Moronidae or Serranidae) (Le Breton 1996; Fig. 1). As mariculture production increases using high-density monoculture, we should expect greater commercial losses from disease outbreaks. The development of fish strains genetically resistant to important pathogens is one approach to addressing the problem of continual and inevitable increases in the number of serious diseases (Chevassus & Dorson 1990). Indeed, Gjedrem & Gjøen (1995) and Gjøen, Refstie Ulla & Gjerde (1997) have indicated that genetic differences exist among Atlantic salmon families for survival to bacterial pathogens.

New export industries

New specialist 'seed' industries, producing eggs and fingerlings for local or overseas markets, can emanate from genetic improvement programmes. High-value, low-volume seed industries may suit economies with small local markets for fish flesh or high labour costs, but with significant regional prospects for the export of seed.

Large returns on investment

Genetic gains compound with each generation of selection. Gains can be 'permanent' if inbreeding is managed, and broodstocks are not outcrossed to inferior strains (a potential problem in developing countries; P B Mather, pers. commun.). The value of genetic gains is proportional to the volume of industry using the improved strains. Hence very favourable returns are likely from investment in genetics. It is possible to calculate with some precision the cost/benefit ratios for genetic breeding programmes. The annual cost/benefit is 1:50 for the UK porcine breeding programme (Mitchell, Smith, Makower & Bird 1982) and 1:15 for Atlantic salmon (Gjedrem 1997).

Filling the gap between demand and supply for seafood products

Total catches from the sea have stabilized at about 100 million tons per year, and future increases in seafood supplies will have to come from aquaculture (Hempel 1993). Thus, world aquaculture, with a total production of 15 million tons in 1990, is predicted to expand fourfold to 62 million tons by 2025 to fill the projected gap between supply from

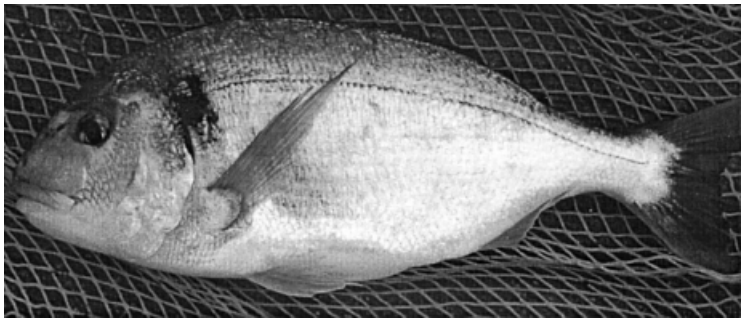


Figure 2 Gilthead seabream *Sparus aurata*.

wild fisheries and demand from world population growth (New 1997). Gjedrem (1997) calculated that the anticipated shortfall can be met simply by the adoption of genetic improvement programmes, yielding an annual growth gain of 4.75%.

Minimize inbreeding in wild and captive populations

Without formal genetic programmes, local industries often attempt selection 'in house' with few broodstock individuals and small effective population sizes. Even without deliberate selection, farm management typically results in severe inbreeding and domestication selection. Inbreeding and domestication selection have implications for loss of genetic diversity in wild populations. Managed genetic programmes usually attempt to minimize inbreeding.

Which objectives?

We can consider that our objective (what we want to achieve) is an increase in overall profit or improvement of any trait or combination of traits that contribute to profit. Choice of objective may vary between specialist seed producers and grow-out companies, although historically most fish selection programmes start with growth as an objective for several reasons (Tave 1995; Knibb, Gorshkova & Gorshkov 1998b), then consider disease resistance, appearance, survival, etc.

Which method?

There are various broad strategies for genetic improvement, which include selection, chromosome set manipulation and genetic engineering. Each broad strategy has various options. For example, selection may be made on strains, families or individuals. How may industry choose among different genetic options? Past successes and failures can indicate the future commercial prospects of a particular strategy. Here, I review different genetic strategies used to improve finfish (mainly seabream; Fig. 2). Genetic programmes for other warm water species, including carp and tilapia, are reviewed elsewhere (Kirpichnikov 1981; Wohlfarth & Hulata 1989; Hulata 1995a).

In 1990, various genetic improvement programmes for seabream commenced at the National Center for Mariculture, Eilat, Israel. The strategic goal was to service emerging local and overseas 'seed' markets. Israel's production of market size seabream was less than 100 tons in 1990 and reached 2500 tons in 1998. Regional (Mediterranean) seabream production is 40 000 tons at present.

Selection among strains

There is no consensus definition of 'strain'. Here, strains are considered to be different wild or captive fish cohorts. Selection pressures and inbreeding levels vary in different natural and artificial environments. Therefore, wild and captive seabream strains may differ for growth and survival performance in captivity. Choice of the best existing strains could equal the genetic gains made by years of within-strain selection using inferior strains. Hence, strain testing should precede within-strain selection (Gunnes & Gjedrem 1978). It seems that performance in captivity can be assessed only by direct experimental trials and cannot be predicted a priori by indirect measures of genetic variation, including allozyme, mitochondrial and microsatellite polymorphisms (Bentsen 1991, 1994).

For seabream, offspring of wild-caught fish had inferior captive performance than a strain main-

Table 1 Which method for industry?

Method	Species	Genetic gain	Cost	$ benefit	$ risk	Major constraints*
Between-strain selection	Carp	+ +	+ +	+ + +	+ +	Disease propagation
	Tilapia	+ +	+ +	+	+ +	
	Seabream	+	+ +	+	+ + +	
Within-strain selection	Carp	+ +	+ + +	+ + +	+ +	Time
	Tilapia	+ +	+ + +	+ + +	+ +	Control lines
	Seabream	+ + +	+ + +	+ + +	+	Competitive rate of gain
Mutations of large effect/ qualitative traits	Carp	+ +	+	+ +	+ + +	Unpredictability
	Tilapia	+ +	+	+ +	+ + +	
	Seabream	+ +	+	+ +	+ + +	
Cross-breeding	Carp	+ +	+ +	+ +	+ +	Disease propagation
	Tilapia	+	+ +	+	+ + +	
	Seabream	+	+ +	+	+ + +	
Interspecific hybridization	Carp	+ +	+	+	+ + +	Reproduction
	Tilapia	+ +	+	+ +	+ + +	Acceptance
	Seabream	+	+	+	+ + +	Unpredictability
Chromosome manipulation	Carp	+	+	+	+ + +	Low GSI values
	Tilapia	+	+	+	+ + +	
	Seabream	+	+	+	+ + +	
Genetic engineering	Carp	+ +	+ +	+	+ + +	Integration
	Tilapia	+ +	+ +	+	+ + +	Acceptance
	Seabream	+	+ +	+	+ + +	Grow-out fitness

+, low; + +, medium; + + +, high.
*Potential constraints common to all: ecological, strain testing and commercial protection.

tained in captivity for many generations (Knibb, Gorshkova & Gorshkov 1996, 1997a). Most finfish species, including carp and tilapia (Wohlfarth & Hulata 1989; Hulata 1995a,b; Bentsen, Eknath, Palada-de-Vera, Danting, Bolivar, Reyes, Dionisio, Longalong, Circa, Tayamen & Gjerde 1998) show some intraspecific strain variation for either growth, disease resistance or cold tolerance, and strain differences have been exploited in commercial farms to varying degrees (Table 1).

Strain testing is moderately expensive, especially under commercial conditions. Costs increase when different environments are used to estimate genotype–environment interactions. Strain testing can be conducted co-operatively among farms and research institutes for several reasons:

- to maximize the number of strains and environments and to assess genotype–environment interactions;
- information on strain rankings and genotype–environment interactions is difficult to monopolize, but has value as public knowledge;
- to share costs, and the high risk that any given strain may not be commercially valuable.

Apart from commercial risk, strain testing risks propagation and transport of diseases, although risk can be ameliorated with accurate and sensitive diagnostic procedures (e.g. Knibb, Colorni, Ankaoua, Lindell, Diamant & Gordin 1993).

Selection within strains

Within-strain selection can be performed for various objectives (economic traits or an aggregate of breeding values weighted by their economic values) using different selection criteria (ways to measure the traits). Individuals can be ranked with information from their phenotype (individual or mass

Table 2 Chronology of sea bream selection

1991–93	Strain evaluation and choice of best strains
1992–95	Selection in tanks and heritability estimated
1994	Collaboration with industry and selection 'at sea'
1997–98	Industry data consistent with laboratory results; all local production based on selected lines
1998	Up to three generations of selection

selection), from their relatives (family or BLUP selection) or from quantitative trait loci or QTLs (marker-assisted selection). A prerequisite for continual selection response is the presence of additive genetic variation. Accordingly, a first step for many fish selection projects is to estimate heritabilities for economic traits (i.e. the proportion of phenotypic variation resulting from additive genetic variation).

Seabream body weight had a moderate heritability and showed response to selection under controlled laboratory conditions (Knibb, Gorshkova & Gorshkov 1997b; unpubl.). Family selection was impractical, as seabream are group spawners (Gorshkov, Gordin, Gorshkova & Knibb 1997). Consequently, an industrial selection programme was started to increase body weight in seabream using mass selection of fish grown under commercial production conditions. Some lines were selected for three generations. Industry records of selected and unselected fish grown separately in sea cages, and of yearly improvements in average growth rates, indicate a selection response of 5–10% per generation. However, as is common, genetic and environmental effects are confounded in available industrial data. Accurate assessment of genetic gains in industry will require the development of new methodologies (see following).

Selection response was achieved for disease resistance, body weight and body proportion in carp (Kirpichnikov 1981; Ankorion, Moav & Wohlfarth 1992), and for body weight in tilapia (Eknath, Dey, Rye, Gjerde, Abella, Sevilleja, Tayamen, Reyes & Bentsen 1998). Most selection experiments for body weight in finfish yielded gains of 10–20% per generation (Refstie, Eknath & Rye 1997). Barton & Turelli (1989) stated that most continuously varying characters in most populations will respond to directional selection. The few instances in which selection failed to yield a response (e.g. in carp and tilapia; Wohlfarth & Hulata 1989) are exceptional and were perhaps caused by inbreeding. These exceptions have a notable legacy in raising doubts (for some aquaculturists) whether fish, unlike terrestrial animals, will respond to selection.

Investment costs, from project conception to delivery of commercial product, are substantial even for mass selection projects. Yet, for those projects reaching industry the commercial benefit is large (Gjedrem 1997).

In view of past successes, there appears to be little risk of commercial failure with selection projects, contingent upon the:

- use of established breeding designs;
- presence of additive genetic variation for commercial traits, which is likely for outbred populations;
- control of reproduction to achieve contemporaneous spawning;
- control of inbreeding.

A specific constraint (Table 1) for within-strain selection is the long investment period before dividend, or until farmers appreciate the gains visually. For seabream (Table 2) and for Atlantic salmon, the lag from concept to industry assimilation was nearly a decade. With controlled experiments, genetic gain can be measured early, from the first round of selection, although initial gains are usually too small to be recognized by industry.

A second constraint is the cost of maintaining unselected control lines. Genetic gain is assessed by comparing control and selected lines. Knowledge of genetic gain is essential for industry and granting agencies to calculate return on investment, to justify further expenditure and to rank the benefits from different research approaches. Wild-caught cohorts are not substitutes for unselected control lines, as wild fish are not exposed to multigeneration domestication selection (although a countervailing view exists that response to domestication selection should be considered as part of the overall selection response and, accordingly, wild fish can be used as controls).

Table 3 Optimize response – for competitive, cost-effective programmes

Maximize correlation between objective (overall $, profit, merit) and criteria (*P*, index):
Select 'on the farm'
Consider sex effects
Minimize age effects
Measure criteria accurately
Consider information from many relatives
Consider information from many traits
Combine all sources of information
Reduce generation time
Increase selection differential (notwithstanding inbreeding)
Minimize inbreeding
Maximize limit to selection
Monitor correlated selection response

See Wei *et al.* (1996); Toro & Lopez-Fanjul (1998).

A third constraint is the need for genetic improvement programmes to be competitive with other national and international programmes. Selection programmes with slow response rates may be superseded by more efficient programmes. Selection projects should be designed to be the best nationally or internationally, as ultimately, commercial pressures will force farmers to purchase their eggs or fingerlings from the best, not second best, strains. There are various approaches to improving the rate of selection gain (Table 3). Most involve maximizing the correlation between the desired improvement (selection objective: merit or profit) and the way fish are measured or ranked for selection (selection criterion: phenotype, or index of information from many relatives and traits).

Maximizing selection response: some practical examples from seabream and seabass

Select fish from commercial production environment

At the Eilat research and commercial facilities, seabream are grown in indoor tanks, inland ponds and sea cages. Technically, it is easier to select fish from ponds and tanks than from sea cages. However, most commercial production of seabream is from sea cages. Pond and sea cage environments may differ and, under each environment, there may be different sets of alleles contributing to growth. Growth in ponds and cages might be considered as different traits. If there is a positive genetic correlation between the traits, then selection from ponds may yield a correlated response from cages, but only under exceptional circumstances (Falconer 1981) will the correlated response be greater than the direct response. With a negative genetic correlation, indirect selection could result in a negative response. Accordingly, until genetic correlations and genotype–environment interactions are known, seabream should be selected after growth in the commercial production environments.

Select on each sex separately (for sexually dimorphic species)

Seabream are protandrous hermaphrodites, most individuals are male at harvest (1.5 years) and, thereafter, an increasing proportion turn into females. At sexual maturity, females tend to be larger than males. Whatever the reasons for the sexual dimorphism (see Knibb *et al.* 1997a), it is possible that males and females represent different hormonal environments. Selection for growth in females (which are the biggest animals at harvest) may yield only correlated responses for growth in male offspring (which are the most common animals at harvest). S Gorshkov and W Knibb (unpubl. data) have shown that growth selection using seabream females yields a response for male offspring, although the conservative approach would be to conduct selection only on males.

Contemporaneous spawning

Ideally, the cohort of individuals for future selection should be the same age (i.e. derive from contemporaneous spawning). Mixing individuals of different ages will increase the environmental contribution to the final phenotypic variation, diminish heritability and reduce selection response. Commercial seabream selection is conducted with cohorts spawned within 24–48 h. Similarly, minimizing other non-genetic sources of variation (environmental) will increase heritability, accuracy of selection and response.

Accurate measurement of selection criteria

Selection for disease resistance may require quantitative measures of pathogen titre in hosts, as well as identification of different pathogen strains (where strains vary for virulence and with geographic region). In this regard, Knibb *et al.* (1993; unpubl.) have shown the utility of DNA diagnostic methods. Where it is possible to produce families, as for Atlantic salmon, a subset of sibs from each family

group can be challenged with specific pathogens and families ranked for survival (Gjedrem & Gjøen 1995).

Information from many relatives, traits and combining sources of information

Seabream are group spawners, and there are technical difficulties in creating families and obtaining information from relatives and for many traits (Gorshkov *et al.* 1997). García de León, Dallas, Chatain, Cannone, Versini & Bonhomme (1995), for seabass, and Magoulas (1998), for seabream, used DNA microsatellite variation to obtain pedigree information, even when fish were produced from mass spawning.

Minimize inbreeding

The increase in inbreeding in a single generation, ΔF, is inversely proportional to the effective population size, N_e, i.e. $\Delta F = 1/2N_e$. Inbreeding is cumulative over generations, so inbreeding at generation t can be calculated as: $F_t = 1-(1-\Delta F)^t$. Performance loss from inbreeding, or inbreeding depression, may increase up to 0.5% (relative to outbred mean) for every 1% increase in the inbreeding coefficient, F (Kincaid 1976a,b, 1983; Gjerde, Gunnes & Gjedrem 1983; Falconer & Mackay 1996). Effective population sizes are low (2–16) for broodstocks of Australian snapper *Pagrus auratus* (Foster, in Block and Schneider), Australian barramundi *Lates calcarifer* (Bloch) and European seabass, so inbreeding depression will be substantial over generations. Inbreeding depression will retard or even negate selection response. For seabream, effective population sizes may be 10% of actual (Knibb *et al.* 1998a), suggesting that about 1000 broodstock are required to limit inbreeding to acceptable levels. Selection, particularly at high intensity, will further reduce N_e. Where it is impractical to maintain a large broodstock (as is common), base populations can be divided and selection imposed on subpopulations. Crossing between subpopulations for commercial production will ameliorate inbreeding depression, as is the practice for industry at Eilat. Other approaches that restrict inbreeding have been outlined by Gjerde, Gjøen & Villanueva (1996).

Monitor correlated selection responses

Because of pleiotropism and scale effects, selection for one trait may result in desirable or undesirable genetic and phenotypic changes in other traits not under direct selection. Accordingly, in selection lines, it is prudent to record a variety of commercial traits, even those not under deliberate selection. In seabream, selection for growth appears to result in changes to food conversion efficiency, gonadal somatic indexes, etc. (Knibb *et al.* 1998a).

In summary, it is generally desirable to optimize accuracy of selection and increase response. However, optimization may incur high costs of tagging and management to obtain pedigree information. Each industry should conduct cost/benefit analyses of different genetic options, calculating total costs and cost per unit of genetic (or dollar) gain from different types of selection. For example, where there is a single trait of primary economic importance with moderate heritability, mass selection can be as efficient as using information from relatives, especially when gains are calibrated against the same levels of inbreeding (Toro & Lopez-Fanjul 1998). The business evaluation of selection options should consider temporal, biological and technical constraints as well as genetic constraints (Table 4). Without feasibility studies, industries should adopt proven genetic technologies, rather than technologies without precedent of commercial success. For some remote and developing regions, availability of genetic management and technical skills should also be considered, especially for complicated genetic programmes requiring extensive and accurate record-keeping (see Tave 1995).

Qualitative traits, major genes and Mendelian mutations

Unlike growth, thought to be caused by many loci of minor effect, some traits are the result of one or few loci. Examples include double muscling in cattle (Hanset & Michaux 1985), lean meat in swine (MacLennan & Phillips 1993) and the Booroola fecundity gene in sheep (Piper & Bindon 1988). Molecular analyses reveal that much of the variation for some traits is due to just several loci of large effect and many loci of minor effect (Georges & Andersson 1996; Falconer & Mackay 1996; Lynch & Walsh 1997). Moreover, specific alleles and new mutations have major, albeit unpredictable, consequences for response to long-term selection (Frankham, Briscoe & Nurthen 1978; Barton & Turelli 1989).

Notwithstanding recent efforts to identify QTLs with molecular approaches, there has been little

Table 4 Business feasibility study of selection options may consider:

Accuracy of selection
Cost of the programme relative to genetic gain
Inbreeding rates
Heritabilities and genetic correlations of commercial traits
Type of organization (international, national, government, private company)
Selection objectives
Selection intensity
Value of market
Volatility of market and investment time frames (is the market long or short term?)
Reproductive constraints
Technical constraints

systematic research on major genes in aquaculture species:

- whether qualitative traits are observed is serendipitous (qualitative traits are evident from new mutations or rare combinations of low frequency recessive alleles);
- whether qualitative traits have commercial value is also serendipitous (new mutations are random genetic changes, usually with negative outcomes for fitness and commercial performance);
- there are strong traditions in aquaculture to discard phenodeviants and potential new mutations (these traditions are contrary to those in *Drosophila*, mice or bacterial genetics).

For the seabream genetics programme at Eilat, there was some attempt to formalize research on qualitative traits and new mutations. Under the umbrella that mutations are a useful resource as genetic markers (e.g. in gynogenetic research) and as elements in genetic protection, we isolated by inbreeding and back-crossing a variety of spontaneous mutations causing visible phenotypes (including yellow and white body colour; Knibb *et al.* 1998a). Candidates for isolation were those phenodeviants of the same type (syndrome) present in many offspring of a particular spawn. Unique phenodeviant individuals were discarded, as they usually result from developmental, not genetic, changes. Particular effort was made to isolate phenodeviant syndromes from lines under selection for growth, as there is a probability (Moav & Wohlfarth 1976), confirmed in the case of *ebony* (Knibb *et al.* 1998a; unpubl.), that this class of mutation can be heterotic for growth. As extreme selection intensities are possible for fecund marine fish, it might be feasible to implement deliberate selective screens for heterotic mutations (M Soller, pers. commun.). Indeed, the key to systematic exploitation of mutations of large effect in seabream would seem to be high fecundity with options for selective screening – procedures more reminiscent of bacterial than large animal genetics. Other approaches might include:

- deliberate inbreeding to recover recessive mutations (e.g. with gynogenesis, as outlined by Gorshkov, Gorshkova, Hadani, Gordin & Knibb 1998);
- screening for major QTLs with molecular approaches.

At present, there are few qualitative traits of commercial value in food fish aquaculture. Examples include *ebony* (for growth and commercial protection) in seabream, red body colour in tilapia (Wohlfarth & Hulata 1989) and scale patterns in carp (Kirpichnikov 1981).

Cross-breeding (intraspecific hybrids)

In terrestrial agriculture, cross-breeding between different strains can yield heterosis and uniformity (Falconer & Mackay 1996). However, cross-breeding among seabream or among Atlantic salmon strains yielded little heterosis, presumably because of lack of inbreeding and genetic differentiation (Gjerde & Refstie 1984; Knibb *et al.* 1997a). In aquaculture, there are few cases in which cross-breeding was integral to commercial production:

- crossing of growth-selected seabream lines to ameliorate potential inbreeding depression (the commercial value of this procedure is unknown);
- crossing of *ebony* and growth-selected seabream lines to exploit the heterosis of the *ebony* mutation (Knibb *et al.* 1998a);
- crossing inbred domesticated carp lines (Wohlfarth 1993).

For most aquaculture applications, cross-breeding should be considered simply as the reverse of inbreeding, rather than as a method of achieving gain over the base outbred population.

Hybrids (interspecific)

Hybrids are created in the hope of:

- combining parental traits, such as fast growth and tolerance to disease or temperature;

- producing monosex hybrid offspring;
- achieving superior performance or heterosis;
- achieving sterility.

Some intergeneric Sparidae hybrids had vestigial gonads that were also sterile (Gorshkov *et al.* 1998), but hybrids failed to show growth acceleration (Knibb *et al.* 1998a). Sterile hybrids might be of commercial interest when production of fertile fish is restricted for ecological reasons.

Considering all species, hybrids usually resemble the average of their parents. Given the large number of attempts to produce new fish hybrids, remarkably few (apparently less than 1%) have resulted in sustained commercial production (Wu 1990; Purdom 1993; Hulata 1995b; Knibb *et al.* 1998a). It seems that commercialization of new hybrids can fail at many points (reproduction, larval growth, grow-out, diseases, markets) and, even if the probability of failure at any one point is moderate, the overall probability of commercial success is low. The high risks of developing new hybrids are reminiscent of the hazards, for similar reasons, of developing new species for aquaculture.

Indeed, unambiguous examples in which interspecific hybrids are of greater commercial value than the parental species are difficult to find. A possible exception was the cross between *Oreochromis niloticus* (L.) and *O. aureus* (Steindachner) tilapia in Israel, once used to produce (nearly) all male offspring, although all male tilapia are now produced with hormonal treatment (Hulata 1995a).

Chromosome manipulations

Potential applications of chromosome set manipulations in fish are diverse and include:

- suppression of gonadal development with triploids;
- monosex offspring using gynogenesis, androgenesis and hormonal treatment;
- rapid inbreeding through gynogenesis;
- genetic mapping using gynogenesis and androgenesis;
- restoration of fish from cryopreserved sperm using androgenesis.

Some triploid Sparidae have small and sterile gonads, but with little or even contrary indication for somatic growth acceleration (Gorshkov *et al.* 1998; Knibb *et al.* 1998a). A similar conclusion was reached for triploid *Cyprinus carpio* (Cyprinidae) (Cherfas, Gomelsky, Ben-Dom, Peretz & Hulata 1994; Cherfas, Hulata, Gomelsky, Ben-Dom & Peretz 1995).

For all fish species, many triploids and hybrid fish triploids were produced but, except for some salmonids, little information exists regarding their commercial performance, and few are used by farmers. Even so, ecological issues might sustain future research on sterile and triploid fish and methods to produce them (e.g. with tetraploid × diploid crosses).

Reviewing together the various combinations of fish hybrids, triploid hybrids and triploids, it seems that gonadal sterility and genetic sterility do not simply translate into superior somatic growth (see also Kerby, Everson, Harrell, Starling, Revels & Geiger 1995). Logically, other factors, most obvious being the overall hybrid or triploid genome, are associated with growth. Of the new species under consideration for culture, those that exhibit an exceptionally large gonadal somatic index (GSI, the proportion of gonadal weight to total weight) during reproduction may be more attractive candidates for triploidy and hybridization experiments.

Genetic engineering

There is no commercial use of genetically engineered fish. In the short–medium term, the prospects of developing commercially valuable fish lines with genetic engineering appear to be remote for various technical and social reasons, including the lack of efficient transformation vectors (Knibb 1997; Knibb, Moav & Elizur 1994, Knibb *et al.* 1998a; Liu, Zohar & Knibb 1999).

General constraints for aquaculture genetics

In addition to specific constraints (Table 1), there are general constraints common to all genetic approaches.

Ecological

There is a public perception that aquaculture genetics belongs with overfishing, release of exotic species, habitat destruction and pollution in the list of negative factors for wild fish

Table 5 Methods of comparing fish strains

Consideration	Rearing condition			
	Industry: each strain separate in replicate sea cages (separate rearing)	**Industry: different strains in the same sea cage (communal rearing)**	**Laboratory: each strain separate in replicate tanks (separate rearing)**	**Laboratory: different strains in the same tank (communal rearing)**
Selection is under commercial production conditions	Yes	? Possible competition and magnification effects	No Possible correlated selection response, G×E effects	No Possible correlated selection response, G×E effects. Possible competition and magnification effects
Procedures are compatible with farm operations	No Industry stocks sea cages sequentially Industry does not have facilities to keep strains separately	Yes When groups can be mixed as eggs No When groups are reared separately before tagging When tags are inedible		
Degree of environmental variation	High Different densities, starting weights, current flows, diseases, fouling, light and feeding rates generate large variations between cages	Negligible When groups can be mixed as eggs Low–moderate When juveniles are raised separately before communal rearing	High–moderate	Negligible When groups can be mixed as eggs Low – moderate When juveniles are raised separately before communal rearing
Statistical power	Low Means of cages available for analysis (few degrees of freedom) Large environmental variation	High Individual fish available for analysis Statistical correction for different starting weights Little environmental variation	Low	High
Requirement for tagging	Unnecessary	Necessary Some tags may be unsuitable Molecular tags (microsatellites, AFLP) will enable eggs of different strains to be mixed	Unnecessary	Necessary Classical tags (e.g. PIT) are used for small-scale experiments
Cost	High Multiple replicates per strain are needed	Moderate	Low	Low

populations. Yet, there is discord between public perception and experimental data (Knibb 1997). Whatever the case, it should be noted that managed genetic breeding programmes, which limit inbreeding, have less risk to wild populations than unmanaged *ad hoc* programmes, which

inevitably develop in farms in the absence of formal programmes and are characterized by high inbreeding levels.

Commercial protection

Without methods to protect improved strains from piracy, research and development investment is lost with the first sale of eggs or fingerlings. Protection technologies will:

- promote continued governmental or industrial investment, as development costs can be recouped;
- facilitate national and international collaboration, as benefit can be distributed according to investment;
- promote long-term industries based on the sale of genetically improved eggs and fingerlings;
- ameliorate public concern over genetic programmes, should protection methods use sterile fish.

Various options have been considered to create seabream strains that were suitable for grow-out (fattening) but unsuitable for reuse as broodstock, including sterile fish (*yellow*, triploids or hybrids), crossing inbred lines and deleterious semi-lethal alleles.

The last option was adopted for industrial application. Fingerlings or eggs, heterozygous for the recessive semi-ethal *ebony* allele, are suitable for grow-out and slaughter. When heterozygotes are used as broodstock, about one-quarter of offspring are *ebony* homozygotes that die from 100 g onwards (i.e. after the farmer has invested in holding and feeding the fish).

Methods for ranking strains and individuals

The ability to rank strains under industrial conditions is elementary to breeding programmes, but all existing methods have deficiencies (Table 5). There are promising new options for tagging fish with molecular markers. García de León *et al*. (1995) used DNA microsatellites to identify different seabass families. Our unpublished data indicate that AFLP polymorphisms can identify different seabream strains grown communally under industrial conditions. Accordingly, eggs from different strains can be mixed, then managed by the farms as a single group.

Conclusions

Theoretically, there are many methods of improving fish strains genetically. In practice, few improved strains have reached the industry and, of these, most were developed with traditional selection exploiting additive genetic variance. Those few strains reaching industry have been of substantial economic value.

Acknowledgments

I thank Sergei Gorshkov and Galina Gorshkov who were indispensable to the genetics programme at Eilat.

References

Ankorion Y., Moav R. & Wohlfarth G.W. (1992) Bidirectional mass selection for body shape in common carp. *Genetics, Selection, Evolution* **24,** 43–52.

Barton N.H. & Turelli M. (1989) Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics* **23,** 337–370.

Bentsen H.B. (1991) Quantitative genetics and management of wild populations. *Aquaculture* **98,** 263–266.

Bentsen H.B. (1994) Genetic effects of selection on polygenic traits with examples from Atlantic salmon, *Salmo salar* L. *Aquaculture and Fisheries Management* **25,** 89–102.

Bentsen H.B., Eknath A.E., Palada-de-Vera M.S., Danting J.C., Bolivar H.L., Reyes R.A., Dionisio E.E., Longalong F.M., Circa A.V., Tayamen M.M. & Gjerde B. (1998) Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. *Aquaculture* **160,** 145–173.

Cherfas N.B., Gomelsky B., Ben-Dom N., Peretz Y. & Hulata G. (1994) Assessment of triploid common carp (*Cyprinus carpio* L.) for culture. *Aquaculture* **127,** 11–18.

Cherfas N.B., Hulata G., Gomelsky B., Ben-Dom N. & Peretz Y. (1995) Chromosome set manipulations in the common carp, *Cyprinus carpio* L. *Aquaculture* **129,** 217.

Chevassus B. & Dorson M. (1990) Genetics of resistance to disease in fishes. *Aquaculture* **85,** 83–107.

Eknath A.E., Dey M.M., Rye M., Gjerde B., Abella T.A., Sevilleja R.C., Tayamen M.M., Reyes R.A. & Bentsen H.B. (1998) Selective breeding of Nile tilapia for Asia. In: *Proceedings of the Sixth World Congress on Genetics Applied to Livestock Production,* Armidale, Australia, 11–16 Jan 1998.

Falconer D.S. (1981) *Introduction to Quantitative Genetics,* 2nd edn. Longman, London.

Falconer D.S. & Mackay T.F.C. (1996) *Introduction to Quantitative Genetics,* 4th edn. Longman, Harlow, UK.

Frankham R.D.A., Briscoe and R.K. & Nurthen. (1978) Unequal crossing over at the rRNA locus as a source of quantitative genetic variation. *Nature* **272,** 80–81.

García de León F.J., Dallas J.F., Chatain B., Canonne M., Versini J.J. & Bonhomme F. (1995) Development and use of microsatellite markers in sea bass, *Dicentrarchus labrax* (Linnaeus, 1758 (Perciformes: Serranidae). *Molecular Marine Biology and Biotechnology* **4,** 62–68.

Georges M. & Andersson L. (1996) Livestock genomics comes of age. *Genome Research* **6,** 907–921.

Gjedrem T. (1997) Selective breeding to improve aquaculture production. *World Aquaculture* **March,** 33–45.

Gjedrem T. & Gjøen H.M. (1995) Genetic variation in susceptibility of Atlantic salmon, *Salmo salar* L., to furunculosis, BKD and cold water vibriosis. *Aquaculture Research* **26,** 129–134.

Gjerde B. & Refstie T. (1984) Complete diallel cross between five strains of Atlantic salmon. *Livestock Production Science* **11,** 207–226.

Gjerde B., Gunnes K. & Gjedrem T. (1983) Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture* **34,** 327–332.

Gjerde B., Gjøen H.M. & Villanueva B. (1996) Optimum designs for fish breeding programmes with constrained inbreeding: mass selection for a normally distributed trait. *Livestock Production Science* **47,** 59–72.

Gjøen H.M., Refstie T., Ulla O. & Gjerde B. (1997) Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture* **158,** 277–288.

Gorshkov S., Gordin H., Gorshkova G. & Knibb W. (1997) Reproductive constraints for family selection of the gilthead seabream *Sparus aurata* L. *Israeli Journal of Aquaculture Bamidgeh* **49,** 124–143.

Gorshkov S., Gorshkova G., Hadani A., Gordin H. & Knibb W. (1998) Chromosome set manipulations and hybridization experiments in gilthead sea bream, *Sparus aurata* (Sparidae). I. Induction of gynogenesis and intergeneric hybridization using males of the red sea bream, *Pagrus major*. *Israeli Journal of Aquaculture Bamidgeh* **50,** 99–110.

Gunnes K. & Gjedrem T. (1978) Selection experiments with salmon. IV. Growth of Atlantic salmon during two years in the sea. *Aquaculture* **15,** 19–33.

Hanset R. & Michaux C. (1985) On the genetic determinism of muscular hypertrophy in the Belgian White and Blue cattle breed. I. *Genetics, Selection, Evolution* **17,** 359–368.

Havenstein G., Ferket P.R., Scheideler S.E. & Rives D.V. (1994) Carcass composition and yield of 1991 vs. 1957 broilers when fed 'typical' 1957 and 1991 broiler diets. *Poultry Science* **73,** 1795–1804.

Hempel E. (1993) Constraints and possibilities for developing aquaculture. *Aquaculture International* **1,** 2–19.

Hulata G. (1995a) A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture* **129,** 143–155.

Hulata G. (1995b) The history and current status of aquaculture genetics in Israel. *Israeli Journal of Aquaculture Bamidgeh* **47,** 142–154.

Kerby J.H., Everson J.M., Harrell R.M., Starling C.C., Revels H. & Geiger J.G. (1995) Growth and survival comparisons between diploid and triploid sunshine bass. *Aquaculture* **137,** 355.

Kincaid H.L. (1976a) Effects of inbreeding on rainbow trout populations. *Transactions of the American Fisheries Society* **105,** 273–280.

Kincaid H.L. (1976b) Inbreeding in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **33,** 2420–2426.

Kincaid H.L. (1983) Inbreeding in fish populations used for aquaculture. *Aquaculture* **33,** 215–227.

Kirpichnikov V.S. (1981) *Genetic Bases of Fish Selection*. Springer-Verlag, Berlin.

Knibb W.R. (1997) Risk from genetically engineered and modified marine fish. *Transgenic Research* **6,** 59–67.

Knibb W.R., Colorni A., Ankaoua M., Lindell D., Diamant A. & Gordin H. (1993) Detection and identification of a pathogenic marine mycobacterium from the European seabass *Dicentrarchus labrax* using PCR and direct sequencing of 16S rDNA sequences. *Molecular and Marine Biology and Biotechnology* **2,** 225–232.

Knibb W.R., Moav B. & Elizur A. (1994) Prevention of chorion hardening in the marine teleost, *Sparus aurata*. *Molecular and Marine Biology and Biotechnology* **3,** 23–29.

Knibb W.R., Gorshkova G. & Gorshkov S. (1996) Potential gains through genetic improvement: selection and transgenesis. In: *International Workshop on Seabass and Seabream Culture: Problems and Prospects*, pp. 175–188. 16–18 October, Verona, Italy. European Aquaculture Society, Oostende, Belgium.

Knibb W.R., Gorshkova G. & Gorshkov S. (1997a) Growth of strains of gilthead seabream *Sparus aurata* L. *Israeli Journal of Aquaculture Bamidgeh* **49,** 43–56.

Knibb W.R., Gorshkova G. & Gorshkov S. (1997b) Selection for growth in the gilthead seabream (*Sparus aurata*). *Israeli Journal of Aquaculture Bamidgeh* **49,** 57–66.

Knibb W.R., Gorshkov S. & Gorshkova G. (1998a) Genetic improvement of cultured marine fish: case studies. In: *Tropical Mariculture* (ed. by S. De Silva), pp. 111–149. Academic Press, Sydney.

Knibb W.R., Gorshkova G. & Gorshkov S. (1998b) Selection and crossbreeding in Mediterranean cultured marine fish. In: *Proceedings of the TECAM Seminar on Genetics and Breeding of Mediterranean Aquaculture Species* (ed. by D.M. Bartley & B. Basurco), pp. 47–60. CIHEAM/FAO, Zaragoza, Spain.

Le Breton A. (1996) An overview of the main infectious problems in cultured seabass *Dicentrarchus labrax* and

seabream *Sparus aurata*: solutions? In: *International Workshop on Seabass and Seabream Culture: Problems and Prospects*, pp. 67–86. 16–18 October, Verona, Italy. European Aquaculture Society, Oostende, Belgium.

Liu X.Y., Zohar Y. & Knibb W. (1999) Association of foreign DNA with sperm of gilthead seabream, *Sparus aurata* after sonication, freezing and DMSO treatments. *Marine Biotechnology* **1**, 175–183.

Lynch M. & Walsh B. (1997) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA, USA.

MacLennan D.H. & Phillips M.S. (1993) Malignant hyperthermia. *Science* **256**, 789–794.

Magoulas A. (1998) Application of molecular markers to aquaculture and broodstock management with special emphasis on microsatellite DNA. In: *Proceedings of the TECAM Seminar on Genetics and Breeding of Mediterranean Aquaculture Species* (ed. by D.M. Bartley & B. Basurco), pp. 153–168. CIHEAM/FAO, Zaragoza, Spain.

Mitchell G., Smith C., Makower M. & Bird P.J.W.N. (1982) An economic appraisal of pig improvement in Great Britain 1. Genetic and production aspects. *Animal Production* **35**, 215–224.

Moav R. & Wohlfarth G. (1976) Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics* **82**, 83–101.

New M. (1997) Aquaculture and the captive fisheries – balancing the scales. *World Aquaculture* **June**, 11–30.

Piper L.R. & Bindon B.M. (1988) The genetics and endocrinology of the Booroola sheep *F* gene. In: *Proceedings of the Second International Conference on Quantitative Genetics* (ed. by B.S. Weir, E.J. Eisen, M.M. Goodman & G. Namkoong), pp. 270–280. Sinauer Associates, Sunderland, MA.

Purdom C.E. (1993) *Genetics and Fish Breeding*. Chapman & Hall, London.

Refstie T., Eknath A.E. & Rye M. (1997) Breeding programs. In: *Second International Symposium on Sustainable Aquaculture,* 2–5 November, Oslo, Norway.

Sweetman J.W. (1993) Perspectives and critical success factors in the present farming of fish. *Aquaculture Europe* **18**, 6–12.

Tave D. (1995) *Selective Breeding Programmes for Medium-sized Fish Farms*. Fisheries Technical Paper, no. 352. FAO, Rome.

Tilseth S., Hansen T. & Moller D. (1991) Historical development of salmon culture. *Aquaculture* **98**, 1–9.

Toro M. & Lopez-Fanjul C. (1998) Recent advances in animal breeding theory and its possible application in aquaculture. In: *Proceedings of the TECAM Seminar on Genetics and Breeding of Mediterranean Aquaculture Species* (ed. by D.M. Bartley & B. Basurco), pp. 31–45. CIHEAM/FAO, Zaragoza, Spain.

Wei M., Caballero A. & Hill W.G. (1996) Selection response in finite populations. *Genetics* **144**, 1961–1974.

Wohlfarth G.W. (1993) Heterosis for growth rate in common carp. *Aquaculture* **113**, 31–46.

Wohlfarth G.W. & Hulata G. (1989) Selective breeding of cultivated fish. In: *Fish Culture in Warm Water Systems: Problems and Trends* (ed. by M. Shilo & S. Sarig), pp. 21–63. CRC Press, Boca Raton, FL.

Wu C. (1990) Retrospects and prospects of fish genetics and breeding research in China. *Aquaculture* **85**, 61–68.

Aquaculture Research, 2000, **31**, 25–33

Genetic improvement of cold-water fish species

T Gjedrem
Institute of Aquaculture Research, AKVAFORSK, 1432 Aas, Norway

Correspondence: T Gjedrem, AKVAFORSK, Postbox 5010, 1432 Aas, Norway

Abstract

Carnivorous fish are two to three times as efficient as pigs and broilers in converting energy and protein to edible food for humans. As the domestication of fish continues, they will become more and more efficient and competitive with these industries. In the future, this will be very important, as more efficient utilization of available food resources is required to supply the growing human population with enough food. Today, about 1% of aquaculture production is based on genetically improved fish and shellfish. For salmonid fishes, we have the necessary knowledge to establish efficient breeding programmes. Large genetic variation has been associated with important economic traits. For growth rate, heritability ranges from 0.2 to 0.3, with a coefficient of variation of 20–30%. This implies that it is possible to obtain large responses from selection for growth rate. In several large-scale experiments and in breeding programmes, 10–15% genetic change has been obtained per generation, which is much higher than that reported for other farm animals. In Norway, extensive breeding experiments with Atlantic salmon and rainbow trout were started in 1971. For the first two generations of selection, the breeding goal was growth rate. Age at sexual maturation (measured as frequency of grilse) was then included. From the fifth generation, disease resistance (measured by challenge test for furunculosis and the virus ISA) and meat quality (measured as fat percentage, fat distribution and flesh colour) were included. Today, Norsk Lakseavl AS (Norwegian Salmon Breeding Company Ltd) or NLA runs the National Breeding Programme and has two breeding stations where 400 full-sib and half-sib families of Atlantic salmon are tested in each of four year classes. For rainbow trout, the number of families totals 120 in each of three year classes. In 1997, the Norwegian production was 310 000 tons of Atlantic salmon and 33 000 tons of rainbow trout. At present, about 65% of the salmon and trout produced in Norway use genetically improved fish from NLA and multipliers. The cost–benefit ratio for the National Breeding Programme in Norway is estimated to be 1:15.

Introduction

The theory of animal and plant breeding is a science developed in this century. The first breeding schemes were started during the 1930s. Today, domesticated and genetically improved farm animals and plants are used. In contrast, fish and shellfish aquaculture breeding programmes have rarely been used, and only between 1% and 2% of production is based on genetically improved stocks (Gjedrem 1997). A large part of aquaculture production relies completely on wild spawn or broodstock, this being true for species such as yellowtail, milkfish, eels and most shellfish species. Species such as carp, catfish, seabass, seabream and several other marine species are periodically 'refreshed' by introducing wild spawners.

Today's aquaculture may be compromised because this worldwide primary production, which is so important for the supply of animal protein, uses wild and undomesticated animals. These animals do not convert available food resources in an efficient way. They do not thrive in captivity and, therefore, they live constantly under stressed conditions.

Before we can plan and start a breeding programme, it is essential to have some basic knowledge about the animal population in question:

- reproduction must be known in order to close the life cycle;
- the breeding goal must be defined;
- there must be genetic variation associated with economic traits;
- the magnitude of phenotypic and genetic variation of these traits must be known.

Breeding goal

The breeding goal must be defined for each species and for each population. In general, all economically important traits should be included in the breeding goal, and each trait must be exactly defined with respect to measurement. Some traits of major economic importance in most breeding populations include:

- feed conversion efficiency: kg food kg^{-1} growth;
- growth rate: body weight at marketing;
- disease resistance: survival rate during lifetime or survival in challenge tests;
- flesh quality: fat content, flesh colour, fat distribution and tenderness;
- age at sexual maturation: frequency of early maturation.

The cost of measuring or recording the trait must be taken into consideration. For example, feed conversion efficiency is not usually included in the breeding goal, because it is so difficult and expensive to record.

Phenotypic and genetic parameters

The most significant phenotypic and genetic parameters are:

- average and standard deviation or coefficient of variation;
- phenotypic and genetic variation;
- heritability for each trait (proportion of genetic to phenotypic variation);
- phenotypic and genetic correlations between the traits in question.

The first estimate of heritability in fish based on half-sib data was published by Aulstad, Gjedrem & Skjervold (1972) for body weight in rainbow trout, and by Kirpichnikov (1972) for weight of fingerlings in common carp. Since then, many estimates of phenotypic and genetic parameters have been published for cold-water fish, particularly for

Table 1 Averages ($\bar{x}$), coefficients of variation (CV), heritabilities ($h^2 \pm$) with standard errors, estimated from sire components of variance

Trait	Species	$\bar{x}$	CV	$h^2 \pm$	Authors
Body weight (kg)	Rainbow trout	3.4	21	0.21	Gjerde & Schaeffer (1989)
	Atlantic salmon	6.6	29	0.35 ± 0.10	Rye & Refstie (1995)
	Coho	0.4	40	0.3 ± 0.10	Hershberger *et al.* (1990)
	Chinook	1.8	26	0.2 ± 0.10	Winkelman & Peterson (1994)
	Arctic char	0.4	37	0.4 ± 0.19	Nilsson (1992)
Age at sexual maturation (%)	Rainbow trout	12.2		0.05	Gjerde (1986)
	Atlantic salmon	21.4		0.15	Gjerde (1986)
Fat percentage	Rainbow trout	14.8	17	0.47	Gjerde & Schaeffer (1989)
	Atlantic salmon	15.6	16	0.30 ± 0.09	Rye & Gjerde (1996)
	Arctic char	20.0	12	0.06 ± 0.08	Elvingson & Nilsson (1992)
Flesh colour					
Score	Rainbow trout	4.3	15	0.27	Gjerde & Schaeffer (1989)
Score	Atlantic salmon	3.3	18	0.09 ± 0.05	Rye & Gjerde (1996)
Photo	Atlantic salmon	7.7	18	0.47 ± 0.13	Rye *et al.* (1994)
Disease					
Survival	Rainbow trout			0.16 ± 0.03	Rye *et al.* (1990)
Survival	Atlantic salmon			0.0 ± 0.02	Rye *et al.* (1990)
Furunculosis	Atlantic salmon			0.4 ± 0.17	Gjedrem *et al.* (1991)
BKD	Atlantic salmon			0.2 ± 0.10	Gjedrem & Gjøen (1995)
Furunculosis	Coho			0.00	Beacham & Evelyn (1992)
Furunculosis	Chinook			0.14 ± 0.11	Beacham & Evelyn (1992)
Fungi	Arctic char			0.34 ± 0.14	Nilsson (1992)

Atlantic salmon and rainbow trout. For species such as coho, Chinook, brown trout and Arctic char, only a few estimates are found in the literature.

In Table 1, some estimates of heritabilities are given for economically important traits in different species. Selected estimates are from studies based on large data sets, using sire components of variance. For body weight, all heritability estimates are above 0.20, and the coefficient of variation is very high for all species. This means that there is large genetic variance for body weight in all species listed. For age at sexual maturation the heritability is low for both rainbow trout and Atlantic salmon. The prospect for genetic improvement is therefore better for body weight than for age at sexual maturation.

Important components of flesh quality are fat content and flesh colour, which show considerable genetic variation in all species, except for fat content in Arctic char. In general, disease resistance shows low genetic variation when expressed as survival rate. However, when resistance is measured by challenge tests, relatively high heritabilities are found, with the exception of furunculosis in coho (Beacham & Evelyn 1992).

Most of the heritability estimates given in Table 1 are based on extensive data. The reliability of the estimates is high for body weight and disease, although somewhat lower for flesh quality traits.

Feed conversion efficiency is a heritable trait but, with present technology, it is not recommended that the trait be measured in a breeding programme. In farm animals, very high genetic correlations are found between growth rate and feed conversion, frequently ranging from –0.80 to –0.95 (Andersen 1977; Vangen 1984; Crawford 1990). Gjøen, Storebakken, Austreng & Refstie (1993) estimated the genetic correlation between growth rate and feed conversion in rainbow trout to be $r_G = -0.78$. When growth rate is included in the breeding goal, which it usually is, a correlated response will be obtained in feed conversion efficiency.

The estimates of genetic correlations are relatively low between the other economically important traits in cold-water fish species and, with few exceptions, they are not antagonistic. The most serious antagonist is a positive genetic correlation between body weight and fat content, averaging $r_G = 0.30$, which suggests that it is not easy to increase growth rate and reduce fat content simultaneously.

In Table 2, genetic correlations between growth rate and measures of survival are given. All seven genetic correlations are positive, averaging 0.31. This is encouraging and shows that, by increasing growth rate, a correlated genetic response will be obtained for disease resistance.

Breeding methods

In cold-water fish, inbreeding depression has been estimated at 3–6% per 10% increase in inbreeding for different traits (Kincaid 1976; Gjerde *et al.* 1983). As inbreeding depression is so high, mating of close relatives must be avoided.

Some cross-breeding experiments have been carried out in salmonids. Gjerde & Refstie (1984) investigated the heterosis effect between five Norwegian strains of Atlantic salmon. They did not find a significant heterosis effect for either growth rate or survival rate. Likewise, Friars, Bailey & Saunders (1979) found no heterosis effect for the growth rate of Atlantic salmon fry. But, in rainbow trout, Gall (1975), Ayles & Baker (1983) and Gjerde (1988) reported significant heterosis for body weight among crosses of rainbow trout strains and inbred lines. It is not possible to draw a general conclusion as to whether cross-breeding should be used in a breeding programme. It must be investigated on a case-by-case basis.

Selection methods

In fish, individual and family selection are of particular interest. Family selection is more efficient compared with individual selection for traits with low heritability, while it is less efficient for traits with high heritability. For all-or-none traits, such as age at sexual maturity and survival, family selection should be chosen. Similarly, for flesh quality traits, which cannot be measured while animals are alive, family selection must be applied. Thus, of the economic traits listed earlier, individual selection is only efficient for growth rate or body weight.

Development of a breeding programme

When AKVAFORSK started breeding experiments in 1971, there was no knowledge of phenotypic and genetic parameters for economically important traits in Atlantic salmon, and only a few were available in rainbow trout. As information became

Table 2 Genetic correlations between growth rate and different measurements of survival

Trait	Genetic correlation	Species	Number		Author
			Sire	Dam	
Overall survival (fingerlings)	0.30	B.t.	32	32	Robison & Luempert (1984)
Cold-water vibriosis (adult)	0.18	A.s.	53	329	Standal & Gjerde (1987)
Overall survival (fingerlings)	0.37	A.s.	187	1404	Rye *et al.* (1990)
Overall survival (fingerlings)	0.23	R.b.	213	1062	Rye *et al.* (1990)
Furunculosis (fingerlings) exp. challenge	0.30	A.s.	25	50	Gjedrem *et al.* (1991)
Overall survival (fingerlings)	0.31	A.s.	100	298	Jonasson (1993)
Fungal infection	0.50	A.c.	36	32	Nilsson (1992)

A.s., Atlantic salmon; R.b., Rainbow trout; B.t., Brook trout; A.c., Arctic char.

available, a breeding plan was constructed with the following elements:

- form base population;
- define breeding goal;
- choose breeding and selection methods;
- predict breeding values;
- select broodstock;
- measure response to selection;
- commercialize the breeding programme.

Base population

To form the base population, eggs and milt were collected from different rivers and populations. Over a 5-year period, samples were collected from 40 river systems for Atlantic salmon and five strains of rainbow trout. In order to eliminate possible inbreeding in the available strains and to secure a broad genetic base, it was decided to produce synthetic populations by crossing the available strains. Our research facilities allowed the testing of 200 families of each species each year. Records of different traits were taken during both the freshwater and the marine phase. These data were accumulated over the years and used for estimating phenotypic and genetic parameters.

Four year classes of Atlantic salmon and three of rainbow trout were formed by crossing the different river strains of Atlantic salmon and the different strains of rainbow trout. It was necessary to produce these year classes, because the generation interval of Atlantic salmon under the Norwegian farming system is 4 years. For rainbow trout, the generation interval is 3 years.

Breeding goal

During the first two generations of selection, growth rate was recorded as body weight at slaughter and was the only trait selected for. From generation three, low frequency of early sexual maturation was included in the breeding goal. From the fifth generation, disease resistance [measured by challenge test for furunculosis and the virus infectious salmon anaemia (ISA)] and meat quality (measured as fat content, fat distribution and flesh colour) were also included in the breeding goal.

Breeding and selection methods

As reliable results from inbreeding and cross-breeding experiments became available, it was quite obvious that pure breeding had to be chosen as the breeding method. Family selection was used for all traits in the breeding goal, while individual selection was used within families for growth rate.

Extensive experiments were carried out to study the magnitude of genotype-by-environment interaction. Gunnes & Gjedrem (1978, 1981) estimated the interaction to account for 1.2–3.7% of the total phenotypic variance. They concluded that, for Norwegian farming conditions, selection of one population for Atlantic salmon and one for rainbow trout was sufficient. On the other hand, there are some estimates that show contrary results in the literature. Sylven, Rye & Simianer (1991) and Ayles & Baker (1983) reported genotype-by-environment interaction for growth rate in rainbow trout in Sweden and Canada respectively. This means that it is difficult to draw a general conclusion concerning

genotype-by-environment interaction, and it should therefore be investigated on a case-by-case basis.

Prediction of breeding values

The main objective of a breeding programme is to find the animals that produce the best offspring in the following generation or the animals with the highest breeding value. Our task is therefore to develop a system for testing the ability or potential of many animals and selecting the best as broodstock to produce progeny for the next generation. The comparison and testing of potential broodstock must take place under equivalent environmental conditions, and the animals should be reared under environmental conditions similar to those used by the industry. Although low genotype-by-environment interaction has been observed, this procedure is a precaution to avoid possible unforeseen interactions.

Testing and recording

Because of the high fecundity in salmonids, the breeding, testing and selection can be centralized at a small number of breeding stations. The Norwegian National Breeding Programme has two breeding stations. Here, a combined individual and family selection procedure is used. A hierarchical mating system is practised, using milt from one male to fertilize eggs from 3–5 females. In this way, both full-sib and half-sib families are produced. Because it is impossible to mark newly hatched fry, each full-sib family is reared separately during hatchery and first feeding periods until the fingerlings reach 10–20 g. At that size, a sample of fingerlings is marked, and the marked fish from all families are reared communally in freshwater at the breeding station until their release into sea cages. Salmon are reared in sea cages until they reach 3–5 kg, and rainbow trout are harvested at a size of 2–4 kg.

Marking of fish and shellfish is a problem. AKVAFORSK used combined cold branding and finclipping for many years, but now uses electronic PIT tags inserted in the body cavity. At marking, additional samples of each family are sent to three or four test stations, which are private farms, to allow testing under ordinary farming conditions. Records from the test stations, together with records from the breeding stations, are used to estimate breeding values for families and for potential breeders. As the breeding stations mark all fish, it is possible to keep pedigree records over many generations, which makes it possible to avoid mating of close relatives, reducing inbreeding in the population.

Methods for recording the traits included in the breeding goal must be worked out and defined precisely. At present, the following procedures are used. (1) Growth rate is recorded on individual fish as body weight at slaughter. Selection criteria are family averages and individual records within the selected families. (2) Frequency of early sexual maturation is recorded for Atlantic salmon after 1 year in sea cages, and after 8 months in sea cages for rainbow trout. Family averages are used as selection criteria. (3) Disease resistance is measured as the average survival rate of families after challenge tests for one bacterial (furunculosis) and one virus (ISA) disease. Family averages are used as selection criteria. (4) Flesh quality is measured by computerized tomography for fat content and fat distribution, and by Minolta Chroma Meter CR-300 for flesh colour. Family averages are used as selection criteria.

Selection of broodstock

The first selection at AKVAFORSK took place in the autumn of 1975. By 1996, Atlantic salmon had been selected for five generations and rainbow trout for seven generations. The different year classes have nearly been closed, without any crossing or immigration. A complete pedigree is available from the start of the breeding programme. This pedigree is used to estimate relationships among potential broodstock before mating. By avoiding mating of close relatives, it is possible to keep the inbreeding stable and low.

When all records from the test stations are gathered, the families are ranked according to breeding values by combining all records from all traits using selection indices. In the index, each trait is weighted by its variance, heritability and economic value. At the breeding station, these family indices are used to select potential broodstock. Usually, males are selected from the 10–15 highest ranking families and females from the top 15–20 families. Final selection of individual fish for breeding is made when all data, including those from the breeding station, are taken and individual indices are calculated for all potential broodstock.

Response to selection

Some selection experiments, yielding positive responses, were started in the USA as early as the 1920s to improve resistance against furunculosis in brook trout (Embody & Hyford 1925) and to increase early spawning, egg number and yearling weight in rainbow trout (Lewis 1944). However, the Israeli scientists Moav & Wohlfarth (1973, 1976) found no response to selection for growth rate in common carp. These results were later confirmed by another Israeli group, Hulata, Wohlfarth & Haley (1986), and by researchers from the USA, Teichert-Coddington & Smitherman (1988), selecting for growth rate in tilapia. The results in common carp and tilapia left the impression that fish are different from farm animals and that selection does not work in fish and shellfish. This negative attitude to selection was very strong among aquaculturists when AKVAFORSK started breeding research in salmonids in 1971. Even at the first Symposium of Genetics in Aquaculture in Galway, Ireland, in 1982, there was considerable scepticism about the effects of selection, even though data were presented to show that, of 18 known selection experiments dealing with growth rate, 15 gave positive results, while three gave zero response to selection (Gall 1983). Purdom (1993) leaves little hope for improvement through selective breeding in fish. However, in Table 3, some results from large-scale selection experiments and breeding programmes are presented and show a response to selection above 10% per generation.

Genetic gain was estimated recently for Atlantic salmon in the Norwegian Breeding Programme by using selection differentials. The data used were the average of three generations, each consisting of four year classes at the breeding station AkvaGen, in addition to the average of two generations, each of four year classes, at the breeding station at Kyrksæterøra. For growth rate, the genetic gain was estimated to be 11% per generation and 22% per generation for reduced frequency of early maturation (Gjøen & Gjerde 1997). For disease resistance and flesh quality, there are no available estimates of genetic gain.

A comparison of improved Atlantic salmon from the fourth generation of selection and wild fish from the river Namsen showed that the genetically improved fish grow 77% faster than the wild fish. This makes a genetic gain per generation above 15%. It was also found that the improved fish showed a better feed conversion ratio, 0.86 compared with 1.08 for the wild fish. The two groups of fish had about the same digestibility ratio for both energy and protein, while the retention rate for improved fish was 43.9% for protein and 62.4% for energy. Corresponding figures for wild fish were 35.1% for protein and 48.5% for energy. These differences were significant (Gjerde *et al.* 1997).

Organization of the breeding programme

The National Norwegian Breeding Programme started as a research project by AKVAFORSK. The data from the first generation were used to estimate phenotypic and genetic parameters (Gunnes & Gjedrem 1978, 1981; Gjerde & Schaeffer 1989). Gradually, the breeding project was converted into a breeding programme, which AKVAFORSK developed and ran until 1986. At that time, AKVAFORSK invited the Fish Farmers Sales Organization and Norwegian Fish Farmers

Table 3 Response to selection in growth rate

Species	$\bar{x}$ weight	Gain per generation (%)	Number of generations	Author
Coho	250 g	10.1	4	Hershberger *et al.* (1990)
Rainbow trout	4.0 kg	13.0	2	Gjerde (1986)
Atlantic salmon	4.5 kg	14.4	1	Gjerde (1986)
Atlantic salmon	5.7 kg	10.6–14.2*	1	B. Gjerde, pers. commun.
Channel catfish	–	12.0–18.0	1	Dunham (1987)
Channel catfish	67 g	20	1	Bondary (1983)
Tilapia	100 g	15	6	F.A.E.E. Eknath, pers. commun.

*Estimated from selection differentials.

Association to be partners. For security reasons, they built an additional breeding station at Kyrksæterøra. AKVAFORSK transferred samples of fertilized eggs from their breeding station at Sunndalsøra to the new breeding station at Kyrksæterøra, now called the Norwegian Fish Farmers Breeding Station (NFA). In 1992, the breeding programme was reorganized. Norsk Lakseavl AS (Norwegian Salmon Breeding Company Ltd) was established and became the owner of the two breeding stations.

In order to meet the requests for improved eggs, NLA co-operates with seven private hatcheries, which serve as multiplier stations. Each year, NLA transfers improved smolts from the last selection to the multiplier stations. These smolts are used to produce eggs that are sold to private hatcheries. Production of eggs should be sufficient to cover requests from the whole industry. In 1997, NLA supplied about 65% of the industry with eyed eggs. To cover the expense of the breeding work at the breeding stations, a duty of AUD$3 per 100 eggs is put on the sale of eggs from the multipliers.

Cost–benefit ratio for the breeding programme

The annual cost of the Norwegian breeding programme for Atlantic salmon and rainbow trout is estimated at AUD$4.2 million. In this programme, around 360 families are tested each year. With an annual production of 220 000 tonnes, the estimated economic profit from this programme is AUD$63 million per year. This gives a cost–benefit ratio of 1:15 (Gjedrem 1997). The cost–benefit estimate for the Norwegian Breeding Programme is similar to corresponding estimates from breeding programmes with traditional farm animals.

Conclusion

Selection programmes in several fish species have shown a response of 10–15% increase in growth rate per generation, and there are indications that similar responses can be obtained for other economically important traits in fish.

The economic benefits for the industry in using improved animals are obvious:

- production will be increased as a result of higher growth rate;
- production costs will be dramatically reduced because of shorter turnover rate and improved food conversion rate;
- domesticated animals will be better adapted to captivity, which will reduce stress and mortality;
- available food and land resources will be used more efficiently because of higher growth rate and higher retention of protein and energy;
- increased disease resistance will reduce the use of chemotherapeutics and contribute to better animal welfare and environmental management;
- improved flesh quality will stimulate the consumption of fish by consumers.

Today, only 1–2% of farmed fish and shellfish in the world result from modern and efficient breeding programmes, while this figure is 65% in Norway. Therefore, there is much to be gained by applying this powerful tool in aquaculture.

References

Andersen B.B. (1977) *Genetic Studies concerning Growth Rate in Cattle, Body Development and Feed Efficiency (Genetiske undersøkelser vedrørende kvaegets tilvaekst, kropsudvikling og foderudnyttelse).* Report no. 488. National Institute of Animal Science, Copenhagen.

Aulstad D., Gjedrem T. & Skjervold H. (1972) Genetic and environmental sources of variation in length and weight of rainbow trout (*S. gairdneri*). *Journal of the Fisheries Research Board of Canada* **29,** 237–241.

Ayles G.B. & Baker R.F. (1983) Genetic differences in growth and survival between strains and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes in Canadian prairies. *Aquaculture* **33,** 269–280.

Beacham T.D. & Evelyn T.P.T. (1992) Genetic variation in disease resistance and growth in chinook, and chum salmon with respect to vibriosis, furunculosis, and bacterial kidney disease. *Transactions of the American Fisheries Society* **121,** 456–485.

Bondary K. (1983) Response to bidirectional selection for body weight in channel catfish. *Aquaculture* **33,** 73–81.

Crawford R.D. (1990) *Poultry Breeding and Genetics.* Elsevier, Amsterdam.

Dunham R.A. (1987) American catfish breeding programs. In: *Proceedings of the World Symposium on Selection. Hybridisation, and Genetic Engineering in Aquaculture.* Bordeaux 27–30 May, 1986. Berlin. **11,** 407–416.

Elvingson P. & Nilsson J. (1992) *Phenotypic and Genetic Parameters of Body and Compositional Traits in Arctic Char (*Salvelinius alpinus*).* Doctoral Thesis, Swedish University of Agricultural Sciences, pp. 1–13.

Embody G.C. & Hyford C.D. (1925) The advantage of rearing brook trout fingerlings from selected breeders.

Transactions of the American Fisheries Society **55,** 135–138.

Friars G.W., Bailey J.K. & Saunders R.L. (1979) Considerations of a method of analyzing diallel crosses of Atlantic salmon. *Canadian Journal of Genetics and Cytology* **21,** 121–128.

Gall G.A.E. (1975) Genetics of reproduction in domesticated rainbow trout. *Journal of Animal Science* **40,** 19–28.

Gall G.A.E. (1983) Genetics of fish: a summary of discussion. *Aquaculture* **33,** 383–394.

Gjedrem T. (1997) Selective breeding to improve aquaculture production. *World Aquaculture* **March 1997,** 33–45.

Gjedrem T. & Gjøen H.M. (1995) Genetic variation in susceptibility of Atlantic salmon, *Salmo salar L.* to furunculosis, BKD and coldwater vibriosis. *Aquaculture Research* **26,** 129–134.

Gjedrem T., Salte R. & Gjøen H.M. (1991) Genetic variation in susceptibility of Atlantic salmon to furunculosis. *Aquaculture* **97,** 1–6.

Gjerde B. (1986) Growth and reproduction in fish and shellfish. *Aquaculture* **57,** 37–55.

Gjerde B. (1988) Complete diallel cross between six inbred groups of rainbow trout, *Salmo gairdneri. Aquaculture* **75,** 71–87.

Gjerde B. & Refstie T. (1984) Complete diallel cross between five strains of Atlantic salmon. *Livestock Production Science* **11,** 207–226.

Gjerde B. & Schaeffer L.R. (1989) Body traits in rainbow trout. II. Estimates of heritabilities and phenotypic and genetic correlations. *Aquaculture* **80,** 25–44.

Gjerde B., Gunnes K. & Gjedrem T. (1983) Effect of inbreeding on survival and growth rate. *Aquaculture* **34,** 327–332.

Gjerde B., Grisdale-Helland B., Helland S.J., Thodesen J. & Korsvoll S.A. (1997) *Vekstkurver som utvalskriterium for tilvekst og fôrutnytting (Growth Curves as Selection Criteria for Growth and Feed Utilisation).* Report No. 39/97. AKVAFORSK, NFR/NLA-project no. 109214/122.

Gjøen H.M. & Gjerde B. (1997) *Selection Differentials for Atlantic Salmon. Year Classes 1980–92 (Seleksjonsdifferanser for laks. Årsklasse 1980–92).* Report No. 31/97. AKVAFORSK.

Gjøen H.M., Storebakken T., Austreng E. & Refstie T. (1993) Genotypes and nutrient utilization. In: *Fish Nutrition in Practice,* Biarritz, France, 24–27 June 1991. Ed. INRA, Paris (Les Colloques, no. 61), pp. 19–26.

Gunnes K. & Gjedrem T. (1978) Selection experiments with salmon. IV. Growth of Atlantic salmon during two years in the sea. *Aquaculture* **15,** 19–23.

Gunnes K. & Gjedrem T. (1981) A genetic analysis of body weight and length in rainbow trout reared in sea water for 18 months. *Aquaculture* **24,** 161–174.

Hershberger W.K., Meyers J.M., McAuley W.C. & Saxton A.M. (1990) Genetic changes in growth of coho salmon (*Oncorhynchus kisutch*) in marine netpens, produced by ten years of selection. *Aquaculture* **85,** 187–197.

Hulata G., Wohlfarth G.W. & Haley A. (1986) Mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **57,** 177–184.

Jonasson J. (1993) Selection experiments in salmon ranching: I. Genetic and environmental sources of variation in survival and growth in freshwater. *Aquaculture* **109,** 225–236.

Kincaid H.L. (1976) Effects of inbreeding on rainbow trout populations. *Transactions of the American Fisheries Society* **105,** 273–280.

Kirpichnikov V.S. (1972) Theory of fish selection. In: *Genetics, Selection and Hybridization of Fish* (ed. by B.I. Cherfas). Academy of Sciences of the USSR, Israel Program for Scientific Translations, Jerusalem.

Lewis R.C. (1944) Selective breeding of rainbow trout at Hot Creek Hatchery. *California Fish and Game* **30,** 95–97.

Moav R. & Wohlfarth G.W. (1973) Fish breeding in Israel. In: *Agricultural Genetics, Selected Topics* (ed. by R. Moav). J. Wiley, New York.

Moav R. & Wohlfarth G.W. (1976) Two way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics* **82,** 83–101.

Nilsson J. (1992) Genetic variation in resistance of Arctic char to fungal infection. *Journal of Aquatic Animal Health* **4,** 36–47.

Purdom C.E. (1993) *Genetics and Fish Breeding.* Chapman & Hall, London.

Robison O.W. & Luempert L.G. (1984) Genetic variation in weight and survival of brook trout (*Salvelinius fontinalis*). *Aquaculture* **38,** 155–170.

Rye M. & Gjerde B. (1996) Phenotypic and genetic parameters of composition traits and flesh colour in Atlantic salmon. *Aquaculture Research* **27,** 121–133.

Rye M. & Refstie T. (1995) Phenotypic and genetic parameters of body size traits in Atlantic salmon *Salmo Salar L. Aquaculture Research* **26,** 875–885.

Rye M., Lillevik K.M. & Gjerde B. (1990) Survival in early life of Atlantic salmon and rainbow trout: estimates of heritabilities and genetic correlations. *Aquaculture* **89,** 209–216.

Rye M., Storebakken T., Gjerde B. & Ulla O. (1994) Efficient selection for colour in Atlantic salmon (Effektiv seleksjon for innfarging hos laks). *Norsk Fiskeoppdrett* **11A,** 22–24.

Standal M & Gjerde B. (1987) Genetic variation in survival of Atlantic salmon during the searearing period. *Aquaculture* **66,** 197–207.

Sylven S., Rye M. & Simianer H. (1991) Interaction of genotype with production system for slaughter weight

in rainbow trout (*Oncorhynchus mykiss*). *Livestock Production Science* **28**, 253–263.

Teichert-Coddington D.R. & Smitherman R.O. (1988) Bidirectional selection for growth in *Tilapia nilotica*. *Transactions of the American Fisheries Society* **117**, 297–300.

Vangen O. (1984) Future breeding program in pigs in a situation with artificial insemination. (Framtidig avlsopplegg på svin i en KS-situasjon). *Aktuelt Fra Statens Fagtjeneste for Landbruket* **1**, 300–306.

Winkelman A., M. & Peterson R.G. (1994) Genetic parameters (heritabilities, dominance ratios and genetic correlations) for body weight and length of Chinook salmon after 9 and 22 months of saltwater rearing. *Aquaculture* **125**, 31–36.

Aquaculture Research, 2000, **31**, 35–44

Genetic improvement of the Pacific oyster *Crassostrea gigas* (Thunberg) in Australia

R D Ward[1,2], L J English[1,2,3], D J McGoldrick[1,2], G B Maguire[2,3]*, J A Nell[2,4] & P A Thompson[2,3]

[1]CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

[2]CRC for Aquaculture, Australia

[3]School of Aquaculture, University of Tasmania, GPO Box 1214, Launceston, Tasmania, Australia

[4]NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW 2316, Australia

Correspondence: R D Ward, CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

***Present address:** Fisheries Western Australia, Research Division, PO Box 20, North Beach, WA 6020, Australia

Abstract

The Pacific oyster industry in Australia is derived from importations from Japan in the late 1940s and early 1950s to Tasmania and is almost completely hatchery based. This makes it a good target for developing and deploying genetically improved strains. An allozyme survey comparing hatchery stocks with self-recruiting Tasmanian stocks and with two collections from Japan found abundant variation and no significant evidence of allele loss. The subsequent selection programme (initiated in the summer of 1996/97) had several strands. We wanted to take advantage of the increased power that marker-assisted selection could bring and, therefore, needed to develop a linkage map and isolate flanking markers around quantitative trait loci (QTLs). Several types of markers (allozymes, microsatellites and AFLPs) were used, and single-pair crosses were set up; QTLs have been detected. Conventional selection programmes, one based on mass selection and one on family selection, have been established. Triploid Pacific oysters produced via chemical means have been available for several years, but rates of triploidy achieved by such means are usually less than 100%. In 1999, we will assess whether our tetraploid 2-year-old broodstock can be crossed with diploids to give 100% triploid offspring.

Introduction

The Pacific oyster industry in Australia, in conjunction with several scientists, has recently embarked on a genetic improvement programme. Here, the programme is reviewed, as it was believed that its background and the early results would be of general interest to aquaculturists. The programme is in its very early stages, and forthcoming papers will present further results as they become available.

Pacific oysters *Crassostrea gigas* (Thunberg) are not native to Australia. They were introduced into Tasmania from Japan in the late 1940s and early 1950s (Thomson 1952, 1958, 1988) to start a new aquaculture industry; at that time, there was little left of the local oyster industry, which had been based on harvesting of the native oyster *Ostrea angasi* (Sowerby). Pacific oyster spat were imported by boat in 1947 and 1948 and by air in 1951 and 1952. The bulk of the importations derived from the Sendai and Hiroshima regions, with some spat coming from the southern region of Kumamoto [*Crassostrea sikamea* (Amemiya)]. A total of 185 cases of oyster spat were imported. These spat were mostly set out at Pittwater Bay in SE Tasmania – attempts to on-grow spat at Oyster Harbour in Western Australia were unsuccessful.

The oysters survived and grew well at Pittwater, but spatfall was limited. In 1953, the majority were transferred to Port Sorrell in the north of the state. It was believed that there, the maintenance of warm summer temperatures for a longer time would encourage spawning and spat settlement. Indeed, spatfall proved more regular at Port Sorrell. A few years later, Pacific oysters were recorded in the

Mersey and Tamar Rivers, respectively, to the west and east of Port Sorrell. It is believed that this arose by larval drift from Port Sorrell.

From 1959 to 1964, the Pacific oyster population in the Tamar River underwent a massive population explosion. Farms, based on wild spatfall, were established in the Tamar, but these ultimately proved unsuccessful. The Pacific oyster industry in Tasmania did not get onto a firm footing until the first commercial hatchery began operations in 1981, using oysters from the Tamar River (C. Sumner, pers. commun.). Further hatcheries were established to support the rapidly expanding industry (Graham 1991) and, recently, Pacific oyster farming based on Tasmanian-supplied spat started in South Australia (Olsen 1994). There are now several naturalized populations in Tasmania and in New South Wales (Medcof & Wolf 1975; Holliday & Nell 1986), the latter possibly resulting from deliberate (although illegal) transplants from Tasmania (Ayres 1991). The entire industry in Tasmania and South Australia, currently worth around $17.5 m per year, is now based on hatchery production of spat (Brown, Van Landeghem & Schiele 1997). The much smaller New South Wales production of Pacific oysters is based on natural spatfall.

The Pacific oyster industry in Australia, being hatchery based (unlike, for example, much of the larger French industry), is in an excellent position to benefit from the development of genetically improved strains (for a good general review of the commercial applications of bivalve genetics, focusing on oysters, see Allen 1998). Furthermore, the growout system using baskets or subtidal trays allows easy separation of stocks or families, facilitating a selective breeding programme. Before embarking on such a programme, however, the industry wished to be assured that genetic diversity remained in the Tasmanian oyster stock. In addition to this diversity/selective breeding approach, the industry is also using triploid stock, and tetraploid animals have been recently produced.

Genetic diversity

The industry had some concerns that stock losses in the early years of the Tasmanian industry, together with more recent hatchery production from relatively small numbers of parents, could have led to bottlenecks depleting variation. The production of small numbers of morphologically abnormal oysters, 'curl-backs', was taken as a possible indication of inbreeding effects, although the aetiology of this abnormality remains to be discovered. Because of disease risks, continuing introductions of stock from a parental population are discouraged (Treadwell, McKelvie & Maguire 1992). Losses of genetic diversity in hatchery-produced Pacific oysters have been documented (Gosling 1982; Hedgecock & Sly 1990), and an assessment of the genetic diversity of the Tasmanian hatchery stocks was therefore requested.

This assessment was carried out using allozyme electrophoresis (English, Maguire & Ward 1999). There had been an earlier study of genetic variation in Tasmanian Pacific oysters, but this study (Deupree 1993) had been of a only a single sample of oysters. This concluded that the typically deep-cupped Tasmanian Pacific oysters were not derived from the similarly deep-cupped Kumamoto oysters (*Crassostrea sikamea*; see Ahmed 1975; Banks, McGoldrick, Borgeson & Hedgecock 1994), which were also originally imported into the state, but were genetically more akin to the more shallow-cupped Miyagi (Sendai) strain. However, there were no side-by-side comparisons with Japanese stocks.

We collected samples from three Tasmanian farmed populations (Smithton, Dunalley and Pittwater), three Tasmanian naturalized populations (two sites along the Tamar River – Beauty Point and Swan Bay – and Bridport), one site in New South Wales (Port Stephens) and two endemic sites in Japan (Sendai and Hiroshima). Seventeen loci were screened in about 100 oysters taken from each of the nine sites, and allele frequencies were determined (English *et al.* 1999).

Differences in levels of variation between population types, naturalized, hatchery and endemic, were minimal and insignificant (Table 1). Abundant variation was present with, on average, 3.2 alleles per locus and average heterozygosities close to 30% per locus. Gene frequencies were also very similar between populations, with only about 1% of the genetic variation attributable to population differences. The full data are in English *et al.* (1999).

The concern of industry and researchers that the Tasmanian hatchery populations might have lost substantial variation subsequent to their domestication in Tasmania appears to be unjustified. One of the first consequences of inbreeding is the loss of rare alleles. While the Tasmanian hatchery samples had slightly fewer alleles per locus per sample

Table 1 Genetic variability at 17 allozyme loci in all populations (after English *et al.* 1999)

Population	Type	Mean sample size per locus	Mean no. of alleles per locus	Percentage loci polymorphic	Mean heterozygosity	
					Observed	Expected
Swan Bay	Naturalized	108.0 ± 1.6	3.2 ± 0.3	70.6	0.254 ± 0.078	0.278 ± 0.050
Beauty Point	Naturalized	106.7 ± 1.5	3.5 ± 0.4	76.5	0.271 ± 0.080	0.303 ± 0.051
Bridport	Naturalised	99.2 ± 0.5	3.5 ± 0.4	70.6	0.282 ± 0.085	0.302 ± 0.056
Port Stephens	Naturalized	92.5 ± 5.1	3.1 ± 0.4	76.5	0.262 ± 0.078	0.277 ± 0.050
Smithton	Hatchery	98.0 ± 1.2	3.1 ± 0.3	76.5	0.303 ± 0.093	0.310 ± 0.056
Pittwater	Hatchery	98.3 ± 0.5	3.1 ± 0.4	70.6	0.290 ± 0.088	0.304 ± 0.055
Dunalley	Hatchery	94.4 ± 1.4	2.8 ± 0.3	64.7	0.296 ± 0.090	0.296 ± 0.055
Sendai	Endemic	97.6 ± 1.6	3.5 ± 0.4	76.5	0.287 ± 0.086	0.315 ± 0.054
Hiroshima	Endemic	97.0 ± 1.3	3.4 ± 0.3	70.6	0.267 ± 0.082	0.295 ± 0.054
Means for population types						
Hatchery ($n = 3$)		290.7 ± 1.1	3.0 ± 0.3	70.6	0.285 ± 0.055	0.303 ± 0.055
Naturalized ($n = 4$)		406.4 ± 0.8	3.3 ± 0.4	73.5	0.267 ± 0.134	0.290 ± 0.052
Endemic ($n = 2$)		194.6 ± 1.4	3.5 ± 0.4	73.6	0.291 ± 0.051	0.305 ± 0.054
Mean ($n = 9$)		99.1 ± 1.7	3.2 ± 0.2	72.7	0.279 ± 0.093	0.298 ± 0.004

A locus is considered polymorphic if the frequency of the most common allele is < 0.95. Heterozygosity estimates are unbiased Hardy–Weinberg estimates (Nei 1978).

(3.0 ± 0.3) than the Japanese samples (3.5 ± 0.4), the difference was not statistically significant.

Most samples showed good agreement with Hardy–Weinberg expectations, but some significant deviations, all resulting from significant heterozygote deficiencies, were observed. The causes of these deviations are not understood, and may differ from case to case, but could, in principle, include gel scoring errors, null alleles, selection, inbreeding or population admixture. Others have recorded similar deficits in *C. gigas* (Buroker, Hershberger & Chew 1979a,b; Fujio 1979; Gosling 1982; Smith, Ozaki & Fujio 1986; Moraga, Osada, Lucas & Nomura 1989; Deupree 1993).

Preliminary analysis of data from four DNA microsatellite loci (L. English, unpubl.) also indicates that most of the variation in the two Japanese populations has been maintained in the Tasmanian hatchery populations. Unlike the allozyme data, deviations from Hardy–Weinberg equilibria were common and, overall, there were very significant deficiencies in heterozygotes. We believe that this reflects the probable presence of null alleles (non-amplifying alleles) at appreciable frequencies; we have evidence from breeding experiments of such alleles in Pacific oysters (discussed later).

It is clear that the introduction of Pacific oysters into Tasmania and their subsequent domestication has not substantially eroded their genetic base. The breeding practices of the hatcheries, which typically use several dozen males and females per spawning run, often from different farms and different hatcheries and sometimes with the introduction of naturalized oysters, appear to have maintained high levels of genetic variation.

These results were sufficiently encouraging that the industry in Tasmania and South Australia agreed to set in place a breeding programme to improve the quality of farmed Pacific oysters in Australia.

The breeding programme

There are several prerequisites for a selection program: (1) one or more distinct traits must be chosen for improvement; (2) these traits must show variation; and (3) part of this variation must result from genetic variation.

Pacific oyster growers in Tasmania were forwarded a questionnaire by the Tasmanian Oyster Research Council (TORC, an industry body). Sixteen 'desirable' characteristics were listed, and farmers were asked to allot points according to their preferences. The characteristic receiving the highest score was meat yield, followed by growth rate and

shell shape. These characteristics will be the ones we will focus on in the selection programmes. All are certainly highly variable in Tasmania and while, at the start of the programme, we had not established the heritabilities for these traits, all are expected to be in the region of 0.2–0.3 (based on work elsewhere, e.g. Lannan 1972; Sheridan 1997).

The breeding programme relies on the list of commercial traits from the questionnaire, selection criteria for those traits and a way of monitoring and removing any possible unwanted effects that arise through genetic correlations among traits. Currently, the breeding programme is in its first generation, but has two components, one based on individual or 'mass' selection and one that relies on the production of pedigreed lines. Upon analysis of the intrafamily correlation (a measure of the proportion of total phenotypic differences attributable to differences among family means), the programme can decide how to proceed most effectively with a combined selection, between-family selection or within-family selection strategy. Currently, between-family selection is expected to be useful. In addition, the programme intends to investigate early selection criteria within the breeding objectives, such as size at 1 year rather than 2 years and the use of molecular markers, a strategy that will progress the traits of interest faster than would be possible otherwise.

Pedigreed lines

The breeding programme is dependent on the production of pedigreed lines, so that genetic variation can be taken advantage of most rapidly and to allow the increased power that molecular markers bring to bear. Our first pedigreed lines, six single-pair crosses of unrelated animals, were established in the summer of 1996/7. Principal component analyses of these lines, using a variety of growth characters, have suggested major clusters within families, implying the segregation of a major gene. Variance for shell trait and size characteristics among family means is high, resulting largely from a family with a significantly larger size-at-age than the others. These families have been used to verify the inheritance of our molecular markers, as reference material for future work, and for the mapping of major genes for some of our commercial traits, such as growth rate. It appears that these lines will be useful to the breeding programme, as the analysis suggests that they contain major genes that are flanked by genetic markers. These first six crosses performed well in the commercial growout site. Each cross yielded enough animals for linkage and QTL mapping and, as a group, yielded novel characteristics, such as shell colour variants of potential commercial importance.

Use of molecular markers in the breeding programme

Molecular markers are variable genes or regions of DNA. There are many types of such markers, including allozymes, microsatellites and AFLPs. Currently, we have about 20 variable allozyme loci, about 40 variable microsatellite loci and about 150 variable AFLP loci. These markers have multiple uses for commercial breeding programmes. For example, they can be used in pedigree analysis to minimize the unwanted loss of effective population size over the course of selection. Secondly, they can be used to identify stocks. Thirdly, they are used to derive a linkage map onto which major genes can be mapped statistically using a modern quantitative genetic technique known as composite interval mapping (Zeng 1993; Jansen & Stam 1994). Lastly, they can be used to introduce marked genes of major effect rapidly (such as disease resistance or growth genes) from pedigreed lines into commercial broodstock, while minimizing the introduction of other unwanted effects.

Pacific oysters have 10 pairs of chromosomes, and three or more markers have been identified on each of these chromosomes after typing about 100 progeny from the highest performing cross. Mapping growth characteristics onto the linkage map revealed several chromosomal segments with major effects on growth. If all these growth effects can be validated and assembled in commercial animals, large gains would be expected. In addition, the total amount of phenotypic variation for meat weight and shell weight explained by the markers exceeds the heritability of these traits, suggesting possible non-additive genetic effects.

We do, however, approach these newer results with caution. Before proceeding with markers as selection criteria, we must first verify our predictions. Specifically, we must confirm the inheritance of these major genes and check for an interaction of these marked genes in another genetic background in living oysters. We have established two crosses to test the transmission of marked major genes and

will be ready to analyse both their genotypes and their phenotypes in early 1999.

One of the interesting findings to come out of these studies was the verification of segregating null alleles at several microsatellite loci in parents and progeny (McGoldrick 1997; Baoprasertkul 1998; D. J. McGoldrick & R. D. Ward, unpubl.). These are non-amplifying alleles that do not yield a product that can be observed on a gel; they are recessive in a manner analogous to the O allele in the human ABO blood system. A minority of segregating loci, even after allowing for the likely existence of null alleles, showed progeny proportions that deviated significantly from Mendelian expectations. It is likely that these cases are caused by selection at a linked locus.

Status of the breeding programme

The breeding programme currently uses two different approaches to selective breeding, mass selection and family selection. These programmes have only recently been initiated, and we have little in the way of data at present. However, we outline here which crosses have been established to date.

Mass selection is particularly appropriate when a single trait is chosen for improvement, especially if it does not have low heritability. We are seeking to improve growth rate by mass selection, and several strategies are planned for minimizing inbreeding or loss of heterozygosity in these lines. Two separate groups of oysters were spawned in the summer of 1993/4, yielding two separate cohorts. In the summer of 1996/7, the upper and lower 20% size tails of each cohort (all of the same age within a cohort and, therefore, variation in size reflecting variation in growth rate) were selected to form the parents of high (fast-growing) and low (slow-growing) lines. The tails of each cohort were pooled as broodstock; about 70 oysters were used to found each of these lines. Each line was subdivided to form three replicates. There was sometimes substantial variation between replicates within lines (Table 2), which could arise from the larval phase, the land-based nursery phase or the sea-based nursery phase. There were large and significant differences in mean size at days 147 and 275, in the appropriate directions, between the high and low progeny from both the small and large size-graded spat (Table 2). This suggests significant genetic variation for growth that can be selected. The juveniles from the large size-graded spat were substantially larger than those from the small size-graded spat, for both high and low lines, at days 147 and 275.

At the same time as running these selection lines, a line termed 'commercial' was established. This line was derived from unrelated parents ($n = 57$) according to normal commercial practice, whereby the hatchery operator chooses oysters with broadly desirable characters, for example a 'good' shape. These parents were not from the same cohort used to form the high and low lines and, presumably, have a different genetic structure. Arguably, it would have been preferable to have broodstock from the cohort from which the high/low lines were derived to act as a control, but available resources did not permit this. In any case, to have commercial value, selected lines must surpass normal hatchery lines; from this standpoint, using a commercial spawning as a quasi-control was appropriate.

The 'commercial' progeny performed similarly to the high selected animals in both size grades (Table 2), suggesting that high selection had had limited effect, and that low selection was more effective than high selection. However, this evidence for response asymmetry may be partially confounded by the commercial animals coming from a different (although most probably very similar) gene pool. Selection for increased fitness usually gives a slower response than selection for decreased fitness (Falconer & MacKay 1996), which in our case is likely to equate to the high and low lines respectively. However, it is notable that high-growth selection lines of the Chilean oyster *Ostrea chilensis* (Philippi) responded more than low lines (live weight realized heritabilities of 0.43–0.69 and 0.29–0.35 respectively) and, in this case, the control lines did come from the same gene pool (Toro, Sanhueza, Winter, Aguila & Vergara 1995).

The apparently greater response of our low line could reflect an increase in the frequency of defective recessive genes, leading to slow growth or 'dwarfing'. At day 275, the high lines were 56% heavier than the low lines for the 25–50% size grade, yet only 15% heavier for the 75–100% grade. This is consistent with the accumulation of defective genes in the low lines, as individuals with such genes might be expected to be more abundant in the smaller size grade. If major recessive dwarfing genes are uncovered, they may have value for protecting intellectual property.

In the family selection programme, 40 single-pair crosses were established in the summer of 1997/98, together with one (mass-spawned) commercial

Table 2 Summary data from mass selection lines

Group	Replicate	Number of bags	Mean weight (g) ± SE* Day 147	Mean weight (g) ± SE* Day 275
25–50% Size grade				
Mass–high	1	13	294.5 ± 7.4	1154.2 ± 171.8
Mass–high	2	9	567.9 ± 26.1	1766.6 ± 332.2
Mass–high	3	11	448.1 ± 31.7	1522.5 ± 223.2
Overall high		33	420.3 ± 23.4	1443.9 ± 138.1
Mass–low	1	12	234.3 ± 8.6	1006.2 ± 173.5
Mass–low	2	13	208.9 ± 5.6	924.3 ± 144.4
Mass–low	3	15	159.2 ± 8.2	855.7 ± 142.1
Overall low		40	197.9 ± 6.6	923.2 ± 86.3
Commercial	1	7	357.5 ± 11.8	1175.3 ± 240.0
Commercial	2	4	695.0 ± 10.7	1904.0 ± 521.4
Commercial	3	7	307.4 ± 9.6	1193.6 ± 228.2
Overall commercial		18	413.0 ± 37.4	1344.3 ± 176.0
75–100% Size grade				
Mass–high	1	10	832.3 ± 31.3	2261.7 ± 307.2
Mass–high	2	8	979.1 ± 46.7	2151.1 ± 277.6
Mass–high	3	10	926.5 ± 32.2	2225.9 ± 263.0
Overall high		28	907.9 ± 23.1	2217.3 ± 158.9
Mass–low	1	10	774.5 ± 14.2	1963.3 ± 209.7
Mass–low	2	8	808.5 ± 33.3	1848.9 ± 253.5
Mass–low	3	12	742.8 ± 37.6	1968.9 ± 263.9
Overall low		30	770.9 ± 18.2	1935.0 ± 139.1
Commercial	1	7	868.9 ± 19.6	2011.4 ± 294.7
Commercial	2	5	1157.0 ± 44.2	2661.8 ± 428.0
Commercial	3	6	960.3 ± 39.3	2293.2 ± 356.1
Overall commercial		18	979.4 ± 33.7	2286.0 ± 200.9

*Mean weight per bag. Each bag contains 100 oysters.

control. Traits such as shell shape may be better selected from family comparisons than from mass selection. Most of these crosses were derived from outbred, presumably unrelated, animals chosen by individual farmers, and most were established in pairs using one male to fertilize the ova of two females to give both full-sib and half-sib families. Three crosses were inbred single-pair families derived from full-sib matings from three of the six crosses established in the summer of 1996/97. One family was derived from two curl-back individuals to provide some information on the heritability of this shell trait.

Where sufficient stock are available, mass selection and family lines are being grown at five sites, three in Tasmania and two in South Australia, including intertidal and subtidal sites. This is being done to assess genotype-by-environment interactions and, thereby, to assess whether different sites require different genetic lines for optimum performance.

In 1999, we plan to establish the next generation of mass selection high lines, cross the three inbred families with one another (a diallel cross) to establish nine families, check on the inheritance of some shell colour traits and produce three inbred lines from the three crosses of the six established in 1996/97 that have not yet given inbred lines.

Ploidy manipulations – triploids and tetraploids

Bivalves are particularly amenable to various forms of chromosome number manipulation. Diploid females usually spawn tetraploid eggs (four sets of chromosomes per cell, 4N), which, after interaction with haploid (1N) sperm, undergo sequential reductions in maternal chromosome number (4N

to 2N and 2N to 1N) through release of the first (2N) and second (1N) polar bodies. Triploid oysters have about 2% of the fecundity of a diploid (2N), while the fertility of a 3N × 3N cross is only 0.0008% of that for a 2N × 2N cross (Guo & Allen 1994a). Triploids have several potential advantages over diploids: inhibition of spawning; faster growth rate through diversion of energy to somatic growth rather than to reproductive output; greater heterozygosity, particularly through blocking of polar body 1 rather than polar body 2; higher glycogen content (although the physiological and economic advantages of this are uncertain); reduction or absence of spatfall (overcatch increases labour costs); greater resistance to diseases (Beaumont & Fairbrother 1991); larger adductor muscle (valuable in some bivalves) (Gardner, Maguire & Kent 1996); and protection of intellectual property in genetically modified individuals (for example, sale of triploid selectively bred animals that cannot readily be used as broodstock).

In 1990, triploid Pacific oysters (meiosis 2) were produced by University of Tasmania staff with the assistance of the industry and Washington State University staff (Nell & Maguire 1994). These were evaluated on three oyster leases in Tasmania. In contrast to Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley) in New South Wales (Nell, Cox, Smith & Maguire 1994) and Pacific oysters in Japan (Akashige & Fushimi 1992), triploid Pacific oysters in Tasmania only exhibited a growth rate advantage over diploid controls (about 23%) at 2–3 years old in the spring as they approached commercial size, and the diploids underwent substantial gonadogenesis. They did, however, maintain commercially appropriate meat condition during summer/autumn, while control diploids spawned and remained unmarketable for several months after spawning. The major problem was that, according to flow cytometry, only 76% of the triploids were actually triploids, the remainder being diploids (Nell & Maguire 1994).

In subsequent projects (Maguire 1997), attempts were made to improve the percentage triploidy by refining chemical stress procedures for inducing triploidy and by producing tetraploids (4N); the latter, in theory, could subsequently be crossed with diploids to produce all triploid offspring. Attempts at producing tetraploids by blocking both polar bodies from newly spawned eggs (4N) and avoiding contribution by haploid sperm (1N) were unsuccessful for both Sydney rock and Pacific oysters (Nell, McMahon & Hand 1998). However, it was possible to condition triploid Pacific oyster broodstock in Tasmania at elevated temperatures indoors (25 °C) or in outdoor pools, strip spawn these eggs (in theory at the 6N stage) and block polar body 1 but not polar body 2. Then, 4N embryos could be produced by fertilizing triploid eggs with haploid sperm from normal diploid males (Guo & Allen 1994b). Tetraploids were detected at low levels in resultant spat, but chromosome counting, in contrast to flow cytometry, indicated a high incidence of mosaicism (individuals containing diploid, triploid and tetraploid nuclei). In the summer of 1998/99, individual pair crosses are planned among tetraploid broodstock (identified by extensive flow cytometry) and between tetraploids and diploids (hopefully) to produce non-mosaic tetraploid and triploid lines respectively (Guo, DeBrosse & Allen 1996). Unfortunately, it has not proved feasible to count chromosomes reliably in broodstock Pacific oysters, confirming the experience of US researchers (S. Allen, pers. commun., 1998).

Refining chemical stress procedures to produce triploids from diploid broodstock was not as rewarding as had been hoped (Nell, Hand, McAdam & Maguire 1996). Numerous trials with Pacific oysters were affected by poor survival of triploid larvae or ciliate infestations during metamorphosis. The latter phase is significant, as percentage triploidy is usually higher in larvae than in spat, presumably through differential survival or efficiency of attachment to cultch.

Other factors assessed in this second phase of research on triploid Pacific oysters (Maguire 1997) were the production and evaluation of meiosis 1 triploids, evaluation of triploids in warmer South Australian waters where diploids were expected to divert more energy to reproduction (thus leading to better growth rate advantages for triploids (Shpigel, Barber & Mann 1992) and evaluation of successive batches of triploids based on different broodstock. In contrast to the production of meiosis 2 triploids, in which developmental rate and, hence, appropriate timing of application of chemical stress can be determined by observing the appearance of the first polar body, production of meiosis 1 triploid Pacific oysters usually involves crude application of chemical stress 5 min after the addition of sperm. Embryo and larval mortality rates can also be very high with meiosis 1 triploids. Surprisingly, spat produced in this way did not grow faster than meiosis 2 stock from the same pool of gametes in Tasmania and

South Australia. Meiosis I triploids are expected to show greater heterozygosity than meiosis 2 triploids (Stanley, Hidu & Allan 1984; Hawkins, Day, Gérard, Naciri, Ledu, Bayne & Héral 1994), but subsequent allozyme analysis of the two Australian triploid groups indicated little difference (L. English, unpubl, data). This suggests that few eggs that had not released the first polar body by 5 min after fertilization survived the chemical stress in the meiosis 1 triploids, so that survivors were probably mostly meiosis 2 triploids and diploids. The relative value of meiosis 1 and 2 triploids remains unresolved under Australian conditions.

Despite higher water temperatures, meiosis 2 triploid Pacific oysters in South Australia did not exhibit a larger growth rate advantage over diploids than did oysters from the same pool of gametes at Tasmanian sites. Indeed, the tendency for triploids to have slightly lower meat condition than diploids (before spawning) (Nell & Maguire 1994) is probably a major disincentive at sites in South Australia during periods, before spawning, when meat condition is below market quality standards. Successive batches of triploids based on different broodstock performed similarly in Tasmania, and the diploid controls provided cohorts from which slow- and fast-growing broodstock were obtained for subsequent mass selection research (see above).

Commercial reaction to triploid Pacific oysters in Australia has been mixed. Some Tasmanian farmers have found that they can be used to overcome marketing problems in summer/autumn after diploids have spawned. However, other Australian farmers have tried to rely solely on triploids when it may be preferable only to harvest them when diploids are unmarketable. Meat discoloration can also be a problem (Nell & Maguire 1994) and often occurs in seasons when a farmer can readily market diploids (Hand, Nell & Maguire 1998a). Although it was not evident in the above studies with Pacific oysters, the relative growth rates of diploids and triploids can vary between sites (Hand *et al.* 1998a). Overseas experience has been that commercial supplies of triploid Pacific oysters can have variable percentage triploidy and, in one trial in South Australia, triploids within a commercial batch (identified by flow cytometry on individual oysters from two farms) grew more slowly than diploids. In general, the potential for commercial reliance on triploids seems to be greater for Sydney rock oysters than for Pacific oysters because of growth rate advantages from an earlier age (Hand *et al.* 1998a) and recent indications of much better disease resistance in triploids (Hand, Nell, Smith & Maguire 1998b).

Conclusions

We are seeking to apply an integrated approach to the genetic improvement of Pacific oysters in Australia. Various tools and approaches have been adopted, and we are seeking to determine which are the most promising. There is clearly abundant genetic diversity in Pacific oysters in Tasmania. Selection programmes have been established that aim to harness some of this variability to produce better yielding strains. High- and low-growth rate lines differ significantly in growth rate after one generation of selection. About 40 family lines now add to the six initial lines that were established in 1996/7. We have numerous molecular markers, a preliminary linkage map and a map of major genes that promises to be useful in the application of marker-assisted selection. We are in the early stages of the genetic improvement programme, but we are confident that the selection programme will lead to significant gains in the traits of concern to the oyster industry and that the industry will propagate and maintain the most valuable lines. Triploidy has proved attractive to some farmers, and delivery of batches of 100% triploids produced via tetraploid × diploid crosses is being investigated.

Acknowledgments

We thank the Cooperative Research Centre for Aquaculture, the Fisheries Research and Development Corporation, the South Australian Oyster Growers' Association and the Tasmanian Oyster Research Council for helping to fund this work. Marine Shellfish Hatcheries, the Tasmanian Aquaculture and Fisheries Institute (Taroona) and John Poke hosted larval and spat production, and five commercial farmers in Tasmania and South Australia are hosting the growout trials. Dr Cam McPhee provided advice on the design of the breeding programme, and Colin Sumner of TORC gave advice on behalf of the industry. Greg Kent, Bronwyn Innes, Ian Duthie, Geraldine McMahon, Lynda Bellchambers, Mark Cozens, Kylie Freeman and Brenton Chatfield provided expert technical assistance. The first author thanks the Fisheries Research and Development Corporation and the Aquaculture Development Council of Western

Australia for sponsoring his attendance at the Genetics in Aquaculture meeting in Perth, Western Australia. Dr John Benzie kindly commented on an earlier version, and an anonymous referee provided constructive criticism of parts of the paper.

References

Ahmed M. (1975) Speciation in living oysters. *Advances in Marine Biology* **13**, 375–397.

Akashige S. & Fushimi T. (1992) Growth, survival, and glycogen content of triploid Pacific oyster *Crassostrea gigas* in the waters of Hiroshima, Japan. *Nippon Suisan Gakkaishi* **58**, 1063–1071.

Allen S.K. Jr (1998) Commercial applications of bivalve genetics: not a solo effort. *World Aquaculture* **March**, 38–43.

Ayres P. (1991) Introduced Pacific oysters in Australia. In: *The Ecology of* Crassostrea gigas *in Australia, New Zealand, France and Washington State.* (ed. by M. Leffler, M. & J. Greer), pp. 2–6. Proceedings of the Oyster Ecology Workshop, October 28–30, Annapolis, MD. Maryland Sea Grant College, University of Maryland, College Park, MD.

Banks M.A., McGoldrick D.J., Borgeson W. & Hedgecock D. (1994) Gametic incompatibility and genetic divergence of Pacific and Kumamoto oysters, *Crassostrea gigas* and *Crassostrea sikamea*. *Molecular Biology* **121**, 127–135.

Baoprasertkul P. (1998) *DNA Microsatellite Analysis of Mendelian Inheritance in Pacific Oyster Families.* Unpublished M. Appl. Sci. Thesis, University of Tasmania.

Beaumont A.R. & Fairbrother J.E. (1991) Ploidy manipulation in molluscan shellfish: a review. *Journal of Shellfish Research* **10**, 1–18.

Brown D., Van Landeghem K. & Schiele M. (1997) *Australian Aquaculture: Industry Profiles for Selected Species*. Research Report 97.3. ABARE, Canberra.

Buroker N.E., Hershberger W.K. & Chew K.K. (1979a) Population genetics of the family Ostreidae. I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis*. *Marine Biology* **54**, 157–169.

Buroker N.E., Hershberger W.K. & Chew K.K. (1979b) Population genetics of the Family Ostreidae. II. Interspecific studies of *Crassostrea gigas* and *Saccostrea commercialis*. *Marine Biology* **54**, 171–184.

Deupree R.H. Jr (1993) *Genetic Characterization of Deep-cupped Pacific Oysters (*Crassostrea gigas*) from Tasmania, Australia.* Unpublished Masters Thesis, University of Washington, Washington, USA.

English L.J., Maguire G.B. & Ward R.D. (1999) Genetic variation of wild and hatchery populations of the Pacific oyster, *Crassostrea gigas* (Thunberg), in Australia. *Aquaculture* (in press).

Falconer D.S. & MacKay T.F.C. (1996) *Introduction to Quantitative Genetics*, 4th edn. Longman, Harlow, UK,.

Fujio Y. (1979) Enzyme polymorphism and population structure of the Pacific oyster, *Crassostrea gigas*. *Tohoku Journal of Agricultural Research* **30**, 32–42.

Gardner N.C., Maguire G.B. & Kent G. (1996) Studies on triploid oysters in Australia. VII. Assessment of two methods for determining triploidy in Pacific oysters, *Crassostrea gigas* (Thunberg): adductor muscle diameter and nuclear size. *Journal of Shellfish Research* **15**, 609–615.

Gosling E.M. (1982) Genetic variability in hatchery-produced Pacific oysters (*Crassostrea gigas* Thunberg). *Aquaculture* **26**, 273–287.

Graham M.A. (1991) *Situation Analysis of the Australian Oyster Industry with Particular Reference to the Tasmanian Pacific Oyster.* A Report for the Tasmanian Aquaculture Co-operative Society and the Business School. University of Tasmania, Tasmania.

Guo X. & Allen S.K. Jr (1994a) The reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). *Biological Bulletin* **187**, 309–318.

Guo X. & Allen S.K. Jr (1994b) Viable tetraploids in Pacific oysters (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs from triploids. *Molecular Marine Biology and Biotechnology* **3**, 42–50.

Guo X.M., DeBrosse G.A. & Allen S.K. Jr (1996) All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. *Aquaculture* **142**, 149–161.

Hand R.E., Nell J.A. & Maguire G.B. (1998a) Studies on triploid oysters in Australia. X. Growth and mortality of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Journal of Shellfish Research* **17**, 1115–1127.

Hand R.E., Nell J.A., Smith I.R. & Maguire G.B. (1998b) Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters *(Saccostrea commercialis)* through outbreaks of winter mortality caused by *Microcytos roughleyi* infestation. *Journal of Shellfish Research* **17**, 1129–1135.

Hawkins A.J.S., Day A.J., Gérard A., Naciri Y., Ledu C., Bayne B.L. & Héral M. (1994) A genetic and metabolic basis for faster growth among triploids induced by blocking meiosis I but not meiosis II in the larviparous European flat oyster, *Ostrea edulis* L. *Journal of Experimental Marine Biology and Ecology* **184**, 21–40.

Hedgecock D. & Sly F. (1990) Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. *Aquaculture* **88**, 21–38.

Holliday J.E. & Nell J.A. (1986) Concern over Pacific oyster in Port Stephens. *Australian Fisheries* **44** (11), 29–31.

Jansen R.C. & Stam P. (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* **136**, 1447–1455.

Lannan J.E. (1972) Estimating heritability and predicting

response to selection for the Pacific oyster. *Proceedings of the National Shellfish Association* **62,** 62–66.

Maguire G.B. (1997) Triploids, tetraploids and other Pacific oyster research topics. Proceedings of the Tasmanian Oyster Research Council Workshop, Hobart, Australia. *TORC News* **7,** 46–50.

McGoldrick D.J. (1997) *An Experimental Investigation of the Genetic Basis of Heterosis in the Pacific Oyster* Crassostrea gigas *Thunberg.* Unpublished PhD Thesis, University of California, Davis, USA.

Medcof J.C. & Wolf P.H. (1975) Spread of Pacific oyster worries NSW culturists. *Australian Fisheries* **34** (7), 32–38.

Moraga D., Osada M., Lucas A. & Nomura T. (1989) Génétique biochemique de populations de *Crassostrea gigas* en France (côte atlantique) et au Japon (Miyagi) (Biochemical genetics of *Crassostrea gigas* populations from France (Atlantic coastline) and from Japan (Miyagi). *Aquatic Living Resources* **2,** 135–143.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89,** 583–590.

Nell J.A. & Maguire G.B. (1994) *Evaluation of Triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania.* Final Report to the Fisheries Research and Development Corporation. Project Report 89/63. FRDC, Canberra.

Nell J.A., Cox E., Smith I.R. & Maguire G.B. (1994) Studies on triploid oysters in Australia. 1. The farming potential of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Aquaculture* **126,** 243–255.

Nell J.A., Hand R.E., McAdam S.P. & Maguire G.B. (1996) Studies on triploid oysters in Australia. V. Evaluation of cytochalasin B and 6-dimethylaminopurine for triploidy induction in Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Aquaculture Research* **27,** 689–698.

Nell J.A., McMahon G.A. & Hand R.E. (1998) *Tetraploidy Induction in Sydney Rock Oysters.* Aquaculture CRC Project D.4.2. Final Report Series, 9. NSW Fisheries, Taylors Beach, Australia.

Olsen A.M. (1994) The history of the development of the Pacific oyster, *Crassostrea gigas* (Thunberg) industry in South Australia. *Transactions of the Royal Society of South Australia* **118,** 253–259.

Sheridan A.K. (1997) Genetic improvement of oyster production – a critique. *Aquaculture* **153,** 165–179.

Shpigel M., Barber B.J. & Mann R. (1992) Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. *Journal of Experimental Marine Biology and Ecology* **161,** 15–25.

Smith P.J., Ozaki H. & Fujio Y. (1986) No evidence for reduced genetic variation in the accidentally introduced oyster *Crassostrea gigas* in New Zealand. *New Zealand Journal of Marine Research* **20,** 569–574.

Stanley J.G., Hidu H. & Allen S.K. Jr (1984) Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. *Aquaculture* **37,** 147–155.

Thomson J.M. (1952) The acclimation and growth of the Pacific oyster (*Gryphaea gigas*) in Australia. *Australian Journal of Marine and Freshwater Research* **3,** 64–73.

Thomson J.M. (1958) The naturalisation of the Pacific oyster in Australia. *Australian Journal of Marine and Freshwater Research* **10,** 144–149.

Thomson J. (1988) A new boy in the division. In: *CSIRO at Sea: 50 Years of Marine Research* (ed. by V. Mawson D.J. Tranter & A.F. Pearce), pp. 55–65. CSIRO Marine Labs, Hobart, Australia.

Toro J.E., Sanhueza M.A., Winter J.E., Aguila P. & Vergara A.M. (1995) Selection response and heritability estimates for growth in the Chilean oyster *Ostrea chilensis* (Philippi, 1845). *Journal of Shellfish Research* **14,** 87–92.

Treadwell R., McKelvie L. & Maguire G.B. (1992) *Potential for Australian Aquaculture.* Australian Bureau of Agricultural and Resources Economics Research Report. ABARE, Canberra.

Zeng Z.-B. (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. *Proceedings of the National Academy of Sciences, USA* **90,** 10972–10976.

Aquaculture Research, 2000, **31**, 45–49

The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) breeding programme: progress and goals

J A Nell[1,2], I R Smith[1,2] & C C McPhee[1,3]

[1]CRC for Aquaculture, Australia

[2]NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW 2316, Australia

[3]Queensland Department of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland 4105, Australia

Correspondence: J A Nell, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW 2316, Australia

Abstract

In 1990, NSW Fisheries initiated a mass selection programme in Port Stephens, NSW, with the aim of breeding faster growing Sydney rock oysters *Saccostrea glomerata* (Gould 1850). After two generations of selection, an average weight for age advantage of 18% (range 14–23% per breeding line) was achieved. This equates to a reduction of 3 months in the time taken to reach market size. Experiments are planned to determine how much of this 3 months advantage is additive to the 6 months advantage this laboratory has already obtained using triploid *S. glomerata*. A parallel set of *S. glomerata* breeding lines was established on the Georges River, NSW, to include selection for resistance to the protistan parasite *Mikrocytos roughleyi*, the causal agent of winter mortality. The programme was disrupted by the outbreak of QX disease *Marteilia sydneyi*, another protistan parasite, in 1994. In 1997, the breeding programme was reorganized and expanded. New lines were bred from oysters that had survived both QX and winter mortality. After one generation, a small improvement in resistance to QX has been recorded; however, the exposure of further generations to *M. sydneyi* will be required to confirm an increase in resistance.

Introduction

A breeding programme for the Sydney rock oyster *Saccostrea glomerata* (Gould 1850) was established by NSW Fisheries in 1990 (Nell, Sheridan & Smith 1996a). This initiative was taken in response to a gradual decline in production (Nell 1993) from around 14 million dozen year^{-1} in the 1970s to around 8 million dozen in 1996/97, a fall of 40%. This decline resulted from increasing costs of production of the slow-growing oyster exacerbated by compounding factors, including increased overcatch problems (spat settling on oyster and growing equipment) associated with the introduction of Pacific oysters to Port Stephens, NSW, in 1984. The regular occurrence of winter mortality, caused by the protistan parasite *Mikrocytos roughleyi* (Nell 1993), and the spread of QX disease, caused by the protistan parasite *Marteilia sydneyi*, were major factors increasing the cost of production. QX disease has caused a 90% decline in the production of Georges River since the early 1990s. Until the outbreak in the Georges River in 1994, QX disease was thought to be limited to northern NSW and unable to spread further south because the parasite could not survive the lower temperatures.

Although production of Sydney rock oysters has increased in some NSW estuaries, this has only partially offset the large decline in Port Stephens and Georges River. The oyster breeding programme was developed by NSW Fisheries in Port Stephens and the Georges River to address the combined problems of slow growth and disease, which affect the economic viability of the Sydney rock oyster industry. The specific aims of the programme in Georges River were to improve survival by 10% per generation and maintain or enhance growth rates.

It is expected that selection for disease resistance may reduce the selection pressure for fast growth in Georges River. If improved survival rates are achieved, smaller increases in growth rates would be acceptable for the Georges River breeding line oysters.

The specific aims for the programme in Port Stephens were to reduce time taken to market by 1 month per generation (current reduction around 3 months), maintain or improve survival rates, maintain meat yield and assess how much of the faster growth from triploidy is additive to that from selective breeding. The breeding goals for Port Stephens are based on the assumption that the average reduction in time taken to market size can be reduced by 1 month per generation (Nell, Smith & Sheridan 1998). It is expected that meat yield and survival rates for the Port Stephens breeding line oysters will not be adversely affected, but both of these parameters will nevertheless be monitored in each generation. If either or both parameters deteriorate, corrective measures will be required.

At the start of the breeding programme, it was assumed that Sydney rock oysters in NSW would have largely lost any genetic variation between estuaries, if any existed in the first place. Around 1888, in the early days of the industry, New Zealand rock oysters *S. glomerata* were imported to replenish the depleted oyster stocks in NSW (Roughley 1922). As the New Zealand rock oyster belongs to the same species as the Sydney rock oyster (Buroker, Hershberger & Chew 1979; Anderson & Adlard 1994), it is likely that they would have interbred with the local oysters. Furthermore, improved road transport during the 1970s and 1980s allowed easier transfer of oysters from one estuary to another to use the best characteristics of each (Nell 1993). Thus, the Sydney rock oyster has a high level of genetic variation (Buroker *et al.* 1979), with no detectable genetic difference in oyster populations from different estuaries in Australia (Buroker *et al.* 1979).

In consideration of this genetic information, it was decided neither to look at separate strains nor to evaluate the performance of oysters from different estuaries. Instead, equal numbers of oysters from the four major growing estuaries, namely Wallis Lake, Port Stephens and the Hawkesbury and Georges Rivers (Fig. 1), were chosen as a base breeding population (Nell *et al.* 1996a). No control selection line was established. Instead, it was decided to use hatchery-produced spat from non-selected oysters obtained from the same estuaries as the base population as controls. Four selection lines were first established for fast growth in Port Stephens in 1990. The second, third and fourth generation breeding lines for Port Stephens were established in 1993, 1995 and 1998 respectively.

In the Georges River, another four selection lines were first established for fast growth and also to select for winter mortality resistance in 1990. The second, third, fourth and fifth generation breeding lines for Georges River were established in 1992, 1994, 1997 and 1999 respectively. For logistical reasons, oysters from the two estuaries are bred in alternate years. After the appearance of QX disease in the Georges River in 1994, the third-generation breeding lines in this estuary were rearranged into separate lines for QX, winter mortality (WM) and QX plus winter mortality (QX/WM) resistance.

Selection for faster growth in oysters has been demonstrated in *Crassostrea virginica* (Gmelin, 1791) (Paynter & Dimichele 1990), *Ostrea edulis* (Linnaeus, 1750) (Newkirk & Haley 1983; Toro & Newkirk 1990) and *Ostrea chilensis* (Philippi, 1845) (Toro & Newkirk 1991). More recently, selection has also been successful in *S. cucullata* (Born, 1778) (Jarayabhand & Thavornyutikarn 1995) and Sydney rock oysters (Nell *et al.* 1996a). For a review of genetic improvement of oyster production, see Sheridan (1997) and Allen (1998).

Selection for resistance to protistan parasites has been successful in other oyster species. In the American oyster *C. virginica*, selection for resistance to the parasite *Minchinia nelsoni*, more commonly known as MSX, reduced mortality from 93% in controls to 56% after three generations (Haskin & Ford 1979) and to 35% after six generations (Allen 1998). A similar response was obtained with selection for *Bonamia* resistance in flat oysters *Ostrea edulis* in France, with mortality reduced from 87% in controls to 41% after three generations of selection for disease resistance (Naciri-Gravin, Martin, Baud, Renault & Gérard 1998).

Growing and selection methods

Parents to the base population for the breeding lines were taken from Wallis Lake, Port Stephens and the Hawkesbury and Georges Rivers. A series of eight separate natural mass spawnings and fertilizations were carried out in February 1990 (Nell *et al.* 1996a). Each spawning/fertilization event used 100 oysters, comprising 25 from each of the four

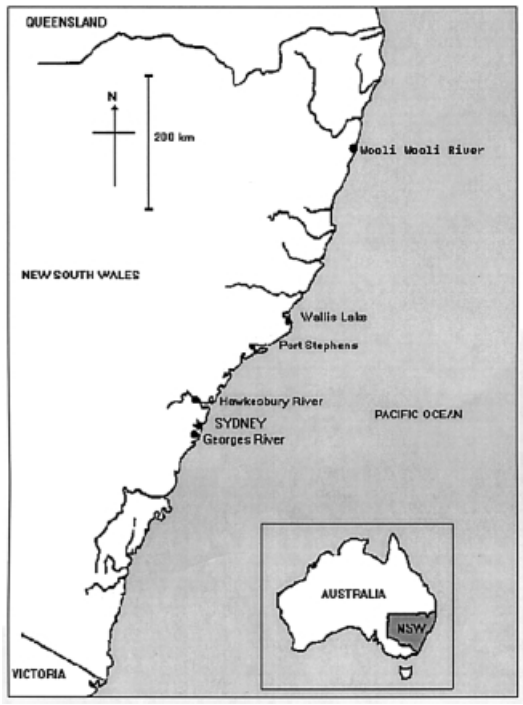


Figure 1 Map of oyster growing estuaries in NSW.

estuaries. Equal numbers of zygotes from each mass spawning/fertilization were stocked into a 20 000-L hatchery tank. Larvae were set on scallop shell chips and spat reared in upwellers in an outdoor nursery. When spat reached a shell height of 12 mm (range 12–20 mm) in August 1990, they were divided randomly into four breeding lines for the Georges River and four more for Port Stephens.

Each breeding line comprised three trays (0.9×1.8 m) of oysters at each of three sites, nine trays in all. Of the four lines for each estuary, two were raised initially by the 'loose' (cultchless) method of culture and two by the 'slat' method on PVC substrate (Smith, Sheridan & Nell 1995). After the first two generations of selection, no measurable advantage was found for the more costly 'slat' method so, after 1997, the 'loose' method was used for all lines (Nell *et al.* 1998). Initial tray stocking rates for the two methods were ≈ 1000 loose spat/tray and 280 spat on slats/tray. For each generation, the 24 fastest growing 'loose' and 'slat' oysters for each replicate tray were selected on a whole weight basis. 'Loose' oysters were selected on a whole tray, of around 350 oysters, basis, i.e. for the Port Stephens line ≈ 7% of oysters were selected. 'Slat' oysters were selected on a within-tray compartment basis (four compartments with seven slats each and 10 oysters per slat). This was ≈ 10% of surviving 'slat' oysters (Nell *et al.* 1998).

For each successive generation, 216 of the largest survivors were selected for breeding on a within-tray basis. Four separate mass spawnings/fertilizations were used per line. Oysters are induced to spawn rather than 'strip' spawned because the quality of 'strip'-spawned eggs is more variable (Nell, Hand, Goard, McAdam & Maguire 1996b). As soon as an oyster commences spawning, it is taken off the spawning table and placed in a separate beaker of clean sea water. For each spawning group, gametes are pooled, eggs and sperm separately, before fertilization.

Allozyme analysis of a representative Georges River breeding line was carried out on second- and third-generation specimens to test for possible inbreeding effects. No substantial loss of genetic variation was found in this (slat 2) line (L J English, pers. commun., 1998). Further generations will be analysed similarly to record any changes in the level of inbreeding.

Results for Port Stephens breeding lines

After one generation of selection (second-generation breeding lines), an average weight increase of 4% (range 0–9%) relative to controls was recorded for the four breeding lines harvested after 18 months cultivation on commercial oyster leases (Nell *et al.* 1996a). These results provided encouragement for the continuation of the programme. After two generations of selection (third-generation breeding lines), an average weight increase of 18% (range 14–23%) per line, which would equate to 3 months earlier to market, was recorded in May 1997 (Nell *et al.* 1998). The presumed 3 months reduction in culture time, is based on the average 3.5 years normally taken to market size (40–60 g whole weight) for Sydney rock oysters. Selected oysters fed more quickly and grew with greater metabolic efficiency than controls (B L Bayne *et al.*, in press).

In all experiments, control spat produced from non-selected wild stock (Nell *et al.* 1996a) were hatchery reared alongside selected oysters. Parent oysters selected from the four Port Stephens third-generation breeding lines and two control groups were spawned and fertilized in February 1998. In addition to the diploid larvae, triploid siblings will be produced for two of the selection lines and both

control groups. These will be used to assess how much of the 3 months reduction in time taken to market achieved by selective breeding (third generation) (Nell *et al.* 1998) is additive to the 6 months reduction obtained using triploidy (Nell, Cox, Smith & Maguire 1994; Hand, Nell & Maguire 1998a; Hand, Nell, Smith & Maguire 1998b).

An average winter mortality kill over three sites [high (downstream), medium (midstream) and low (upstream) kill] of between 15% (August–September 1993) and 50% (August–September 1996) was recorded for the first and second generations of selection respectively.

Results for Georges River breeding lines

While all breeding lines were selected for fast growth, the four Georges River lines were also exposed to winter mortality. Average mortality attributable to *M. roughleyi,* for all breeding lines during the first and second generation was around 15%/generation (August–September 1991 and 1993 respectively).

QX disease first appeared in the Georges River in 1994, but did not affect breeding lines, which were elsewhere in the nursery stage. In the 1995 and 1998 episode, however, QX killed 85% of the experimental oysters at two (midstream and upstream) out of the three growing sites, severely disrupting the breeding programme. In January 1997, the program was re-established and modified to incorporate selection for resistance to both QX and winter mortality diseases. Survivors of the old third-generation lines were used as parents to create new fourth-generation selection lines (three generations of selection for fast growth). Broodstock parents for new 'QX survivor' lines were chosen by selecting the 216 largest oysters remaining at the two upstream sites that had contained 12 trays, each of around 350 'loose' oysters, and another 12 trays, each of around 250 'slat' oysters, before the onset of losses (85%) in February/March 1995.

In the next generation, similarly, 216 parent oysters for 'winter mortality survivors' were selected by taking the largest survivors (on a within-tray basis) from the six trays of 'loose' and six trays of 'slat' oysters that were subjected to heavy winter mortalities (50%) at the downstream site in August and September 1996. An additional 108 QX and another 108 winter mortality survivors were chosen as above to be crossed with one another as in group 3 below. Oysters were spawned, and matings of disease survivors were organized into three groups: (1) QX males × QX females (216 QX survivors to spawning table); (2) WM males × WM females (216 WM survivors to spawning table); (3) WM males × QX females and WM females × QX males (108 QX parents and 108 WM survivors to spawning table). Approximately 45% of these oysters spawned and were used as parents for the fourth generation.

Washed fertilized eggs were transferred to the nearby NSW Fisheries Research Institute, Cronulla, for rearing and successfully set in March 1997. Each of the three groups of spat were split into two parallel breeding lines to form six 'new' breeding lines (fourth generation) on leases in Georges River in August 1997 with six trays of > 1000 oysters each: (1) two for QX resistance at Lime Kiln Bar (upstream); (2) two for QX disease/WM resistance at Woolooware Bay (midstream); (3) two for WM resistance at Quibray Bay (downstream).

A growth and survival comparison experiment of the six breeding lines, plus two controls (spat from Wooli Wooli River matched for size and weight) was carried out at Woolooware Bay (for winter mortality) and at Lime Kiln Bar (for QX exposure) from August 1997 to April 1999. There were four half-trays of 500 oysters each per line at each site.

At the completion of the experiment, there was little difference in mortality between the selection lines and controls, but the two QX resistance lines were ≈ 19% heavier than the controls. However, the exposure of further generations will be required to show a definite trend in selection for disease resistance.

It is expected that survivors from the fourth-generation Georges River breeding lines will be bred in early 1999 to produce fifth-generation offspring. These will be used to continue selection for disease resistance.

Acknowledgments

We would like to thank the Fisheries Research and Development Corporation (FRDC 96/357) for their support of this programme. We are also greatly indebted to those oyster farmers who have offered lease space and assisted the programme in many other ways.

References

Allen S.K. Jr (1998) Commercial applications of bivalve genetics: not a solo effort. *World Aquaculture* **29** (1), 38–43.

Anderson T.J. & Adlard R.D. (1994) Nucleotide sequence of rDNA internal transcribed spacer supports synonymy of *Saccostrea commercialis* and *S. glomerata. Journal of Molluscan Studies* **60**, 196–197.

Bayne B.L., Svensson S. & Nell J.A. (1999) The physiological basis for faster growth in the Sydney rock oyster *Saccostrea glomerata. Biological Bulletin* (in press).

Buroker N.E., Hershberger W.K. & Chew K.K. (1979) Population genetics of the family Ostreidae. I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis. Marine Biology* **54**, 157–169.

Hand R.E., Nell J.A. & Maguire G.B. (1998a) Studies on triploid oysters in Australia. X. Growth and mortality of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley). *Journal of Shellfish Research* **17**, 1115–1127.

Hand R.E., Nell J.A., Smith I.R. & Maguire G.B. (1998b) Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. *Journal of Shellfish Research* **17**, 1129–1135.

Haskin H.H. & Ford S.E. (1979) Development of resistance to *Minchinia nelsoni* (MSX) mortality in laboratory-reared and native oyster stocks in Delaware Bay. *Marine Fisheries Review* **41**, 54–63.

Jarayabhand P. & Thavornyutikarn M. (1995) Realised heritability estimation on growth rate of oyster, *Saccostrea cucullata* Born, 1778. *Aquaculture* **138**, 111–118.

Naciri-Gravin Y., Martin. A.G., Baud J.P., Renault T. & Gérard A. (1998) Selecting the flat oyster *Ostrea edulis* (L.) for survival when infected with the parasite *Bonamia ostreae. Journal of Experimental Marine Biology and Ecology* **224**, 91–97.

Nell J.A. (1993) Farming the Sydney rock oyster (*Saccostrea commercialis*) in Australia. *Reviews in Fisheries Science* **1**, 97–120.

Nell J.A., Cox E., Smith I.R. & Maguire G.B. (1994) Studies on triploid oysters in Australia. I. The farming potential of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Aquaculture* **126**, 243–255.

Nell J.A., Sheridan A.K. & Smith I.R. (1996a) Progress in a Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding program. *Aquaculture* **144**, 295–302.

Nell J.A., Hand R.E., Goard L.J., McAdam S.P. & Maguire G.B. (1996b) Studies on triploid oysters in Australia. Evaluation of cytochalasin B and 6-dimethylaminopurine for triploidy induction in Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). *Aquaculture Research* **27**, 689–698.

Nell J.A., Smith I.R. & Sheridan A.K. (1998) Third generation evaluation of Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) breeding lines. *Aquaculture* **170**, 195–203.

Newkirk G.F. & Haley L.E. (1983) Selection for growth rate in the European oyster, *Ostrea edulis*: response of second generation groups. *Aquaculture* **33**, 149–155.

Paynter K.T. & Dimichele L. (1990) Growth of tray-cultured oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay. *Aquaculture* **87**, 289–297.

Roughley T.C. (1922) *Oyster Culture on the Georges River, New South Wales.* Technical Education Series 25. Technological Museum, Sydney.

Sheridan A.K. (1997) Genetic improvement of oyster production-a critique. *Aquaculture* **153**, 165–179.

Smith I.R., Sheridan A.K. & Nell J.A. (1995) Evaluation of growing methods for use in a Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley) selective breeding program. *Aquaculture* **131**, 189–195.

Toro J.E. & Newkirk G.F. (1990) Divergent selection for growth rate in the European oyster *Ostrea edulis*: response to selection and estimation of genetic parameters. *Marine Ecology Progress Series* **62**, 219–227.

Toro J.E. & Newkirk G.F. (1991) Response to artificial selection and realised heritability estimate for shell height in the Chilean oyster *Ostrea chilensis. Aquatic Living Resources* **4**, 101–108.

Aquaculture Research, 2000, **31**, 51–59

Genetic improvement programmes in abalone: what is the future?

N G Elliott
CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001

Correspondence: N G Elliott, CSIRO Marine Research, GPO Box 1538, Hobart, Tasmania 7001, Australia

Introduction

One aim of this paper is to discuss the options available for genetic improvement programmes in the culture of Australian abalone. In presenting specific areas of genetics that I consider promising, I will briefly review abalone research in these areas, highlighting, where appropriate, what has or is being done in Australia. The other aim of the paper is to engender further interest and enthusiasm in establishing an industry-wide genetic improvement programme to ensure a viable and efficient Australian abalone culture industry.

Abalone are univalve (single-shelled) marine gastropods from the genus *Haliotis*. There are close to 100 species found worldwide in both tropical and temperate waters (Hahn 1989). In their natural habitat, they are slow-feeding nocturnal herbivores, with the adults grazing predominantly on seaweeds and the juveniles on microalgae and diatoms found on the surfaces on which they settle.

These animals are highly prized for their large adductor muscle (or foot), and aboriginal people have harvested them for thousands of years. Hahn (1989) reports an early reference to abalone divers in Japan from around 30 AD, and of early North American Indians harvesting abalone. Commercial fisheries now exist for over 30 species, and the products are sold in live, fresh, frozen, canned or in dried form, with the main markets being in China, Japan, Hong Kong (and south-east Asia), USA, Mexico, Korea and Europe (Oakes & Ponte 1996). Australia is a major supplier of abalone products, with an estimated 81% of the fresh and frozen product and 67% of the canned product in the international markets (Brown 1997). The majority of this product is from commercial dive fisheries.

The worldwide popularity of abalone has led to the decline of many fisheries through commercial overfishing, excessive poaching and large recreational catches; Australian fisheries are, however, in relatively good condition. Such declines, closures of fisheries and high market prices have seen extensive interest in the culture of abalone in many countries; over 20 species are either in production or in the experimental stages (Landau 1992). Research into hatchery rearing methods for abalone began in Japan in about 1960 and spread to California and Taiwan in the 1960s, Korea, France and Ireland in the 1970s and New Zealand and Australia in the 1980s (Hahn 1989). Other countries now involved in some way with abalone culture include China, Mexico, Chile, South Africa, Iceland, Thailand and Israel. World production of cultured abalone has grown slowly but, over the past decade, has more than doubled from an estimated 1453 tonnes in 1987 to 3349 tonnes in 1996 (FAO 1998). The bulk of the production comes from Taiwan, and unconfirmed sources suggest that the figure of 3000 tonnes is a very conservative estimate of current world production, with Taiwan alone producing well over this amount and increasing. Other countries, including Australia, are only now starting to produce notable quantities; the forecast for Australia is to reach over 300 tonnes around the year 2000, a rise from only 10 tonnes in 1995/96 (Brown 1997).

In Australia there are four species of interest: the blacklip abalone *Haliotis rubra* (Leach); the greenlip abalone *H. laevigata* (Donovan); the donkey's ear *H. asinina* L.; and Roe's abalone *H. roei* (Gray).

H. rubra and *H. laevigata* are both temperate species fished commercially off southern Australia. There is already considerable interest and infrastructure in the southern states for culturing these

species and their hybrid. Research on culturing the tropical *H. asinina* has only been evident in Australia recently, although there has been interest in this species overseas for some years (e.g. in Thailand; Jarayabhand & Paphavasit 1996). The industry for culturing *H. roei* in Western Australia is very much in its infancy.

Abalone are attractive aquaculture species as, in most instances, they already have a recognized status and attract a high price in the international marketplace. Cultured abalone fit a different market niche to the commercial fishery products, as they are generally harvested at a smaller size, 50–70 mm compared with over 130 mm for the main temperate wild-harvested Australian species. As world production of the culture product increases, there is likely to be a decrease in prices with increased market competition. Therefore, the Australian industry needs to examine improvement programmes to ensure its continued viability and competitiveness. The exploitation of the as yet untapped genetic gains that are possible through well-designed genetic improvement programmes offer a logical and low-risk solution.

Over the past decade, the Australian industry has made significant progress in mastering the production cycle. The lifecycle in culture is closed, and farms are at the stage of a rapid increase in output. Research to date has focused on the 'environment' of the animal – culture techniques, tank design and nutrition. There are two areas that the industry now needs to concentrate on. One is an analysis of the markets, establishing what traits or characteristics particular markets prefer, e.g. size, presentation, tenderness, taste and colour of the mantle and foot. The second area, and the subject of this paper, is the exploitation of the biological potential of the species through the establishment of a genetic improvement programme. The goal of the programme is to provide production gains to the industry. As presented in other papers at this meeting, production gains of between 5% and 15% per generation have been recorded for other aquaculture species through genetic improvement programmes.

Genetic improvement

What do I mean by genetic improvement? In the context of this paper, genetic improvement means a gain in the cultured production of an abalone species through the exploitation or manipulation of the genetic variation present within the particular species. In general, there are four 'inputs' to a culturing system that a manager can alter to improve production gain – farm size, management practices (including husbandry, tank design, etc.), nutrition and genetics. The first three are all associated with the environment in which the animals live and are the areas usually targeted first with any culture venture. Genetics, on the other hand, is associated with the biological potential of the species to exploit the particular environment. The aim of a genetic improvement programme then is to increase the production gain by exploiting the inherent biological potential of the cultured population.

So what can genetics provide? In most genetic improvement programmes, the first trait of interest is usually growth, with the aim of producing faster growing individuals and reducing the production time and cost for a market size individual. Growth is certainly of importance with abalone, as many people describe a slow growth rate as a hindrance to their aquaculture potential. Genetic changes to the red abalone *H. rufescens* (Swainson) have been reported to provide 50% to 100% increases in growth rates (Powers, Kirby & Gomez-Ghiarri 1996; Anon 1997).

Genetics can potentially do more than just increase growth rates; it can assist in the provision of abalone strains that: are better suited to particular growout environments; are disease resistant; have higher survival rates; have better food conversion ratios; have desirable market traits (e.g. colour, texture, taste, tenderness); return a better meat yield; have a higher fecundity with age; have a higher or lower age at maturity; are sterile; are single sex only; will enhance pearl production.

One of the first steps in a genetic improvement programme is for the industry to decide what are the main characters of economic importance that need to be targeted.

The long-term future for genetic improvement in abalone culture will be a combination of biotechnology (ploidy and gene manipulation) and the more traditional selective breeding techniques (including the use of marker-assisted selection). However, to function within either or both of these areas, there is a requirement for some underpinning strategic and applied research in areas such as: broodstock conditioning and cryopreservation; genetic variation; genetic markers; hybrids; ploidy; gene manipulation; selection.

Broodstock conditioning and cryopreservation

One problem with spawning abalone in culture is the uncertainty of individuals spawning at the required time. Commercially, it can be expected that as few as 40% of potential broodstock individuals will actually spawn. This can be compensated for in a commercial mass spawning by increasing the number of potential broodstock. However, the industry may wish to extend the spawning period (out of season or earlier in the season) to take advantage of particular growout conditions. In addition, when the industry begins to use selected progeny for broodstock, including the production of hybrids, and for research needs, the spawning and fertilization of particular individuals will be required. This could be achieved through either control of the 'conditioning' or maturation of the individuals and/or the use of cryopreserved sperm or ova. The application of cryopreserved sperm or ova would also be advantageous for potentially increasing genetic vigour with a low inbreeding risk, establishing local genetic pools, transference of genetic material between states (otherwise restricted because of disease concerns) and for use in ploidy and single sex production studies.

Hahn (1994) and Moss (1998) have shown that gonad maturation (condition) of abalone can be achieved through the controlled manipulation of water temperature. Abalone sperm has been preserved successfully through cryopreservation, and the treated sperm has achieved 95% fertilization after storage for 20 days and 89% after 365 days (Tsai & Chao 1994). At present, there is no research within Australia in either field, but proposals have been prepared, as there is significant interest from certain sections of the industry.

Genetic variation

There are two aspects of genetic variation that I would like to touch on – genotypic and phenotypic. The genotypic variation is related to the genetic make-up of the individual and is the variation at the DNA level, or gene level. This area is usually examined through the use of various molecular genetic markers, such as allozymes, mitochondrial DNA, or nuclear DNA markers, such as microsatellites, and genetic tools, such as sequencing, restriction analysis and electrophoresis. The phenotypic variation, on the other hand, is the physical expression of what the particular gene or set of genes (the genotype) will produce under particular environmental conditions, and these traits or phenotypes are described (qualitatively, e.g. colour, sex) or measured (quantitatively, e.g. weight, survival). An understanding of the mean and range of a phenotype (or trait) within the population will help to determine the extent of any improvement that may be likely through selective breeding programmes. For Australian abalone species, this information is not yet available.

Development of domesticated and genetically improved strains is made possible through hatchery production. Propagation of genetically closed populations may, however, lead to loss of genetic diversity and inbreeding, with negative production effects. This could be an issue with abalone, given their high fecundity and variable fertility, which may lead to just a few individuals in a mass spawning contributing to the next generation. Despite hatcheries holding and conditioning relatively large numbers of broodstock, many individuals may not contribute to the hatchery seed, resulting in small effective breeding numbers – so increasing the rate of inbreeding and the loss of genetic variation. Inbreeding may also be caused by differential survival of families or interfamily competition.

Changes in allozyme gene frequencies, losses of rare alleles and lower than expected effective breeding numbers have been reported for hatchery stocks of abalone (Smith & Conroy 1992; Mgaya, Gosling, Mercer & Donlon 1995; Gaffney, Rubin, Hedgecock, Powers, Morris & Hereford 1996; I. Kawahara, pers. commun.). Smith & Conroy (1992) recommended that, for *H. iris* (Gmelin) in culture, hatcheries needed to use 10–13 males and 25–50 females in spawning tanks to produce an effective minimum breeding size of five females, and this would reduce the risk of losing genetic variation. An alternative approach to this problem recommended by Gaffney *et al.* (1996) was for the pooling of larvae from multiple small spawning groups, while Mgaya *et al.* (1995) suggested periodic introduction of wild broodstock into the hatchery population.

Genetic markers

Genetic markers are vital for many aspects of genetic improvement programmes, including quantification of genetic variation, inbreeding and effective breeding numbers, assessing polyploids,

assisting in pedigree analysis and for locating genes or quantitative trait loci (QTLs; markers associated with particular phenotypic traits that are used to assist selection programmes) in genome mapping projects. Variation at allozyme (protein) markers has received the most attention with abalone to date, e.g. Brown (1991); Brown & Murray (1992a,b); Fujino (1992); Smith & Conroy (1992); Mgaya *et al*. (1995); Gaffney *et al*. (1996). In recent years, attention has moved towards the production and assessment of nuclear DNA markers, such as microsatellites and minisatellites (e.g. Huang, Chai, Hanna & Gough 1997; Huang & Hanna 1998; Kirby, Villa & Powers 1998; Muchmore, Moy, Swanson & Vacquier 1998). In Australia, there are three laboratories involved in the production and assessment of microsatellite markers for abalone – Deakin University (Hanna and colleagues), University of Queensland (Degnan and colleagues) and my laboratory in CSIRO Marine Research.

Hybrids

The production of interspecies hybrid abalone has the potential to provide production gains through faster growth, adaptation to particular environmental conditions and desired market qualities. Hybridization is particularly useful if the heritability of a trait (the genetic component of the variation observed in the trait) is low, as within species selection may be inefficient. Several species of abalone have been crossed experimentally to produce hybrids.

Leighton & Lewis (1982) conducted a series of experimental crosses with four species – red abalone *H. rufescens*, pink abalone *H. corrugata* (Wood), green abalone *H. fulgens* (Philippi) and white abalone *H. sorenseni* (Bartsch). They report that crosses with female red abalone and sperm from the other three were the most successful. In addition, findings included the production of fertile F1s yielding F2 progeny from a *H. rufescens* (female) with *H. fulgens* (male) cross, better growth rates in the *H. rufescens* × *H. sorenseni* and *H. rufescens* × *H. fulgens* hybrids than in parental forms and higher survival rates for hybrids from a *H. rufescens* × *H. corrugata* cross compared with the normal *H. corrugata* spawning.

Hoshikawa, Sakai & Kijima (1998) are attempting to improve low water temperature tolerance and growth rate of the local ezo abalone *H. discus hannai* (Ino) in northern Japan by crossing it with the Alaskan pinto abalone *H. kamtschatkana* (Jonas). They have achieved a hybrid between the female ezo abalone and the male pinto abalone, but not in the other direction. The growth rate of the hybrid was significantly higher than either parental species at 18 °C. At the lower temperature of 8 °C, the hybrid performed better than the native ezo abalone, but not as well as the pinto abalone.

The two main Australian temperate abalone species, *H. rubra* and *H. laevigata*, are known to hybridize naturally, and allozyme data from natural populations suggest that the hybrid is fertile and has, in some instances, back-crossed with both parental species (Brown 1995). There is significant commercial interest in southern Australia for the production of cultured hybrids, with the assumed advantages of faster growth in the particular environmental conditions. Rigorous assessment of the hybrids is required. There are 11 abalone species in Australia, and further experimental trials may establish suitable hybrid strains for particular farm sites and/or markets. One difficulty with the production of hybrids is the controlled spawning of both species, and this work will be greatly benefited by research into broodstock condition and cryopreservation.

Single sex production

Female *H. laevigata* (Shepherd & Hearn 1983) are reported to grow up to 25% faster than males in the wild. Single sex production within aquaculture is not uncommon for reasons of faster growth and (avoiding) precocious maturity. Single sex production of cultured abalone is an option yet to be examined.

The gynogenetic production of female *H. discus hannai* has been reported through the application of UV-irradiated sperm (Arai, Naito, Sasaki & Fujino 1984), although the resulting haploids were deformed and non-viable. Despite this, the authors suggested that genetic improvement in abalone might be possible through the application of induced gynogenesis followed by diploidization of the resulting zygote. This would provide rapid establishment of inbred lines with a high degree of homozygosity, sex control, and a possibly accelerated loss of deleterious genes. Gynogenetic diploids have since been produced by cold shock treatment of zygotes after insemination with UV-irradiated sperm (Fujino, Arai, Iwadare, Yoshida & Nakajima 1990). While there was low survival, there was 50–

60% induction of gynogenetic diploids, and the resulting offspring, when compared with normal diploid siblings, gave greater variances in shell length and mean values that, in one experiment, were greater and, in another, smaller.

Triploids

The assessment of triploid animals in aquaculture is undertaken for two perceived advantages – faster growing and infertile animals. Ploidy manipulation is probably the most comprehensive area of abalone genetics examined thus far, with numerous studies reporting the production of triploids through the inhibition of both the first and second polar body (e.g. Arai, Naito & Fujino 1986; Wang, Zhang, Wang, Wu, Yang, Zhu, Chang, Xia, Wang *et al*. 1990; Kudo, Arai & Fujino 1991; Sun, Song, Li, Zhao & Guan 1992; Curatolo & Wilkins 1995; Powers *et al*. 1996; Okumura, Furukawa, Sugie, Sekimiya, Toda & Yamamori 1996; Stepto & Cook 1998; Zhang, Wang, Chang, Song, Ding, Wang & Wang 1998). Induction has been achieved through cold and heat shock, pressure shock, use of cytochalasin B, caffeine and 6-dimethylaminopurine (6-DMAP); induction rates generally vary from 50% to 87%. Levels of survival varied from a low 10% to 90%, and normality of larvae varied within and between studies. A doubling of growth rate for triploid *H. rufescens* has been reported (Anon 1997), while Sun *et al*. (1992) observed no growth difference in triploid *H. discus hannai* during the first year or the early part of the second year, but significant increases in shell length (10%) and total weight (20%) from late in the second year. Triploid abalone (*H. discus hannai*) were found to be high temperature tolerant, surviving significantly longer than normal diploids (Fujino 1992).

Stepto & Cook (1998) observed up to 34% production of tetraploid larvae using cytochalasin B with *H. midae* L., but these died within 5 days, possibly because of starvation, as the amount of yolk may not be sufficient to support the increased metabolism required with the extra DNA. The successful production and maturation of tetraploid abalone would allow for possible commercial production of triploids by fertilization of gametes from a tetraploid and a diploid parent, thus eliminating the use of chemicals that may have undesirable market acceptance.

There is Australian industry interest in an assessment of triploid production and performance. Production of triploids in Australian species commenced only recently with work on the greenlip abalone *H. laevigata* in South Australia, where chemical treatment resulted in low production of both triploids and tetraploids (X. Li, pers. commun.); this work is continuing. There needs to be further validation of the technique and transfer of the technology to other commercial species, including the assessment of tetraploids for future triploid production. Triploid lines need to be produced on a commercial scale to evaluate the survival and normality of triploid larvae, growth characteristics, maturity and, of course, market acceptance.

Gene manipulation

Interest in the possibility of accelerating growth rate in abalone through the manipulation of growth hormones began with the discovery that, like other molluscs, abalone have growth hormone-like molecules (Kawauchi 1991) referred to as insulin-like peptides. Two such growth hormone-like substances have been purified from *H. discus hannai* (Moriyama, Atsuta, Kobayashi & Kawauchi 1989).

Enhanced growth of the post-larval stage of *H. rufescens* was achieved by treatment of whole animals with mammalian insulin and growth hormone (Morse 1981). Likewise, growth in juvenile *H. discus hannai* was increased by treatment with recombinant salmonid growth hormone (Kawauchi & Moriyama 1991). However, when Taylor, Donovan, McLean, Donaldson & Carefoot (1996) injected recombinant bovine or porcine growth hormones, somatostatin or bovine serum albumin into 70 mm *H. kamtschatkana*, they observed no change in growth. This response was attributed either to the method of introduction or to a lack of response of larger, more mature animals, because of either the dosage level or growth at this maturity stage being controlled by the reproductive cycle and not influenced by exogenous hormones.

The argument for gene transfer technology is that the more traditional selective breeding approaches to genetic improvement will take several generations to provide the large gains potentially possible through transgenics. While increases of over 400% in growth in salmon have been documented through such technology (Aqua Bounty Farms Update, June 1996), more moderate increases of between 50% and 100% have been reported for abalone (Anon 1997). The technology does have other advantages, particularly for the incorporation of genes associated with disease resistance. Improvement with this

technology is usually a one-off gain, while cumulative gains (10–15%) in each generation are usually achievable through selective breeding. A negative side of the gene manipulation area is the current public concern about such products and the issue of intellectual property and safeguarding modified strains. Once these issues are overcome, there is great scope for potential improvement via this technique, especially if considered in conjunction with other genetic improvement techniques, including the triploid manipulation of transgenic animals (Powers *et al.* 1996).

There are two aspects in relation to genome manipulation – gene transfer technology and gene identification. The means to transport a gene construct efficiently into abalone has been examined using either sperm as a carrier or by direct introduction to the ova or embryo. Sin, Mukherjee, McKenzie & Sin (1995) with *H. iris* and Tsai, Lai & Yang (1997) with *H. divorsicolor suportexta* (*sic*) (Lischke) have demonstrated that electroporation of sperm is a suitable vector for transferring genes into abalone embryos. It is described as a relatively simple mass gene transfer procedure for shellfish, because it is efficient enough to enable the processing of several hundred thousand eggs at one time. The success rate of Tsai *et al.* (1997) of 65% is comparable with those of Powers, Kirby, Cole & Hereford (1995) for fertilized egg electroporation in red abalone (72%). The latter reported larval survival of 70–84% of that of non-electroporated siblings. Working with the tropical species *H. asinina*, Counihan, Preston, Baule & Degnan (1997; pers. commun.) from the University of Queensland have also proved the use of electroporation as an efficient method for introducing DNA into abalone eggs and embryos.

Gene identification in abalone has shown some success in recent years, e.g. Degnan, Degnan, Fentenany & Morse (1997) discovered homeobox genes expressed in larvae during metamorphosis, and Wang & Hanna (1998) report the isolation of an egg-laying hormone. Research is ongoing in this area at the University of Queensland for regulatory genes for growth and reproduction in *H. asinina* (E. O'Brien and B. Degnan, pers. commun.).

Selection

Despite the success of selective breeding programmes in many areas of primary production, the application of such technology for genetic improvement in aquaculture production, apart from Atlantic salmon, has been slow. There are few reports of research in this area for abalone (e.g. Jonasson, Stefansson, Gudnason & Steinarsson 1997; J. Jonasson, pers. commun.; Kawahara, Noro & Omori 1997a; I. Kawahara, pers. commun.). However, a number of studies have assessed abalone growth in culture and suggested that there are genetic factors involved in growth and that selective breeding could improve this trait (e.g. Hara 1990; Kobayashi & Fujio 1994, 1996).

Third-generation mass selection for faster growth in *H. discus hannai* produced a 21% increase in daily growth rate in animals of shell size 20–30 mm and 65% increase in those from 30 to 70 mm, compared with the maximum growth of control commercial animals (Hara & Kikuchi 1992). As well as demonstrating significant increases (10–15% per generation) in growth in *H. discus hannai* through selection, Kawahara, Noro, Omori, Hasekura & Kijima (1997b) also established a significant correlation between shell length at the juvenile stage (13 months) and shell length and whole body weight at 3.5 years, thus potentially enabling selection to occur at an early production stage.

In Iceland, a large research programme on the red abalone *H. rufescens* was started in 1996 to study genetic variation in survival, growth rate, meat yields and age at maturity (Jonasson *et al.* 1997; J. Jonasson, pers. commun.). They have produced 100 families using a hierarchical mating system of one male to 2–6 females. Results to date have returned heritabilities (genetic proportion of the observed variation) of 0.11 for survival at 4 months of age, 0.08 for shell length at 8 months (≈ 13 mm) and a very promising value of 0.30 for shell length after 17 months growth (≈ 36 mm). The results suggest that these traits could be improved through selection in abalone.

To understand fully the genetic variation present for various economic traits, it is necessary to establish and evaluate family lines for the species of interest. To achieve this, hatchery and nursery facilities need to be established to allow the growout to tagging size of individual crosses. Once animals are individually tagged, they would be grown under commercial conditions at various farms and industry-nominated traits evaluated. Such facilities could be at commercial or research sites; either way, research lines need to be established and their integrity maintained. An alternative, and more expensive, method of producing the families would be to use pedigree analysis (using genetic markers,

such as microsatellites) of progeny from commercial mass spawnings. However, it is highly likely that future breeding programmes will be based on family lines and, therefore, it is suggested that the establishment of commercial and research facilities enabling the production of family lines of abalone would be the most suitable and beneficial first step.

What is the future?

The long-term future for genetic improvement in abalone culture is the production of various strains of pure species and hybrids, produced and maintained by a combination of biotechnology (ploidy and gene manipulation) and selection programmes (including the use of genetic markers to assist in broodstock selection). Gene manipulation must be seen as a long-term possibility for the industry, whereas improvements through well-designed selection programmes and the production of triploids could provide production gains for the industry within one or two generations.

I recommend that a genetic improvement project for abalone be designed with the aim of providing production gains through the establishment of genetically improved strains. Specific objectives would include the establishment and evaluation of family lines and the evaluation on a commercial scale of triploid individuals. At the same time, research in the area of gene manipulation and the development and application of molecular genetic markers for future marker-assisted broodstock selection should be progressed.

Progress with such a genetic improvement programme for abalone requires strong national industry collaboration. The issue of ownership and protection of technology and/or selected/modified lines needs to be clarified. This is an issue not specific to abalone, but to all aquaculture species under genetic improvement.

A genetic improvement programme is not to be considered as a one-off piece of research, but rather an industry programme providing continual and long-term returns to the industry. The Australian industry is at a suitable stage of development, where it should be commencing a genetic improvement programme to ensure its long-term viability by embracing traditional and modern technology to provide significant and continued production gains.

Acknowledgments

I express my gratitude to the following for access to unpublished material, advice or support in preparing this paper: Jonas Jonasson (Stofnfiskur Ltd, Iceland), Ikue Kawahara (Iwate Fisheries Technology Center, Japan), Hiroshi Hoshikawa (Hokkaido Central Fisheries), Xiaxou Li (SARDI), Regina Counihan, Liz O'Brien and Bernie Degnan (University of Queensland) and Brad Evans, Bob Ward and Graeme Dunstan (CSIRO Marine Research). I also acknowledge the Fisheries Research and Development Corporation and the Aquaculture Development Council of Western Australia for sponsoring my attendance at the Genetics in Aquaculture meeting in Perth, Western Australia.

References

Anonymous (1997) Genetics turned on abalone. *Fish Farming International* **24, June 1997,** 6.

Aqua Bounty Farms Update (1996) **1** (1). June 1996, 4.

Arai K., Naito F., Sasaki H. & Fujino K. (1984) Gynogenesis with ultraviolet ray irradiated sperm in the Pacific abalone. *Bulletin of the Japanese Society of Scientific Fisheries* **50,** 2019–2023.

Arai K., Naito F. & Fujino K. (1986) Triploidization of the Pacific abalone with temperature and pressure treatments. *Bulletin of the Japanese Society of Scientific Fisheries* **52,** 417–422.

Brown D. (1997) *Australian Aquaculture: Industry Profiles for Selected Species. ABARE Report to the Fisheries Resources Research Fund.* Australian Bureau of Agricultural and Resource Economics, Canberra. Research Report 97.3.

Brown L.D. (1991) Genetic variation and population structure in the blacklip abalone, *Haliotis rubra*. *Australian Journal of Marine and Freshwater Research* **42,** 77–90.

Brown L.D. (1995) Genetic evidence for hybridisation between *Haliotis rubra* and *H. laevigata*. *Marine Biology* **123,** 89–93.

Brown L.D. & Murray N.D. (1992a) Genetic relationships within the genus *Haliotis*. In: *Abalone of the World* (ed. by S.A. Shepherd, M.J. Tegner & S.A. Guzman Del Proo), pp. 19–23. Blackwell Scientific Publications Ltd, Oxford.

Brown L.D. & Murray N.D. (1992b) Population genetics, gene flow, and stock structure in *Haliotis rubra* and *Haliotis laevigata*. In: *Abalone of the World* (ed. by S.A. Shepherd, M.J. Tegner & S.A. Guzman Del Proo), pp. 24–33. Blackwell Scientific Publications Ltd, Oxford.

Counihan R., Preston N., Baule V. & Degnan B. (1997) Analysis of abalone and *Drosophila* gene promoters electroporated into eggs and zygotes of the tropical

abalone *Haliotis asinina* [abstract]. Third International Abalone Symposium, Monterey, California, October 1997.

Curatolo A. & Wilkins N.P. (1995) Induzione della triploidia per mezzo della pressione idrostratica nell'abalone giapponese (*Haliotis discus hannai*) [abstract]. *Biologia Marina Mediterranean* **2,** 315–316.

Degnan B.M., Degnan S.M., Fentenany G. & Morse D.E. (1997) A mox homeobox gene in the gastropod mollusc *Haliotis rufescens* is differentially expressed during larval morphogenesis and metamorphosis [abstract]. *FEBS Lett* **411,** 119–122.

FAO (1998) *1987–96 Aquaculture Production Statistics.* FAO Fisheries Circular no. 815. Revision 10. FAO, Rome.

Fujino K. (1992) Review of genetics and stock management of Pacific abalone. In: *Abalone of the World* (ed. by S.A. Shepherd, M.J. Tegner & S.A. Guzman Del Proo), pp. 491–503. Blackwell Scientific Publications Ltd, Oxford.

Fujino K., Arai K., Iwadare K., Yoshida T. & Nakajima S. (1990) Induction of gynogenetic diploid by inhibiting second meiosis in the Pacific abalone. *Nippon Suisan Gakkaishi* **56,** 1755–1763.

Gaffney P.M., Rubin V.P., Hedgecock D., Powers D.A., Morris G. & Hereford L. (1996) Genetic effects of artificial propagation: signals from wild and hatchery populations of red abalone in California. *Aquaculture* **143,** 257–266.

Hahn K.O. (1989) *Handbook of Culture of Abalone and Other Marine Gastropods.* CRC Press, Boca Raton, Florida.

Hahn K.O. (1994) Gametogenic cycle of the Japanese abalone (ezoawabi), *Haliotis discus hannai,* during conditioning with effective accumulative temperature. *Aquaculture* **122,** 227–236.

Hara M. (1990) The effect of genetics on growth in three groups of abalone seeds. *Bulletin of the Tohoku National Fisheries Research Institute* **52,** 73–77.

Hara M. & Kikuchi S. (1992) Increasing the growth rate of abalone, *Haliotis discus hannai,* using selection techniques. *NOAA Technical Report NMFS* **106,** 21–26.

Hoshikawa H., Sakai Y. & Kijima A. (1998) Growth characteristics of the hybrid between pinto abalone, *Haliotis kamtschatkana* Jonas, and ezo abalone. *H. discus hannai* Ino, under high and low temperature. *Journal of Shellfish Research* **17,** 673–677.

Huang B. & Hanna P.J. (1998) Identification of three polymorphic microsatellite loci in blacklip abalone, *Haliotis rubra* (Leach), and detection in other abalone species. *Journal of Shellfish Research* **17,** 795–799.

Huang B., Chai Z., Hanna P.J. & Gough K.H. (1997) Molecular sequences of two minisatellites in blacklip abalone *Haliotis rubra*. *Electrophoresis* **18,** 1653–1659.

Jarayabhand P. & Paphavasit N. (1996) A review of the culture of tropical abalone with special reference to Thailand. *Aquaculture* **140,** 159–168.

Jonasson J., Stefansson S.E., Gudnason A. & Steinarsson A. (1997) Genetic variation for survival and body size during the first life stages of red abalone, *Haliotis rufescens,* in Iceland [abstract]. Third International Abalone Symposium, Monterey, California, October 1997.

Kawahara I., Noro T. & Omori M. (1997a) Growth characteristics of abalone, *Haliotis discus hannai*: an examination of genetic variation and interaction of family groups with different ambient temperatures [abstract]. Third International Abalone Symposium, Monterey, California, October 1997.

Kawahara I., Noro T., Omori M., Hasekura O. & Kijima A. (1997b) Genetic progress for growth in different selected populations of abalone, *Haliotis discus hannai,* at different hatcheries. *Fish Genetics and Breeding Science* **25,** 81–90.

Kawauchi H. (1991) Characterization of abalone growth hormone-like protein [abstract]. *Program and Abstracts Second International Marine Biotechnology Conference, Baltimore, Maryland, October 1991,* p. 66.

Kawauchi H. & Moriyama S. (1991) Characterization of abalone growth hormone-like protein. In: *Short Communications of the 1991 International Marine Biotechnology Conference,* Vol. II. Developments in Industrial Microbiology. Extended Abstract Series, Wm. C. Brown, pp. 535–541. [Cited in Taylor B.E., Donovan D.A., McLean E., Donaldson E.M. & Carefoot T.H. (1996) Effect of recombinant vertebrate growth hormones on growth of adult abalone, *Haliotis kamtschatkana. Aquaculture* **140,** 153–158.).

Kirby V.L., Villa R. & Powers D.A. (1998) Identification of microsatellites in the California red abalone, *Haliotis rufescens. Journal of Shellfish Research* **17,** 801–804.

Kobayashi M. & Fujio Y. (1994) Genetic study on metric traits of the Pacific abalone *Haliotis discus hannai. Tohoku Journal of Agricultural Research* **44,** 59–66.

Kobayashi M. & Fujio Y. (1996) Genetic study on shell shape and growth-related traits in the Pacific abalone *Haliotis discus hannai. Tohoku Journal of Agricultural Research* **46,** 141–147.

Kudo M., Arai K. & Fujino K. (1991) Triploidization of *Haliotis diversicolor diversicolor* by cold shock. *Nippon Suisan Gakkaishi* **57,** 1263–1267.

Landau M. (1992) *Introduction to Aquaculture*. John Wiley & Sons, New York.

Leighton D.L. & Lewis C.A. (1982) Experimental hybridization in abalones. *International Journal of Invertebrate Reproduction* **5,** 273–282.

Mgaya Y.D., Gosling E.M., Mercer J.P. & Donlon J. (1995) Genetic variation at three polymorphic loci in wild and hatchery stocks of the abalone, *Haliotis tuberculata* Linnaeus. *Aquaculture* **136,** 71–80.

Moriyama S., Atsuta S., Kobayashi M. & Kawauchi H. (1989) Growth hormone-like substance of abalone *Haliotis discus hannai*. In: *XIth International Symposium on Comparative Endocrinology,* Malaga, 14–20 May 1989

(ed. by A. Epple, C.G. Scanes & M.H. Stetson). [Cited in Taylor B.E., Donovan D.A., McLean E., Donaldson E.M. & Carefoot T.H. (1996) Effect of recombinant vertebrate growth hormones on growth of adult abalone, *Haliotis kamtschatkana*. *Aquaculture* **140**, 153–158].

Morse D.E. (1981) *Biochemical and Genetic Control of Critical Physiological Processes in Molluscan Life-Cycles. Basic Mechanisms, Water-Quality Requirements and Sensitivities to Pollutants*. University of California Press, La Jolla, CA. [Cited in Taylor B.E., Donovan D.A., McLean E., Donaldson E.M. & Carefoot T.H. (1996) Effect of recombinant vertebrate growth hormones on growth of adult abalone, *Haliotis kamtschatkana*. *Aquaculture* **140**, 153–158].

Moss G.A. (1998) Effect of temperature on the breeding cycle and spawning success of the New Zealand abalone, *Haliotis australis*. *New Zealand Journal of Marine and Freshwater Research* **32**, 139–146.

Muchmore M.-E., Moy G.W., Swanson W.J. & Vacquier V.D. (1998) Direct sequencing of genomic DNA for characterization of a satellite DNA in five species of Eastern Pacific abalone. *Molecular Marine Biology and Biotechnology* **7**, 1–6.

Oakes F.R. & Ponte R.D. (1996) The abalone market: opportunities for cultured abalone. *Aquaculture* **140**, 187–195.

Okumura S., Furukawa S., Sugie T., Sekimiya D., Toda A. & Yamamori K. (1996) Triploid larvae of Pacific abalone, *Haliotis discus hannai* (Archaeogastropoda: Haliotidae) induced by suppression of meiotic division using caffeine treatment. *Chromosome Information Service* **61** (4).

Powers D.A., Kirby V.L., Cole T. & Hereford L. (1995) Electroporation as an effective means of introducing DNA into abalone (*Haliotis rufescens*) embryos. *Molecular Marine Biology and Biotechnology* **4**, 369–375.

Powers D.A., Kirby V. & Gomez-Ghiarri M. (1996) Genetic engineering abalone: gene transfer and ploidy manipulation. *Journal of Shellfish Research* **15**, 477.

Shepherd S.A. & Hearn W.S. (1983) Studies on southern Australian abalone (genus *Haliotis*). IV. Growth of *H. laevigata* and *H. ruber* (*sic*). *Australian Journal of Marine and Freshwater Research* **34**, 461–475.

Sin F.Y.T., Mukherjee U.K., McKenzie J.C. & Sin I.L. (1995) Electroporation of abalone sperm enhances sperm–DNA association. *Journal of Fish Biology* **47** (Suppl. A), 20–28.

Smith P.J. & Conroy A.M. (1992) Loss of genetic variation in hatchery-produced abalone, *Haliotis iris*. *New Zealand Journal of Marine and Freshwater Research* **26**, 81–85.

Stepto N.K. & Cook P.A. (1998) Induction of triploidy in the South African abalone using cytochalasin B. *Aquaculture International* **6**, 161–169.

Sun Z., Song Z., Li N., Zhao Y. & Guan X. (1992) A preliminary study on the growth of triploid abalone (*Haliotis discus hannai* Ino) [abstract]. *Transactions of Oceanology and Limnology* **4**, 70–75.

Taylor B.E., Donovan D.A., McLean E., Donaldson E.M. & Carefoot T.H. (1996) Effect of recombinant vertebrate growth hormones on growth of adult abalone, *Haliotis kamtschatkana*. *Aquaculture* **140**, 153–158.

Tsai H.-P. & Chao N.-H. (1994) Cryopreservation of small abalone (*Haliotis diversicolor*) sperm – technique and its significance [abstract]. *Journal of the Fisheries Society of Taiwan* **21**, 347–360.

Tsai H.-J., Lai C.-H. & Yang H.-S. (1997) Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (*Haliotis diversicolor supor-texta*). *Transgenic Research* **6**, 85–95.

Wang L. & Hanna P.J. (1998) Isolation, cloning and expression of a DNA sequence encoding an egg-laying hormone of the blacklip abalone (*Haliotis rubra* Leach). *Journal of Shellfish Research* **17**, 789–793.

Wang Z., Zhang G., Wang Y., Wu L., Yang H., Zhu L., Chang Y., Xia F., Wang Q. *et al*. (1990) Triploidization of the Pacific abalone with cytochalasin B and temperature shock [abstract]. *Journal of Dalian Fisheries College* **5**, 1–8.

Zhang G., Wang Z., Chang Y., Song J., Ding J., Wang Y. & Wang R. (1998) Triploid induction in Pacific abalone *Haliotis discus hannai* Ino by 6-dimethylaminopurine and the performance of triploid juveniles. *Journal of Shellfish Research* **17**, 783–788.

Aquaculture Research, 2000, **31**, 61–67

A review of genetic improvement in growth rate in redclaw crayfish *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae)

C M Jones[1], C P McPhee[2] & I M Ruscoe[1]

Queensland Department of Primary Industries, [1]Freshwater Fisheries and Aquaculture Centre, Walkamin, Q 4872, Australia, and [2]Animal Research Laboratories, Yeerongpilly Q 4105, Australia

Correspondence: C M Jones, Principal Fisheries Scientist, Department of Primary Industries, Freshwater Fisheries and Aquaculture Centre, Kennedy Highway, Walkamin Q 4872, Australia

Abstract

This paper provides a review and summary of strain comparison and selective breeding work carried out on redclaw crayfish *Cherax quadricarinatus* (von Martens) at the Queensland Department of Primary Industries, Freshwater Fisheries and Aquaculture Centre, Walkamin. Redclaw crayfish are a highly marketable, environmentally tolerant, freshwater crayfish with a moderately fast, but variable, growth rate. Five strains of the species were assessed morphologically and in terms of age at maturity. A non-replicated production trial was also carried out before two strains, from the Gilbert and Flinders rivers in North Queensland, were chosen to be part of an experimental selective breeding programme for improved growth. Initially, 14 families of each strain were randomly mated and grown out. At this point, a size-related selection took place, with the largest animals from each family forming a selected line, and individuals from around the mean forming a control line. Within-family selection and reciprocal mating between families was performed. Data were collected after each of two generations of growout and analysed by analysis of variance. Significant differences ($P < 0.05$) were measured between strains, sexes and between selected and control lines. Selected individuals grew 9.5% faster than the controls. Recommendations for selective breeding research involving freshwater crayfish are presented.

Introduction

Redclaw *Cherax quadricarinatus* (von Martens) is a tropical species of freshwater crayfish native to the ephemeral catchments of the Gulf of Carpentaria in northern Australia.

Research by Jones (1990) and Jones & Ruscoe (1996) indicates that redclaw's aquaculture attributes are comprehensive. It is a gregarious and non-aggressive species, amenable to stocking at relatively high densities, with no significant difference in survival up to 15 m^{-2} (Jones & Ruscoe 1996). As a result of its extreme natural environment, it is physically robust with broad tolerances to environmental variables. In common with other freshwater crayfish species, it has favourable reproductive characteristics, including moderate fecundity, simple life cycle and maternal incubation of eggs through to hatching of highly developed juveniles. Growth rate is good compared with other freshwater crayfish and will allow market-size animals, approaching 100 g, to be grown within 12 months. Redclaw will perform well in earthen ponds on a relatively low-protein, and therefore low-cost, supplemental diet. Redclaw is highly marketable, with strong demand expressed for this type of crustacean in both domestic and export markets.

Commercial redclaw aquaculture was initiated in Queensland in 1987, and the industry has grown slowly as farming technologies have been developed. There are currently around 35 commercial

farms, collectively generating ≈60 tonnes of live product per year (Lobegeiger 1998). This represents a value of just over $AUS 800 000 based on a farmgate price averaging $13–14 per kilogram. The major difficulty the industry faces is lack of production volume and, consequently, an inability to exploit lucrative export market opportunities.

Genetically improving redclaw's growth rates would benefit individual enterprises and attract greater industry investment through an increase in profitability. Faster growth rate or larger size for age will provide a shorter production period, or alternatively, the option of growing stock to a larger size over a fixed period, both of which will increase profitability. Economic assessment (Hinton & Jones 1997) indicates that redclaw bioeconomics are sensitive to growth rate or time to reach market size and, consequently, growth rate has been identified as the most important characteristic on which to investigate genetic improvement.

Other traits, such as increasing the size at maturity, are also of interest and may benefit from a genetic selection programme. An increase in size at maturity would be advantageous by minimizing reproduction in growout ponds and reducing resource competition between generational cohorts. At this time, however, genetic improvement efforts for redclaw have been confined to growth rate. In 1993, a strain evaluation project was undertaken, which was followed by experimental (1994) and commercial-scale (1997) selective breeding programmes. This paper describes past and current research and development activities applied to genetic selection of redclaw to improve growth rate.

Strain evaluation

The origin of the redclaw stocks now present on farms throughout Queensland is unknown, but is believed to represent several of the Gulf of Carpentaria catchments. Initiation of a redclaw genetic improvement programme was based on comparison of natural populations (strains) of redclaw within that distribution (Jones & Ruscoe 1996). The greatest abundance and diversity of redclaw is within the five major catchments at the base of the Gulf of Carpentaria, which lie primarily in Queensland. From east to west, these are the Mitchell, Gilbert, Flinders, Leichhardt and Gregory rivers. Although the natural distribution of redclaw extends considerably farther into far north Queensland and into the Northern Territory, several studies (Herbert 1987; Austin 1996) and unpublished survey information indicate that stocks in these areas were precocious breeders and displayed a small maximum size, and were therefore less suitable for aquaculture.

Stocks representing the five major catchments were assessed at the Freshwater Fisheries and Aquaculture Centre, Walkamin, through a programme of captive reproduction and pond growout. One 1000-m^2 pond of each strain was identically stocked and managed. In the absence of replication, production performance data were viewed as indicative only. Nevertheless, morphological and reproductive characteristics, including size at maturity and fecundity, were based on hundreds of individuals of each strain. Several methods have been used to assess the separateness of discrete redclaw strains, including linear and meristic morphometrics and biochemical genetic methods (Macaranas, Mather, Hoeben & Capra 1995; Austin 1996). The present study concurred with those of Austin (1996) and Macaranas *et al.* (1995), in that there was substantial variation in characteristics within strains but little variation between strains. Morphologically, there was significant sexual dimorphism within each strain, with mature females having longer, wider and deeper tails than the males ($P < 0.05$). Males have longer and wider chelae ($P < 0.05$).

Three methods were used to determine size at sexual maturity: broken-stick analysis on morphological characteristics; ovarian development and red patch attainment in males. The estimates based on these methods were so variable that useful comparison between strains proved impossible, highlighting the lack of confidence when using a single variable as a determinant of maturity.

Allozyme electrophoresis in redclaw (Macaranas *et al.* 1995) revealed a high degree of homogeneity, typical of many crustaceans (Busack 1988). Craig & Wolters (1988) demonstrated the genetic base and significant heritability of several important traits from different populations of the crayfish *Procambarus clarkii* (Girard), despite the homogeneous nature of its allozymes (Busack 1988). Based on that evidence, there was cause for some optimism that the measured differences in production characteristics in redclaw had a genetic base and could therefore be selected for. The production characteristics were markedly different between strains. The Flinders river strain displayed a 38% greater yield than the next closest strain. Population

structures at harvest were generally similar. However, the Gilbert river strain yielded a greater proportion of larger, more valuable animals in its size frequency distribution. The Flinders and Gilbert river strains in particular had the most favourable attributes for aquaculture. They were both chosen for subsequent selective breeding.

Captive breeding programme for selection within strains

The captive breeding programme was started in 1994 with funding from the Cooperative Research Centre for Aquaculture Ltd. Its primary objectives were to develop selection procedures and obtain a measure of heritability of growth rate. Full methods and results are presented in McPhee & Jones (1997) and are summarized below.

Facilities used to operate the breeding programme included a system of tanks used as breeding chambers, supplied with recirculating water, which was necessary for controlled matings and incubation. As it was not always possible to run this programme in synchrony with natural reproductive seasonality, the system allowed for heating of the water and artificial increase in day length and, on this basis, captive reproduction on demand.

In addition to the tank system, an earthen pond was used for the production of juveniles (in 1994 and 1995) and the growout phases. Redclaw growth and survival in tank systems is always poor (Jones 1995) and, despite a concerted effort, this was also the case in the first breeding season of the selection programme. The provision of a pond environment in all subsequent seasons permitted good growth and survival, which were equivalent to best practice commercial operations (Jones & Ruscoe 1996).

To maintain discrete families and genetic lines, pens were established in the pond. These consisted of heavy-duty plastic mesh enclosures, the design and specification of which evolved considerably over the life of the project.

Within-family selection (Falconer & Mackay 1996) and mating between families was performed. Individuals hatched in the same tank/pen were defined as family members, as they were more likely to be related than individuals from different tanks/pens. Selection was based on the weight difference between an individual and the family mean. This minimized the effect of environmental differences between tanks/pens, which might have obscured the weight differences between individuals from different pens and increased the proportion of phenotypic variation attributable to genetic variation. The same number of individuals from each family was selected to ensure that all the parents of one generation had the same chance of leaving offspring in the next generation. Reciprocal (male/female) mating of animals selected from one family with those selected from another was applied to reduce the chance of mating related animals, and thus reduce inbreeding in the short term. The prospects of gain from selection were seen as good because of the high phenotypic variability of size at age within the pens (CV = 38%; McPhee & Jones 1997).

Gilbert and Flinders river strains were maintained as discrete lines. In the first year, crayfish were mated randomly within family groups, and the juveniles were grown out to maturity. At this point, the first selection took place, whereby the largest stock were separated to form a selected line, and those from around the mean size were separated to form a control line. These two lines, selected and control, were then mated independently in the second year, and juveniles were again produced and grown out to maturity. A second mating of the selected and control lines was achieved, and their progeny were grown out to maturity before the project finished. At this point, for each strain, there were 10 families from the selected lines and four from the control.

Reproduction involving the mating of adults was managed in the tanks (Table 1). On average, four males and 10 females were stocked per tank and left to mate over 3 weeks. The second stage, involving incubation of eggs, then proceeded by holding the berried females in the tanks. On average, 70% of females mated successfully and produced eggs. Males and unberried females were removed, and the berried females were maintained in the tanks for around 3 weeks to allow eggs to incubate to within 2–3 weeks of hatching. Stage three involved the transfer of the berried females to the pond pens, where hatching and juvenile release could occur without disturbance. Juveniles were left for 3 months in the pens to progress to a robust size of around 5–10 g before they were harvested. Generally, in excess of 500 juveniles were retrieved from each pen, representing ≈ 70 advanced juveniles for each female stocked. Of the juveniles harvested, 150 from around the mean size for each cage were returned to the cage for growout. These

Table 1 Stages and their respective details for each generation of a selective breeding programme for increased growth rate in redclaw

Stage	Process	Facility	Statistics	Time
Reproduction	Matings	Tanks	Four male and 10 female/tank	3 weeks
Incubation	Holding berried females	Tanks	Seven females/tank	3 weeks
Juvenile release	Stocking berried females to pens	Pond pens	Seven females/pen	1 week
Juvenile production	Harvest advanced juveniles/parents	Pond pens	500+/pen	3 months
Growout	Stock juveniles	Pond pens	150/pen	4 months
Maturation	Harvest adults	Pond pens	100/pen	1 day

Statistics provided are averages over all tanks/pens and over two selected generations.

Table 2 Comparison of weights of redclaw on the basis of river stock, selection line and sex after two generations of selective breeding, as reported by McPhee & Jones (1997)

	Comparison	Weight (g) (at 7 months)	Difference (%)
Stock	Flinders vs. Gilbert	46.3 vs. 43.1	7.4
Line	Selected vs. Control	46.7 vs. 42.7	9.5
Sex	Male vs. female	47.7 vs. 41.7	14.4

All differences were significant at the 0.05 level.

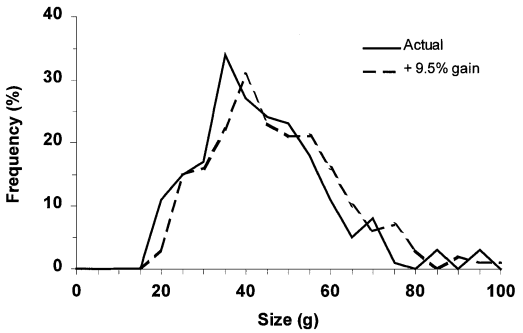


Figure 1 The result of a simulated 9.5% increase in weight applied to each crayfish from actual harvest data. Original data represent a harvest density of 12.5 m^{-2} from a 6-month growout period.

were left for 4 months to grow to maturity, at which time around 100 adults were retrieved from each cage, from which the breeding stock was chosen for the next generation.

Data were collected and combined for generations two and three and analysed by analysis of variance with effects being river strain, line, sex and generation. Body weight was corrected by covariance for number harvested per family, and error was estimated from family means. Significant differences ($P < 0.05$) in weight at harvest between the two river strains, and between male and female, were measured. However, the most important significant difference was that measured between the selected and control lines, which represented a 9.5% increase in size at age (Table 2). The selection differential and selection response were 19.4 ± 1.1 g and 4.7 ± 1.9 g. The ratio of the latter over the former gave a measure of realized heritability (Hadley, Dillon & Manzi 1991) of 0.24 ± 0.06. That is, 24% of the variability in growth rate was of genetic origin. This is at the low end of the range of values reported previously for redclaw (Gu, Mather & Capra 1995) and other crustaceans (Carr, Fjalestad, Godin, Swingle, Sweeney & Gjedrem 1996; Benzie, Kenway & Trott 1997; Benzie 1998). However, because of a variety of constraints through this programme, there is some likelihood that a higher value will be measured in subsequent breeding programmes.

Figure 1 is a diagrammatic representation of this 9.5% increase in growth, as applied to individuals from a typical commercial crop at harvest. It should be noted, however, that variance would probably increase with an increase in mean size, and the figure is therefore indicative only. However, simplistically applying this predicted size distribution to the size class price structure indicates that a 12.5%

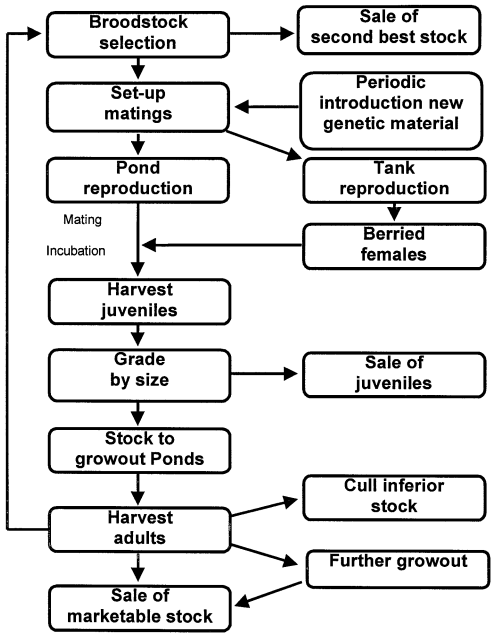


Figure 2 Flowchart of processes for the managed aquaculture of redclaw involving selective breeding by farmers.

increase in crop value would result. This occurs as smaller individuals move into larger, more valuable size classes.

Constraints during the captive breeding programme

A number of constraints occurred in the initial breeding programme, which may provide valuable lessons for future breeding programmes involving freshwater crayfish. The first was the escape of stock from pond pens and, therefore, the potential of genetic leakage between strains and between lines. Owing to their propensity for climbing and their ability to move out of water, redclaw are difficult to contain. The pen design used evolved considerably as the project progressed to minimize the extent of escape. By using a robust mesh of < 2 mm, escape through the mesh was eliminated. Furthermore, a 100-mm-diameter PVC pipe barrier around the top margin of the cage prevented escape over the top.

A second constraint was the small size of the original parent population. It numbered less than 150 parents of each strain. For our short-term research purposes, this was sufficient. However, in consideration of potential production of improved stock for transfer to industry, the medium- to long-term potential for inbreeding depression is high (Tave 1986).

Exacerbating the small breeding base was poor survival of juveniles from the original matings, when juvenile production was managed in the tank system. Jones (1995) has indicated that, in order to attain reasonable survival and growth of juveniles, the production should be managed in a pond environment, where production of zooplankton in particular can be maximized as a food source for the juveniles.

Recommendations

On the basis of our experience, we can now declare some fundamental requirements for selective breeding research for freshwater crayfish. First, the nature of the facilities used is of paramount importance. These facilities need to provide sufficient capacity for a breeding base of substantial genetic breadth, and they need to be sufficiently secure to prevent escape or mixing and to eliminate predation.

Specifically, we recommend that matings be managed in a tank system, where appropriate control of both environment and stock can be applied, but that juvenile production and growout be performed in pond facilities, where growth and survival are optimized. The specifications and design of pen enclosures are particularly important.

The advantages of producing juveniles in a pond environment rather than in a tank have been identified clearly in our work to date. Although hatch rates in tank systems are very high, the attrition rate through to a size of 5–10 g is unacceptably high, because of the high densities (up to $50\,m^{-2}$) and cannibalism during frequent moulting (Jones 1995). Handling small juveniles to transfer them to alternative production facilities also results in high mortalities. The best option is to generate berried females in a tank system and transfer them to a pond environment before hatching, allowing juveniles to release without disturbance and then to grow on to a robust size of 5–10 g.

From an industry perspective, the key to effective selective breeding is intensive stock management, particularly knowledge of the age of crayfish, which demands good record-keeping. The introduction of new genetic material on a regular basis is essential.

These recommendations and the procedures suggested are summarized in Fig. 2 and fully detailed in a breeding manual published for crayfish farmers (Jones, McPhee & Ruscoe 1998).

Hybridization and further selection

In 1997, a new breeding programme was initiated to build on the results of the previous one. The objectives are to cross the selected lines of the two strains, to minimize inbreeding and evaluate hybrid vigour. Furthermore, this programme aims to generate sufficient quantities of improved stock for transfer to industry.

However, because of serious reservations about the degree of relatedness in the stocks of the first breeding programme as a result of the constraints discussed, a new population base will be used. Approximately 500 crayfish (100 males and 400 females) representing unselected stocks of Flinders and Gilbert river strains will be reciprocally crossed to create a 'Walkamin' strain. Alongside these, an additional 500 crayfish (100 males and 400 females) representing domesticated commercial stocks from selected, well-managed farms will also be crossed to create a commercial strain, against which the Walkamin strain will be compared. The development of genetic markers (microsatellites) for redclaw to enable the establishment of marker-assisted selection programmes is progressing. Five microsatellites have been identified by colleagues at Queensland University of Technology and CSIRO, and it is envisaged that they will play an increasing role in future selection programmes.

Conclusion

Improvements in economically valuable traits of redclaw can be achieved through simple genetic selection. In a preliminary study, a 9.5% gain in growth rate was achieved within two selected generations. With the establishment now of a broader breeding base, more highly developed methodologies and facilities, crossing of strains and the application of genetic markers, further improvements may follow.

References

Austin C.M. (1996) Systematics of the freshwater genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia: electrophoretic and morphological variation. *Australian Journal of Zoology* **44,** 259–296.

Benzie J. (1998) Penaeid genetics and biotechnology. *Aquaculture* **164,** 23–47.

Benzie J., Kenway M. & Trott L. (1997) Estimates for the heritability of size in juvenile *Penaeus monodon* prawns from half-sib matings. *Aquaculture* **152,** 49–53.

Busack C.A. (1988) Electrophoretic variation in the red swamp (*Procambarus clarkii*) and white river (*P. astacus*) (Decapoda: Cambaridae). *Aquaculture* **69,** 211–226.

Carr W.H., Fjalestad K.T., Godin D., Swingle J., Sweeney J.M. & Gjedrem T. (1996) Genetic variation in weight and survival in a population of specific pathogen-free shrimp *Penaeus vannamei*. In: *World Aquaculture '96. Book of Abstracts.* World Aquaculture Society, Baton Rouge, USA.

Craig R.J. & Wolters W.R. (1988) Sources of variation in body size traits, dressout percentage and their correlations for the crayfish *Procambarus clarkii*. *Aquaculture* **72,** 49–58.

Falconer D.S. & Mackay T.F.C. (1996) *Introduction to Quantitative Genetics,* 4th edn. Longman Group, Essex, UK.

Gu H., Mather P. & Capra M.F. (1995). Juvenile growth performance among stocks and families of redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture* **134,** 29–36.

Hadley N.H., Dillon R.T. & Manzi J.J. (1991) Realized heritability of growth rate in the hard clam *Mercinaria mercenaria. Aquaculture* **93,** 109–119.

Herbert B. (1987) Note on *Cherax quadricarinatus* from the Jardine River, Cape York Peninsula. *Queensland Naturalist* **28,** 19–21.

Hinton A.W. & Jones C.M. (1997) Redclaw crayfish farming, an economic perspective. In: *Redclaw Crayfish Aquaculture. New Opportunities for the Atherton Tablelands. Choices Seminar Series 1*, pp. 2–10. Queensland Department of Primary Industries, Mareeba.

Jones C.M. (1990) *The Biology and Aquaculture Potential of the Tropical Freshwater Crayfish,* Cherax quadricarinatus. Queensland Department of Primary Industries Information Series QI90028. Queensland Department of Primary Industries, Brisbane.

Jones C.M. (1995) Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture* **138,** 221–238.

Jones C.M., McPhee C.P. & Ruscoe I.M. (1998) *Breeding Redclaw. Management and Selection of Broodstock.* Queensland Department of Primary Industries Information Series QI98016. Queensland Department of Primary Industries, Brisbane.

Jones C.M. & Ruscoe I. (1996) *Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*).* Final Report

FRDC Project 92/119. Fisheries Research and Development Corporation, Canberra.

Lobegeiger R. (1998) *Queensland Australia, Aquaculture Information Series. Aquaculture Production Survey 1996/97; Report to Farmers.* Queensland Department of Primary Industries, Brisbane.

Macaranas J.M., Mather P.B., Hoeben P. & Capra M.F. (1995) An investigation of the population genetics of the freshwater crayfish *Cherax quadricarinatus* using allozyme and RAPD-PCR markers. *Marine and Freshwater Research* **46,** 1217–1228.

McPhee C. & Jones C. (1997) Selection for weight gain in redclaw crayfish. In: *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12, 81**–84.

Tave D. (1986) *Genetics for Fish Hatchery Managers.* AVI Publishing Company, Westport CT.

Aquaculture Research, 2000, **31**, 69–82

Genetic improvement of marron *Cherax tenuimanus* Smith and yabbies *Cherax* spp. in Western Australia

C S Lawrence & N M Morrissy
Fisheries WA, WA Marine Research Laboratories, North Beach, Western Australia

Correspondence: C S Lawrence, Fisheries WA, WA Marine Research Laboratories, PO Box 20, North Beach, WA 6020, Australia

Abstract

Marron and yabbies are farmed commercially in Western Australia and are native and introduced species respectively. Genetic improvement of farmed stock for aquaculture can be achieved by choosing particular strains, by selective breeding or by producing hybrids of different strains. Studies into the taxonomy and growth of both marron and yabbies have shown variation between geographically isolated populations. This variation may offer potential for selecting strains that demonstrate desirable traits for aquaculture, such as improved growth rates. In addition to selection of wild strains that demonstrate desirable traits for aquaculture, further advances in production may be achieved by selection of characteristics in captive stock that are heritable. In marron, moderate proportions of the phenotypic variation in growth rate, tail and chelae size have been shown to be heritable ($h^2 = 0.3–0.6$). Recent research into yabby genetics involved assessing growth and reproductive characteristics of geographically isolated populations of yabbies inhabiting a range of ecotypes throughout Australia. Growth rates and size at sexual maturity varied among populations. However, there was no significant difference in the sex ratio between these geographically isolated groups. The main focus of the yabby genetics research has been to develop a method to prevent or reduce reproduction of yabbies. Uncontrolled reproduction in this species results in high densities and smaller animals because growth is density dependent. Preventing reproduction and thereby controlling densities in yabby ponds or dams can be achieved by stocking monosex populations. The stocking of monosex populations results in an estimated 70% increase in gross income. Hybidization of yabby populations was investigated as a method of controlling reproduction. Sterile hybrids were produced by crossing yabbies from geographically isolated populations. These experiments have also revealed a hybrid cross that consistently produces only male progeny. If this all-male hybrid strain performs as well as all-male monosex pond stock, achieved by manual sexing, the production of large, and therefore higher value, yabbies will be increased in the commercial aquaculture industry.

Introduction

Marron *Cherax tenuimanus* are a large freshwater crayfish native to the main permanent rivers in the forested, high-rainfall south-west of Western Australia. Their distribution has been extended by translocation to streams near Esperance and north of Geraldton. International interest in marron farming has led to introductions of this species to South Africa, Zimbabwe, Japan, USA, China, Chile and the Caribbean.

The marron is one of the largest freshwater crayfish in the world, and it has consequently been the subject of continued aquaculture interest (Lawrence 1998a). There are currently 140 marron farms in Western Australia producing 47 tonnes in 1996/97 and, recently, there has been a very rapid expansion in the number of farms. Research into the feeding, rearing and farming of marron has been undertaken by Fisheries WA (see Morrissy 1979, 1980, 1982, 1990, 1992a,b; Morrissy, Walker & Moore 1995). However, there is limited information on the genetic improvement of this highly sought after species.

The common name yabby is derived from one of the numerous aboriginal terms used to describe a group of freshwater crustaceans found in south-eastern and central Australia (Olszewski 1980). The potential of yabbies for aquaculture has received considerable interest, both within their natural distribution and in Western Australia where they were introduced in 1932 (Smallridge 1990; Brown, Van Landeghem & Schuele 1997; Lawrence 1998b).

The majority of yabby production is focused upon *Cherax albidus* in Western Australia and either *Cherax albidus* or *Cherax destructor* in central and Eastern Australia. The majority of yabby production comes from extensively managed farm dam populations. Current estimates suggest that around 4000 farm dams in Western Australia are harvested for yabbies, with an annual production of 107 tonnes in 1996/97. However, farm dam production fluctuates markedly depending on rainfall. In addition, as farm dams cannot be drained, there is no control over reproduction or population densities. A smaller proportion of yabby production comes from semi-intensive purpose-built ponds in South Australia, New South Wales, Victoria and Western Australia, which produced around 56 tonnes in 1996/97.

Although the taxonomy of yabbies and, to a lesser extent, marron has been reviewed a number of times, in general, these studies have focused upon whether different varieties of these animals are the same or separate species. (i.e. *Cherax albidus*, *C. destructor*, *C. cuspidatus*, *C. rotundus*). From an aquaculture perspective, it is perhaps more important to recognize that there are a number of 'strains' of yabbies and marron, some of which may be more suitable for farming than others.

Review of marron genetics research

Electrophoretic studies with marron provide evidence that different river populations are geographically isolated and genetically different (Austin & Knott 1996). More recently polymorphic microsatellite markers have been used to differentiate marron populations from several geographically distinct regions in Western Australia (Imgrund, Groth & Wetherall 1997).

A study comparing production characteristics of marron populations from four geographically isolated river systems in the south-west of Western Australia has shown genetic variation, which may be of use in selecting marron stocks for aquaculture (Henryon 1996).

In particular, marron from different rivers showed variation in growth rate (30%) and tail yield (10%) (Henryon 1996). Furthermore, marron from the Donnelly river grew 20% and 30% faster than those from the Warren and Deep rivers respectively (Henryon 1996). However, the slower growing marron populations (Warren and Deep rivers) had a tail yield that was 10% greater than the faster growing population (Donnelly) (Henryon 1996).

In a comparison of the breeding success of marron (i.e. percentage of females mated), the Margaret river population had a lower rate of breeding success (11%) than the other three populations (Warren, Deep and Donnelly rivers; 38–64%) (Henryon 1996).

Along with selection of strains that demonstrate desirable traits for aquaculture, further advances in production may be achieved by selection of characteristics that are heritable. In marron, a moderate proportion of the phenotypic variation in growth rate, tail and chelae size has been shown to be heritable ($h^2 = 0.3–0.6$) (Henryon 1996).

The genetic variation reported for marron highlights the potential to develop an improved commercial strain of this species for aquaculture. Increased economic returns can be achieved by selecting the population with the most suitable production characteristics (i.e. growth), and then further gains may be achieved by selective breeding for improved growth, tail and chelae yields (Henryon 1996). Use of the faster growing marron strain (Donnelly) results in a 40% and 80% increase in profit compared with Warren or Deep populations respectively (Henryon 1996). Furthermore, selection for faster growth in marron has a greater significance on profit than selection for tail or chelae yields (Henryon 1996).

In addition to strain selection, one method of improving growth is crossing populations to obtain hybrid vigour, or heterosis. However, population crosses for four strains of marron from geographically isolated river systems failed to detect heterosis for any of the production characteristics of growth, survival, tail yield or chelae size (Henryon 1996).

Marron genetics research at Fisheries WA

Based upon the results of previous studies and industry reports, Fisheries WA researchers have

expressed concern regarding the conservation of geographically isolated marron populations, particularly Margaret River, Donnelly River, Warren river (tiger) and Deep river (blue) marron strains. This is because of the current uncontrolled translocation of stocks between river systems and farms and is likely to result in hybridization between strains. In order to preserve the genetic integrity of marron stocks for conservation and future application in breeding programmes by commercial marron farms, a number of 'genetically pure' populations have been established at the Fisheries WA South-west Freshwater Research and Aquaculture Centre at Pemberton. These stocks may also potentially provide a basis for further studies into selection and cross-breeding of marron for aquaculture. In addition, a captive breeding programme has commenced at Pemberton to preserve the vulnerable Margaret River marron strain.

At the South-west Freshwater Research and Aquaculture Centre, pond trials comparing growth rates of Warren River (black) and blue marron cohorts are being completed. Preliminary results indicate that there is no difference in growth or size variation between blue and black marron.

Currently, commercial marron farms in WA predominantly stock faster growing marron. However, they have yet to establish formal selection and breeding programmes to maximize the genetic potential of this species. Future research directions for the Fisheries WA marron research programme include establishing a formal selective breeding programme for marron, comparing the faster growing Donnelly river marron with 'domesticated' commercial farm stock and evaluation of geographically isolated populations and hybrids of marron from the major river systems in the south-west of Western Australia in ponds similar to those in use by commercial producers.

Review of yabby genetics research

The 'yabby complex' has been the subject of ongoing taxonomic studies and debate since 1878, when it was reported that the nomenclature of Australian crayfish required a thorough revision (Huxley 1878). Over 100 years later, Sokol (1988) and subsequently Campbell, Geddes & Adams (1994) and Austin (1996) still felt that the taxonomy of some *Cherax* species was confused. In support of their argument, they highlighted the issue of the taxonomic validity of a number of the species of yabbies and, in particular, those that make up the 'destructor complex' (Sokol 1988; Campbell *et al.* 1994; Austin 1996).

Reik (1969) categorized *Cherax* in Australia into five broad groups: *Astaconephrops*, *quinquecarinatus*, *destructor*, *punctatus* and *dispar*. After removal of animals that originated from WA (*quinquecarinatus* group), northern Queensland and Northern Territory (northern members of the *dispar* group and *Astaconephrops* group), the remaining species from the *destructor* group, the *punctatus* group and the *dispar* group represent the 'yabby complex'. Within the 'yabby complex', a number of species have been described; these include *C. rotundus* (Clark 1941), *C. rotundus-setosus* (Reik 1951), *C. punctatus* (Clark 1936), *C. neopunctatus* (Reik 1969), *C. depressus* (Reik 1951), *C. cuspidatus* (Reik 1969), *C. dispar* (Reik 1951) and the 'destructor group' (Reik 1969) consisting of *C. destructor* (Clark 1936), *C. albidus* (Clark 1936), *C. davisi* (Clark 1941) and *C. esculus* (Reik 1956).

Since yabbies were originally described, a number of authors have subsequently redefined the synonymy of species within the yabby complex using morphology (Clark 1936, 1941; Reik 1951, 1969), quantitative morphology (Sokol 1988), serological testing of antigens (Clark & Burnet 1942), haemocyanin electrophoresis and immunochemistry (Patak & Baldwin 1984) and electrophoresis and morphology (Campbell *et al.* 1994; Austin 1996).

Consequently, the taxonomic validity of a number of the species and the categories originally described by Reik (1969) (Fig. 1), particularly those within the 'destructor complex', has recently been disputed by Sokol (1988) (Fig. 2), Campbell *et al.* (1994) and Austin (1996) (Fig. 3).

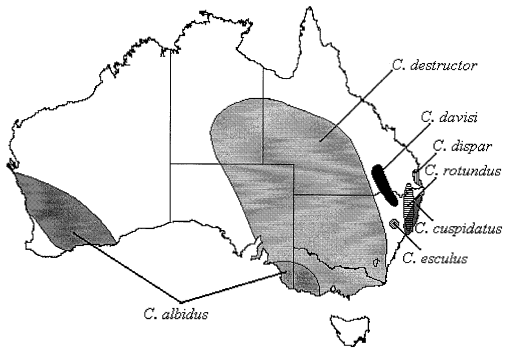


Figure 1 Distribution of yabbies according to Reik (1969).

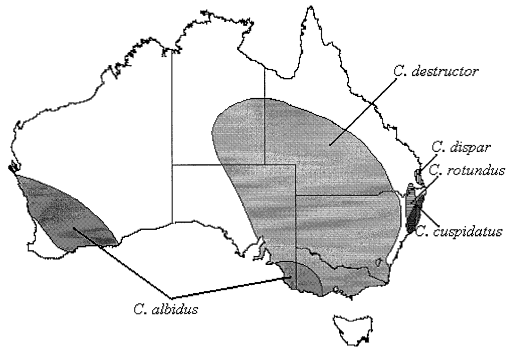


Figure 2 Distribution of yabbies according to Sokol (1988).

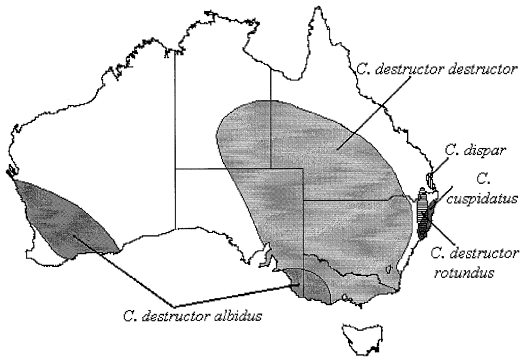


Figure 3 Distribution of yabbies according to Austin (1996).

It is clear that at least part of the confusion regarding the taxonomy of the 'yabby complex' may be attributed to the high degree of both morphological and electrophoretic variation reported (Austin 1996).

While we do not intend to enter the debate regarding the taxonomy of the yabby complex in this paper, what is clear from the results of previous studies is that, within the 'yabby complex', species, subspecies or strains exist that: (1) are separated by geographical barriers; (2) demonstrate morphological variation between geographically isolated groups; (3) may have variation in traits of interest to aquaculture (i.e. growth rate); and (4) may demonstrate full or partial reproductive isolation and, therefore, their hybrids may have some potential for aquaculture.

In contrast to the aforementioned taxonomic studies, relatively few researchers have compared production characteristics such as growth.

In a study comparing *Cherax destructor* from three geographically discrete populations, there was no marked variation in growth between populations (Geddes, Mills & Walker 1988). In contrast, a more recent study by Austin, Jones, Stagnitti & Mitchell (1997) has shown a significant difference in growth rates between two populations of *C. destructor* and *C. albidus*, with both the '*destructor*' populations growing faster than the '*albidus*' population.

International markets distinguish between *C. destructor* and *C. albidus* on the basis that the '*destructor*' yabby has a propensity to burrow deeply. Abundance and morphology of *C. albidus* burrows are currently being assessed in commercial ponds and dams in Western Australia (C S Lawrence, unpublished data).

Yabby genetics research at Fisheries WA

There is a large number of closely related yabby species, subspecies and strains in the wild, which are adapted to a very wide range of environmental conditions (desert to mountain) (Sokol 1988; Austin 1996). The type localities of the so-called *destructor* complex have been well documented (Clark 1936, 1941; Reik 1951, 1956, 1969; Sokol 1988). Based on the taxonomy of Reik (1969), within the broad distribution of yabbies, we identified those strains throughout Australia that were thought to offer the most potential for contributing to stock diversity (Lawrence, Morrissy, Bellanger & Cheng 1998). Yabby populations were selected for sampling according to the following factors:

(1) populations representative of the broad range of ecotypes of yabbies adapted to diverse environments;

(2) genetically 'pure' populations, as opposed to populations that are the result of recent translocations;

(3) putative species of *Cherax* that may have been isolated for sufficient time to have developed reproductive incompatibility with each other;

The yabby genetic stocks were collected during a 6-week expedition in September–October 1995. The yabbies were collected from environments as diverse as central Australian mound springs surrounded by desert, to the alpine Snowy Mountain region and warm-temperate northern NSW (Fig. 4).

We obtained 14 different populations of yabbies representing strains of both *Cherax destructor* and *Cherax albidus*, along with a number of closely related species, such as *C. dispar*, *C. rotundus*, *C. cuspidatus* and one as yet undescribed yabby

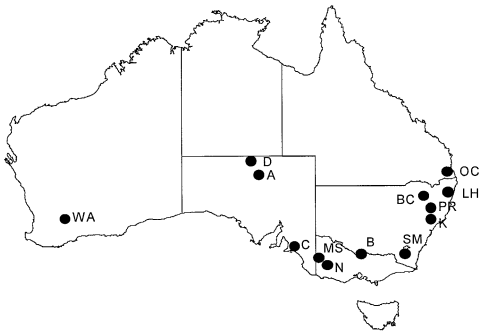


Figure 4 Collection localities. D, Dalhousie Springs; A, Algebuckina; C, Clayton; MS, Merwyn Swamp; N, Nurrabiel; B, Barmah Forest; SM, Murrumbidgee River; PR, Nundle, Peel River; BC, Barrack Creek; LH, Lake Hiawatha; OC, Oxley Creek, WA, Narembeen; K, the location and identity of this strain is currently under commercial caveat.

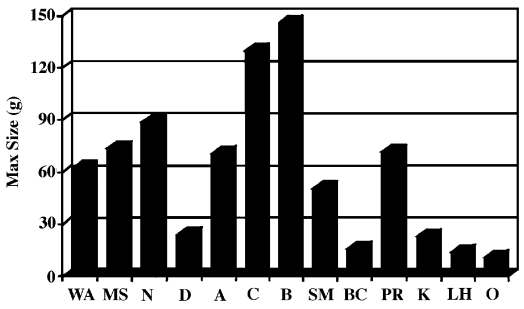


Figure 5 Maximum size of yabbies from collection localities.

thought to be a new species of *Cherax*. [Note: since the collecting expedition Austin (1996) has published a revised taxonomy of the yabby complex; see Figs 1–3].

The yabbies were transported alive to a newly established Reproduction and Genetics Laboratory in Perth provided by the University of Western Australia, constructed to quarantine standards, where they formed the basis of a gene pool for investigating the genetics and reproduction of *Cherax* spp. that make up the 'yabby complex'.

The size of yabbies from different localities throughout Australia

Most previous studies on the morphology of different 'varieties' of yabbies have concentrated upon the taxonomy of the genus *Cherax* using either morphological or biochemical variation to discriminate between species (Clark 1936, 1941; Reik 1951, 1969; Sokol 1988; Campbell *et al.* 1994; Austin 1996).

Species are often subdivided into local populations or demes, and substantial genetic differences may evolve among demes that have limited gene exchange (Hedgecock, Stelmach, Nelson, Lindenfelser & Malecha 1979). Previous studies have shown that isolated populations of freshwater crayfish of the same species (*Astacus astacus*) from the same ancestral population can show changes in morphology after being separated for less than a century (Fevolden & Hessen 1989).

From the yabby aquaculture industry's point of view, the major factor overlooked by previous taxonomic studies is whether the size of yabbies varies according to different species or geographical strains. Body weight is the main factor of importance to the aquaculture industry, as yabbies are graded according to weight and condition. Although weight grades and prices vary around Australia, yabbies below 30 g are considered by industry to be of no commercial value. Processors pay higher prices for larger yabbies, which reflects the demand for larger crayfish and the greater difficulty in producing large yabbies (Lawrence 1998b).

The maximum weight of yabbies from a number of collection localities was below 30 g, the minimum weight grade considered to be of commercial value (Fig. 5). Consequently, commercial harvesting of yabbies from these localities is unlikely to be economically viable.

The growth rates of yabby strains and hybrids

Previous studies have shown that geographically isolated species and strains of freshwater crayfish may show different growth rates. For example, a comparison of strains of redclaw *Cherax quadricarinatus* from four different regions has shown a variation in growth between stocks from different regions (Jones & Ruscoe 1996). Similarly, a study of marron *Cherax tenuimanus*, although based upon young animals, has shown that populations of this species have different growth rates, with faster growing strains showing up to 30% better growth than the slower growing populations (Henryon 1996).

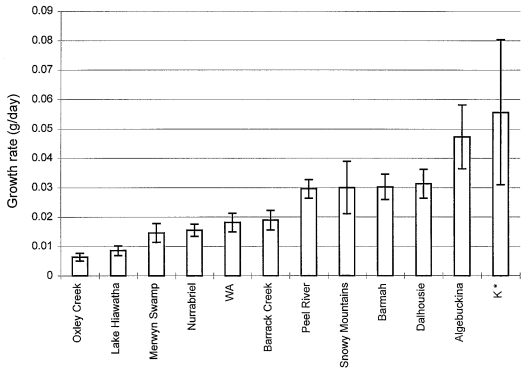


Figure 6 Growth rate ($g\,day^{-1}$) of cohorts from 12 populations of yabbies. K*, the location and identity of this strain is currently under commercial caveat.

Often, the aim of hybridizing two species is to produce a faster growing animal as a result of heterosis or hybrid vigour (Lutz 1997). In aquaculture, a number of species that have been hybridized, including tilapia, carp and catfish, have resulted in a faster growing animal because of heterosis (Tave, Smitherman, Jayaprakas & Kuhlers 1990; Thien & Trong 1995; Bakos & Gorda 1995; Rahman, Bhadra, Begum, Islam & Hussain 1995).

The growth rates of yabbies collected from different geographical regions in Australia were significantly different (Fig. 6). The strain K (*C. rotundus*) had the highest growth rate ($0.056 \pm 0.024\,g\,day^{-1}$), which was over nine times higher than the strain with the lowest growth rate, Oxley creek (*C. dispar*) ($0.006 \pm 0.001\,g\,day^{-1}$).

Strains with the lowest growth rates belong to the *dispar* group, whereas strains with the highest growth rates belong to the *rotundus* and *destructor* groups. The *destructor* group showed the greatest variation in growth rates, particularly between geographically isolated catchments. Within the *destructor* group, growth of the four strains from the Murray–Darling catchment was slower than the Algebuckina strain from the Lake Eyre catchment (Fig. 6)

Morrissy & Cassells (1992) hypothesized that yabbies in Western Australia were translocated from Merwyn Swamp, Victoria. This small founding population of 10 animals formed the basis of the highly successful yabby industry in Western Australia. The low initial number of animals has, however, led to claims that the yabbies in Western Australia are stunted because of inbreeding. In our experiment, there was no significant difference in the growth rate of *albidus* populations (*C. albidus*) from Merwyn swamp, Nurrabiel and Western Australia ($P = 0.67$) (Fig. 6). This supports the hypothesis of Morrissy & Cassells (1992) and contradicts the claims that stunting of yabbies in farm dams is caused by inbreeding.

The majority of the hybrids (nine hybrids) grew faster than both parent strains. Seven of the hybrids had growth rates intermediate between the parent strains. Two hybrids showed slower growth than either parent strain.

Genetic potential for controlling reproduction and density

In general, the major problem faced when farming many species of freshwater crayfish is the production of large numbers of small animals as a result of high uncontrolled densities (Avault, Bretonne & Huner 1975; Huner & Romaire 1979; Morrissy 1979; Francesconi, Bird, Fellows & Morrissy 1995; Jarboe & Romaire 1995)

As with many crustaceans, stunting of freshwater crayfish is related to population density; the higher the number of animals m^{-2}, the lower the growth rate and the smaller the average size becomes. This has been demonstrated in a number of crayfish species, including marron (*C. tenuimanus*) (Morrissy 1979, 1980; 1992b; Morrissy, Walker & Moore 1995), *O. virilis* (Brown, Wilson, Wetzel & Hoene 1995), crawfish (*P. clarkii*) (McClain 1994), *C. destructor* (Mills & McCloud 1983) and *C. albidus* (Lawrence *et al.* 1998).

In contrast to wild populations, in permanent water such as ponds or farm dams, population growth of yabbies is not controlled by mortality resulting from periodic drought. Consequently, yabbies can rapidly reach a population density at which individual growth rates are greatly reduced, as growth is density dependent, with a resultant low number of market-size animals (Lawrence *et al.* 1998).

In farm dams and ponds, yabbies *Cherax albidus* have multiple spawnings from early spring to midsummer. Yabbies spawn so readily that there is generally no need to purchase juveniles, with most farms producing more stock than they require. In fact, the major problem faced by yabby farmers is the production of large numbers of animals that are below market size (< 30 g) (Francesconi *et al.* 1995; Lawrence 1998b). This is particularly significant

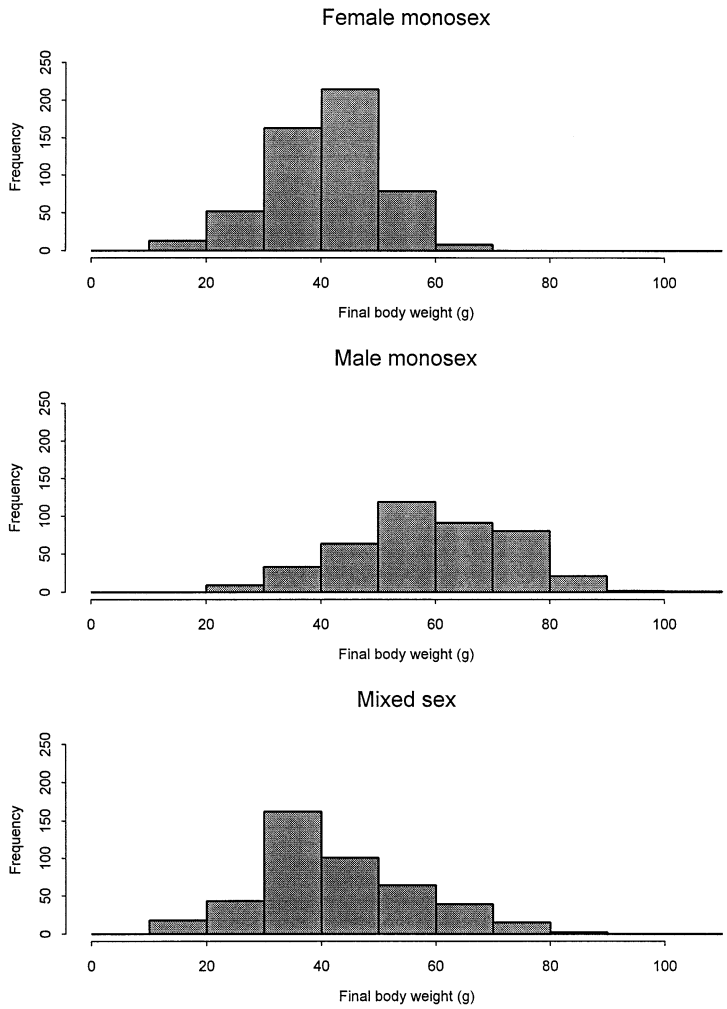


Figure 7 The frequency distribution of female monosex ($n = 6$ ponds), male monosex ($n = 6$ ponds) and mixed-sex yabbies ($n = 6$ ponds) (juveniles excluded < 5 g).

economically, as larger yabbies receive higher market prices per kilogram (Lawrence 1998b).

One way to control population density is to prevent reproduction. However, juvenile yabbies are produced over the warmer months of the year (September–May) by broodstock contained within farm dams, where there is currently little or no control by most farmers over yabby reproduction or density.

Therefore, this study investigated a number of options for controlling density by preventing or reducing reproduction in farm dams and ponds.

Monosex culture

Stocking ponds with animals of only one sex to prevent reproduction. In comparison with female and mixed-sex ponds, those stocked with only male yabbies resulted in a greater proportion of animals in the larger, and therefore more valuable, size classes at harvest. The frequency distribution of adult yabbies in female monosex, male monosex and mixed sex treatments is shown in Fig. 7.

Male monosex populations grew, on average, 68% faster than the female monosex animals and 53% faster than the males and females combined in mixed-sex populations. Female yabbies in monosex culture grew 9% slower than males and females combined in mixed sex (50:50) populations. Both males (17%) and females (31%) in monosex culture grew faster than males and females in mixed-sex culture. Stocking ponds with only male yabbies resulted in a 70% greater gross value of animals produced than normal mixed-sex production.

However, while manual sexing of yabbies to stock monosex populations in wheatbelt farm dams and

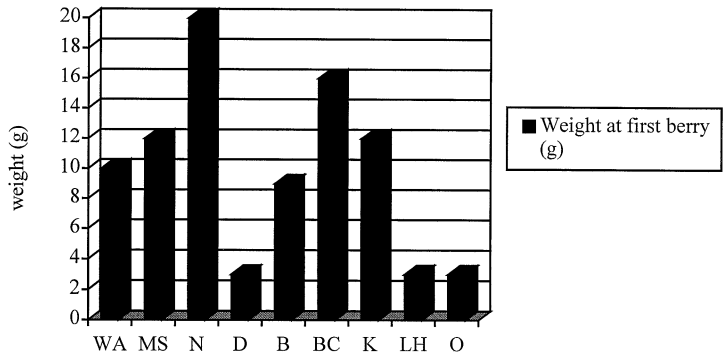


Figure 8 Minimum size at sexual maturity recorded from different yabby populations.

semi-intensive ponds is becoming more common in Australia, it is very time-consuming, prone to error and labour intensive.

Size at sexual maturity of yabby strains

A 'variety' of yabby in which the female did not become sexually mature until market size could be harvested before reproducing.

In contrast to permanent water bodies such as ponds or farm dams, fluctuating natural environments favour r-strategist species or strains, in which age and size at first reproduction are, respectively, lower and smaller (Stearns 1976). When yabbies are translocated from their natural ecotypes to aquaculture ponds and farm dams, their high rate of reproduction is not controlled by periodic drought-induced mortality, resulting in increased densities and, consequently, stunted populations.

One approach to controlling reproduction of yabbies in farm dams and ponds would be to farm a 'variety' of yabby in which the female did not become sexually mature until market size (> 30 g) and therefore could be harvested before reproducing. Size at sexual maturity has been reported to vary between populations of other freshwater crayfish species, including *P. clarkii* (Huner & Romaire 1979) and *Euastacus bispinosus* (Honan & Mitchell 1995).

Evidence exists supporting the hypotheses that the size of sexual maturity of yabbies, as determined by the smallest berried females recorded in a population, varies between localities (Woodland 1967; Lewis 1976; Johnson 1979; Reynolds 1980; Faragher 1983; Mills & McCloud 1983; Sokol 1987). In a population of *C. destructor* from western NSW, the minimum size recorded for berried females was an OCL of 38.8 mm (Reynolds 1980). The smallest recorded size at sexual maturity for a yabby is for the strain from Dalhousie Springs (OCL 19.8 mm) (Sokol 1987).

A study of farm dams in the Pingelly region of the WA wheatbelt showed that, on average, female *C. albidus* yabbies mature at ≈ 20 g (5 se) (N M Morrissy, unpubl. data); this equates to an OCL of around 32 mm (Lawrence *et al.* 1998).

As size at sexual maturity in decapods may be caused by genetic and/or environmental factors, the age and minimum size at sexual maturity of strains from different localities were compared under homogeneous conditions in a controlled environment within the Reproduction and Genetics Laboratory at Perth.

As it has been shown for other species of decapods that morphological changes do not always correlate with functional sexual maturity (Aiken & Waddy 1980; Wenner, Page & Siegel 1985), and to permit comparison with the results of previous researchers, the minimum age/size at sexual maturity was recorded as the age/size at which females in mixed-sex population tanks first became berried.

Size and age at sexual maturity was recorded by measuring weight and OCL of F1 generation berried females from breeding populations of each strain over a period of 3 years.

The minimum age and size of berried females varied between populations under controlled homogeneous conditions. However, no population reached market size (30 g) before sexual maturity (Fig. 8).

Therefore, under homogeneous conditions, yabbies from a variety of localities reach sexual maturity at different sizes.

The WA yabby strain matured at a smaller size than animals from the ancestral locality (MS). This may be a result of fluctuating environments, such as farm dams, favouring an early maturing r-strategy, inbreeding or high selection pressure as a result of

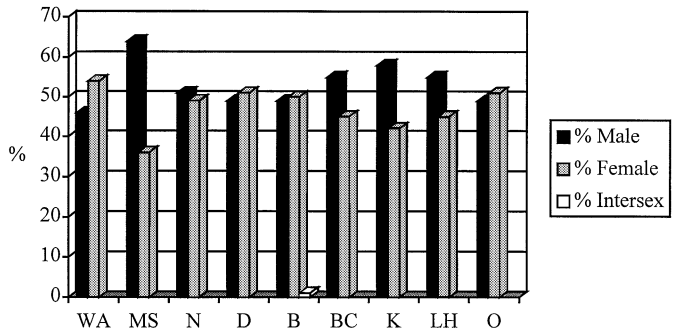


Figure 9 Sex ratio of juveniles from nine strains of yabbies.

trapping increasing the proportion of smaller yabbies contributing to the reproductive effort of the population. In salmonids, selective breeding programmes have increased the age at which sexual maturity occurs. The variation in age and size of sexual maturity shown for yabbies in this study may indicate potential for selective breeding for this trait.

Sex ratio of strains

A strain that offered a skewed sex ratio in favour of males would limit the reproductive potential of a population and capitalize upon the faster growth of male yabbies.

The sex ratio of yabbies in WA farm dams is biased in favour of females (1 male:1.2 females). This is thought to be due to trapping, which removes more males than females (Lawrence *et al.* 1998). In addition, male yabbies grow faster than female yabbies. Therefore, a variety of yabby that produces more male than female offspring could have potential benefits for industry.

Previous studies on the sex ratio of yabbies from farm dams recorded sex ratios of 1 male:1.14 females (Woodland 1967) and 1 male:1 female (Reynolds 1980). However, the data of Reynolds (1980) are likely to under-represent the proportion of females in these dams because of sampling bias using baited traps.

In their natural environment, the sex ratio of both *C. dispar* from Brisbane and Japanese crayfish *Cambroides japonicus* was 1:1 (Reik 1967; Kawai, Hamano & Matsuura 1995).

While data on the primary sex ratio of freshwater crayfish are limited, in a recent study by Jones & Ruscoe (1996) of five stocks of redclaw *Cherax quadricarinatus*, all showed similar sex ratios, close to 1:1 (ranging from 1 male:0.94 females to 1 male:1.15 females), with all but one strain having slightly more females than males.

In this study, sex ratios ranged from 1:0.56 to 1:1.17 (Fig. 9).

The sex ratio of juveniles from the WA strain of yabbies in this aquarium laboratory study did not vary significantly from 1:1. However, the sex ratio of yabbies from Western Australian farm dams has been shown to be significantly skewed towards females (Lawrence *et al.* 1998). It is likely that the sex ratio of yabbies in WA farm dams is affected by environmental or management practices after juveniles have been released from females rather than by strain characteristics (Lawrence *et al.* 1998)

Hybrid sterility

Allopatric populations of other species have shown full or partial hybrid sterility.

A method for controlling the overpopulation of yabbies would be to farm an animal that cannot reproduce. Hybridization by mating different species or subspecies often results in the production of sterile offspring or animals that posses a greatly reduced reproductive potential, when compared with their parent types (Tave 1993; Gardner 1997). Therefore, one method proposed to prevent unwanted reproduction in culture systems is to produce sterile offspring by hybridization of two species (Naevdal & Dalpadado 1986; Dunham 1990; Lutz 1997). Although sterility may not be complete, with at least one report of F1 hybrid females producing eggs while males are sterile (Hamaguchi & Sakaizumi 1992), the resultant diminished fecundity may still limit population density.

Sterile hybrids have been reported for fish species of interest to aquaculture (Tave 1993; Knibb 1994; Varadi, Hidas, Varkonyi & Horvath 1995; Stoumboudi & Abraham 1996). However, the scientific literature on hybridization among crustaceans of aquacultural importance is sparse. Among

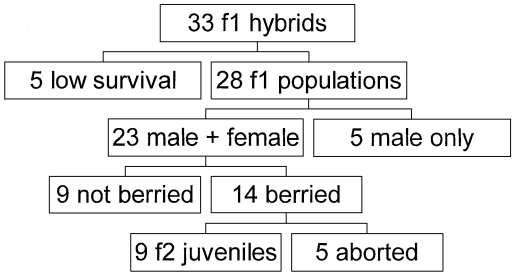


Figure 10 Fertility of hybrids between geographically isolated populations.

the decapods, marine shrimp, freshwater prawns and clawed lobsters have been hybridized artificially (Hedgecock 1987).

Unlike many fish species in which gametes may be obtained by stripping broodstock, the artificial hybridization of crustaceans is constrained by the lack of control over their reproductive processes. Subsequently, the most important potential benefits of interspecific hybridization, hybrid vigour and sterility, have not yet been realized in commercial crustacean culture (Hedgecock 1987).

Allopatric populations of other species have shown full or partial hybrid sterility as a result of genetic divergence, resulting from geographic isolation (Ganz & Burton 1995). There are a large number of closely related species, subspecies and strains of yabbies that have been separated by geographical barriers since the Tertiary period (Sokol 1988). It is therefore possible that allopatric populations of yabbies that have been segregated for sufficient time may have diverged and acquired reproductive incompatibility with each other.

Differing levels of reproductive isolation have been shown between reciprocal crosses in fish. In a number of cases, while hybrids can be produced, the reciprocal cross results in deformed or non-surviving juveniles (Hester 1970; Tave 1993; Rahman *et al.* 1995). Therefore, to permit reciprocal crosses (male × female and female × male) for each of nine populations in this experiment, 81 separate aquaria were established.

For each of the populations and potential hybrid crosses, matings were repeated three times, resulting in a total of 243 matings. The hybrid crosses were completed between October 1995 and January 1998. The F1 hybrids were kept in separate aquariums, and sexual maturity was determined by observing populations daily for berried females. In general, most hybrids demonstrated a low survival rate from egg to juvenile. Of the 32 populations of F1 hybrids produced, five crosses had survival too low for analysis in this study. This resulted in 27 populations of hybrids. Of these 27 hybrid populations, 12 mated and berried. From these matings, eight populations produced juveniles, while four aborted eggs before release. The remaining 15 populations of hybrids did not mate during this study (Fig. 10).

As matings of K × WA, K × N and K × MS produced only male F1 hybrids, the fertility of these crosses has not been determined. Similarly, WA × B and BC × D produced only male progeny but had very low survival (Lawrence *et al.* 1998).

This leaves 10 hybrids that, although considered to be sexually mature, failed to reproduce during the period of this study.

Hybrids produced in this study have, at this stage, shown preliminary evidence of hybrid sterility. However, it is not known whether this was because of their being sterile hybrids or merely that they had not yet reached sexual maturity. If this is the case, a number of the hybrids in this study do not mature until a larger size than the parent populations.

Hybrid sterility does, to some extent, conform to the taxonomy proposed by Austin (1996) and, in addition, supports the hypothesis that animals separated by greater distances are more likely to show full or partial hybrid sterility as a result of genetic divergence resulting from geographic isolation (Sokol 1988; Austin 1996).

Further investigations to confirm the sterility, fecundity and age/size of sexual maturity of the hybrids developed during this study are likely to result in sterile hybrids with commercial potential for aquaculture.

Sex ratio of hybrids

Hybrids between allopatric populations may have single-sex offspring.

The control of reproduction, overpopulation and consequent density-induced stunting may be achieved by sex control of yabby populations. The problem of early maturity and pond breeding during growout, and consequently stunted populations, has been encountered in a fish species that is now farmed worldwide, i.e. tilapia. With tilapia, a major step in stock improvement to overcome the breeding problem was to investigate hybrids of closely related species, resulting in single-sex progeny and the development of more efficient strains (Lovshin 1982).

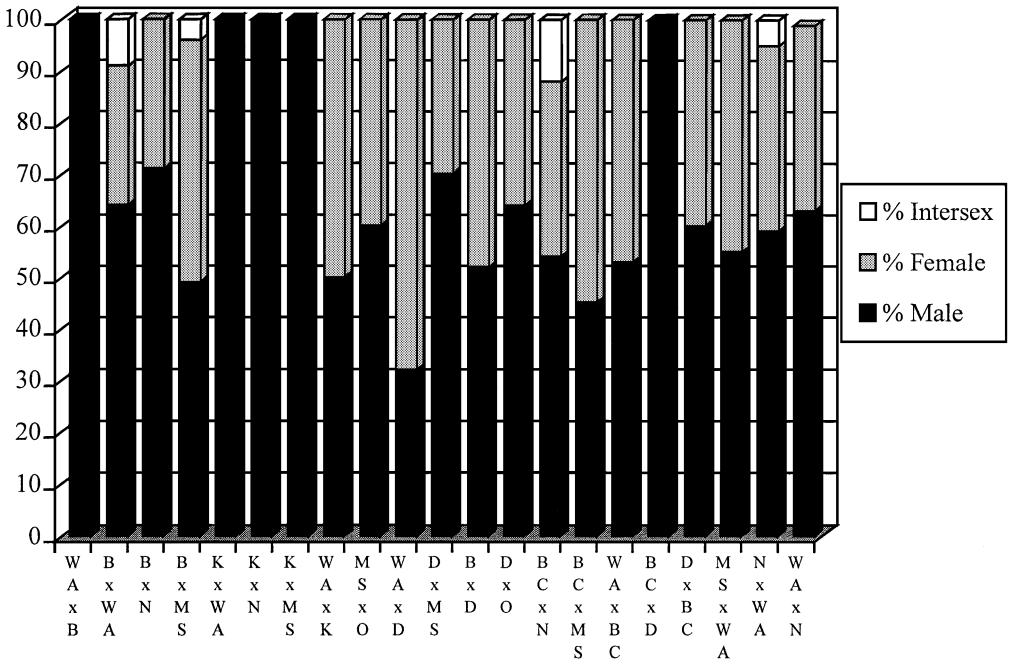


Figure 11 Sex ratio of hybrids between geographically isolated populations.

Hybrids between allopatric populations may demonstrate single-sex offspring (Tave 1993; Gardner 1997). Upon removal of physical barriers by translocation, post-mating reproductive isolating mechanisms, such as those that exist for a number of other hybrid aquatic species (Gardner 1997), may be used to contribute to aquaculture production of yabbies.

The hypothesis investigated used the allopatric isolation theory applied to yabbies. The taxonomy of sister group yabbies suggests that discrete genetic populations of yabbies exist and, consequently, sufficient variation has been established between gene pools for reproductive isolating mechanisms to occur.

Reciprocal crosses (male × female and female × male) were established for nine strains of yabbies collected in 1995. The hybrid crosses were completed between October 1995 and January 1998.

Of the 81 possible reciprocal mating combinations, F1 progeny were produced from the nine strains along with sufficient numbers of 21 hybrids to determine sex ratios (Fig. 11). The sex ratio of six additional hybrids was not discernible at the time of this publication.

The K × WA, K × MS and K × N (*C. rotundus* × *C. albidus*) hybrid is of particular significance to the aquaculture of yabbies in farm dams, as juveniles produced from 11 matings resulted in only male progeny (Fig. 11) (Lawrence *et al.* 1998).

Although male-only populations were produced from WA × B and BC × D matings, the low number of surviving animals reduces the commercial potential of these crosses for producing male-only progeny. The discovery of male-only progeny by hybridizing WA × B (*C. albidus* × *C. destructor*) was first reported by Lawrence *et al.* (1998) and subsequently corroborated by Austin and Meewan (C. Austin, pers. commun.).

The growth and production of the male-only hybrid is being evaluated under quarantine conditions in ponds at the Genetics and Reproduction Laboratory in Perth, along with a study into the reproductive physiology and sex determination of the strains.

Should the all-male hybrid yabbies demonstrate similar or superior growth to yabbies currently in farm dams, the commercial application of this technology will solve the overpopulation and consequent stunting difficulties currently faced by industry. This relatively simple technique, which does not require expensive or specialized equipment, can easily be applied by farmers and remove the current labour-intensive practice of hand-sorting male and female yabbies for monosex culture.

Acknowledgments

This work was funded by the Fisheries Research and Development Corporation (FRDC) project no. 94/75. Facilities were supplied by the University of Western Australia and Fisheries WA. We wish to thank M Stuckey, J Bellanger, N Cole, technical officers, Fisheries WA, for assistance in maintaining the experiments, Y W Cheng for assistance with data analyses and Dr Greg Maguire, Dr P Vercoe and Dr I Williams for comments on the manuscript.

References

Aiken D.E. & Waddy S.L. (1980) Reproductive biology. In: *The Biology and Management of Lobsters 1* (ed. by J.S. Cobb & B.F. Phillips), pp. 215–276. Academic Press, New York.

Austin C.M. (1996) Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia: electrophoretic and morphological variation. *Australian Journal of Zoology* **44,** 259–296.

Austin C.M. & Knott B. (1996) Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in southwestern Australia: electrophoretic, morphological and habitat variation. *Australian Journal of Zoology* **44,** 223–258.

Austin C.M., Jones P.L., Stagnitti F. & Mitchell B.D. (1997) Response of the yabby, *Cherax destructor* Clark, to natural and artificial diets: phenotypic variation in juvenile growth. *Aquaculture* **149,** 39–46.

Avault J.W., Bretonne L.W. & Huner J.V. (1975) Two major problems in culturing crayfish in ponds: oxygen depletion and overcrowding. In: *Freshwater Crayfish II* (ed. by J.W. Avault), pp. 139–144. Louisiana State University, LA.

Bakos J. & Gorda S. (1995) Genetic improvement of common carp strains using intraspecific hybridisation. *Aquaculture* **129,** 183–186.

Brown D., Van Landeghem K. & Schuele M. (1997) *Australian Aquaculture Industry Profiles for Selected Species.* ABARE Research Report 97.3. ABARE, Canberra.

Brown P.B., Wilson K.A., Wetzel J.E. & Hoene B. (1995) Increased densities result in reduced weight gain of crayfish *Orconectes virilis. Journal of the World Aquaculture Society* **26,** 165–171.

Campbell N.J., Geddes M.C. & Adams M. (1994) Genetic variation in yabbies *Cherax destructor* and *C. albidus* (Crustacea: Decapoda: Parastacidae), indicates the presence of a single highly sub-structured species. *Australian Journal of Zoology* **42,** 745–760.

Clark E. (1936) The freshwater and land crayfishes of Australia. *Memoirs of the National Museum Melbourne, Memoirs of the National Museum Victoria* **X,** 5–58.

Clark E. (1941) New species of Australian freshwater and land crayfishes (Family Parastacidae) *Memoirs of the National Museum Melbourne* **12,** 31–41.

Clark E. & Burnet F.M. (1942) The application of serological methods to the study of crustacea. *Australian Journal of Experimental Biology and Medical Science* **20,** 89–95.

Dunham R.A. (1990) Production and use of monosex or sterile fishes in aquaculture. *Reviews of Aquatic Science* **2,** 1–17.

Faragher R.A. (1983) Role of the crayfish *Cherax destructor* Clark as food for trout in Lake Eucumbene, New South Wales. *Australian Journal of Marine and Freshwater Research* **33,** 407–417.

Fevolden S.E. & Hessen D.O. (1989) morphological and genetic differences among recently founded populations of noble crayfish (*Astacus astacus*) *Hereditas* **110,** 149–158.

Francesconi K.A., Bird C., Fellows C.J. & Morrissy N.M. (1995) Fenvalerate as a stock management agent in extensive aquaculture for the freshwater crayfish *Cherax albidus. Journal of Applied Aquaculture* **5,** 11–20.

Ganz H.H. & Burton R.S. (1995) Genetic differentiation and reproductive incompatibility among Baja California populations of the copepod *Tigriopus californicus. Marine Biology* **123,** 821–827.

Gardner J.P.A. (1997) Hybridisation in the sea. In: *Advances in Marine Biology 31* (ed. by J.H.S. Blaxter & A.J. Southward), pp. 2–78. Academic Press, San Diego.

Geddes M.C., Mills B.J. & Walker K.F. (1988) Growth in the Australian freshwater crayfish *Cherax destructor* Clark, under laboratory conditions. *Australian Journal of Marine and Freshwater Research* **39,** 555–568.

Hamaguchi S. & Sakaizumi M. (1992) Sexually differentiated mechanisms of sterility in interspecific hybrids between *Oryzias latipes* and *O. curvinotus. Journal of Experimental Zoology* **263,** 323–329.

Hedgecock D. (1987) Interspecific hybridisation of economically important crustaceans. In: *Selection, Hybridisation, and Genetic Engineering in Aquaculture,* Vol. 2. *Proceedings of a World Symposium Sponsored and Supported by the European Inland Fisheries Advisory Commission of FAO and the International Council for the Exploration of the Sea,* Bordeaux, June 27–30, 1986 (ed. by K. Tiews), pp. 61–69. Heenemann Verlagsgesellschaft mbH, Berlin.

Hedgecock D., Stelmach D.J., Nelson K., Lindenfelser M.E. & Malecha S.R. (1979) Genetic divergence and biogeography of natural populations of *Macrobrachium rosenbergii. Proceedings of the World Mariculture Society* **10,** 873–879.

Henryon M. (1996) *Genetic Variation in Wild Marron can be used to develop an Improved Commercial Strain.* PhD Thesis, University of Western Australia.

Hester F.E. (1970) Phylogenetic relationships of sunfishes as demonstrated by hybridisation. *Transactions of the American Fisheries Society* **99,** 100–104.

Honan J.A. & Mitchell B.D. (1995) Reproduction of *Euastacus bispinosus* Clark (Decapoda: Parastacidae), and trends in the reproductive characteristics of freshwater crayfish. *Marine and Freshwater Research* **46,** 485–499.

Huner J.V. & Romaire R.P. (1979) Size at sexual maturity as a means of comparing populations of *Procambarus clarkii* (Girrard) (Crustacea: Decapoda) from different habitats. In: *Freshwater Crayfish IV, France 1978* (ed. by P. Laurent), pp. 53–64. Institut National De la Recherche Agronomique, France.

Huxley T.H. (1878) On the classification and the distribution of the crayfishes. *Proceedings of the Zoological Society of London* **1878,** 752–788.

Imgrund J., Groth D. & Wetherall J. (1997) Genetic analysis of the freshwater crayfish *Cherax tenuimanus*. *Electrophoresis* **18,** 1660–1665.

Jarboe H.H. & Romaire R.P. (1995) Effects of density reduction and supplemental feeding on stunted crayfish *Procambarus clarkii* populations in earthen ponds. *Journal of the World Aquaculture Society* **26,** 29–37.

Johnson H.T. (1979) *Reproduction, Development and Breeding Activity in the Freshwater Crayfish* Cherax destructor *Clark*. MSc Thesis, School of Biological Sciences, University of Sydney, NSW.

Jones C.M. & Ruscoe I.M. (1996) *Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*)* Final report. Project 92/119. Fisheries Research and Development Corporation. DPI Queensland. Freshwater Fisheries and Aquaculture Centre, Walkamin, Australia.

Kawai T., Hamano T. & Matsuura S. (1995) Sex ratio of the Japanese Crayfish *Cambroides japonicus* (de Haan 1841) (Crustacea, Decapoda, Astacoidea) in a stream and a small lake in Hokkaido. *Journal of the National Fisheries University* **44,** 21–23.

Knibb W.R. (1994) Genetic improvement of warmwater fish and their environmental impact. In: *Proceedings of the 3rd International Marine Biotechnology Conference* (ed. by International Advisory Committee of the International Marine Biotechnology Conference 1994), Tromsoe, Norway. 7–12 August 1994, p. 90. University of Tromsoe, Norway.

Lawrence C.S. (1998a) Marron. In: *The New Rural Industries – a Handbook for Farmers and Investors* (ed. by K. Hyde), pp. 114–119. RIRDC, Canberra.

Lawrence C.S. (1998b) Yabbies. In: *The New Rural Industries – a Handbook for Farmers and Investors* (ed. by K. Hyde), pp. 147–152. RIRDC, Canberra.

Lawrence C.S., Morrissy N.M., Bellanger J.E. & Cheng Y.W. (1998) *Enhancement of Yabby Production from Western Australian Farm Dams.* Final Report, FRDC Project 94/075, Fisheries Research Report 112. Fisheries Western Australia.

Lewis R.B. (1976) *Aspects of the Life History, Growth, Respiration and Feeding Efficiencies of the Yabby, Cherax destructor Clark with regard to Potential for Aquaculture.* BSc Thesis, Dept of Zoology, University of Adelaide, South Australia.

Lovshin L.L. (1982) Tilapia hybridisation. In: *The Biology and Culture of Tilapias* (ed. by R.S.V. Pullin & R.H. Lowe-McConnell), pp. 279–307. ICLARM Conference Proceedings 7.

Lutz C.G. (1997) What do you get when you cross. *Aquaculture Magazine* **23,** 84–90.

McClain W.R. (1994) Investigations of crayfish density and supplemental feeding as factors influencing growth and production of *Procambarus clarkii*. In: *Freshwater Crayfish X* (ed. by M.C. Geddes, D.R. Fielder & A.M. Richardson), pp. 512–520. Louisiana State University, LA.

Mills B.J. & McCloud P.I. (1983) Effects of stocking and feeding rates on experimental pond production of the crayfish *Cherax destructor* Clark (Decapoda: Parastacidae). *Aquaculture* **43,** 51–72.

Morrissy N.M. (1979) Experimental pond production of marron, *Cherax tenuimanus* (Smith) (Decapoda: Parastacidae). *Aquaculture* **16,** 319–344.

Morrissy N.M. (1980) Production of marron in Western Australian farm dams. *Fisheries Research Bulletin* **24,** 79.

Morrissy N.M. (1982) A standard reference diet for crustacean nutrition research. IV Growth of freshwater crayfish *Cherax tenuimanus*. *Journal of the World Aquaculture Society* **20,** 114–117.

Morrissy N.M. (1990) Optimum and favourable temperatures for growth of *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae). *Australian Journal of Marine and Freshwater Research* **41,** 735–746.

Morrissy N.M. (1992a) Feed development for marron *Cherax tenuimanus*, in Western Australia. In: *Proceedings of the Aquaculture Nutrition Workshop, Salamander Bay*, 15–17 April 1991 (ed. by G.L. Allan & W. Dall), pp. 72–76. NSW Fisheries.

Morrissy N.M. (1992b) Density-dependant pond growout of single year-class cohorts of a freshwater crayfish *Cherax tenuimanus* (Smith) to two years of age. *Journal of the World Aquaculture Society* **23,** 154–168.

Morrissy N.M. & Cassells G. (1992) Spread of the introduced yabby *Cherax albidus* (Clark 1936) in Western Australia. *Fisheries Research Report* **92,** 1–27. Fisheries Department of Western Australia, Perth.

Morrissy N.M., Walker P. & Moore W. (1995) Predictive equations for managing semi-intensive grow-out of a freshwater crayfish (Marron), *Cherax tenuimanus* (Smith 1912) (Decapoda: Parastacidae), on a commercial farm. *Aquaculture Research* **26,** 71–80.

Naevdal G. & Dalpadado P. (1986) Intraspecific hybridisation – cold water species. In: *Selection, Hybridisation, and Genetic Engineering in Aquaculture,* Vol. 2. *Proceedings of a*

World Symposium Sponsored and Supported by the European Inland Fisheries Advisory Commission of FAO and the International Council for the Exploration of the Sea, Bordeaux, 27–30 June 1986 (ed. by K. Tiews), pp. 23–33.

Olszewski P. (1980) *A Salute to the Humble Yabby.* Angus & Robertson Publishers, Sydney.

Patak A. & Baldwin J. (1984) Electrophoretic and immunochemical comparisons of haemocyanins from Australian fresh-water crayfish (Family Parastacidae): Phylogenetic implications. *Journal of Crustacean Biology* **4,** 528–535.

Rahman M.A., Bhadra A., Begum N., Islam M.S. & Hussain M.G. (1995) Production of hybrid vigour through cross breeding between *Clarias batrachus* L. and *Clarias gariepinus* Bur. *Aquaculture* **138,** 125–130.

Reik E.F. (1951) The freshwater crayfish (Family Parastacidae) of Queensland. *Records of the Australian Museum* **22,** 368–388.

Reik E.F. (1956) Additions to the Australian freshwater crayfish. *Records of the Australian Museum* **XXIV,** 1–7.

Reik E. (1967) *Synopsis of Biological Data on the Freshwater Crayfish* Cherax dispar *Reik.* Australian/New Zealand Meeting on Decapod Crustacea, Sydney, 24–28 October 1967.

Reik E.F. (1969) The Australian freshwater crayfish Crustacea: Decapoda: Parastacidae), with definitions of new species. *Australian Journal of Zoology* **17,** 855–918.

Reynolds K.M. (1980) *Aspects of the Biology of the Freshwater Crayfish* Cherax destructor *in Farm Dams in Far-Western NSW.* MSc Thesis, School of Zoology, University of NSW.

Smallridge M. (1990) *Biology and Farming of the Yabby.* South Australian Department of Fisheries, Adelaide.

Sokol A. (1987) Yabbies at Dalhousie Springs, northern South Australia: morphological evidence for long term isolation. *Transcripts of the Royal Society of South Australia* **3,** 207–209.

Sokol A. (1988) Morphological variation in relation to the taxonomy of the *destructor* group of the genus *Cherax. Invertebrate Taxonomy.* **2,** 55–79.

Stearns S.C. (1976) Life history tactics: a review of the ideas. *The Quarterly Review of Biology* **51,** 3–47.

Stoumboudi M.T. & Abraham M. (1996) The spermatogenetic process in *Barbus longiceps, Capoeta damascina* and their natural sterile hybrid (Teleostei, Cyprinidae). *Journal of Fish Biology* **49,** 458–468.

Tave D. (1993) *Genetics for Fish Hatchery Managers,* 2nd edn. Van Nostrand Reinhold, New York.

Tave D., Smitherman R.O., Jayaprakas V. & Kuhlers D. (1990) Estimates of additive genetic effects, maternal genetic effects, individual heterosis, maternal heterosis, and egg cytoplasmic effects for growth in *Tilapia nilotica.* L. *Journal of the World Aquaculture Society* **21,** 263–270.

Thien T.M. & Trong T.D. (1995) Genetic resources of common carp in Vietnam. *Aquaculture* **129,** 216.

Varadi L., Hidas A., Varkonyi E. & Horvath L. (1995) Interesting phenomena in hybridization of carp (*Cyprinus carpio*) and rosy barb (*Barbus conchonius*). *Aquaculture* **129,** 211–214.

Wenner A.M., Page H.M. & Siegel P.R. (1985) Variation in size at onset of egg production. In: *Crustacean Issues 3: Factors in Adult Growth* (ed. by A.M. Wenner), pp. 149–164. Balkema, Rotterdam.

Woodland D.J. (1967) *Population Study of a Freshwater Crayfish* Cherax albidus *Clark: with Particular Reference to the Ecoenergetics of a Population.* PhD Thesis, University of New England, Armidale NSW.

Aquaculture Research, 2000, **31**, 83–94

Genetic implications of translocation and stocking of fish species, with particular reference to Western Australia

T F Cross

Aquaculture Development Centre, Department of Zoology and Animal Ecology, National University of Ireland, Cork, Ireland

Correspondence: T F Cross, Aquaculture Development Centre, Department of Zoology and Animal Ecology, National University of Ireland, Lee Maltings, Prospect Row, Cork, Ireland

Abstract

Species or strains of fish may be translocated for farming, where the only access to the wild is via inadvertent escapes, or for stocking, where deliberate releases are undertaken. In either case, it is important that the translocated animals are representative of the donor population(s) in terms of genetic composition and level of variability. Many studies have shown that this ideal is difficult to achieve, the major reason being the use of inadequate numbers or composition of broodstock as founders of a strain. Also, where more than one conspecific population is involved, there may be outbreeding depression problems. In the case of farming, measures to improve the introduced strain genetically are likely to be undertaken, e.g. breeding programmes, manipulation of sex and ploidy, transgenic techniques. Such approaches are necessary economically, but can alter genetic make-up. Thus, stringent attempts must be made to minimize escapes or reduce their impact should they occur. With stocking, genetic change during captive rearing should be avoided. No strain manipulation should be undertaken, and other agents of change should be minimized. Stocking may result in hybridization with related species or with endemic populations of the same species. In either case, there can be detrimental genetic effects on the native forms. To be able to identify subsequently any genetic changes in reared strains, whether intended for farming or stocking, wild population composition should be determined, using appropriate molecular techniques. Such molecular methods will demonstrate the degree of interpopulation differentiation and, thus, reproductive isolation. The same markers should then be used in each subsequent generation (in the hatchery and after escape or reintroduction to the wild) to monitor any changes in genetic composition or variability. Markers should include microsatellite DNA loci, but the inclusion of more than one type of marker is recommended. However, as the aforementioned markers are not considered to be influenced by natural selection, they give no information on the adaptive nature of such differences. For this reason, it is suggested that markers influenced by selection should be investigated. Monitoring a strain subsequent to deliberate or inadvertent release can be undertaken using genetic markers, either deliberately enhanced by breeding or occurring naturally. Highly variable minisatellite DNA loci have been used as family markers in farmed escape studies with Atlantic salmon. These investigations have demonstrated significantly superior survival of native strains compared with farmed salmon in natural stream conditions. These latter results, demonstrating fitness differences, were strongly indicative of local adaptation. Thus, methods exist to monitor the genetic effects of translocation and stocking. However, a holistic approach should be taken to such exercises, where genetics forms part of a wider suite of considerations.

Introduction

In the past, many species have been introduced (or translocated) into Australia, often with disastrous ecological consequences. Prominent examples include the European rabbit *Oryctolagus cuniculus* L. (Elton 1958) or, in the case of fish, the common carp *Cyprinus carpio* L. (Morison & Hume 1990). Thus, any suggestions of further translocations, either into the country or between regions, must be treated with extreme caution. Nevertheless, there has been recent pressure for translocation of fish species, particularly into isolated regions such as Western Australia (Lawrence 1993). These translocation requests are either for species or strains to be used in commercial aquaculture, or for stocking into freshwater impoundments, estuaries or coastal waters to boost sport fishing. In Western Australia, there is a depauperate freshwater fish fauna, and the native species are regarded as unsuitable for stocking for recreational angling, because of their small size (Prokop 1995).

In the past, most concern in relation to translocations has focused on the ecological and disease transfer implications for native species (Langdon 1990; Lever 1998). More recently, the genetic consequences of this activity have also been considered. In this review, the possible genetic consequences of translocation for aquaculture or stocking are discussed, as are the genetic principles that should be observed in the choice of suitable donor populations. The genetic measures that should be taken to maximize the success of stocking exercises and to minimize the effect of stocked fish on wild conspecifics or on other species are also considered.

Genetic considerations in translocation

Translocation (defined here as the movement of species or strains outside their native range; Pollard 1990) can be either for aquaculture or for stocking. It is often assumed that translocations for aquaculture inevitably result in the escape of reared animals into the wild, where there will be subsequent interaction with other native species or with natural populations of the same species (Lawrence 1993). If this concept were to be accepted, then strains used for aquaculture would have to be regarded in the same way as those intended for stocking. Therefore, it has been suggested that attempts should be made to minimize genetic alteration of strains intended for aquaculture.

Instead, it is argued in the present review that different strategies should be adopted when breeding for aquaculture and stocking, as genetic improvement of aquaculture strains is an economic imperative, without which it is impossible for industry to compete. Thus, every effort must be made to minimize escapes from aquaculture activities. The methods of genetic improvement used in aquaculture will be mentioned below and are described in detail elsewhere in this volume (Gjedrem 1999; Knibb 1999). While some escapes of aquaculture animals will inevitably occur, despite the most stringent efforts at containment, escapes should form only a small proportion of the total number of that species in a particular location (if, indeed, the species occurs naturally in the area). Unless farmed production considerably exceeds wild population numbers (as it does with Atlantic salmon in Norway; Gjoen & Bentsen 1997), occasional small escapes should have less drastic effects on wild populations (Youngson, Hansen & Windsor 1998). However, it is impossible to generalize, and field experiments, such as that described below, are necessary to assess the effect of any particular situation.

There are two major genetic considerations in relation to translocation: (1) to invoke genetic methods to choose the optimum strain for translocation, whether intended for aquaculture or for stocking; (2) to reduce impact on wild populations or species, thus preserving biodiversity.

The latter point relates to stocking or to farmed escapes, where these occur.

Genetic considerations common to translocation for aquaculture or stocking

Whether translocation is intended for aquaculture or stocking, two genetic points should be considered. These relate to choice of donor strain and to avoiding inbreeding effects during hatchery propagation.

Choice of donor strain

In the past, many translocations used the most convenient hatchery strain. Instead, consideration should be given to the population structure of the species when deciding the most appropriate donor. (Here, a population is defined as a group of conspecific animals, which is largely reproductively

isolated from other such populations.) In general, molecular studies have demonstrated far greater population structuring in freshwater and anadromous fish species than in marine species (Ward, Woodwark & Skibinski 1994), but each novel species should be investigated using the most appropriate molecular methods (see below; Carvahlo & Pitcher 1995; Carvahlo 1998). Often, nearby populations are more similar to each other genetically than are geographically distant ones, but this is not always the case.

Where large differences occur between groups of populations (sometimes termed races), as is the case with barramundi *Lates calcarifer* (Bloch) in northern Australia (Shaklee & Salini 1985; Doupé, Horwitz & Lymbery 1999; Keenan 1999) and *Salmo salar* L. from the eastern Atlantic and Baltic Sea (Stahl 1987; Cross 1989), translocation between groups is not recommended.

An excellent example of the dangers of such transfers was where Atlantic salmon were moved from a river entering the Baltic Sea to stock rivers in western Norway. In this exercise, the donor strain carried the ectoparasitic monogenetic trematode *Gyrodactylus salaris* Malmberg (Johnsen & Jensen 1986). While the donor strain was genetically resistant to this parasite, the local populations were susceptible, and huge mortality of native salmon resulted (Bakke 1991).

In choosing a suitable donor population for translocation for stocking, biological aspects other than genetics should be considered, such as environmental aspects of the donor and recipient habitats (Cross 1989). Furthermore, the idea that translocation legislation general to all species can be based on a certain degree of molecular genetic difference between donor and recipient groups is spurious, as will be immediately obvious from consideration of the great variation in differences that occur between populations of different species (see Ward *et al*. 1994; Johnson 1999).

Avoiding genetic changes in the hatchery

The second major consideration with donor strains that are used in translocation is that the majority are of hatchery origin. Even one generation of artificial spawning and hatchery rearing can cause large changes in genetic make-up (often detrimental to fitness) in terms of genetic composition and level of genetic variability (Allendorf & Ryman 1987; Cross 1999). In order to avoid genetic change in hatchery breeding (Tave 1986), it is important to collect broodstock randomly in numbers sufficient to avoid so-called inbreeding effects (at least 50 of each sex in equal numbers, in species in which separate sex individuals occur). All of these individuals must then participate in spawning, preferably using single pair mating (Gharrett & Shirley 1985). Where single pair mating is not possible, molecular genetic methods should be used to assess parental contribution (Cross, Galvin & Dillane 1998a). When inadequate numbers of broodstock are used, genetic variability is lost, and genetic composition is changed because of the random process, genetic drift (Tave 1986). This process becomes exponentially more prevalent as broodstock numbers are reduced or when there is a large departure from equal sex ratio. In the extreme case of one male and one female, 25% of genetic variability is lost in each generation. This calculation assumes non-overlapping generations. Reduction in broodstock numbers can have even more profound effects in species with overlapping generations (Ryman 1997).

Loss of genetic variability can have detrimental effects (Kincaid 1976; but see Avise 1994), whether the translocated strain is intended for aquaculture or stocking. In the former case, performance of the aquaculture strain may be reduced, as may be the potential to respond to artificial selection. In the case of stocking, the use of inbred strains can lead to reduced performance in the wild. Furthermore, genetic composition can vary unpredictably between year classes of the reared strain.

Dangers of crossing strains: outbreeding depression

It is sometimes suggested that the donor strain in translocation exercises be made up of two or more natural populations, in order to maximize the amount of genetic variability. The implications here are that local populations differ considerably, and also that the populations making up the donor strain will be crossed deliberately, in the case of aquaculture, or breed randomly in the wild, in the case of stocking. Without a molecular survey, the amount of interpopulation difference cannot be determined (see below). Furthermore, the crossing of locally adapted natural populations can cause outbreeding depression, which can result in reduced fitness.

The mode of action of outbreeding depression is as follows. The assumption is that local adaptation leads to the possession by members of a population of a particular arrangement of alleles at different

loci, termed co-adapted gene complexes. Hybridization between two different populations or reared strains may then lead to a breakdown of these complexes, resulting in reduced fitness. This effect has been demonstrated in pink salmon *Oncorhynchus gorbuscha* (Walbaum) by Gharrett & Smoker (1991). A difficulty with demonstrating outbreeding depression is that its severity is evident only in the second and subsequent generation hybrids. Few experiments have continued to monitor interactions for long enough in the wild (e.g. the experiments of Jorstad, Naevdal, Paulsen & Thorkildsen 1994 with stocked Atlantic cod *Gadus morhua* L., or McGinnity, Stone, Taggart, Cooke, Cotter, Hynes, McCamley, Cross & Ferguson 1997 with farmed escaped and wild *Salmo salar*, which will be described in detail below, only considered first-generation hybrids). Also, the concept of local adaptation, where it is assumed that the genetic composition of a population has been moulded by natural selection to perform optimally in the suite of environmental conditions pertaining in its particular habitat, is itself difficult to prove (but see Donaghy & Verspoor 1997).

Because of the possibility of outbreeding depression, it is recommended that strains translocated for stocking do not consist of the progeny of inter-population crosses. This does not preclude comparing more than one population in the first generation of a stocking exercise, provided they are separately tagged and so can be separated for spawning. In the case of translocations for aquaculture, the geneticists involved must decide whether the benefits in terms of increased genetic variation, resulting from crossing of populations, outweigh any later detrimental effects on fitness resulting from outbreeding depression.

Special considerations with translocations for aquaculture

Efforts at genetic improvement will usually be made with strains translocated for aquaculture. These methods are described in detail elsewhere in this volume; the commonest approach being to set up a breeding programme with selection for traits such as fast growth and disease resistance (see Gjedrem 1999; Knibb 1999).

Selective breeding

In well-designed breeding programmes, inbreeding is avoided as far as possible, and chosen traits are also targeted, while attempting to avoid changes to what is termed genetic background (Gjoen & Bentsen 1997). Escaped animals from a selection programme might therefore be expected to have relatively high fitness in the wild. However, as most selection programmes involve a closed rearing system, the effects of domestication selection (see below) will become more profound with each succeeding generation, probably making the animals progressively less fit in the wild.

Manipulation of sex and ploidy

Other genetic techniques, such as the manipulation of sex and ploidy, are being used experimentally or have already been introduced into the commercial culture of certain species. For example, all-female rainbow trout *Oncorhynchus mykiss* (Walbaum) are used extensively in Europe (Ingram 1988) to allow a greater market size to be reached before sexual maturity. (In rainbow trout farming in temperate regions, on average, males mature for the first time at 2 years, but females as 3 year olds. As market value declines drastically with maturity, all-female production is economically advantageous.) Induced triploidy is also used to produce sterile trout, which continue to grow somatically when sexual maturity results in cessation of growth in diploid rainbow trout (Ingram 1988).

Production of all-female flatfish, such as turbot *Scophthalmus maximus* (L.) and halibut *Hippoglossus hippoglossus* (L.), is also being proposed in Europe, as females grow considerably faster than males after the first year. (The sex determination mechanism has not been fully resolved in these flatfish species, so all-female production is not yet feasible.)

Transgenic production

The technique of transgenesis, in which genes from one species are introduced into another species to produce genetically modified organisms (using the most universally accepted definition of a GMO), has been publicized as having major potential in aquaculture. For example, Devlin, Yesaki, Blagl, Donaldson, Swanson & Chan (1994) have demonstrated much faster growth in coho salmon *Oncorhynchus kisutch* (Walbaum) into which growth hormone genes from chinook salmon *O. tshawytscha* (Walbaum) have been introduced. However, there is a great deal of concern about the possible environmental impact of these transgenic organisms, if they should escape from contained culture facilities (Cross & Galvin 1996). Thus, very stringent

legislation controls their use in Europe and North America, making it unlikely that they will be used extensively in commercial aquaculture in these areas in the near future.

Methods of minimizing escapes or their effects on natives

As argued above, genetic improvement is economically vital in aquaculture. Thus, instead of prohibiting such improvements, legislation should focus on minimizing escapes. Several measures have been proposed, and some are in force (either as legislation or as a voluntary industry code of practise) to minimize escapes of Atlantic salmon from farms in Europe (Youngson *et al.* 1998).

Matching equipment to site

The most feasible measure relates to matching equipment, such as sea cages and moorings, to the environmental conditions of the site. This measure is used widely and is successful in reducing the proportion of fish escaping. However, the Atlantic salmon industry is continuing to grow rapidly, so the actual number of escapes in each year remains similar.

Sanctuary zones

Another useful measure has been to introduce sanctuary zones near particular wild spawning or nursery areas, where aquaculture is prohibited. The logistics of this approach depend on the mobility of the species at various stages of the life cycle, but it can be effective.

Use of sterile animals in culture

Sterile salmonid fish can be produced in a two-generation process. Sex-reversed genetic females, which act as functional males, are first produced by hormone therapy. Milt from these individuals is then used to fertilize eggs from normal females, resulting in all-female progeny. These progeny are physically shocked early in development using either temperature or pressure, resulting in a high yield of sterile triploid all-female fish. This process is widely used in the rainbow trout industry to eliminate sexual maturity, where large size is required for processing, such as smoking. It is currently being explored in a major European project as a device to achieve sterility in farmed Atlantic salmon. R. Johnstone (pers. commun.) indicates that there is poorer performance at certain stages in the life cycle of triploids compared with untreated diploids, so industry uptake may be slow. Furthermore, the industry feels that customers may be reluctant to buy salmon that have been genetically altered in this way.

Farming of natives

The farming of native populations has been suggested as a way to minimize the effect of escapes on natural populations. However, this measure can be impractical if the species is highly structured and if breeding programmes, which are very expensive undertakings, exist only for a limited number of strains. This is the case for Atlantic salmon in Norway, where farm production now exceeds 350 000 tons per year.

One of the best measures for the protection of native populations from adverse effects resulting from escapes of cultured conspecifics is to have healthy wild stocks, which have not been reduced by habitat deterioration or overfishing. In this respect, co-operation is required between the management of aquaculture and fisheries. By using the most practical of these measures to prevent escapes or minimize their effects, the impact on wild populations of any particular species can be reduced.

Genetic considerations in stocking

With stocking, as stated earlier, the genetic objectives should be to maximize effectiveness, while simultaneously minimizing any detrimental effects on native populations or species. Measures to maximize effectiveness of stocking exercises are detailed in Palmer (1995) and Cross (1999) and are only briefly described here, where the primary consideration is the effect of cultured fish on native species and populations. Cowx (1998) defined three major forms of stocking, which are relevant in the present context.

These are:

- introduction, which is similar to translocation of non-native species for deliberate release;
- reintroduction, which is the replacement of a species or strain into an area where it was previously present but became extinct as a result of human activities;
- enhancement, where wild populations are present and the objective is to increase productivity (Welcomme & Bartley 1998). This objective is the most difficult to achieve, but is the most relevant in the present context.

Stocking with natives

In the majority of cases, stocking involves translocation, where an introduced species or strain is used as donor. However, stocking of natives can be used, for example in an attempt to rebuild a population rapidly that has been depleted as a result of habitat deterioration or overfishing, and indeed is the only technique recommended for enhancing depleted Atlantic salmon populations (Cross, McGinnity & Galvin 1998b).

If sufficient reproductively capable members of an endangered population still remain (say about 500), it might be intrinsically less risky from a genetic point of view to use an adequate number of these (see considerations of inbreeding effects) in a local hatchery programme, rather than using a non-native strain (even if the latter can be obtained conveniently from an existing hatchery).

If less than say 100 adults remain, it might be decided to bring in translocated stock. However, as this decision implies abandoning the remnant population, it should only be taken after prolonged discussion. The decision will be specific to the particular situation and will be influenced by aspects such as the degree of population structuring observed in the species, the status of any other populations (endangered or 'healthy') and the biogeography of the species. If a small population were the last remnant of a species facing extinction, then different considerations would become important. In what follows, the assumption is that the natural population to be enhanced outnumbers the non-native strain used for stocking.

Translocation for stocking

Where non-native reared fish are introduced into natural waters, several consequences for other native fish species or for native populations of the same species may result (Carvahlo & Cross 1998).

There can be an indirect genetic effect, by which the introduced species outcompetes one or more native species, causing the latter to be greatly reduced in number. The result is that inbreeding can become a problem in the affected native species.

There can also be direct genetic effects, where interbreeding (termed hybridization) occurs between the wild species or strains, and native fish. Hybridization can be of two types, between species (termed interspecific) or between strains of the same species (referred to as intraspecific).

Interspecific hybridization

In the wild, interspecific hybridization only occurs between species that are closely related. Examples are hybridization between native brown trout *Salmo trutta* L. and Atlantic salmon *S. salar* in Europe, and between rainbow trout *Oncorhynchus mykiss* and cutthroat trout *O. clarkii* (Walbaum) in western North America. In the former case, hybridization increases in frequency when there are farmed escaped salmon present (Youngson, Webb, Thompson & Know 1993), whereas hybridization between rainbow and cutthroat trout is particularly common when rainbow trout are stocked into areas where they were formerly absent (Campton 1987). Specific Western Australian examples of hybridization are given by Thorn (1995).

Successful hybridization between species is less likely where forms are more distantly related in an evolutionary sense. Even with closely related species, first-generation hybrids are rarely fully fertile (Verspoor & Hammar 1991), so complete merging (introgression) of two species is unlikely (but see Avise 1994). Nonetheless, the presence of large numbers of first-generation hybrids may be unacceptable from the public point of view.

Intraspecific hybridization

When translocation involves the introduction of a different strain of an existing species into the range of a native population, what is termed intraspecific hybridization will usually occur. This assumes that the object of the exercise is to produce a larger self-sustaining population. This ideal is difficult to achieve (Cowx 1998; Howell, Moksness & Svasand 1999; but see Palmer 1995). What is much simpler biologically is to produce hatchery fish in each generation, as in salmon ranching (Thorpe 1980), and to use returning reared adults as broodstock for the next generation.

Where a larger self-sustaining population is the aim, the important element is the fitness of the hybrids relative to natives. Relative fitness of hybrids in second and subsequent generations might be expected to be lower than natives, if local adaptation is presumed, and outbreeding depression occurs (see above). Thus, field experiments, such as that described below with Atlantic salmon, but spanning two or more generations are recommended to assess

the results of stocking and should be implicit in any future stocking programmes.

Apart from the effects of genetic drift during hatchery propagation, as described above, selection is also a potential source of genetic change during hatchery rearing. In organisms intended for stocking, no deliberate artificial selection should be applied (for example for traits important in aquaculture, such as fast growth or a particular body conformation, but see Jonasson, Gjedre & Gjedrem (1997), where selection was applied for return rate in ranched Atlantic salmon). Even in the absence of deliberate selection, inadvertent hatchery selection (often termed domestication selection) occurs. This mode of selection to the hatchery environment makes the strain more suitable to hatchery conditions in each subsequent generation. Unfortunately, domestication selection appears to have the opposite effect on ability to survive in the wild (R.R. Reisenbichler, pers. commun.). At present, the process is insufficiently understood for its effects to be controlled. It is therefore suggested that strains to be used in stocking be kept in the hatchery for no more than one generation.

What has been suggested above assumes that one strain derived from a single wild unit population is involved in the translocation exercise, as outbreeding depression may result if two or more populations are used.

Molecular methods

Molecular methods have been mentioned several times in this review and are described in detail by Carvahlo & Pitcher (1995) and Carvahlo (1998). Here, a brief overview is given, and the relevance of the different classes of marker to translocation and stocking is considered.

Allozymes

Before the 1970s, the only molecular technique available to population geneticists was protein electrophoresis. In this technique, proteins, usually enzymes, which were the products of specific gene loci, were screened (Utter, Aebersold & Winans 1987). What has been termed the allozyme technique more recently was used extensively in fish for many applications, including directing and monitoring translocation and stocking exercises. However, there are distinct disadvantages to the allozyme method compared with the direct DNA screening techniques presently available. The allozyme technique requires either fresh or deep frozen (preferably at –40 °C or colder) tissues from an array of organs, e.g. liver, heart, brain and eye, as well as blood and skeletal muscle. The latter requirement means that samples often cannot be taken non-destructively, prohibiting use with valuable broodstock or with members of endangered species or populations. The allozyme technique also fails to demonstrate large amounts of the underlying genetic variability. Despite these shortcomings, the allozyme technique continues to be used in fisheries and aquaculture, and often provides sufficiently precise answers to particular questions.

Mitochondrial DNA

Direct DNA techniques first became available in the mid-1970s, when mitochondrial DNA screening was initiated. The small size of the mitochondrial genome (many orders of magnitude smaller than the nuclear genome) meant that it could be screened using the techniques available in the 1970s, while nuclear DNA could not. Mitochondrial DNA differs in several respects from nuclear DNA, in that it is haploid and almost exclusively maternally inherited. This haploid mode of inheritance makes it four times more susceptible to reductions in population size (so-called bottlenecks) than nuclear DNA. This means that it is good for comparing levels of genetic variability in wild populations and reared strains, or in progressive generations of the latter. It is also useful in detecting ancient relationships (so-called phylogeny) because of another property of the mitochondrial genome, the lack of genetic recombination (see Carvahlo & Pitcher 1995). However, there are shortcomings, such as the fact that only female behaviour is traced (because of the mode of inheritance) and that many sections of the mitochondrial genome lack variability, so that it is vital to choose a variable part of the mitochondrial genome for a particular study.

Nuclear DNA: microsatellite loci

Since the mid-1980s, it has been possible to study nuclear DNA directly. The most useful parts of the nuclear genome in the present context are the so-called microsatellite loci (O'Connell & Wright 1997; Estoup & Angers 1998). Microsatellites are a simple sequence motif, usually of two to four nucleic acids, tandemly repeated; the number of repeats defining

the particular allele. They are highly variable, in that most microsatellite loci are polymorphic, and many have large numbers of alleles. Microsatellite loci are common throughout the genome, so many can be isolated, and they can be detected and amplified using the polymerase chain reaction (PCR) technique. The latter means that sampling requirements are relaxed. Alcohol-preserved or dried samples of most tissues can be used. Microsatellite DNA can also be extracted from archival samples, such as scales or otoliths, so in reintroductions, it is often possible to screen the original population, which is currently extinct in a particular area (see Neilsen, Hansen & Loeschcke 1997 for an excellent description of this usage in Atlantic salmon reintroduction in Denmark). More rapid screening is possible using DNA sequencers and multiplexing (where more than one locus is screened in a single process). While there are still some problems with microsatellites, such as the finding of fewer heterozygotes than expected in unit populations, possibly because of so-called null alleles, microsatellites are undoubtedly the genetic loci of choice for current studies of translocation and stocking.

Use of a combination of molecular methods

Total reliance on one marker type is not recommended. Many authors suggest that an appropriate combination of methods (microsatellites, mtDNA and perhaps allozymes) be applied to any particular case, if time and funding allows. In the past, it has been presumed that most of these markers were functionally neutral (unaffected by the influence of natural selection and, thus, not indicative of adaptively important interpopulation/strain differences).

Choice of marker is likely to be influenced by natural or artificial selection

While the presumption of neutrality may not be true for all the aforementioned loci (see Verspoor & Jordan 1989), it has been suggested that loci likely to be influenced by selection be sought deliberately (Ferguson 1995). Most likely candidates are the so-called transcribed sequence genes, coding for example for proteins such as haemoglobin, serum transferrin and elements of the major histocompatibility complex (MHC). By investigating such genes in populations and strains involved in translocation and stocking, it may be possible in the future to advise on the functional importance of retaining or eliminating certain genotypes.

Outputs from studies using molecular markers

Using one, or preferably more than one, of the molecular marker types listed above, several aspects of translocation and stocking exercises can be investigated, namely:

- wild population structure can be determined (Avise 1994; Carvahlo & Pitcher 1995);
- the choice of a donor population can be facilitated;
- genetic make-up of wild populations and reared strains can be compared, particularly in relation to level of variability (Cross & King 1983);
- if progressive generations of hatchery rearing are involved, any genetic changes over generations can be detected;
- interspecific hybrids can be detected after stocking or escape of aquaculture strains (but see Cross *et al.* 1998a in relation to using mtDNA for this purpose);
- intraspecific hybrids can be identified, whether intended, as in most enhancement exercises (Taggart & Ferguson 1986), or accidental, as in escapes (Clifford, McGinnity & Ferguson 1998) (in one such approach, genetic manipulation is required, as described below);
- in general, the fate of reared animals after deliberate or inadvertent release to the wild can be monitored.

There are very few cases where all of these aspects have been investigated. However, in order to achieve the twin aims of translocation for stocking, maximizing performance of the donor strain and minimizing detrimental effects on the wild recipient population, all should be included in future exercises.

Monitoring after release

The effects of the majority of previous enhancement stocking exercises have not been adequately monitored. Often, increase in catch in a particular area has been used as an indication of the success of such an exercise. Such a measure is inaccurate without a great deal of information on factors such as effort, environmental conditions and the effects of such conditions on the catchability of the species in question.

Limitations of physical tagging

A more accurate indication of relative performance can be obtained by physically tagging reared animals before release (Palmer 1995). However, external tags can increase mortality, so internal tags, usually so-called coded wire tags, are used with ranched anadromous salmonids in both the north Pacific and the north Atlantic, and have been used in Australia with barramundi (Russell, Hales & Ingram 1991). The latter are effective if it is assumed that all surviving fish will home to their release area and be recaptured. In such a ranching scheme, hatchery intervention is required in each generation. As stated earlier, this is not the objective of most enhancement schemes, which aim at larger self-sustaining populations. To monitor such an exercise, genetic 'tags' are required, as these are detectable in offspring. Such marks can identify either group or family.

Genetic marking to group

In a minority of cases, donor strain and wild population naturally have very different genetic compositions (e.g. Taggart & Ferguson (1986) with *Salmo trutta* in Northern Ireland; Krieg & Guyomard (1985) with the same species in France). More usually, a rearing step is required. The objective of this step is to increase the frequency of a previously very rare allele in fish intended for stocking (Palmer 1995). This process was used by Knut Jorstad and colleagues with Atlantic cod *Gadus morhua* in Norway, which were being released into fjords to enhance local populations (see Jorstad *et al.* 1994). A rare allozyme allele at the glucosephosphate isomerase locus was chosen. Heterozygote animals were crossed yielding, as expected, $\approx 25\%$ rare homozygotes. These homozygotes were identified by biopsy and then released. Any subsequent catches of rare homozygotes were direct releases or their progeny, whereas the vast majority of heterozygotes were first-generation progeny of mating between stocked and wild cod.

Genetic identification to family

Identification of offspring to family has been made possible by the availability of DNA loci with many alleles, such as microsatellites or the related minisatellites (where the repeat motifs consist of 10–100 basepairs). There are several advantages to this system over group marking, the chief being that no element of manipulation is involved (the 'markers' occur naturally and do not have to be induced by breeding). Also, the information provided on individual families is advantageous.

Methodology of a field experiment using molecular methods to identify to family

An example of this system is an experiment carried out by M^{c}Ginnity *et al.* (1997) in the Burrishoole river system in the west of Ireland. This experiment was designed to compare the fitness of native and farmed Atlantic salmon and their hybrids under natural stream conditions. In 1992, a natural spawning stream was closed off 1.7 km downstream of an impassable waterfall by the construction of complete trapping facilities. Thus, native spawners were denied access and instead removed and stripped artificially. Simultaneously, the same number of farmed fish from a strain of Norwegian origin, which was then the most popular strain in the Irish industry, were also stripped.

Eggs and milt from wild and farmed fish were crossed in order to produce 60 families, 15 each of wild $\times$ wild, farmed $\times$ farmed and both hybrids (farmed $\times$ wild and wild $\times$ farmed). The majority of the eggs from each family were spread randomly throughout the stream in specially designed egg containers. Hatchery controls were also retained using the remaining eggs. The parents were typed for a sufficient number of minisatellite loci to enable nearly unambiguous identification ($> 95\%$) to family. (Determination of the number of loci required used computer modelling.)

Results from the Burrishoole experiment

Eight months after the eggs were placed in the stream, ≈ 300 juveniles were recovered by electrofishing and typed for the same minisatellite loci. While the expectation would be of equal survival, the native fish in fact survived significantly better than expected and the farmed progeny significantly less well, whereas the hybrids were close to or, in one case, exceeded expectation. The whole experiment was repeated in the following year with similar results. In contrast, no difference in hatchery survival between the various groups was observed in either year. Thus, native fish exhibited higher survival only in the more exacting conditions of the wild. First-generation hybrids showed intermediate survival. However, as discussed earlier, there are theoretical reasons to expect that the full effects of

outbreeding depression, if it were to occur in this case, would not be evident until the second and subsequent generations.

This combination of field experiment and molecular techniques demonstrated that native salmon were more fit under natural conditions, and thus productivity could be detrimentally affected by incursions of farmed escapes. Similar experiments looking at enhancement stocking, particularly in marine species, are urgently required in order to judge the efficacy of stocking (Howell 1994). Previous experiments, in which individual molecular identification was involved, used confined systems in which all offspring could potentially be identified. In the open situation of coastal waters, only the cultured animals would be identifiable. Computer simulations are required to determine the number and level of variability of microsatellite or minisatellite loci required in such circumstances.

Conclusions

In the foregoing review, the genetic aspects of translocation and stocking of fish species have been considered from the twin viewpoints of maximizing performance of translocated and stocked strains, but also minimizing the environmental impact of these practices. It is important, however, not just to consider the genetic aspects of a particular translocation or stocking proposal. All other aspects, e.g. ecology, disease implications, management implications, should also be considered before reaching a consensus. To achieve such a consensus, a team consisting of experts in all of these areas should consider each application. Only then can maximal environmental protection be achieved.

If it is decided that the aquaculture industry is to grow in Western Australia, then the granting of certain translocation requests is an inevitable consequence. This does not mean, however, that such requests should be granted without due consideration.

Requests for translocations for stocking are considerably more problematic, both because the potential benefits in economic and social terms are harder to quantify and because the possible adverse environmental effects can be grave. Many attempts at enhancement of fish species worldwide have failed to achieve beneficial effects, particularly in the marine environment (Palmer 1995; Howell, Moksness & Svasand 1999). With the relatively pristine environment that exists in Western Australia, extreme caution is required.

Acknowledgments

I wish to thank the Aquaculture Development Council of Western Australia and the Australian Fisheries Research and Development Corporation for providing financial support to attend a workshop in Perth, from which this paper emerged. I am also grateful to Dr A J Lymbery, Agriculture Western Australia, and to Dr R C Lenanton, Fisheries Department of Western Australia, for inviting me to attend the workshop and for a great deal of discussion and advice on the Western Australian situation.

References

Allendorf F.W. & Ryman N. (1987) Genetic management of hatchery stocks. In: *Population Genetics and Fishery Management* (ed. by N. Ryman & F. Utter), pp. 141–160. University of Washington Press, Seattle.

Avise J.C. (1994) *Molecular Markers, Natural History and Evolution.* Chapman & Hall, New York, London.

Bakke T.A. (1991) A review of the inter- and intraspecific variability in salmonid hosts to laboratory infections with *Gyrodactylus salaris* Malmberg. *Aquaculture* **98,** 303–310.

Campton D.E. (1987) Natural hybridisation and introgression in fishes: methods of detection and genetic implications. In: *Population Genetics and Fishery Management* (ed. by N. Ryman & F. Utter), pp. 161–192. University of Washington Press, Seattle.

Carvahlo G.R. (1998) Molecular ecology: origins and approaches. In: *Advances in Molecular Ecology* (ed. by G.R. Carvahlo), pp. 1–24. IOS Press, Amsterdam, Berlin, Oxford, Tokyo, Washington, DC.

Carvalho G.R. & Cross T.F. (1998) Enhancing fish production through introductions and stocking: genetic perspectives. In: *Stocking and Introductions of Fish* (ed. by I.G. Cowx), pp. 329–337. Fishing News Books, Oxford.

Carvahlo G.R. & Pitcher T.J. (eds) (1995) *Molecular Genetics in Fisheries.* Chapman & Hall, London.

Clifford S.L., McGinnity P. & Ferguson A. (1998) Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *Journal of Fish Biology* **52,** 118–127.

Cowx I.G. (ed.) (1989) *Stocking and Introductions of Fish.* Fishing News Books, Oxford.

Cross T.F. (1989) *Genetics and the Management of the Atlantic Salmon.* Atlantic Salmon Trust, Pitlochry, UK.

Cross T.F. (1999) Genetic considerations in stock en-

hancement and sea ranching of invertebrates, marine fish and salmonids. In: *Stock Enhancement and Sea Ranching* (ed. by B.R. Howell, E. Moksness & T. Svasand), pp. 37–48. Fishing News Books, Oxford.

Cross T.F. & Galvin P. (1996) *The Nature and Current Status of Transgenic Atlantic Salmon.* Marine Resource Bulletin No. 1. Irish Marine Institute, Dublin.

Cross T.F. & King J. (1983) Genetic effects of hatchery rearing in Atlantic salmon. *Aquaculture* **33**, 33–40.

Cross T.F., Galvin P. & Dillane E. (1998a) The use of molecular markers in aquaculture genetics. In: *ICES CM Doc. 1998/M: 6, Symposium K, The Use of Genetics in Aquaculture.* ICES, Copenhagen.

Cross T., McGinnity P. & Galvin P. (1998b) Genetic considerations in stocking Atlantic salmon, *Salmo salar*. In: *Stocking and Introductions of Fish* (ed. by I.G. Cowx), pp. 355–370. Fishing News Books, Oxford.

Devlin R.H., Yesaki T.Y., Blagl C.A., Donaldson E.M., Swanson P. & Chan W.-K. (1994) Extraordinary salmon growth. *Nature* **371**, 209–210.

Donaghy M.J. & Verspoor E. (1997) Egg survival and timing of hatch in two Scottish Atlantic salmon stocks. *Journal of Fish Biology* **51**, 211–214.

Doupé R.G., Horwitz P. & Lymbery A.J. (1999) Mitochondrial genealogy of Western Australian barramundi: applications of inbreeding coefficients and coalescent analysis for separating temporal population processes. *Journal of Fish Biology* **54**, 1197–1209.

Elton C.S. (1958) *The Ecology of Invasions by Animals and Plants.* Methuen, London, New York.

Estoup A. & Angers B. (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: *Advances in Molecular Ecology* (ed. by G.R. Carvahlo), pp. 55–86. IOS Press, Amsterdam, Berlin, Oxford, Tokyo, Washington, DC.

Ferguson A. (1995) Molecular genetic in fisheries: current and future perspectives. In: *Molecular Genetics in Fisheries* (ed. by G.R. Carvahlo & T.J. Pitcher), pp. 111–116. Chapman & Hall, London.

Gharrett A.J. & Shirley S.M. (1985) A genetic examination of spawning methodology in a salmon hatchery. *Aquaculture* **47**, 245–256.

Gharrett A.J. & Smoker W.W. (1991) Two generations of hybrids between even- and odd-year pink salmon (*Oncorhynchus gorbuscha*): a test for outbreeding depression. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1744–1749.

Gjedrem T. (1999) Genetic improvement of cold water fish species. *Aquaculture Research* **31**, 25–33.

Gjoen H.M. & Bentsen H.B. (1997) Past, present and future of genetic improvement in salmon aquaculture. *ICES Journal of Marine Science* **54**, 1009–1014.

Howell B.R. (1994) Fitness of hatchery-reared fish for survival in the sea. *Aquaculture and Fisheries Management* **25**, 3–17.

Howell B.R., Moksness E., Svasand T. (eds) (1999) *Stock Enhancement and Sea Ranching.* Fishing News Books, Oxford.

Ingram M. (1988) Farming rainbow trout in fresh water tanks and ponds. In: *Salmon and Trout Farming* (ed. by L.M. Laird & T. Needham), pp. 155–189. Ellis Horwood, Chichester.

Johnsen B.O. & Jensen A.J. (1986) Infestations of Atlantic salmon, *Salmo salar*, by *Gyrodactylus salaris* in Norwegian rivers. *Journal of Fish Biology* **29**, 233–241.

Johnson M.J. (1999) Measuring and interpreting genetic structure to minimize genetic risks of translocations. *Aquaculture Research* **31**, 133–143.

Jonasson J., Gjedre B. & Gjedrem T. (1997) Genetic parameters for return rate and body weight in sea-ranched Atlantic salmon. *Aquaculture* **154**, 219–231.

Jorstad K.E., Naevdal G., Paulsen O.I. & Thorkildsen S. (1994) Release and recapture of genetically tagged cod fry in a Norwegian fjord system. In: *Genetics and Evolution of Marine Organisms* (ed. by A.R. Beaumont), pp. 519–528. Chapman & Hall, London, New York.

Keenan C. (1999) Should we allow human-induced migration of the Indo-West Pacific fish, barramundi *Lates calcarifer* (Bloch) within Australia? *Aquaculture Research* **31**, 121–131.

Kincaid H. (1976) Inbreeding in rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada* **33**, 2420–2426.

Knibb W. (1999) Genetic improvement of marine fish – which method for industry? *Aquaculture Research* **31**, 11–23.

Krieg F. & Guyomard R. (1985) Population genetics of French brown trout (*Salmo trutta* L.): large geographical differentiation of wild populations and high similarity of domesticated stocks. *Genetic Selection and Evolution* **17**, 225–242.

Langdon J.S. (1990) Disease risks of fish introductions and translocations. In: *Introduced and Translocated Fishes and Their Ecological Effects* (ed. by D.A. Pollard), pp. 98–107. Australian Government Publishing Service, Canberra.

Lawrence C. (1993) *The Introduction and Translocation of Fish, Crustaceans and Molluscs in Western Australia.* Fisheries Management Paper, no. 57. Fisheries Department of Western Australia, Perth.

Lever C. (1998) Introduced fishes: an overview. In: *Stocking and Introductions of Fish* (ed. by I.G. Cowx), pp. 143–152. Fishing News Books, Oxford.

McGinnity P., Stone C., Taggart J.B., Cooke D., Cotter D., Hynes R., McCamley C., Cross T. & Ferguson A. (1997) Genetic impact of escaped farm Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farm and hybrid progeny in a natural environment. *ICES Journal of Marine Sciences* **54**, 998–1008.

Morison A. & Hume D. (1990) Carp (*Cyprinus carpio* L.) in Australia. In: *Introduced and Translocated Fishes and Their*

Ecological Effects (ed. by D.A. Pollard), pp. 110–113. Australian Government Publishing Service, Canberra.

Neilsen E.E., Hansen M.M. & Loeschcke V. (1997) Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years. *Molecular Ecology* **6,** 487–492.

O'Connell M. & Wright J.M. (1997) Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* **7,** 331–363.

Palmer P.J. (1995) *Enhancing Non-impounded Fisheries: a Review of the Current Literature with Relevance to Southern Queensland.* Department of Primary Industries, Brisbane.

Pollard D.A. (1990) *Introduced and Translocated Fishes and Their Ecological Effects.* Australian Government Publishing Service, Canberra.

Prokop F.B. (1995) What translocation can do for recreational fishing enhancement: benefits v costs. In: *Translocation Issues in Western Australia* (ed. by F.B. Prokop), pp. 30–36. Fisheries Management Paper No. 83. Fisheries Department of Western Australia, *Perth.*

Russell D.J., Hales P.W. & Ingram B.A. (1991) Use of coded-wire tags in enhancing coastal barramundi stocks. *Austasia Aquaculture* **5,** 26–27.

Ryman N. (1997) Minimising adverse effects of fish culture: understanding the genetics of populations with overlapping generations. *ICES Journal of Marine Science* **54,** 1149–1159.

Shaklee J.B. & Salini J. (1985) Genetic variation and population subdivision in Australian barramundi, *Lates calcarifer* (Bloch). *Australian Journal of Marine and Freshwater Research* **36,** 203–218.

Stahl G. (1987) Genetic population structure of Atlantic salmon. In: *Population Genetics and Fisheries Management* (ed. by N. Ryman & F. Utter), pp. 121–140. University of Washington Press, Seattle and London.

Taggart J.B. & Ferguson A. (1986) Electrophoretic evaluation of a supplemental stocking programme for brown trout (*Salmo trutta*). *Aquaculture and Fisheries Management* **17,** 155–162.

Tave D. (1986) *Genetics for Fish Hatchery Managers.* AVI Publishing Company, Westport, CN.

Thorn C. (1995) Translocation policy and controls. In: *Translocation Issues in Western Australia* (ed. by F.B. Prokop), pp. 12–27. Fisheries Management Paper No. 83. Fisheries Department of Western Australia, Perth.

Thorpe J.E. (ed.) (1980) *Salmon Ranching.* Academic Press, London.

Utter F., Aebersold P. & Winans G. (1987) Interpreting genetic variation detected by electrophoresis. In: *Population Genetics and Fisheries Management* (ed. by N. Ryman & F. Utter), pp. 21–45. University of Washington Press, Seattle and London.

Verspoor E. & Hammar J. (1991) Introgressive hybridisation in fishes: the biochemical evidence. *Journal of Fish Biology* **39** (Suppl. A), 309–334.

Verspoor E. & Jordan W.C. (1989) Genetic variation at the Me-2 locus in Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology* **35** (Suppl. A), 205–213.

Ward R.D., Woodwark M. & Skibinski D.O.F. (1994) A comparison of genetic diversity levels in marine, freshwater and anadromous fish. *Journal of Fish Biology* **44,** 213–232.

Welcomme R.M. & Bartley D.M. (1998) Current approaches to the enhancement of fisheries. *Fisheries Management and Ecology* **5,** 351–382.

Youngson A.F., Webb J.H., Thompson C.E. & Knox D. (1993) Spawning of escaped farmed Atlantic salmon (*Salmo salar*): hybridisation of females with brown trout (*Salmo trutta*). *Canadian Journal of Fisheries and Aquatic Sciences* **50,** 1986–1990.

Youngson A.F., Hansen L.P. & Windsor M.L. (1998) *Interactions between Salmon Culture and Wild Stocks of Atlantic Salmon: the Scientific and Management Issues.* Norwegian Institute for Nature Research (NINA), Trondheim, Norway.

Aquaculture Research, 2000, **31**, 95–119

Population genetic structure in penaeid prawns

J A H Benzie
Australian Institute of Marine Science, PMB No. 3, Townsville MC 4810, Queensland, Australia

Correspondence: J A H Benzie, Australian Institute of Marine Science, PMB No. 3, Townsville MC 4810, Queensland, Australia

Abstract

Genetic data are available for 27 species of penaeid prawn. Collected largely for fisheries purposes, they include information on several species of importance to aquaculture. Most studies used allozymes, but a small number have used mtDNA, random amplified polymorphic DNAs (RAPDs) and/or microsatellites. The DNA-based markers have revealed far greater levels of variation compared with the allozyme data. However, in the few cases for which joint information is available, the mtDNA and microsatellite information tended to confirm the spatial patterns of variation detected by allozymes. These revealed little genetic variation over long distances (thousands of kilometres) for many species, but relatively major shifts in gene, or genotype, frequencies over relatively short distances (hundreds of kilometres). Much of the genetic structure in wild populations appears to reflect historical events on large biogeographical scales, rather than resulting from patterns of present-day dispersal. Genetic variation in cultured stocks is generally less than in wild ones, the extent of the reduction being dependent upon broodstock management procedures. There is no conclusive evidence that aquaculture escapees have altered the genetic constitution of wild stocks of *Penaeus monodon* in Thailand. Nevertheless, the occurrence of strong patterns of geographic variation in wild stocks suggests that more detailed planning will be required to maintain this diversity, and to determine how best to capture its benefits for aquaculture.

Introduction

Penaeid prawns provide the basis for large aquaculture industries worldwide (Fast & Lester 1992). These industries have developed rapidly over the last 30 years and are dominated by five species [*P. monodon* Fabricius 1798 (60% of world production); *P. vannamei* Boone 1931 (27%); *P. chinensis* (Osbeck 1765) (8%); *P. stylirostris* Stimpson 1874 (3%); *P. japonicus* Bate 1888 (< 1%); several other species account for the remaining 2% of production, principal among which are *P. penicillatus* Alcock 1905; *P. merguiensis* De Man 1888; *P. indicus* H. Milne Edwards, 1837; *M. ensis* (De Haan 1844) (Rosenberry 1997)]. These have proved particularly useful out of the 20 or so that were investigated originally for their aquaculture potential. Many more penaeid prawn species have been subject to fishing for a long time (Dall, Hill, Rothlisberg & Staples 1990), and most of the published genetic studies on penaeid prawns have been aimed at determining stock structure for fisheries.

This information is still of considerable use for aquaculture purposes. Wild populations remain the dominant source of broodstock for some of the most important aquaculture species, and sound management of the wild resource is therefore as vital for the aquaculture industry as for wild fisheries. These studies also provide useful information on the genetic diversity available in nature and for the future planning of the source of broodstock for closed-cycle breeding programmes (Hedgecock & Malecha 1991).

Much of the early allozyme work generally emphasized the lack of significant geographic structure in wild populations. However, it was noted that penaeids, like many decapods, have very little allozyme variation (Hedgecock, Tracey & Nelson 1982). The possibility that the lack of spatial differentiation among wild populations observed using allozyme techniques was simply a result of the low resolution of the technique led to interest in

using potentially more variable markers, such as mtDNA and microsatellites. The growing interest in aquaculture of prawns also led to the development of highly variable DNA markers for use in paternity testing, confirmation of progeny identity and for tracking families in culture systems (reviewed by Benzie 1998a). Recent work has shown that prawn populations can have considerable genetic structure, and this has implications for the management of these species and aquaculture development.

The present paper reviews the available data on penaeid prawns to assess the level of genetic diversity present in wild and cultured populations, and the spatial and temporal structure of wild populations.

Variation in the wild

The available data

Data have been published on eight *Metapenaeus* species and 19 *Penaeus* species in a total of 30 studies (Table 1). Reports for 20 species come from a small number of publications that examined only one or two populations for a variety of taxa (Mulley & Latter 1980; Ko, Pasteur, Bonhomme, Aquacop, Seafdec & Liao 1983; Ko 1984; Tam & Chu 1993). However, multiple studies have been reported for 13 species, providing a range of estimates of genetic diversity for these.

By far the majority of the reports have been of allozyme variation (58 out of the 71 studies listed in Table 1). This contrasts with the three reports for mtDNA, three for RAPDs and six for microsatellites estimated on the same basis.

Most of the studies have been concentrated in Australia, followed by Hong Kong, the Gulf of Mexico, the east coast of the USA and Thailand. Other regions sampled, usually only one region for a given species, have been Japan, south-east Asia, South Africa and Madagascar, and the Mediterranean. Sampling has therefore been patchy, but major areas of the species range have been covered for 11 species: *M. bennetae* Racek & Dall 1965; *M. macleayi* (Haswell 1879); *P. aztecus* Ives 1891; *P. duorarum* Burkenroad 1939; *P. esculentus* Haswell 1879; *P. kerathurus* (Forskal 1775); *P. latisulcatus* Kishinouye 1896; *P. monodon*; *P. plebejus* Hess 1865; *P. setiferus* (Linnaeus 1767); and *P. vannamei*.

The studies also cover a range of life history types described for penaeids (Dall *et al.* 1990), from those in which species reproduce and complete development within estuaries and tidal lakes to those in which species spend all their adult life in offshore waters and whose larvae develop in open inshore waters rather than in estuaries.

Genetic diversity

Information on the genetic diversity within prawn populations is highly variable with respect to the measure of genetic diversity reported and the sample size of both loci and number of individuals upon which the estimates were based (Table 2). The number of individuals sampled for a given locus ranges from one to more than 200, but most fall within a range of 20–60 per population. Similarly, the number of loci sampled ranges from one to 40, but most studies used between 15 and 35.

Excluding those studies aimed at determining spatial patterns of variation, which therefore used only polymorphic loci, the proportion of polymorphic loci per population (where the frequency of the most common allele does not exceed 0.95) ranges from 0.022 to 0.333 (average 0.179). Where the frequency of the most common allele was defined not to exceed 0.99, the proportion of polymorphic loci reported ranges from 0.059 to 0.294 (average 0.175). The mean number of alleles per locus ranges tightly from 1.06 to 1.56 (average 1.25). Observed heterozygosities range from 0.006 to 0.089 (average 0.034) and, although showing a tendency to be smaller than the estimates of expected heterozygosity, differ little from these in most cases.

There is no obvious difference in genetic diversity between species with respect to the level of variation detected (Table 2). Most assessments of the mean number of alleles, or of observed heterozygosity, overlap the standard errors of the estimates for other taxa, or the range of estimates from different studies of one taxon overlap the range of estimates for other taxa. The estimates of allozyme variation from the present review are similar to those reported by Hedgecock *et al.* (1982) for decapods. As the data then available for penaeids were limited to five species (*P. aztecus*, *P. brasiliensis* Latreille 1817, *P. duorarum*, *P. merguiensis* and *P. setiferus*), Hedgecock *et al.* (1982) pooled information from these with some caridean shrimp. The mean numbers of alleles per locus (and range) over 11 species they reported was 1.64 (1.18–2.17), the proportion of polymorphic loci (using a 0.99 cut-off) was 0.267

Table 1 Details of the geographic extent of sampling, sample size and type of markers used in each genetic study of wild populations of penaeid prawns for which data have been published (from the genera *Metapenaeus* and *Penaeus*), together with information on the biology of the species concerned.

Species information			Genetic data collected			Source
Species	**Geographical range**	**Life history**	**Geographical extent of study**	**Type of genetic marker**	**No. of populations sampled**	
M. affinis (H. Milne Edwards 1837)	Northern Indian Ocean, SE Asia (Arabia, India, SE Asia east to Java, Borneo and S. China)	Type 2. Adults nearshore to 92 m	Hong Kong	Allozymes	1	Tam & Chu (1993)
M. bennettae Racek and Dall 1965	Eastern Australia	Type 1. Juveniles estuarine or upriver. Adults same and nearshore to 14 m	East Australia	Alloyzymes	2	Mulley & Latter (1980)
			East Australia	Allozymes	2–4	Mulley & Latter (1981a)
			East Australia	Allozymes	6	Salin (1987)
M. eboracensis Racek and Dall 1965	Northern Australia Papua New Guinea	Type 2. Inshore, rivers and estuaries to 30 m	North Australia	Allozymes	1	Mulley & Latter (1980)
M. endeavouri (Schmitt 1926)	Northern Australia Papua New Guinea	Type 3. Juveniles estuarine. Adults inshore to 50 m	North and West Australia	Allozymes	2	Mulley & Latter (1980)
			North and West Australia	Allozymes	5	Mulley & Latter (1981b)
M. ensis (De Haan 1844)	Indo-West Pacific (Eastern India to SE Asia, Japan and Australia)	Type 2. Juveniles estuarine. Adults inshore to 65 m	North and East Australia	Allozymes	2	Mulley & Latter (1980)
			Japan	Allozymes	3	Taniguchi & Han (1989)
			North and East Australia	Allozymes	2	Mulley & Latter (1981b)
			Hong Kong	Allozymes	1	Tam & Chu (1993)
			Japan	RAPDs	1	Meruane *et al.* (1997)
M. insolitus Racek and Dall 1965	Northern Australia	Type 2. Inshore, creeks and estuaries to 33 m	North Australia	Allozymes	1	Mulley & Latter (1980)
M. joyneri (Miers 1880)	Indo-China to Japan	Type 2. Inshore, rivers and estuaries to 30 m	Hong Kong	Allozymes	1	Tam & Chu (1993)
M. macleayi (Haswell 1879)	Eastern Australia	Type 2. Juveniles estuarine. Adults inshore to 60 m	East Australia	Allozymes	3	Mulley & Latter (1980)
			East Australia	Allozymes	11	Mulley & Latter (1981a)
P. aztecus Ives 1891	East and South USA, Mexico. (Atlantic coast New Jersey to Florida, Gulf of Mexico)	Type 2. Juveniles estuarine, marine. Adults inshore 4 to 160 m	Gulf of Mexico	Allozymes	3	Proctor *et al.* (1974)
			Gulf of Mexico	Allozymes	1	Lansford *et al.* (1976)
			Gulf of Mexico	Allozymes	4	Lester (1979)
			South Carolina	Microsatellites	1	Ball *et al.* (1998)

Table 1 Continued

Species information			Genetic data collected			Source
Species	**Geographical range**	**Life history**	**Geographical extent of study**	**Type of genetic marker**	**No. of populations sampled**	
P. brasiliensis Latreille 1817	Western Atlantic. (Florida, Caribbean, east coast of South America to S. Brazil)	Type 3. Juveniles estuarine. Adults inshore mostly 45–65 m, to 365 m	Gulf of Mexico	Allozymes	1	Lansford *et al.* (1976)
P. chinensis (Osbeck 1765)	China and Korea	Type 2. Juveniles estuarine, marine. Adults offshore 90 to 180 m	Hong Kong	Allozymes	1	Tam & Chu (1993)
P. duorarum Burkenroad 1939	East and South USA, Mexico. (Atlantic coast from Maryland to Florida, Gulf of Mexico)	Type 2/3. Juveniles brackish water. Adults inshore, 2 to 70 m	Florida	Alloyzymes	1	Marvin *et al.* (1977)
			Gulf of Mexico	Alloyzymes	4	Lester (1979)
			Gulf of Mexico	Alloyzymes	4	Lester (1983)
			Florida	Microsatellites	1	Ball *et al.* (1998)
P. esculentus Haswell 1879	North, East and West Australia	Type 3. Juveniles estuarine, marine. Adults offshore 10 to 200 m	North and West Australia	Allozymes	2	Mulley & Latter (1980)
			North East and West Australia	Allozymes	4	Mulley & Latter (1981b)
P. indicus H. Milne Edwards 1837	Indo-West Pacific (South Africa to India, SE Asia and north Australia)	Type 2. Juveniles estuarine, marine. Adults inshore 2 to 90 m	Kenya	Allozymes	1	Ko *et al.* (1983), Ko (1984*)
P. japonicus Bate 1888	Indo-West Pacific (South Africa to India, SE Asia, Japan, north and east Australia)	Type 3. Juveniles estuarine, marine. Adults inshore to 90 m	Japan	Allozymes	4	Taniguchi & Han (1989)
			Hong Kong	Allozymes	1	Tam & Chu (1993)
			Japan	RAPDs	1	Meruane *et al.* (1997)
P. kerathurus (Forskal 1775)	Europe and Africa (Atlantic coast of Angola to France, all Mediterranean)	Type 3. Juveniles estuarine, marine. Adults inshore to 40 m	Adriatic	Allozymes	1	DeMatthaeis *et al.* (1983)
			France	Allozymes	1	Ko *et al.* (1983); Ko (1984*)
			Spain, France, Italy, Tunisia	Allozymes	6	Mattoccia *et al.* (1987)
P. latisulcatus Kishinouye 1896	Indo-West Pacific (Mozambique to India, SE Asia, north, east, south and west Australia)	Type 3. juveniles estuarine, marine. Adults inshore to 90 m	North, West and South Australia	Allozymes	3	Mulley & Latter (1980)
			North West and South Australia	Allozymes	8	Mulley & Latter (1981b)
			South Australia	Alloymes	6	Richardson (1982)
			Hong Kong	Allozymes	1	Tam & Chu (1993)

Table 1 Continued

Species information			Genetic data collected			Source
Species	**Geographical range**	**Life history**	**Geographical extent of study**	**Type of genetic marker**	**No. of populations sampled**	
P. longistylus Kubo 1943	West Pacific (South China Sea to Australia)	Type 3. Juveniles estuarine, marine. Adults inshore 35 to 55 m	North and East Australia	Allozymes	2	Mulley & Latter (1980)
P. merguiensis De Man 1888	Indo-West Pacific (Persian Gulf and India to SE Asia and Australia)	Type 2. Juveniles estuarine, marine. Adults inshore 10 to 45 m.	North and East Australia	Allozymes	2	Mulley & Latter (1980)
			North Australia (Gulf of Carpentaria)	Allozymes	1	Redfield *et al.* (1980)
				Allozymes	2	Mulley & Latter (1981b)
			North and East Australia Hong Kong	Allozymes	1	Tam & Chu (1993)
P. monodon Fabricius 1798	Indo-West Pacific (South Africa to India, SE Asia, Japan, north, east and west Australia)	Type 2. Juveniles estuarine, marine. Adults inshore to 110 m.	North and East Australia	Allozymes	2	Mulley & Latter (1980)
			Taiwan, Fiji, Philippines	Allozymes	3	Ko *et al.* (1983), Ko (1984*)
			Thailand	Allozymes	4	Sodsuk *et al.* (1992)
			North, East and West Australia	Allozymes	7	Benzie *et al.* (1992)
			Hong Kong	Allozymes	1	Tam & Chu (1993)
			South West Indian Ocean	Allozymes	5	Forbes *et al.* (1999)
			East and West Australia	mtDNA	3	Benzie *et al.* (1993)
			SW Indian Ocean, SE Asia and Australia	mtDNA	13	Benzie *et al.* (2000)
			SE Asia and Africa	mtDNA	10	Klinbunga *et al.* (1998)
			Thailand	RAPDs	3	Tassanakajon *et al.* (1997)
			Thailand	Microsatellites	1	Tassanakajon *et al.* (1998a)
			Thailand	Microsatellites	5	Tassanakajon *et al.* (1998b)
			North, East and West Australia	Microsatellites	5	Brooker *et al.* (1999)
P. notialis Perez Farfante 1967	Atlantic (coasts of west and central Africa, southern Brazil, north to Cuba and South Mexico in the Caribbean)	Type 2/3. Juveniles estuarine. Adults inshore to 100 m.	Cuba	Allozymes	3	Villaescusa *et al.* (1984)

Table 1 Continued

Species information			Genetic data collected			Source
Species	**Geographical range**	**Life history**	**Geographical extent of study**	**Type of genetic marker**	**No. of populations sampled**	
P. penicillatus Alcock 1905	Northern Indian Ocean and West Pacific (Madagascar, Gulf of Arabia, India, SE Asia east to Java, Borneo and S. China)	Type 2. Juveniles marine. Adults inshore to 90 m.	Hong Kong	Allozymes	1	Tam & Chu (1993)
P. plebejus Hess 1865	Eastern Australia	Type 3. Juveniles estuarine. Adults offshore to 220 m.	East Australia	Allozymes	4	Mulley & Latter (1980)
			East Australia	Allozymes	8–11	Mulley & Latter (1981a)
P. semisulcatus De Hann 1844	Indo-West Pacific (South Africa to India, SE Asia, Japan, north, east and west Australia)	Type 3. Juveniles shallow marine. Adults inshore to 130 m.	North and East Australia	Allozymes	2	Mulley & Latter (1980)
			North and East Australia	Allozymes	2	Mulley & Latter (1981b)
			Japan	Allozymes	1	Taniguchi & Han (1989)
			Hong Kong	Allozymes	1	Tam & Chu (1993)
P. setiferus (Linnaecus 1767)	East and South USA, Mexico. (Atlantic coast from New Jersey to Florida, Gulf of Mexico)	Type 2. Juveniles estuarine. Adults inshore to 90 m.	Texas, Louisiana and South Carolina	Allozymes	5	Marvin & Caillouet (1976)
			Louisiana and Mississippi	Allozymes	2	Marvin *et al.* (1977)
			Gulf of Mexico	Allozymes	4	Lester (1979)
			Gulf of Mexico	Allozymes	4	Lester (1983)
			South Carolina	Microsatellites	1	Ball *et al.* (1998)
P. stylirostiris Stimpson 1874	Eastern Pacific (Mexico to central Peru)	Type 2. Juveniles estuarine. Adults inshore to 27 m.	Mexico, Ecuador.	Allozymes	3	Lester (1983)
P. vannamei Boone 1931	Eastern Pacific (Sonora, Mexico to northern Peru)	Type 2. Juveniles shallow marine. Adults inshore to 72 m.	Costa Rica, Ecuador	Allozymes	2	Lester (1983)
			Mexico, Panama and Ecuador	Allozymes	3	Sunden & Davis (1991)

*Ko (1984) was referenced in error as Lioe (1984) by Mattoccia *et al.* (1987), and subsequently by Benzie *et al.* (1992) and Benzie (1998) (Author's full name is Ko, Gin Lioe). Information on the range and life histories was obtained from the references cited or, more usually, from Grey *et al.* (1983), Dore & Frimodt (1987) and Dall *et al.* (1990). The dash (–) indicates no data were available from the paper cited.

Table 2 Genetic variation observed in each genetic study of wild populations of penaeid prawns (from the genera *Metapenaeus* and *Penaeus*), for which data have been published

Species	Genetic data collected								Source
	No. of populations sampled	Mean no of individuals sampled per population	No. of loci, (no. of polymorphic loci)	Proportion of polymorphic loci 95%	99%	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected	
Allozymes									
M. affinis	1	1.0–26.0	17 (2)	0.059	0.118	1.18 ± 0.13	0.029 ± 0.025	0.027 ± 0.023	Tam & Chu (1993)
M. bennettae	2	48.5	40 (8)	0.200	–	–	0.020 ± 0.009	–	Mulley & Latter (1980)
	2–4	83.3–84.5	3 (3)	–	–	–	–	–	Mulley & Latter (1981a)
	6	172.3	8 (8)	–	–	–	–	–	Salini (1987)
M. eboracensis	1	44.0	40 (7)	0.175	–	–	0.019 ± 0.013	–	Mulley & Latter (1980)
M. endeavouri	2	49.5	40 (8)	0.200	–	–	0.030 ± 0.015	–	Mulley & Latter (1980)
	5	34.8–132	4 (4)	–	–	–	–	–	Mulley & Latter (1981b)
M. ensis	2	33.5	40 (8)	0.200	–	–	0.013 ± 0.006	–	Mulley & Latter (1980)
	2	35.0–37.5	2 (2)	–	–	–	–	–	Mulley & Latter (1981b)
	3	55–59	15 (4)	0.133–0.266		1.27–1.53	0.025–0.038	0.041–0.046	Taniguchi & Han (1989)
	1	18.0–34.0	17 (1)	0.059	0.059	1.06 ± 0.06	0.020 ± 0.020	0.029 ± 0.029	Tam & Chu (1993)
M. insolitus	1	29.0	40 (4)	0.100	–	–	0.010 ± 0.006	–	Mulley & Latter (1980)
M. joyneri	1	2.0–26.0	17 (3)	0.118	0.177	1.18 ± 0.10	0.022 ± 0.014	0.020 ± 0.012	Tam & Chu (1993)
M. macleayi	3	50.0	40 (7)	0.175	–	–	0.026 ± 0.015	–	Mulley & Latter (1980)
	11	78.0	1 (1)	–	–	–	–	–	Mulley & Latter (1981a)
P. aztecus	3	200.0	1 (1)	1.000	1.000	–	–	–	Proctor *et al.* (1974)
	1	310.0	1 (1)	1.000	1.000	–	–	–	Lansford *et al.* (1976)
	4	39.0	24 (11)	0.330	–	–	0.089 ± 0.028	–	Lester (1979)
P. brasiliensis	1	390.0	1 (1)	1.000	1.000	–	–	–	Lansford *et al.* (1976)
P. chinensis	1	27.0–37.0	17 (2)	0.118	0.118	1.12 ± 0.08	0.041 ± 0.028	0.045 ± 0.032	Tam & Chu (1993)
P. duorarum	1	206	1 (1)	1.000	1.000	–	–	–	Marvin *et al.* (1977)
	4	32.6	24 (10)	0.330	–	–	0.084 ± 0.030	–	Lester (1979)
	4	32.6	24 (10)	0.33	–	–	0.08	–	Lester (1983)
P. esculentus	2	46.5	37 (9)	0.243	–	–	0.033 ± 0.016	–	Mulley & Latter (1980)
	4	46.8–76.0	4 (4)	1.000	1.000	–	–	–	Mulley & Latter (1981b)
P. indicus	1	20	29 (4)	0.138	–	–	0.026	–	Ko *et al.* (1983); Ko (1984*)
P. japonicus	4	51–60	15 (6)	0.385–0.533		1.62 ± 1.93	0.031–0.060	0.036–0.069	Taniguchi & Han (1989)
	1	22.0–26.0	17 (4)	0.235	0.235	1.24 ± 0.11	0.047 ± 0.029	0.068 ± 0.036	Tam & Chu (1993)

Table 2 Continued

Species	Genetic data collected								Source
	No. of populations sampled	Mean no of individuals sampled per population	No. of loci, (no. of polymorphic loci)	Proportion of polymorphic loci 95%	99%	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected	
P. kerathurus	1	18.0–68.0	34 (8)	–	0.265	1.27	0.049	0.055	DeMatthaeis *et al.* (1983)
	1	21	29 (5)	0.172	–	–	0.051	–	Ko *et al.* (1983); Ko (1984*)
	4	41	18 (10)	0.022–0.333$^{0.99}$		1.28–1.56	0.052–0.089	0.046–0.095	Mattoccia *et al.* (1987)
P. latisulcatus	3	55.7	37 (5)	0.135	–	–	0.032 ± 0.019	–	Mulley & Latter (1980)
	8	97.1–146.1	(3)	–	–	–	–	–	Mulley & Latter (1981b)
	6	86.6–88.2	18 (2)	0.111	0.111	–	0.028	–	Richardson (1982)
	1	22.0–28.0	17 (3)	0.118	0.177	1.18 ± 0.10	0.049 ± 0.031	0.058 ± 0.037	Tam & Chu (1993)
P. longistylus	2	6.5	37 (3)	0.081	–	–	0.006 ± 0.003	–	Mulley & Latter (1980)
P. merguiensis	2	34.5	37 (6)	0.162	–	–	0.008 ± 0.004	–	Mulley & Latter (1980)
	1	216.0	25 (4)	0.040	0.160	1.40 ± 0.21	0.008 ± 0.003	0.008 ± 0.003	Redfield *et al.* (1980)
	2	68.0	1 (1)	1.000	1.000	–	–	–	Mulley & Latter (1981b)
	1	24.0–32.0	17 (4)	0.118	0.235	1.29 ± 0.14	0.045 ± 0.025	0.057 ± 0.034	Tam & Chu (1993)
P. monodon	2	6.0	37 (3)	0.081	–	–	0.008 ± 0.005	–	Mulley & Latter (1980)
	3	20.3	29 (3)	0.103	–	–	0.000–0.023	–	Ko *et al* (1983); Ko (1984*)
	3	119.3	35 (5)	–	0.14	–	0.029–0.041	–	Sodsuk *et al.* (1992)
	4	97.8	35 (5)	–	0.14	–	0.030–0.040	–	Sodsuk *et al.* (1992)
	7	85.1	8 (8)	0.375–1.000$^{0.95}$		1.40–3.00	0.045–0.103	0.053–0.103	Benzie *et al.* (1992)
	1	14.0–30.0	17 (4)	0.177	0.235	1.35 ± 0.19	0.040 ± 0.022	0.054 ± 0.030	Tam & Chu (1993)
	5	68.6	8 (8)	0.556–0.778$^{0.95}$		1.8–2.4	0.083–0.102	0.111–0.119	Forbes *et al.* (1999)
P. notialis	3	50.0	2 (2)	1.000	1.000	–	–	–	Villaescusa *et al.* (1984)
P. penicillatus	1	10.0–18.00	17 (1)	0.059	0.059	1.06 ± 0.06	0.007 ± 0.007	0.007 ± 0.007	Tam & Chu (1993)
P. plebejus	4	35.0	37 (9)	0.243	–	–	0.022 ± 0.014	–	Mulley & Latter (1980)
	8–11	94.8–111.4	2 (2)	1.000	1.000	–	–	–	Mulley & Latter (1981a)

Table 2 Continued

Species	Genetic data collected							Source
	No. of populations sampled	Mean no of individuals sampled per population	No. of loci, (no. of polymorphic loci)	Proportion of polymorphic loci 95%	99%	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected	
P. semisulcatus	2	37.0	37 (8)	0.216	–	–	0.017 ± 0.007	–	Mulley & Latter (1980)
	2	29.0–40.5	3 (3)	1.000	1.000	–	–	–	Mulley & Latter (1981b)
	1	70	15 (6)	0.400		1.47	0.057	0.058	Taniguchi & Han (1989)
	1	13.0–33.0	17 (5)	0.177	0.294	1.29 ± 0.11	0.044 ± 0.023	0.052 ± 0.031	Tam & Chu (1993)
P. setiferus	5	209.6	1 (1)	1.000	1.000	–	–	–	Marvin & Caillouet (1976)
	2	184	1 (1)	1.000	1.000	–	–	–	Marvin *et al.* (1977)
	4	34.8	24 (10)	0.294	–	–	0.070 ± 0.024	–	Lester (1979)
	4	34.8	24 (10)	0.29	–	–	0.07	–	Lester (1983)
P. stylirostris	3	Not given	20 (4)	0.250	–	–	0.06	–	Lester (1983)
P. vannamei	2	not given	18 (3)	0.16	–	–	0.02	–	Lester (1983)
	3	117.3	13 (10)	0.192–0.269[0.95]		–	0.017–0.020 (0.045–0.054)		Sunden & Davis (1991)
Microsatellites									
P. aztecus	1	9.3	3 (3)	1.000	1.000	–	0.573	–	Ball *et al.* (1998)
P. duorarum	1	9.3	3 (3)	1.000	1.000	–	0.483	–	Ball *et al.* (1998)
P. monodon	1	48.0–62.0	2 (2)	1.000	1.000	21.0	0.425	0.890	Tassanakajon *et al.* (1998a)
	5	35.6–36.8	3 (3)	1.000	1.000	26.3	0.657–0.803	0.933–0.950	Tassanakajon *et al.* (1998b)
	5	60.2–62.4	3 (3)	1.000	1.000	24.7–46.3	0.870–0.988	0.852–0.981	Brooker *et al.* (1999)
P. setiferus	1	40.8	6 (6)	1.000	1.000	–	0.537	–	Ball *et al.* (1998)
RAPDs			Number of bands	Proportion of polymorphic bands		Not applicable	Not applicable	Not applicable	
M. ensis	1	15	11 (11)	1.00		–	–	–	Meruane *et al.* (1997)
P. japonicus	1	11	11 (11)	1.00		–	–	–	Merrruane *et al.* (1997)
P. monodon	3	23.7	70 (40)	0.242	0.478	–	–	–	Tassanakajon *et al.* (1997)

Table 2 Continued

Species	Genetic data collected								Source
	No. of populations sampled	Mean no of individuals sampled per population	No. of loci, (no. of polymorphic loci)	Proportion of polymorphic loci 95%	99%	Mean no. of alleles per locus	Mean heterozygosity observed	Mean heterozygosity expected	
mtDNA			Number of restriction enzymes			Ratio of no. of haplotypes to no. of individuals	Haplotype diversity	Nucleotide diversity ($\times 10^3$)	
P. monodon	3	3.0–6.0	4(4)	–		0.33–0.67	–	–	Benzie *et al.* (1993)
	13	64.8	7(7)	–		0.10–0.38	0.682 ± 0.002	1.283 ± 0.002	Benzie *et al.* (2000)
	10	20.6	5(5)	–		0.08–0.38	0.537 ± 0.006	3.341 ± 0.003	Klinbunga *et al.* (1998)

The dash (–) indicates no data were available from the paper cited.

(0.107–0.458) and expected heterozygosities were 0.051 (0.008–0.089). If anything, the data from a far wider range of penaeids collected in the present review suggest slightly lower levels of polymorphism, perhaps as a result of not including data from other taxonomic groups. The more recent allozyme data confirm that penaeid prawns have lower levels of polymorphism than other taxonomic groups (Hedgecock *et al.* 1982).

In contrast, the limited data for microsatellites show a far higher proportion of loci that are polymorphic (100% to date), although it will be some time before realistic estimates are available given the difficulty of isolating loci from prawns for which primers can be designed that work reliably. This has been specifically a problem in prawns because of the large size of many microsatellites and the degenerate margins of these making primer design difficult (Brooker, Benzie, Blair & Versini 1999). Observed heterozygosities over all loci reported for four species range from 0.425 to 0.964 (average 0.666) and were often less than the expected heterozygosities, which range from 0.890 to 0.959 (average 0.927). These values are similar to those reported for a wide range of vertebrates and invertebrates. Similarly, RAPD data also show levels of polymorphism that are higher than allozymes (range 0.242–1.00). The limited data available for mtDNA diversity, from *P. monodon*, also indicate far higher levels of genetic diversity than were detected using allozymes. Haplotype diversity (about 0.5–0.7) and nucleotide diversity (1.2–3.3) estimates are similar to those from a wide variety of other taxonomic groups (Avise 1994).

The five penaeid species that dominate aquaculture production do not appear to differ from other penaeids with respect to the level of genetic diversity they display in wild populations. The proportion of polymorphic loci per population (where the frequency of the most common allele does not exceed 0.95) ranges from 0.118 to 0.295 (average 0.215) compared with 0.022–0.333 (average 0.179) for the whole data set. The mean number of alleles per locus ranges tightly from 1.12 to 1.99 (average 1.45) compared with 1.06–1.56 (average 1.25) for the whole data set. Observed heterozygosities range from 0.00 to 0.103 (average 0.049) compared with 0.006–0.089 (average 0.034) for the whole data set.

Table 3 Genetic differentiation reported among wild populations of penaeid prawns (from the genera *Metapenaeus* and *Penaeus*).

	Genetic data collected						
Species	**No. of populations sampled**	**Geographical range (km)**	**Genetic distance**	**F_{ST} or G_{ST}(+)**	**Frequency of private alleles**	**Genetic differentiation detected [and the scale (km)]**	**Source**
M. bennettae	2–4	20–800	–	–	0.01	Yes (30–800)	Mulley & Latter (1981a)
	6	30–1300	–	–	None	Yes (900)	Salini (1987)
M. endeavouri	5	100–3500	–	–	None	Yes (3500)	Mulley & Latter (1981b)
M. ensis	2	1300	–	–	0.046	No	Mulley & Latter (1981b)
	3	400–700	0.0007–0.0016	–	None	No	Taniguchi & Han (1989)
M. macleayi	11	30–1500	–	–	None	Yes (100)	Mulley & Latter (1981a)
P. aztecus	3	200–400	–	–	None	No	Proctor *et al.* (1974)
	4	400–1600	0.003 ± 0.001	0.022+*	None	No (but significant on χ^2 test made for the present review)	Lester (1979)
P. duorarum	4	400–1400	0.002 ± 0.001	0.015 + NS	None	No	Lester (1979)
P. esculentus	4	1200–5900	–	–	0.010	No	Mulley & Latter (1981b)
	4	400–700	0.002–0.0008	–	None	No	Taniguchi & Han (1989)
P. kerathurus	4	200–1400	0.001–0.011	0.040 NS	0.040	Yes (1400) (but not significant on χ^2 test made for the present review)	Lester (1979)
P. duorarum	4	400–1400	0.002 ± 0.001	0.015 NS	None	No	Lester (1979)
P. esculentus	4	1200–5900	–	–	0.010	No	Mulley & Latter (1981b)
	4	400–700	0.002–0.0008	–	None	No	Taniguchi & Han (1989)
P. kerathurus	4	200–1400	0.001–0.011	0.040 NS	0.040	Yes (1400) (but not significant on χ^2 test made for the present review)	Mattoccia *et al.* (1987)
P. latisulcatus	8	300–5900	–	–	0.006	Yes (450–5900)	Mulley & Latter (1981b)
	6	30–150	–	–	None	No	Richardson (1982)
P. merguiensis	2	2100	–	–	0.01	No	Mulley & Latter (1981b)
P. monodon	3						Ko *et al.* (1983); Ko (1984*)
	3	150–600	0.001–0.002	0.008 NS	None	No	Sodsuk *et al.* (1992)
	4	150–600	0.000–0.002	0.008 NS	None	No	Sodsuk *et al.* (1992)
	7	400–5000	0.000–0.015	0.031***	0.007	Yes (1400)	Benzie *et al.* (1992)
	5	150–2500	0.000–0.002	0.007 NS	0.015	No	Forbes *et al.* (1999)
P. notialis	3	40–80	–	–	None	No	Villaescusa *et al.* (1984)
P. plebejus	11	60–1500	–	–	0.01	No	Mulley & Latter (1981a)
P. semisulcatus	2	1300	–	–	0.04	No	Mulley & Latter (1981b)

Table 3 Continued

Species	Genetic data collected: No. of populations sampled	Geographical range (km)	Genetic distance	F_{ST} or G_{ST}(+)	Frequency of private alleles	Genetic differentiation detected [and the scale (km)]	Source
P. setiferus	5	200–1800	–	–	None	No	Marvin & Caillouet (1976)
	2	600	–	–	None	No	Marvin *et al.* (1977)
	4	400–1300	0.002 ± 0.001	0.017 NS	None	No	Lester (1979)
P. stylirostris	3	3000	–	–	0.01	No	Lester (1983)
P. vannamei	3	1200–3500	–	0.004 NS	None	No	Sunden & Davis (1991)
RAPDs							
P. monodon	3	150–600	0.032–0.070	–	None	Yes (600)	Tassanakajon *et al.* (1997)
Microsatellites							
P. monodon	5	150–600	–	–	–	Yes (600)	Tassanakajon et al. (1998b)
	5	400–5000	–	0.010**	–	Yes (3300)	Brooker *et al.* (1999)
mtDNA			Nucleotide divergence				
P. monodon	3	400–5000	–	–	–	Yes (1400)	Benzie *et al.* (1993)
	13	250–12 000	0.052 ± 0.00003	–	–	Yes (250)	Benzie *et al.* (2000)
	10	150–9500	1.334 ± 0.001	–	–	Yes (600)	Klinbunga *et al.* (1998)

*$P<0.05$, **$P<0.01$, ***$P< 0.001$; NS, not significant.

Where no genetic distance or F_{ST} value was provided in the paper, genetic differentiation was detected usually by pairwise chi-squared tests of difference in allele frequencies between populations. The dash (–) indicates no data were available from the paper cited.

Life history variation

In the early studies of genetic variation in Australian species of penaeid prawns, Mulley & Latter (1980) noted a relatively close correlation between the life history of a species and the level of genetic differentiation between populations. *M. bennettae*, which reproduces and completes development within tidal estuaries and lakes, demonstrated some significant differences in allele frequencies between populations, whereas other species did not. The *M. bennettae* life history has been classified by Dall *et al.* (1990) as type 1, one of a set of four life history classes they recognized. The other species examined belonged to type 2 and type 3 classes. Type 2 species spend their adult life at sea, spawn in coastal waters, and their larvae migrate inshore, often into estuaries where they develop before moving offshore as they mature. Type 3 spends all its adult life offshore and, although the larvae can move into estuaries, they tend to inhabit inshore regions, perhaps seagrass or algal beds. Finally, type 4 species spend all their life in offshore regions, and some could be described as oceanic. Mulley & Latter (1980) considered that genetic differentiation was progressively less for those species whose life cycles appeared to provide greater opportunities for mixing of populations.

Later work has not revealed as close a relationship between life history type and genetic differentiation between populations (Mulley & Latter 1981a,b; Salini 1987; Benzie, Frusher & Ballment 1992). Salini (1987) did not detect differences in allele frequencies in *M. bennettae* between the geographically close populations for which Mulley & Latter (1981b) had reported differences. Subsequently, type 2 species were shown to be differentiated over similar scales to those Salini (1987) had reported for *M. bennettae* (Benzie *et al.* 1992).

Prawns for which data have been collected fall largely into type 2 (17 species) or type 3 (nine species) life history types, with *M. bennettae* the only type 1 representative (see Table 3). As noted in the previous section, measures of genetic diversity were similar over a range of species, and no obvious trends were observed among life history groups. Surveys of species from all life history groups extended for hundreds to thousands of kilometres, and nearly all in each grouping covered thousands of kilometres, so that there was no obvious bias in the extent of surveys that might influence the chance that genetic differentiation would be detected. The one study of type 1 species detected genetic differentiation but, of the 15 allozyme surveys of spatial variation of type 2 species, 13 (87%) reported no differentiation and two (13%) found spatial variation (Table 3). Of the eight allozyme surveys of spatial variation of type 3 species, four (57%) reported no differentiation, and three (43%) found spatial variation. The distance over which differentiation occurred was similar for each type of life history – hundreds to thousands of kilometres.

The life history types are generalizations and have not been reliably assessed for all species, but the wide range of results within each group suggests that there is no close correlation of life history types with geographic differentiation. Aquaculture species fall largely into type 2 life histories and do not appear to have any distinguishing characteristics with respect to their genetic structure relative to other species in that group.

Geographic variation

Early work emphasized the lack of genetic differentiation among penaeid prawn populations, noting that populations separated by thousands of kilometres showed no significant differences in allele frequencies (e.g. Lester 1979; Mulley & Latter 1980). This observation is confirmed by many of the results listed in Table 3. No spatial variation has been observed for *M. ensis*, *P. aztecus*, *P. duorarum*, *P. esculentus*, *P. merguiensis*, *P. plebejus*, *P. semisulcatus* De Haan 1844, *P. setiferus*, *P. stylirostris* and *P. vannamei*, despite the fact that populations were sampled thousands of kilometres apart. These data all support the view that prawn populations are panmictic and are reinforced by tagging data for several species (summarized by Dall *et al.* 1990, p. 313), which shows considerable movement by individual prawns of hundreds to thousands of kilometres over a few months. The assumption of panmixis when confronted with no significant spatial structuring only follows if the populations are at equilibrium, and many marine species showing little spatial structure have been shown not to be at equilibrium, often observed through the detection of structure using other markers (e.g. using mtDNA) (Benzie 1999). In these cases, the allozyme data reflect the results of intermittent or past episodes of dispersal, such as those associated with range expansion. The lack of gene flow across

regions where there is no obvious barrier to dispersal is discussed later and suggests that many prawn populations may not be at equilibrium.

Where genetic differentiation has been observed, this is often between populations separated by thousands of kilometres (Table 3). However, examination of the data in Table 3 shows that the minimum distance between which populations demonstrate differentiation is often much less (hundreds of kilometres), and these results have been obtained from studies in which there has been a greater density of sampling. Although showing no differentiation for more than a thousand kilometres in the northern part of its range, Salini (1987) found significant differentiation between northern New South Wales and southern Queensland populations of *M. bennettae*, separated by a similar distance. Benzie *et al.* (1992) found Western Australian populations of *P. monodon* at the extreme of the species' range were differentiated, but the nearest population was only 1400 kilometres away. This is despite the fact that the other populations of *P. monodon* demonstrated no significant differentiation, although they were separated by 3000 km or more.

Benzie *et al.* (1992) noted that the population differentiation observed by Mulley & Latter (1981b) among *P. latisulcatus* populations occurred between populations from the north (Gulf of Carpentaria) and those from the west (Shark Bay, Exmouth Gulf, Cockburn Sound) and south (Gulf of St Vincent). Similarly, *M. endeavouri* (Schmitt 1926) populations from the west (Exmouth Gulf) were different from those in the north (Gulf of Carpentaria). The fact that the distances between the populations showing differentiation were some 3000 km or more was a reflection of the sampling regime rather than the real scale at which genetic differentiation might have occurred in prawn populations. Mulley & Latter (1981b) also detected significant differences between two Western Australian populations of *P. latisulcatus* separated by only 450 km, suggesting some differentiation of southern populations. There appears to be a strong biogeographic element to the differentiation between populations of Australian penaeids, given the consistent geographical pattern of differentiation among several species.

A growing body of evidence has been accumulated recently, which indicates that widespread marine species do not necessarily reach their dispersal potential (see Knowlton & Keller 1986 and reviews by Palumbi 1997; Benzie 1998b, 1999). Relatively dense sampling has shown that patterns of gene flow in giant clams, which have a planktonic larval phase, do not correspond to present-day ocean currents (Benzie & Williams 1997). Sharp changes in allele frequencies where there are no obvious geographic or oceanographic barriers have been described for several taxa (fish and molluscs) in Florida (Avise 1994) and for several taxa (fish and echinoderms) between the Indian and Pacific Oceans (Palumbi 1997; Williams & Benzie 1998). Despite the opportunity for dispersal along major ocean currents and for gene exchange over the last 7000 years, gene flow has not occurred across these boundaries, at least not to the extent that allele frequencies have altered significantly.

Data from allozymes (Forbes, Demetriades, Benzie & Ballment 1999), mtDNA (Klinbunga, Penman, McAndrew, Tassanakajon & Jarayabhand 1998; Benzie, Ballment, Forbes, Demetriades, Sugama, Haryanti & Mori 1999) and microsatellites (Tassanakajon, Supangul, Klinbunga, Jarayabhand & Boosaeng 1998b) for *P. monodon* all show marked gene frequency differences between populations from the Indian and Pacific Oceans. The patterns of genetic differentiation are consistent with those described recently for other marine species in the region (Benzie 1999). Allozyme, microsatellite and mtDNA markers demonstrate significant genetic differences between Andaman Sea and Gulf of Thailand populations of *P. monodon*, suggesting that the genetic dysjunction between the Indian Ocean populations and those from the Pacific occurs along the margin of the Malay Peninsula and the Indonesian Islands. (However, they report similar allele frequencies in the west of the Gulf to those found in the Andaman Sea, in places where there have been significant transfers of animals for aquaculture from the Andaman Sea.) Bouchon, Souty-Grosset & Raimond (1994) calculated a 1.68% sequence divergence between cultured *P. monodon* originally derived from Fiji and stocks originally derived from Australia and Malaysia, suggesting that this was because the source populations were genetically differentiated.

Allozymes (Benzie *et al.* 1992), mtDNA (Benzie *et al.* 2000) and microsatellites (Brooker *et al.* 1999) have all demonstrated that Western Australian populations of *P. monodon* are significantly different from other Australian populations. The mtDNA data also suggest that the Western Australian population is different from Indonesian populations

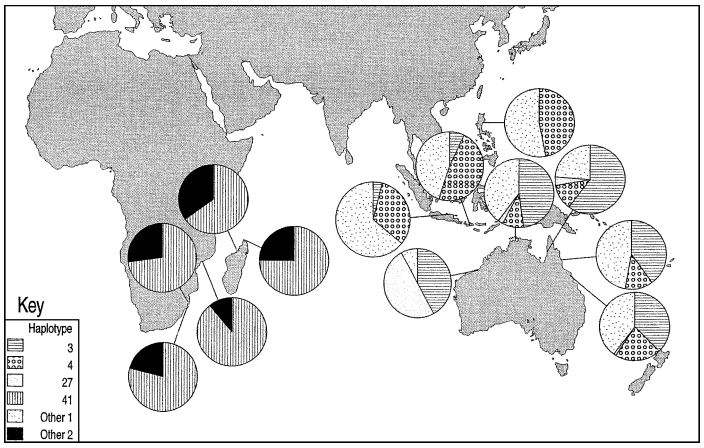


Figure 1 Map showing variation in the proportion of different haplotypes (based on variation in cut sites for the whole mtDNA genome for six restriction enzymes) at several sites in the Indian Ocean and the Indo-West Pacific region for 13 *P. monodon* populations (after Benzie *et al.* 2000). They demonstrate marked differences between the SW Indian Ocean samples and those from the Indo-West Pacific (no variants shared). There are some differences between the Australian and SE Asian samples, and in the distinction (largely in the frequency of haplotype 27) of the Western Australian population, which clearly has greater similarity with other Australian populations than with Indonesian populations.

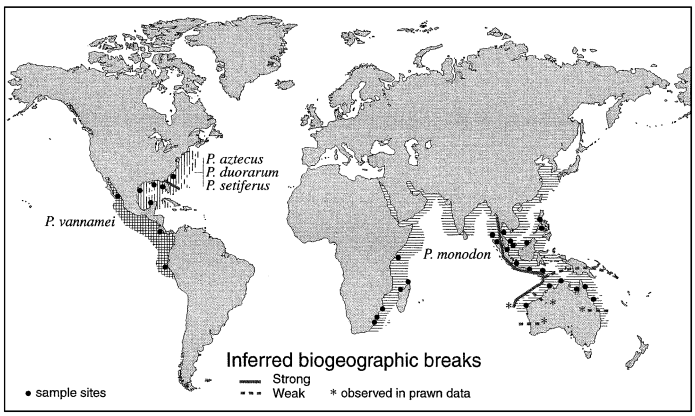


Figure 2 The distribution of several penaeid species for which there are reasonable data sets, the location of sample sites and their relation to zones of major genetic discontinuities described from other marine species or from prawn data.

(Benzie *et al.* 2000). It does, however, cluster with populations having a Pacific Ocean genetic signature and differs markedly from Indian Ocean populations collected from southern Africa and Madagascar. In summary, there is a major genetic discontinuity between the Indo-Pacific populations and those from the Indian Ocean, with less marked but significant genetic differentiation of isolated populations such as that in Western Australia. The pattern of genetic differentiation of *P. monodon* is illustrated in Fig. 1 using mtDNA data from Benzie *et al.* (2000).

The lack of genetic differentiation described for several American species does not mean that biogeographical influences have not affected these species. Although the ranges of *P. aztecus*, *P. duorarum* and *P. setiferus* encompass the region in Florida where a marked genetic dysjunction has been described in a variety marine molluscs, arthropods and fishes (Avise 1994), all genetic surveys of penaeid prawn species have concentrated in the Gulf of Mexico, to the south of this zone of dysjunction, with one exception (Fig. 2). The exception (Marvin & Caillouet 1976), however, reported no significant genetic differentiation at the one locus studied (PGM) between a South Carolina population of *P. setiferus* and four populations collected from Texas and Louisiana. It is pertinent that a test of the significance of G_{ST} levels given by Lester (1979), using equations referenced in Benzie & Williams (1997), was marginally significant for *P. aztecus* (Table 3), suggesting some spatial differentiation, although it is not known between which populations. Lester & Pante (1992) reference unpublished

data from S. Davis, which showed that F_{ST} values based on restriction fragment length polymorphism (RFLP) analysis of DNA from Mexican and Ecuadoran populations of *P. stylirostris* and *P. vannamei* were far higher than those based on allozymes (six- to 20-fold higher), and statistically significant.

The levels of genetic variation in mtDNA within Indonesian populations of *P. monodon* (nucleotide diversity 0.040–0.058) and those from Indonesia, Thailand and Malaysia (mean 0.038) are higher than those seen in the Philippines (0.008–0.010), Sabah (0.007) and Australian populations (0.010) (Klinbunga *et al.* 1998; Benzie *et al.* 2000). Even less variation was observed in isolated populations at the extremes of the species' range in Western Australia (0.002), southern Africa (0.004) and Kenya (0.00) (Benzie *et al.* 2000). In contrast, allozyme data (Forbes *et al.* 1999) did not show reduced allozyme diversity in southern African populations of *P. monodon*. The average number of alleles per locus (1.8–2.4) and observed heterozygosity (0.08–0.10) were similar to those recorded for most Australian populations (average number of alleles per locus = 1.9–2.8; observed heterozygosity = 0.05–0.06). It is possible that historically large population size or the occurrence of a large number of semi-isolated populations in the highly geographically structured Indo-west Pacific region generated, or at least maintained, the diversity in south-east Asia. Alternatively, the low variation in southern Africa and Western Australia may reflect bottlenecks sometime in the last few million years or founder effects following reinvasion after extinction of peripheral populations during the last ice age. Phylogenetic analysis of mtDNA haplotypes demonstrated two monophyletic groups (south-east Asia and Australia separate from Africa) and a discrete group of African derivation that was only observed in south-east Asian samples (Benzie *et al.* 2000). This suggests separation and subsequent divergence of the African and south-east Asian/Australasian populations followed by a reinvasion of the Indonesian region by a group of African (or west Indian Ocean) origin.

In summary, considerable gene flow occurs over wide regions of many penaeid species' ranges, but marked differentiation can occur at what appear to be major biogeographic boundaries. The sampling density of most studies has been insufficient to determine the actual (or minimum) geographical distance between which differentiation occurs, but it is likely to be smaller than the minimum distance recorded so far between populations shown to differ in gene frequencies. In this regard, aquaculture species do not differ from other penaeids. Mean genetic distances among populations within species dominant in aquaculture average 0.0036 compared with an average of 0.0033 in other species, and F_{ST} values for the dominant aquaculture species range from 0.007 to 0.031 (average 0.013) compared with other species, which range from 0.004 to 0.040 (average 0.020). In fact, most information on this topic is available for aquaculture species, particularly *P. monodon*, and populations of this species have been found to be strongly structured, not only with respect to identity of genotypes, but also with respect to levels of genetic variability.

Temporal variation

There has been no study aimed specifically at determining the temporal variation in gene frequencies at several locations for any prawn species, but a small number of studies have determined allozyme variation at some sites more than once. These have revealed a stability of gene frequencies through time for *M. bennettae* (two populations, one sampled over 2 years, another sampled twice over 3 years) (Salini 1987). Sodsuk, McAndrew & Penman (1992) reported significant differences in one or two loci at two out of four populations of *P. monodon* from Thailand, but these values are not significant when a Bonferroni correction is applied to the tests. Richardson (1982) found some significant differences in allele frequencies between sample stations for *P. latisulcatus*. These had been sampled in a different year from other localities. As the data were not obtained from the same places in different years, they did not provide sound evidence for shifts in allele frequency with time. Given the association of these sites with deviations from Hardy–Weinberg equilibrium, the data were thought by Richardson (1982) to indicate the occurrence of stocks that might spawn at different times.

Variation in cultured stocks

The available data

Data have been published on one *Metapenaeus* species and six *Penaeus* species in a total of 13 studies, with most information being provided for

Table 4 Genetic data published on cultured populations of penaeid prawns (from the genera *Metapenaeus* and *Penaeus*).

Species	Nature of sample	Genetic data collected								Source
		Type of genetic marker	No. of populations sampled	Mean no. of individuals sampled per population	No. of loci, bands, restriction enzymes (no. polymorphic)	% Polymorphic loci, bands, REs 95%	99%	Mean no. of alleles per locus	Mean hetero-zygosity observed	Mean hetero-zygosity expected	
M. ensis	33-day-old cultured post-larvae	RAPDs	1	15	1(0) bands	0.00		–	–	–	Meruane *et al.* (1997)
P. chinensis = *P. orientalis*	Tahiti. Cultured population (No details provided. Originally from Hong Kong)	Allozymes	1	12	29(3)	0.103	–	–	0.021	–	Ko *et al.* (1983); Ko (1984*)
	China (crosses from different populations)	Allozymes	2	64–80?	2(1)	–	–	–	–	–	Zhang & Wang (1994)
P. indicus	Tahiti. Cultured population (No details provided. Originally from the Philippines)	Allozymes	1	11	29(1)	0.035	–	–	0.014	–	Ko *et al.* (1983); Ko (1984*)
P. japonicus	Japan (Hatchery-reared post-larvae from wild spawns: no. of parents unknown)	Allozymes	1	18.0–34.0	31(12)	–	0.387	1.49	0.118	0.121	De Matthaeis *et al.* (1983)
	France. Cultured population (No details provided. Originally from Japan)	Allozymes	1	25	29(1)	0.035	–	–	0.015	–	Ko *et al.*(1983); Ko (1984*)
	France. One family, fourth generation in captivity (original stock from Japan)	Allozymes	1	3.5–43.0	37(5)	0.130	–	–	0.071	–	Laubier *et al.* (1984)
	Italy. Seven generations of broodstock (Originally from Japan: two or three broodstock in first generation, 50–300 later years)	Allozymes	7	30.6	20(8)	0.150–0.350$^{0.95}$		1.15–1.40	0.039–0.102	0.049–0.105	Sbordoni *et al.* (1986)
	France (Hatchery population No details provided)	mtDNA	1	20.0	5(0) REs	0		–	–	–	Bouchon *et al.* (1994)

Table 4 Continued

Species	Nature of sample	Genetic data collected: Type of genetic marker	No. of populations sampled	Mean no. of individuals sampled per population	No. of loci, bands, restriction enzymes (no. polymorphic)	% Polymorphic loci, bands, REs 95%	99%	Mean no. of alleles per locus	Mean hetero-zygosity observed	Mean hetero-zygosity expected	Source
P. monodon	Tahiti. Cultured population (No details provided)	Allozymes	1	21	29 (2)	0.069	–	–	0.016	–	Ko *et al.* (1983); Ko (1984*)
	Tahiti (Hatchery population Three stocks originally from Malaysia, Australia and Fiji)	mtDNA	3	2.0	12 (3) REs	0.25	–	–	–	–	Bouchon *et al.* (1994)
	Australia (Six families from wild parents)	RAPDs (14 primers)	1	300	48 (3) bands	0.062	–	–	–	–	Garcia & Benzie (1995)
P. stylirostris	Tahiti. Cultured population (No details provided. Originally from Ecuador)	Allozymes	1	18	29 (4)	0.138	–	–	0.045	–	Ko *et al.* (1983); Ko (1984*)
	Tahiti. Cultured population (No details provided. Originally from Mexico)	Allozymes	1	15	29 (3)	0.104	–	–	0.036	–	Ko *et al.* (1983); Ko (1984*)
	USA, South Carolina. no details provided.)	Micro-satellites	1	29.8	4 (4)	1.000	1.000	–	0.463	–	Ball *et al.* (1998)
P. vannamei	Tahiti. Cultured population (No details provided. Originally from Ecuador)	Allozymes	1	16	29 (2)	0.069	–	–	0.031	–	Ko *et al.* (1983); Ko (1984*)
	Tahiti. Cultured population (No details provided)	Allozymes	1	19	29 (1)	0.035	–	–	0.016	–	Ko *et al.* (1983); Ko (1984*)

Table 4 Continued

Species	Nature of sample	Genetic data collected: Type of genetic marker	No. of populations sampled	Mean no. of individuals sampled per population	No. of loci, bands, restriction enzymes (no. polymorphic)	% Polymorphic loci, bands, REs 95%	99%	Mean no. of alleles per locus	Mean hetero-zygosity observed	Mean hetero-zygosity expected	Source
	USA. Cultured population. (First generation from wild Guatemalan stock)	Allozymes	1	?	28 (9)	0.321	–	–	0.075	–	Harris *et al.* (1990)
	USA. Cultured population. (Seven generations in culture, origin not identified)	Allozymes	1	?	39 (9)	0.231	–	–	0.038	–	Harris *et al.* (1990)
	Texas. Broodstock (originally 500+ individuals from Mexico, Panama and Guatemala: 400+ individuals per generation used as broodstock subsequently)	Allozymes	1	104	12 (6)	0.500	–	–	0.011	–	Sunden & Davis (1991)
	Hawaii. Specific pathogen-free stock. Various times in culture.	Allozymes	3	30	30 (7)	0.033–0.167$^{0.95}$		–	0.160–0.064	–	Garcia *et al.* (1994)
	(1–6 generations)	mtDNA	4	28.5	1 (1) REs	1.000–1.000		0.03–0.04	–	–	Garcia *et al.* (1994)
	[Originally from a small number of families (3 or so) from Mexico and Ecuador]	RAPDs (six primers)	4	28.5	73 (65) bands	0.48–0.77$^{0.95}$		–	–	–	Garcia *et al.* (1994)
		Micro-satellites	6	52	1 (1)	1.000	1.000	47	0.450–1.000	0.388–0.960	Wolfus *et al.* (1997)
	USA, South Carolina. (No details provided)	Micro-satellites	1	9.7	3 (3)	1.000	1.000	–	0.463	–	Ball *et al.* (1998)

The dash (–) indicates no data were available from the paper cited.

the dominant aquaculture species, *P. vannamei*, *P. japonicus*, *P. stylirostris* and *P. monodon* (Table 4). Reports for most species come from a study that examined a number of species held in captivity in Tahiti. (Ko *et al.* 1983; Ko 1984). Otherwise, the bulk of the information comes from cultured populations of *P. vannamei* in the USA.

By far the majority of the reports have been of allozyme variation (16 out of the 25 studies listed in Table 4). This contrasts with the three reports each for microsatellites, RAPDs and mtDNA estimated on the same basis.

Genetic diversity

Data on genetic diversity within cultured prawn populations is highly variable in respect of the measure of genetic diversity reported, the sample size of both loci and number of individuals upon which the estimates were based, and in the nature of the populations surveyed (Table 4). The number of individuals sampled for a given locus ranges from three to more than 300, but most fall within a range of 15–50 per population. Similarly, the number of loci sampled ranges from one to 39, but most studies used between 20 and 31. Some populations sampled were young from ponds stocked with larvae from an unspecified number of wild broodstock, while others were progeny from specific matings of populations held for several generations in culture.

Using all the available data, the proportion of polymorphic loci per population (where the frequency of the most common allele does not exceed 0.95) ranges from 0.000 to 0.500 (average 0.145). Where the frequency of the most common allele was defined not to exceed 0.99, the proportion of polymorphic loci ranges from 0.167 to 0.387 (average 0.277). The mean number of alleles per locus ranges tightly from 1.15 to 1.40 (average 1.28). Observed heterozygosities range from 0.011 to 0.118 (average 0.043) and, although showing a tendency to be smaller than the estimates of expected heterozygosity, differ little from those in the few cases in which they were both calculated. The various estimates for the range of genetic diversity in cultured stocks did not differ, particularly when populations derived from wild broodstock were excluded from the calculations.

There is no obvious difference in genetic diversity between species with respect to the level of variation detected (see Table 4). The range of most assessments of the mean number of alleles, or of observed heterozygosity, for a given species overlaps the range of the estimates for other taxa. The estimates for cultured stocks, over all species, do not appear to be different from those obtained for wild populations. However, when values for wild and cultured populations from particular species are compared, there appears to be a largely consistent trend for genetic diversity levels to be less in cultured populations (Table 5). Where specific comparisons have been made over time, there is clear evidence of a loss of variation in cultured stocks over time (Sbordoni, De Matthaeis, Cobolli-Sbordoni, La Rosa & Mattoccia 1986, Sbordoni,, La Rosa, Mattoccia, Cobolli-Sbordoni & De Matthaeis 1987; Sunden & Davis 1991; see next section for details).

As in the wild populations, the limited data for microsatellites show a far higher proportion that are polymorphic than for allozymes (100% to date). Observed heterozygosities over all loci reported for three species range from 0.450 to 1.000 (average 0.594) and are less than the expected heterozygosities, which range from 0.388 to 0.960 (average 0.674). The RAPD data also show a broad range of polymorphism (0.000–0.770). The limited data available for mtDNA show haplotype diversity of about 0.03–0.04. The data from the DNA-based markers, like the allozymes, show broad overlap with values from wild populations. However, the range falls well below those provided for wild populations, providing some evidence for inbreeding or loss of genetic variation (Table 5).

Given the scatter of the data derived from a large variety of heterogeneous studies, more useful interpretations can be made only from a small number of studies in which some of the set of variables have been controlled and a larger set of populations sampled. The three examples are discussed in the next sections.

Temporal variation

One might expect a reduction in the amount of genetic variation in cultured stocks, simply because of founder or bottleneck effects in the establishment of broodstock populations, and possibly as a result of domestication selection. The results of general surveys of allozyme data (Table 4) did not suggest any general pattern of loss of variation in cultured populations, although particular intraspecific com-

Table 5 Change in genetic diversity in wild and cultured populations of penaeid prawns (from the genus *Metapenaeus* and *Penaeus*)

Species	Genetic diversity measure	Wild populations Mean (range)	Cultured populations Mean (range)	Change in diversity
Allozymes				
P. chinensis	% Polymorphic loci	0.118	0.103	Decrease
= *P. orientalis*	Observed heterozygosity	0.041	0.021	Decrease
P. indicus	% Polymorphic loci	0.138	0.035	Decrease
	Observed heterozygosity	0.026	0.014	Decrease
P. japonicus	% Polymorphic loci	0.235	0.083 (0.035–0.130)	Decrease
	Observed heterozygosity	0.047	0.043 (0.015–0.071)	Mostly decrease
P. monodon	% Polymorphic loci	0.120 (0.081–0.177)	0.159 (0.069–0.250)	Increase
	Observed heterozygosity	0.026 (0.000–0.040)	0.016	Decrease
P. stylirostris	% Polymorphic loci	0.250	0.121 (0.104–0.138)	Decrease
	Observed heterozygosity	0.060	0.041 (0.036–0.045)	Decrease
P. vannamei	% Polymorphic loci	0.195 (0.160–0.261)	0.187 (0.035–0.500)	Mostly decrease
	Observed heterozygosity	0.019 (0.017–0.020)	0.027 (0.011–0.064)	Mostly decrease
RAPDs				
M. ensis	% Polymorphic bands	1.00	0.00	Decrease
P. monodon	% Polymorphic bands	0.242–0.478	0.062	Decrease
mtDNA				
P. monodon	% Polymorphic bands	1.00	0.250	Decrease

parisons did (Table 5). Microsatellite and mtDNA markers, more likely to have a higher resolution because of the greater natural variation they display in prawn populations, showed greater signs of reduced variation in cultured populations.

Sunden & Davis (1991) compared two wild populations and one cultured population of *P. vannamei* that had been in captivity for several years (the precise number of generations was not reported in the paper). Using exactly the same set of allozyme markers, they demonstrated slightly lower levels of heterozygosity and fewer rare alleles in the cultured stock relative to the wild populations, but no evidence of reductions in effective population size.

The only study to follow changes in allele frequencies through time was that conducted over several years on *P. japonicus* stocks that had been introduced into Italy (Sbordoni *et al.* 1986, 1987). They recorded a decline in observed heterozygosity from 0.102 in the F1 generation, through 0.069 and 0.033 in the F4 and F5 generations, respectively, to 0.018 in the F7 generation. The decline in genetic variation was paralleled by a strong decline in mean hatch rates from 50% to 10% as a result of unintended inbreeding. They also found that spatial variation among populations founded from the same initial batch was low but significant ($F_{ST} = 0.04–0.05$, $P < 0.05$). However, variation in allele frequencies among the same stocks over time was greater ($F_{ST} = 0.08–0.14$, $P < 0.001$), indicating low effective population sizes ($N_e < 4$), although large numbers of individuals were used as broodstock.

Studies of four cultured and wild source stocks of *P. vannamei* for the US shrimp programme have revealed a similar pattern, in that stocks held in captivity for a longer time demonstrate reduced genetic variability. Garcia, Faggart, Rhoades, Alcivar-Warren, Wyban, Carr, Sweeney & Ebert (1994) did not provide allozyme data for wild or for cultured stocks in their first generation, but showed the percentage polymorphic loci at three and seven generations to be 3–7% and 16%, respectively, and heterozygosities to be 0.018–0.023 and 0.064. These values are within the range for wild stocks reported by Sunden & Davis (1991). However, the proportion of polymorphic RAPD bands observed in stocks held for one, three and seven generations was 77%, 55% and 48% respectively (Garcia *et al.* 1994). Microsatellite polymorphism was 100%, 97.6% and 45%, respectively, in the three groups. Their observed microsatellite heterozygosity was 0.95–0.96, 0.91 and 0.39, and the number of

alleles found in each was 22–23, 17 and four respectively (Wolfus, Garcia & Alcivar-Warren 1997). DNA markers, with higher resolution, have detected significant loss of variation in populations, admittedly from different sources, but ones that had been in culture for different lengths of time. These cultured populations had been set up initially on a small genetic base (offspring from three females, and a total of 12 matings in the seven-generation case).

Influence of management regimes

There are few data on the effects of different management regimes on genetic diversity. The data from *P. japonicus* populations in Italy have provided a very clear example of the potentially damaging effects of uncontrolled inbreeding and the need to maintain reasonably high levels of effective population size. Despite using large numbers of individuals as broodstock in later generations, an early bottleneck in the first generation, when only a few adults were used, and the mating of close relatives later led to deleterious levels of inbreeding. Information from *P. vannamei* stocks, in which large numbers of males and females from different ponds were used to minimize the likelihood of mating close relatives, showed relatively little loss of genetic variation, suggesting that this approach was a practical solution to the problem (Sunden & Davis 1991). However, other studies, using markers with greater resolution, have revealed loss of genetic variation in different stocks where the original genetic base was limited (Garcia *et al.* 1994; Wolfus *et al.* 1997). There is a need to continue to test whether different broodstock management regimes do achieve their intended goals.

Interactions of aquaculture and wild stocks

There is considerable debate as to the effects of aquaculture escapees on wild fish stocks (Skaala, Dahle, Jørstad & Naevdal 1990; Bentsen 1991; Sheridan 1995). There are concerns that hatchery-reared animals will outcompete wild ones in the short term, because hatchery-reared animals are likely to have been better fed and to be larger than wild animals. Hatchery-reared stocks can differ greatly in gene frequencies, relative to local stocks, even if broodstock are obtained locally, let alone if broodstock are obtained from elsewhere.

It is now clear that, while gene frequencies may not change over large portions of penaeid prawn species' ranges, they can differ between regions, and this change may occur over a relatively short distance. There is, then, a need to plan for the maintenance of wild genetic diversity in prawn populations. It has been suggested that the transfer of large numbers of spawners from the Andaman Sea to farms in the Gulf of Thailand has already resulted in a change in the genetic structure of the wild stocks of *P. monodon* in the Gulf of Thailand (Benzie 1998a). There is no published data on escapees, but anecdotal data on loss of prawns from production ponds. The suggestion of genetic change stems from circumstantial evidence that the amount of genetic differentiation between *P. monodon* populations from the Andaman Sea and populations east of the Thai–Malaysian peninsula is relatively small compared with that observed in other marine species. Further evidence in support of this view is the fact that allele frequencies at some loci in the Surat populations (east Gulf of Thailand) are intermediate to those of the Andaman Sea and Trat (east Gulf of Thailand) in the 1992 populations sampled by Sodsuk *et al.* (1992). However, gene frequencies in some of these populations differed in 1991 samples, and subsequent mtDNA analysis has shown no distinction between east and west Gulf populations, and that both are highly differentiated from Andaman Sea populations (Klinbunga *et al.* 1998). Microsatellite data have also indicated significant differences between the Andaman Sea and Gulf populations, but no analysis of differences within the Gulf have been reported (Tassanakajon *et al.* 1998b). Sodsuk *et al.* (1992) reported some evidence for differentiation of Gulf populations, but current evidence to support the view that this difference is the result of the introduction of genetic material from the Andaman Sea is weak. There are also no before and after data to help interpret any results.

Although there is no strong evidence currently available to confirm any effects of introductions on the genetic structure of wild populations, there is need for care in the distribution of cultured stocks that might endanger wild genetic resources. In the case of *P. monodon*, there is now also evidence that the greatest levels of genetic variation are found in south-east Asia, the region where the greatest growth of prawn farming has taken place to date. This is also where the greatest growth is likely to

occur in the near future, and where the potentially greatest threat of loss of wild genetic diversity exists.

Conclusions

Much of the genetic information available for penaeid prawns comes from studies of allozyme variation, although studies of mtDNA and nuclear DNA markers, such as microsatellites, are beginning to appear. Work, not yet published, examining variation in the introns of nuclear genes is promising to detect even greater levels of variation and of population subdivision (S. R. Palumbi, pers. commun.).

The recent surveys have shown that high levels of gene flow appear to occur over large areas of many penaeid species' ranges, but marked differentiation can occur at what appear to be major biogeographic boundaries. The recent findings also raise questions as to why prawns have not dispersed between regions, given their ability to move over far greater distances within regions. In fact, in the taxon for which most information is available, *P. monodon*, populations are strongly structured geographically, not only with respect to identity of genotypes, but also with respect to levels of genetic variability. High levels of genetic diversity have been observed in south-east Asia, and reduced levels in the Philippines and Australia, and even less at the extremes of the species range.

The fact that the regional variation appears to be largely historical suggests that regional genetic diversity will not be readily replaced if it is lost. Given our lack of knowledge of the processes maintaining genetic diversity, and the possibility that present-day gene flow is not as great between populations, there is a need to develop management strategies for wild genetic diversity. This is particularly so in south-east Asia, given the rapid development in this region of greatest penaeid genetic diversity.

Evidence from cultured populations is patchy but, in suitably detailed studies, tends to suggest that there is loss of variation in cultured stocks, although this is highly variable, and there are few tests of the efficacy of different broodstock management regimes. There is a need for more structured, co-ordinated research to clarify more clearly the regional differences between prawn populations, to test the effectiveness of broodstock and hatchery management regimes and the likely interaction of wild and aquaculture stocks.

Acknowledgments

I would like to thank all those who provided access to their unpublished data, or papers in press, which allowed a more up-to-date review to be achieved, and Bill Dall for his comments on the life histories of the species examined. This is contribution number 963 from the Australian Institute of Marine Science.

References

Avise J.C. (1994). *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Ball A.O., Leonard S. & Chapman R.W. (1998) Characterization of $(GT)_n$ microsatellites from native white shrimp (*Penaeus setiferus*). *Molecular Ecology* **7,** 1251–1253.

Bentsen H.B. (1991) Quantitative genetics and management of wild populations. *Aquaculture* **98,** 263–266.

Benzie J.A.H. (1998a) Penaeid genetics and biotechnology. *Aquaculture* **184,** 23–47.

Benzie J.A.H. (1998b) Genetic structure of marine organisms and SE Asian biogeography. In: *Biogeography and Geological Evolution of SE Asia* (ed. by R. Hall & J.D. Holloway), pp. 197–209. Backhuys Publishers, Leiden, The Netherlands.

Benzie J.A.H. (1999) Genetic structure of coral reef organisms – ghosts of dispersal past. *American Zoologist* **39,** 131–145.

Benzie J.A.H. & Williams S.T. (1997) Gene flow among giant clam (*Tridacna maxima*) populations in the west Pacific is not consistent with dispersal by present-day ocean currents. *Evolution* **51,** 768–783.

Benzie J.A.H., Frusher S. & Ballment E. (1992) Geographical variation in allozyme frequencies of *Penaeus monodon* (Crustacea: Decapoda) populations in Australia. *Australian Journal of Marine and Freshwater Research* **43,** 715–725.

Benzie J.A.H., Ballment E. & Frusher S. (1993) Genetic structure of *Penaeus monodon* in Australia: preliminary data from allozymes and mtDNA. *Aquaculture* **111,** 89–93.

Benzie J.A.H., Ballment E., Forbes A.T., Demetriades N.T., Sugama K., Haryanti & Mori S. (2000) mtDNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology* (in press).

Bouchon D., Souty-Grosset C. & Raimond R. (1994) Mitochondrial DNA variation and markers of species identity in two penaeid shrimp species: *Penaeus monodon* Fabricius and *P. japonicus* Bate. *Aquaculture* **127,** 131–144.

Brooker A.L., Benzie J.A.H., Blair D. & Versini J.-J. (1999) Population structure of the giant tiger prawn, *Penaeus monodon*, in Australian waters using microsatellite markers. *Marine Biology* (in press).

Dall W., Hill B.J., Rothlisberg P.C. & Staples D.J. (1990)

The biology of the Penaeidae. *Advances in Marine Biology* **27,** 1–489.

De Matthaeis E., Allegruchi G., Caccone A., Cesaroni D., Cobolli-Sbordoni M. & Sbordoni V. (1983) Genetic differentiation between *Penaeus kerathurus* and *P. japonicus* (Crustacea, Decapoda). *Marine Ecology – Progress Series* **12,** 191–197.

Dore I. & Fridmont C. (1987). *An Illustrated Guide to Shrimp of the World*. Van Nostrand Reinhold, New York.

Fast A.W. & Lester L.J., eds. (1992) Marine shrimp culture: principles and practices. *Developments in Aquaculture and Fisheries Science* **23,** 1–862.

Forbes A.T., Demetriades N.T., Benzie J.A.H. & Ballment E. (1999) Allozyme frequencies indicate little geographic variation amongst stocks of the giant tiger prawn, *Penaeus monodon*, in the south-east Indian Ocean. *South African Journal of Marine Biology* **21,** 271–277.

Garcia D.K. & Benzie J.A.H. (1995) RAPD markers of potential use in penaeid prawn (*Penaeus monodon*) breeding programs. *Aquaculture* **130,** 137–144.

Garcia D.K., Faggart M.A., Rhoades L., Alcivar-Warren A., Wyban J., Carr W.H., Sweeney J.N. & Ebert K.M. (1994) Genetic diversity of cultured *Penaeus vannamei* shrimp using three molecular genetic techniques. *Molecular Marine Biology and Biotechnology* **3,** 270–280.

Grey D.L., Dall W. & Baker A. (1983). *A Guide to the Australian Penaeid Prawns*. Northern Territory Government, Darwin, Australia.

Harris S.E.G., Dillion R.T. Jr, Sandifer P.A. & Lester L.J. (1990) Electrophoresis of isozymes in cultured *Penaeus vannamei*. *Aquaculture* **85,** 330.

Hedgecock D. & Malecha S.R. (1991) Prospects for the application of biotechnology to the development and improvement of shrimp and prawns. In: *Shrimp Culture in North America and the Caribbean* (ed. by P.A. Sandifer), pp. 161–200. Advances in World Aquaculture, Vol. 4. World Aquaculture Society, Baton Rouge, LA, USA.

Hedgecock D., Tracey M.L. & Nelson K. (1982) Genetics. In: *The Biology of Crustacea* (ed. by L.G. Abele), pp. 284–403. Academic Press, New York.

Klinbunga S., Penman D.J., McAndrew B.J., Tassanakajon A. & Jarayabhand P. (1998) Genetic variation, population differentiation, and gene flow of the giant tiger shrimp (*Penaeus monodon*) inferred from mt DNA-RFLP data. In: *Advances in Shrimp Biotechnology* (ed. by T.W. Flegel), pp. 51–59. National Center for Genetic Engineering and Biotechnology, Bangkok.

Knowlton N. & Keller B.D. (1986) Larvae which fall far short of their potential: highly localized recruitment in an alpheid shrimp with extended larval development. *Bulletin of Marine Science* **39,** 213–223.

Ko G.L. (1984) *La Variabilite et la Differentiation Genetique de Quelques Especes Peneides*. PhD Thesis, Universite des sciences et techniques du Languedoc, Montpellier, France.

Ko G.L., Pasteur N., Bonhomme F., Aquacop, Seafdec & Liao D. (1983) Variabilite genetique de quelques especes de crevettes peneides d'interet aquicole. Bases biologiques de l'aquaculture, Montpellier 1983. *Actes de Colloques* **1,** 175–180.

Lansford L.M., Caillouet C.W. & Marvin K.T. (1976) Phosphoglucomutase polymorphism in two penaeid shrimp, *Penaeus brasiliensis* and *Penaeus aztecus subtilis*. *Fisheries Bulletin* **74,** 453–457.

Laubier A., Pasteur N. & Moriyasu M. (1984) Estimation du polymorphisme enzimatique d'une population de *Penaeus japonicus* maintenue en elevage depuis quatre generations. *Oceanologica Acta* **7,** 451–456.

Lester L.J. (1979) Population genetics of penaeid shrimp from the Gulf of Mexico. *Journal of Heredity* **70,** 175–180.

Lester L.J. (1983) Developing a selective breeding program for penaeid shrimp mariculture. *Aquaculture* **33,** 41–50.

Lester L.J. & Pante Ma. J.R. (1992) Genetics of *Penaeus* species. In: *Marine Shrimp Culture: Principles and Practices* (ed. by A.W. Fast & L.J. Lester), pp. 29–52. Elsevier Science Publishers, Amsterdam, The Netherlands.

Marvin K.T. & Caillouet C.W. (1976) Phosphoglucomutase polymorphism in white shrimp, *Penaeus setiferus*. *Comparative Biochemistry and Physiology B* **53B,** 127–131.

Marvin K.T., Benton R.C., Lansford L.M. & Caillouet C.W. (1977) Polymorphism in phosphohexose isomerase in white shrimp, *Penaeus setiferus* Linnaeus, and pink shrimp, *P. duorarum duorarum*. *Comparative Biochemistry and Physiology B* **57B,** 95–97.

Mattoccia M., La Rosa G., De Matthaeis E., Cobolli-Sbordoni M. & Sbordoni V. (1987) Patterns of genetic variability in Mediterranean populations of *Penaeus kerathurus* (Crustacea, Decapoda). In: *Selection, Hybridization and Genetic Engineering in Aquaculture* (ed. by K. Tiews), pp. 131–142. Heenemann-Verlag, Berlin.

Meruane J., Takagi M. & Taniguchi N. (1997) Species identification and polymorphisms using RAPD-PCR in penaeid prawns *Penaeus japonicus* and *Metapenaeus ensis*. *Fisheries Science* **63,** 149–150.

Mulley J.C. & Latter B.D.H. (1980) Genetic variation and evolutionary relationships within a group of thirteen species of penaeid prawns. *Evolution* **34,** 904–916.

Mulley J.C. & Latter B.D.H. (1981a) Geographic differentiation of eastern Australian prawn populations. *Australian Journal of Marine and Freshwater Research* **32,** 889–895.

Mulley J.C. & Latter B.D.H. (1981b) Geographic differentiation of tropical Australian prawn populations. *Australian Journal of Marine and Freshwater Research* **32,** 895–906.

Palumbi S.R. (1997) Molecular biogeography of the Pacific. *Coral Reefs* **16,** S47–S52.

Proctor R.R., Marvin K.T., Lansford L.M. & Benton R.C. (1974) Phosphoglucomutase polymorphism in brown

shrimp, *Penaeus aztecus*. *Journal of the Fisheries Research Board of Canada* **31**, 1405–1407.

Redfield J.A., Hedgecock D., Nelson K. & Salini J.P. (1980) Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis. *Marine Biology Letters* **1**, 303–313.

Richardson B.J. (1982) Geographical distribution of electrophoretically detected protein variation in the Australian commercial fishes. III. Western king prawn, *Penaeus latisulcatus* Kishinouye. *Australian Journal of Marine and Freshwater Research* **33**, 933–937.

Rosenberry R. (1997) *World Shrimp Farming 1997*. Shrimp News International, San Diego.

Salini J.C. (1987) Genetic variation and population subdivision in the greentail prawn *Metapenaeus bennettae*. *Australian Journal of Marine and Freshwater Research* **38**, 339–349.

Sbordoni V., De Matthaeis E., Cobolli-Sbordoni M., La Rosa G. & Mattoccia M. (1986) Bottleneck effects and the depression of genetic variability in hatchery stocks of *Penaeus japonicus* (Crustacea, Decapoda). *Aquaculture* **57**, 239–251.

Sbordoni V., La Rosa G., Mattoccia M., Cobolli-Sbordoni M. & De Matthaeis E. (1987) Genetic changes in seven generations of hatchery stocks of the Kuruma prawn, *Penaeus japonicus* (Crustacea, Decapoda). In: *Selection, Hybridization and Genetic Engineering in Aquaculture* (ed. by K. Tiews), pp. 143–155. Heenemann-Verlag, Berlin.

Sheridan A.K. (1995) The genetic impacts of human activities on wild fish populations. *Reviews in Fisheries Science* **3**, 91–108.

Skaala Ø., Dahle G., Jørstad K.E. & Naevdal G. (1990) Interactions between natural and farmed fish populations: information from genetic markers. *Journal of Fish Biology* **36**, 449–460.

Sodsuk S., McAndrew B.J. & Penman D.J. (1992) Genetic population structure of the giant tiger prawn (*Penaeus monodon* Fabricius, 1798) in the gulf of Thailand and the Andaman Sea. In: *Proceedings of the First ASEAN-EEC Aquaculture Development and Coordination Program* (ed. by D. Penman, N. Roogratri & B. McAndrew), pp. 161–165. AADCP, Stirling, UK.

Sunden S.L.F. & Davis S. (1991) Evaluation of genetic variation in a domestic population of *Penaeus vannamei* (Boone): a comparison with three natural populations. *Aquaculture* **97**, 131–142.

Tam Y.K. & Chu K.H. (1993) Electrophoretic study of the phylogenetic relationships of some species of *Penaeus* and *Metapenaeus* (Decapoda: Penaeidae) from the South China Sea. *Journal of Crustacean Biology* **13**, 697–705.

Taniguchi N. & Han H. (1989) Population genetic analysis of fish and shellfish by isozymes. *Japanese Fisheries Resource Conservation Association Report* **1989**, 275–281.

Tassanakajon A., Pongsomboon S., Rimphanitchayakit V., Jarayabhand P. & Boosaeng V. (1997) Random amplified polymorphic DNA (RAPD) markers for determination of genetic variation in wild populations of the black tiger prawn (*Penaeus monodon*) in Thailand. *Molecular Marine Biology and Biotechnology* **6**, 110–115.

Tassanakajon A., Tiptawonnukul A., Supungul P. & Rimphanitchayakit V. (1998a) Isolation and characterization of microsatellite markers in the black tiger prawn *Penaeus monodon*. *Molecular Marine Biology and Biotechnology* **7**, 55–61.

Tassanakajon A., Supangul P., Klinbunga S., Jarayabhand P. & Boosaeng V. (1998b) Microsatellite variation in wild populations of the black tiger prawn, *Penaeus monodon*, in Thailand. In: *Agricultural Biotechnology: Laboratory, Field and Market* (ed. by P. Larkin), pp. 255–257. Proceedings of the 4th Asia-Pacific Conference on Agricultural Biotechnology. CPN Publications, Canberra.

Villaescusa A., Camacho A. & Rivalta V. (1984) Polimorfismo de la fosfoglucosa isomerasa y de la fosfoglucomutasa en el camaron rosado (*Penaeus notialis*). *Ciencias Biologicas* **12**, 23–30.

Williams S.T. & Benzie J.A.H. (1998) Evidence of a biogeographic break between populations of a high-dispersal starfish: congruent regions within the Indo-West Pacific defined by colour morphs, mtDNA and allozyme data. *Evolution* **52**, 87–99.

Wolfus G.M., Garcia D.K. & Alcivar-Warren A. (1997) Application of the microsatellite technique for analyzing genetic diversity in shrimp breeding programs. *Aquaculture* **152**, 35–47.

Zhang Z. & Wang Y. (1994) Preliminary studies on LDH and MDH isozymes of two *Penaeus orientalis* species groups. *Tropical Oceanology* **13**, 87–90.

Aquaculture Research, 2000, **31**, 121–131

Should we allow human-induced migration of the Indo–West Pacific fish, barramundi *Lates calcarifer* (Bloch) within Australia?

C P Keenan
QDPI, Bribie Island Aquaculture Research Centre, PO Box 2066, Woorim, Queensland 4507, Australia

Correspondence: C P Keenan, QDPI, Bribie Island Aquaculture Research Centre, PO Box 2066, Woorim, Queensland 4507, Australia

Abstract

Some biologists have expressed concerns about the possible genetic impacts of translocation between stocks of barramundi *Lates calcarifer* (Bloch) in Australia. Recent genetic, biogeographical studies have provided an understanding of the evolution of the currently observed population structure in Australian barramundi by assessing the impacts of ice-age, sea-level changes on their distribution. These studies found that genetic differences between most barramundi populations are extremely small, have arisen in the past 17 000 years, and substantial migration and hybridization between eastern and western populations, isolated for at least 110 000 years, has occurred naturally. Some phenotypic support for these minor genetic differences can be inferred from the lack of adaptation to temperature in growth and survival responses of widely separated stocks (tropical and temperate). Based on a low level of genetic differentiation and high levels of gene flow between populations, with little evidence of local adaptation, translocation between populations should not pose a significant risk or problem.

Introduction

Apart from the transfer of diseases and parasites, most of the impacts caused by human-induced migration, or translocation, of hatchery-reared fish can be classified as either ecological or genetic changes. Possible ecological changes include habitat modification, changes in spatial relationships or changes in trophic relationships. These changes can occur when the introduction is either an exotic or a closely related native species, but are unlikely to occur if the stocked fish are the same species. Genetic interaction, on the other hand, can occur only between related species, subspecies or stocks, and is not usually found when exotic species are translocated.

The question of possible adverse genetic impacts through intentional stocking, or accidental escape from aquaculture, of hatchery-reared fish is one aspect that is particularly controversial. There exists a substantial literature on the subject, primarily theoretical in nature, which supports two opposing views. One theoretical position, developed over the past 15 years, is that existing stock structure should be maintained, regardless of costs, and that any restocking should take place only from fish sourced from the same genetic stock. This is based on the hypothesis that the demonstrated population structure is a consequence of long-term reproductive isolation (Shaklee, Salini & Garrett 1993) and that, therefore, the populations are adapted to local conditions. Furthermore, this position argues for the situation in barramundi that 'if the local adaptations are at all dependent on co-adapted gene complexes, interbreeding between stocks would be expected to result in diminished fitness of the hybridized stock (outbreeding depression)' (Shaklee *et al.* 1993). An alternative theoretical position, and one that is being seriously considered for the conservation of endangered species (Moritz 1994), is that translocation may take place between management units (MUs) within an evolutionary significant unit (ESU), but that translocation between ESUs could cause significant genetic disrup-

tion. Also, where connectivity between populations has been fragmented by human interference, translocation to increase heterozygosity and limit inbreeding should be considered within MUs (Conant 1988; Saccheri, Kuussaari, Kankare, Vikman, Fortelius & Hanski 1998; Moritz, 1999). If, for some unpredicted reason, this resulted in less fit individuals, natural selection would be expected to remove them given enough time (Shaklee *et al.* 1993).

This paper will argue that the genetic diversity and population structure of Australian barramundi has developed recently in evolutionary time, and that they represent a single ESU. Therefore, Australian barramundi have a 'shallow' stock structure that should not produce 'outbreeding depression' if translocation occurs between even the most isolated populations. In contrast to other species with higher levels of genetic distance between populations, there is little evidence of local adaptation through natural selection of barramundi populations in Australia. On the contrary, in two studies in which differences were expected, none were observed. In addition, observed differences in biological parameters between some populations have been attributed to environmental variation, as the phenotypic responses change under different environmental conditions.

What is outbreeding depression?

As discussed above, the most frequent objection to translocation between populations is the possible deleterious effects of genetic introgression, which could result in 'outbreeding depression'. Outbreeding depression 'simply refers to the phenomenon of fitness reduction (usually in either fertility or viability) following hybridization (either in the immediate hybrids, or perhaps delayed until the backcross or later generations)' Templeton (1986). It is generally accepted that outbreeding depression is related to genetic distance between individuals. This was summarized clearly by Waser (1993), who stated 'If one accepts that inbreeding and outbreeding are simplified terms describing a continuum of genetic similarity, and that outbreeding logically extends to the level of differentiation represented by subspecies and closely related species, it follows that postzygotic reproductive barriers in crosses at these taxonomic levels represent 'outbreeding depression' in fitness.'

One argument that 'outbreeding depression' will occur in barramundi is based on the assumption that local selection within each population results in the development of co-adapted gene complexes (see quote from Shaklee *et al.* 1993 above). Just what is a co-adapted gene complex? Darwin coined the term to describe the integrated assemblage of characters that function well in concert to form a distinctive anatomical architecture, for instance a phylum (Dobzhansky, Ayala, Stebbins & Valentine 1977). Co-adaptation is defined as 'the correlated variation and adaptation in two mutually dependent organs' (Henderson & Holmes 1979). In genetic terms, how are co-adapted gene complexes achieved? By definition, a co-adapted gene complex must be a unique combination of alleles from a number of genes that are fixed in the population, i.e. homozygous. These are common at the species level of divergence and are used as a tool in species definition (e.g. Keenan, Davie & Mann 1998), as any polymorphism indicates hybridization. If the alleles that make up the complex were heterozygous, then they would naturally be subject to independent assortment during meiosis, which would rapidly break up any co-adapted complex. Therefore, co-adapted gene complexes show linkage disequilibrium to maintain their co-adapted nature.

While outbreeding depression has not been detected in the majority of studies of hybridization between divergent populations of plants (Barrett & Kohn 1991) or vertebrates (Waldman & McKinnon 1993), there are well-known, naturally occurring examples of linkage disequilibrium involving paracentric inversions of chromosomes in *Drosophila pseudoobscura* (Dobzhansky 1936). These inversions prevent recombination and therefore represent a co-adapted gene complex. In *Drosophila*, paracentric inversions do not cause a decline in fertility because of the absence of crossing over in males. However, in nearly all other species of animal, they usually cause the death of the embryo because of chromosome abnormalities (Hartl 1980). In the literature, discussion about a broader sense of 'outbreeding depression' can be found (e.g. Templeton 1986). There appears to be little direct evidence for this in natural populations of vertebrates (Barrett & Kohn 1991; Shields 1993; Waldman & McKinnon 1993; Knapp & Dyer 1998), and most authors agree that this type of outbreeding depression is a temporary phenomenon that can be rapidly eliminated by natural selection (Templeton 1986) and that superior gene complexes may be achieved through

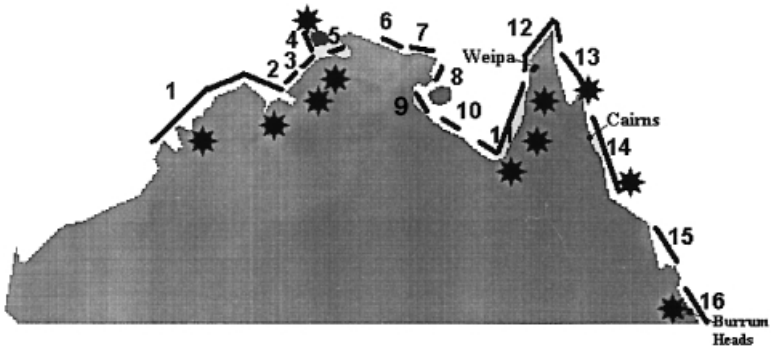


Figure 1 Present understanding of population structure in Australian barramundi based on Keenan (1994), Chenoweth *et al.* (1998a,b) and Doupé *et al.* (1999). Mitochondrial DNA sampling sites of Chenoweth *et al.* (1998a,b) and Doupé *et al.* (1999) are highlighted by a star. Numbers refer to populations: 1, Fitzroy to Ord and Moyle Rivers; 2, Daly and Finniss Rivers; 3, Darwin Harbour and Shoal Bay; 4, Port Hurd; 5, Mary River, NT; 6, Goyder River; 7, Buckingham Bay; 8, Blue Mud Bay; 9, Roper River; 10, McArthur River; 11, south-east Gulf of Carpentaria; 12, Cape York Tip; 13, East Cape York and Princess Charlotte Bay; 14, north east coast; 15, central east coast; 16, south east coast.

heterosis (hybrid vigour) (Fenster & Dudash 1994 in Knapp & Dyer 1998).

For Australian barramundi, it is therefore highly unlikely that 'outbreeding depression' would result from translocation. While available data show very small, although significant, allele frequency differences between populations, there are no observed fixed differences in nuclear DNA. Barramundi populations (Fig. 1) show very small genetic differences (see below), with the largest genetic distance between the East Coast and Arafura Sea metapopulations (Fig. 2). There is very strong evidence for a broad zone of natural hybridization, with no evidence of reduced fitness, between these metapopulations of barramundi in the south-east Gulf of Carpentaria (Keenan 1994), one of the most productive barramundi fisheries in Australia.

To emphasize some of these issues for fish species, an examination of the genetic effects of translocated fish on the receiving population is warranted. With the variety of possible impacts and levels of interactions that may occur, the following evidence derived from Australian and other literature examines situations in which there is a close relationship between the introduction and the native species or stock.

Examples of hybridization at the species level

Genetic hybridization is an effective way of destroying a species' genetic identity. If baseline data are available, hybridization is easy to quantify but, otherwise, can be difficult to detect. Depending on the level of genetic relatedness between potential hybridizing species or stocks, it can be either beneficial or detrimental to a population. For example, in the worst-case scenario, hybridization between different species can result in reduced reproductive potential, reduced fitness of hybrids and sterility, and eventual loss of genotype and extinction (Rowland 1988). On the other hand, hybridization of endangered populations of the same species is recommended by some authors (e.g. Krueger, Gharrett, Dehring & Allendorf 1981; Moritz, 1999) to increase genetic variability and thus reduce the detrimental effects of inbreeding that may occur if there is no intervention.

Natural hybridization between taxa of fish is much more frequent than that found in other vertebrate classes. It is more likely to occur between fish taxa for a number of reasons, e.g. primitive fertilization mechanisms (external, non-specialized sperm and eggs), weak behavioural isolating mechanisms, mass spawning and competition for limited spawning habitat (Allendorf & Leary 1988). Additionally, hybrids between distantly related fish taxa are sometimes viable. This suggests that fish have a degree of developmental compatibility not found in other vertebrate species at comparable levels of genetic divergence.

When introductions are made of species from the same genus/family as native species, the chances of more subtle interactions at both the trophic and the genetic levels are increased. However, the precise impact of the introduction cannot be predicted without some prior knowledge or study of the degree of possible trophic and/or genetic interac-

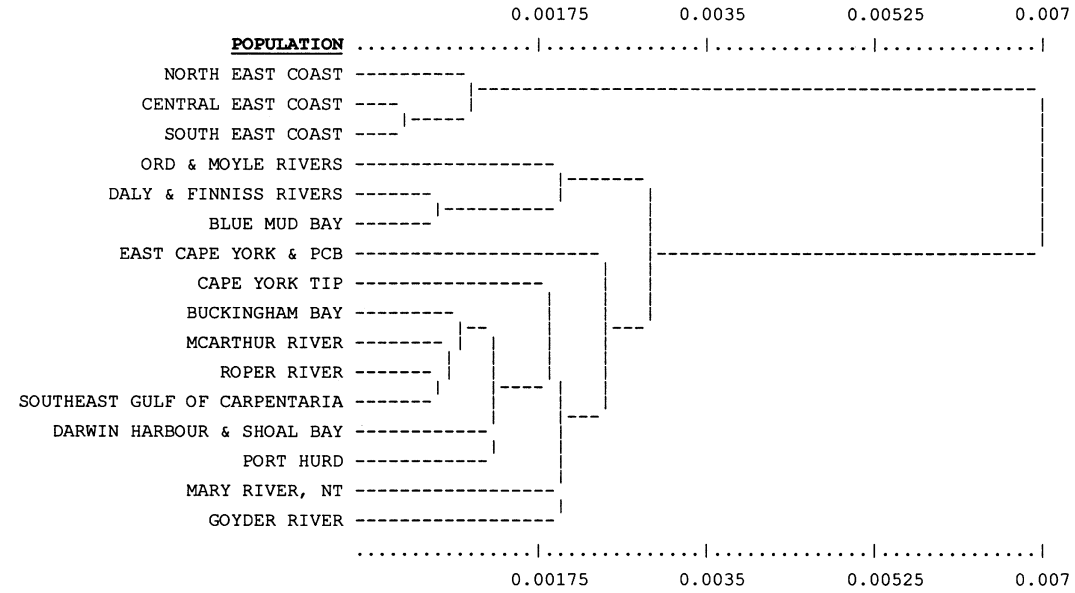


Figure 2 Pool cluster (May & Krueger 1990) dendrogram of Nei's genetic distance for the 16 identified populations of barramundi. While this is a good representation of genetic distances, anomalies associated with the dichotomous nature of this representation can be seen. Such a presentation cannot show post-isolation hybridization clearly, whereas multidimensional scaling (MDS) demonstrates this linkage (see Fig. 5, Keenan 1994).

tion. There are many examples in which, in an extremely short (evolutionary) time, there has been introgressive hybridization, e.g. between species of tilapia (Pullin 1988) and between rainbow and cutthroat trout (Leary, Allendorf, Phelps & Knudsen 1984). In the latter case, this has been a result of the widespread release of large numbers of hatchery-reared rainbow trout throughout the range of the cutthroat trout. The hatchery-reared rainbow trout lost the reproductive isolation of wild fish and unexpectedly interbred with the other species. Allendorf & Leary (1988) inferred that introgression was the most important factor responsible for the loss of native cutthroat trout populations, of which 16 have been recognized in the literature, including one now extinct and two endangered. Experimentally hybridized populations demonstrated lower fitness than the parental taxa, e.g. slower developmental rate, decreased developmental stability and slower growth rates under hatchery conditions. The very presence of many introgressed populations throughout the species range was found to threaten the remaining native populations. It is feared that the only remaining native populations will be those isolated by dispersal barriers.

However, there are many examples of natural interspecies hybridization that form viable hybrids. An example was presented by Rowland (1984), who studied natural hybrids between Australian yellow-fin and black bream, *Acanthopagrus australis* (Gunther) and *A. butcheri* (Munro) respectively. Under certain natural environmental conditions, these species form fertile hybrids, as evidenced by segregation of alternate alleles (Rowland 1984). Despite the production of fertile F_1 hybrids and back-crossing between the species, there was no evidence of a breakdown in species structure (as predicted by Wright's 'island model' if $Nm > 1$, see later). The migration rate of fertile hybrids from their land-locked lakes was apparently insufficient to impact on the widespread populations of these two species. This situation is very similar to that observed with two crappie species in Weiss Lake, AL, USA (Smith, Maceina & Dunham 1994), which is located at the crossing point of sympatric and allopatric populations.

In Australia, a potential problem with interspecies hybridization exists between the Murray cod Maccullochella peelii peelii (Mitchell) and its close relatives, trout cod Maccullochella macquariensis (Cuvier), Eastern freshwater cod Maccullochella ikei (Rowland) and Mary River cod Macullochella peelii mariensis (Rowland). Rowland (1985) discusses experimental work in which a hybrid cross between

Murray cod and Clarence cod produced a high proportion of abnormal larvae. Whether natural hybridization will occur between these two species is unknown. However, natural hybridization within the genus *Macculochella* is known between trout cod and Murray cod in the artificial environment of Cataract Dam, NSW (Wajon 1983) and, recently, (natural) hybrids have been observed in the Murray River.

Hybridization at the population level

At the population level of genetic subdivision, factors that produce and maintain breeding isolation (i.e. species) are not developed. Distinct populations are indicated by statistically significant differences in gene frequencies, and these differences can vary from subspecific to almost negligible. There are now many well-documented examples of Australian species that show distinct population structure. For example, across northern Australia, many species share a common zoogeographic barrier in the Torres Strait (Keenan 1998).

Keenan & Salini (1990) and Shaklee *et al.* (1993) argued that, intuitively, one would expect populations of fish to be adapted to their environment. In many species, population differentiation has occurred over many hundreds of thousands of years or longer, and (presumably) the various isolated populations have evolved and adapted to suit their environment over this time. Comparisons of sister species each side of the Isthmus of Panama, isolated 1.2 million years ago, have provided information relevant to this area of research. Keenan & Salini (1990) stated that it was necessary to consider the timescale that has produced isolated populations, because evolutionary change is a slow and time-dependent process. In 1990, little was understood about the timescale that produced stocks of barramundi, and caution was therefore urged in the mixing of stocks, because they may show local adaptation.

For instance, in China, distinct stocks of silver carp present in each of the major northern, temperate (Changjiang, 29–32°N) and southern, subtropical (Zhujiang ≈ 23°N) rivers have different growth characteristics (Li, Lu, Peng & Zhao 1987). The colder adapted (northern) stock has a significantly higher growth rate, about 10% faster than the warmer adapted stock when grown under the same conditions. Analysis showed that between 29% and 34% of the variance in weight could be attributed to a strong genetic component, the remainder being a result of environmental (pond) differences. Based on this argument, Keenan & Salini (1990) anticipated a similar response to growth between northern and southern stocks of barramundi. (This hypothesis was tested by two experiments discussed below.) Since 1990, there has been a considerable amount of research conducted on barramundi, and our knowledge of both the evolution of stock structure and genotype–environment interactions has increased.

Population structure in barramundi

Keenan (1994) proposed that barramundi most probably colonized Australia either 350 000 or 150 000 years before present (BP) from the eastern Indonesian archipelago during a period of low sea level (Ice Age). They then migrated to the east Australian coast during a period of high sea level (320 000 or 130 000 years BP) when the shallow Torres Strait was open. When sea level receded, for a period of about 110 000 years, east–west isolation developed. Since the last Ice Age only 17 000 years BP (Fig. 3a), barramundi have recolonized inundated rivers across the whole extent of northern Australia. Therefore, the present population structure must have developed during this period.

As sea levels rose (Fig. 3a and b), barramundi populations derived from source populations lost heterozygosity (genetic variation) through inbreeding and genetic drift in the populations at the colonizing front as they migrated into their new habitat. These changes in gene frequency and heterozygosity of barramundi populations were most probably a stochastic function of these massive environmental changes, resulting in significant isolation-by-distance (Keenan 1994). The lack of time for mutations in protein coding genes to arise and large gene frequency differences to establish are clearly seen in the maximum Nei's $D = 0.007$ exhibited between the major eastern and western metapopulations (Fig. 2). Within each metapopulation, the maximum Nei's D is < 0.003.

About 7000 years ago, the Torres Strait opened (and has since remained open), and migration through the Strait was possible once again. Initially east coast stock moved west into the Gulf of Carpentaria and hybridized with the eastern-moving migration front from the west (Keenan 1994). The mitochondrial DNA data of Chenoweth, Hughes, Keenan & Lavery (1998a,b) and Doupé,

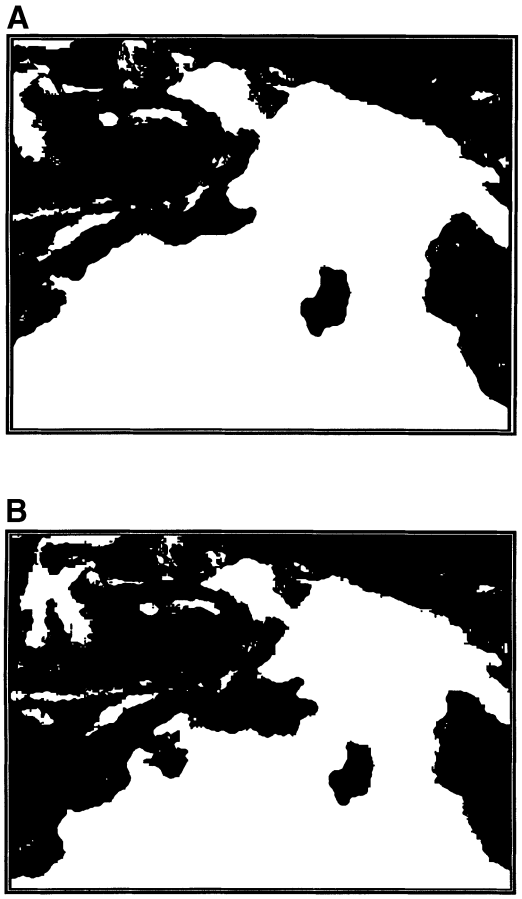


Figure 3 Coastal image of northern Australia as it would have been (A) 17 000 years BP and (B) 12 000 BP. The rapid rise in sea level between 17 000 and 6000 years BP would have had spectacular effects on the marine/land interface and the barramundi's habitat. Marine inundation over this period was ≈ 125 m per year overland, in the area now known as the Arafura Sea. The freshwater Lake Carpentaria was not overtopped by marine water until 11 000 years ago, and fully marine conditions suitable for barramundi breeding may not have been established until 9500 years BP (for details, see Keenan 1994).

Horwitz & Lymbery (1999) support this hypothesis, for all family lineages can be found interspersed throughout the distribution of barramundi across northern Australia, in both metapopulations. Chenoweth *et al.* (1998a,b) and Doupé *et al.* (1999) found ≈ 4% divergence between two major clades of the mitochondrial control region sequence of barramundi. One clade, which predominates in east coast populations, decreases in frequency from the east coast to the western Arafura Sea (Chenoweth *et al.* 1998b). The divergence between lineages can be used to estimate divergence times. Chenoweth *et al.* (1998b) estimated that the clades shared a common maternal ancestor around 335 000 BP, which corresponds closely with another time of high sea level when the Torres Straits would have been open (Keenan 1994). During periods of lower sea levels, when the Torres Straits were closed and migration of barramundi impossible, the sequence differences between these two major clades presumably evolved. Yet, despite the large geographical distance between the eastern and western regions (≈ 5000 km coastal distance), gene flow, through the migration of fish, has mixed the haplotypes from their probable origins across the width of the sampled range (Chenoweth *et al.* 1998b). Chenoweth *et al.* (1998b) calculated (based on a generation dispersal distance of 15.3 km) that only 1580 years were required to transport either clade across the Australian distribution. The data of Doupé *et al.* (1999) clearly show these two major clades and also the mixture of these clades in their samples from wild populations from the Fitzroy and Ord Rivers. The lack of sequence difference and the presence of only a single clade in each of the Cairns and Darwin samples examined by Doupé *et al.* (1999) presumably resulted from low sample sizes (five and ten respectively) and sampling F_1 fish obtained from fish hatcheries (Doupé 1997).

Phenotypic response

Barramundi stocks are known to respond to different environments, but the evidence points to environmental rather than genetically derived differences. For instance, Davis (1985) found that the growth of barramundi varied markedly both within and between rivers, concluding 'that the observed variability was a reflection of the different environmental conditions experienced by barramundi in the different rivers'. Further, different sizes at first maturity of males from Weipa (290 mm total length) and the Mary River NT (600 mm total length) were found to result from a slower growth of fish from Weipa and were not caused by maturation at an earlier age (Davis 1985). Growth rate differences are common in aquacultured barramundi, in which genetically similar full-sib juveniles must be graded regularly to prevent cannibalism. Davis (1985) also found that the timing and duration of the breeding season varied considerably among regions, rivers and years. Davis (1987) concluded that the success

of barramundi in northern Australia could be attributed to its dynamic and flexible biology.

These environmentally induced differences do not persist if fish are translocated into different environments. For instance, Weipa fish stocked into Tinaroo Dam (near Cairns; Fig. 1) grew far more rapidly than in their native environment. Several studies, prompted by possible commercial opportunities to exploit the population genetic differences, examined these genotype–environment interactions in detail for different stocks of barramundi. Rodgers & Bloomfield (1993) studied the growth of juvenile barramundi derived from Cairns (north-east coast stock, 16°45′S) and the Burrum River (south-east coast, 26°00′S, close to the southern limit for this species). Large numbers of fish (> 1000) were ongrown under a variety of density conditions (200–500 fish m^{-3}) in cages in freshwater ponds at Walkamin Research Station. Over two trials, the first of 2 months' duration with falling temperatures (from 22 °C to < 16°C) and the second over 6 months of rising temperatures (from < 16°C to 27°C), there was no significant difference in the specific growth rate of these two stocks, despite the predictions. Also, overwinter mortality began in both strains when average daily temperatures fell below 20 °C, and cumulative mortality rates of 32% and 39% were recorded for Cairns and Burrum River fish, respectively, i.e. the most southerly stock exhibited a slightly higher mortality.

In a separate study, Burke (1994) examined the temperature tolerance of barramundi fry from the Burrum River and Cairns. Both stocks displayed a similar preferred temperature range of 27.5–34.5 °C and a maximum thermal limit of 42.5 °C. At cold temperatures, final death points were ≈ 12 °C for both strains. Critical thermal minima (CTM) of 14.6 ± 0.4 °C were obtained for the juvenile barramundi, with no significant difference in fish reared from Burrum River and Cairns stocks. Burke (1994) developed this research further, repeating the experiment to examine the effects of salinity, size and rearing conditions. Using fingerlings from different parents from the first trial, he was unable to find differences between fish sourced from different populations when they were the same size.

Of course, the results of these experiments are based on a limited number of single-pair matings. However, the similarities between these results for the widely separated barramundi strains are remarkable, given that chance sampling from a population should maximize any differences. One thing is quite clear from these experiments – barramundi do not show the same level of adaptation and genetic-based variation in growth rate that the silver carp stocks from temperate and subtropical China show, and their genetic distance is far lower between populations than the carp.

Genetic models and the estimation of migration rate

In the genetics literature, there is an ongoing argument about the relative importance of 'adaptive selection' vs. 'genetic drift of neutral genes' in the development and maintenance of population structure. While there is strong evidence for selection at particular loci, the neutral gene concept is the basis for the stochastic set of genetic models that incorporate genetic drift, mutation and the influence of migration on population structure. Further, the statistical identification of population structure is based on the assumptions of the neutral model. Yet, many biologists are very happy to interpret this population structure as implying that they represent real differences in quantitative traits. As discussed above, very few studies have tested adequately for differences in life history traits of different populations of barramundi, and those studies that have been conducted have found no evidence of such differences. Another problem with interpretation, which underlies all that has been discussed above, is the influence of migration on population structure.

The potential influence of translocation (or human-induced migration) on the genetic constitution of the receiving population under the neutral allele model is usually considered to be very large. Using Wright's 'island model', it is simple to show that, if there is more than one effective, or breeding, migrant per generation, then population structure, as measured by the level of F_{ST}, or the level of population subdivision, is greatly reduced (e.g. Hartl 1980, p.196). The island model, however, 'is not likely to be exactly realised in nature' (Wright 1943), because it assumes that migration is **equal between all** populations in two-dimensional space for every generation. In the case of barramundi, this would mean that the rate of migration, in both directions, is the same between Darwin and the Ord River, Weipa and the Ord River, Cairns and the Ord River and the Burrum River and the Ord River (see Fig. 1). Obviously, this is not the case for a coastal fish, although it could be possible, although highly unlikely, for birds.

A corollary of Wright's widely accepted model is that, regardless of population size, populations will not diverge by the process of genetic drift through isolation if more than five individuals are exchanged per generation. Therefore, apart from virtually complete geographic isolation, selection is often regarded as the most important force producing genetic structure within a species (Keenan 1994). The definition of a stock as 'a reproductively, and usually geographically, isolated group of fish within a species, that is genetically distinguishable from other groups in the same species' (Harvey, Ross, Greer & Carolsfeld 1998) highlights this interpretation based on the 'island model'.

This 'fact' has been incorporated into textbooks for decades and is incorporated in definitions published by the IUCN (1995) for the protection of endangered species. The general acceptance of this overly simplistic model has misled people in their interpretation of the outcomes that could arise from translocating numbers of fish from one stock into another.

Estimates of migration rate using the mathematically precise and more realistic 'one-dimensional stepping-stone model' (Kimura & Weiss 1964) suggest migration rates between adjacent barramundi stocks of roughly 10%, two orders of magnitude higher than predicted by the 'island model' (for a detailed comparison, see Keenan 1994). The 'stepping stone' group of models, in either one or two dimensions, assume that migration is **equal between adjacent** populations in every generation. This situation is much more like the real situation for barramundi and almost all species of animals or plants.

Heterozygosity

An issue important to the health of a population is the level of heterozygosity, both of the population and of the individuals within the population (e.g. Coltman, Bowen & Wright 1998; Saccheri *et al.* 1998). Both theory (Mitton 1993) and experience demonstrate that fish populations (Quattro & Vrijenoek 1989) or butterfly populations (Saccheri *et al.* 1998) with higher levels of inbreeding and lower levels of genetic variation are less viable, in terms of larval survival, growth and fecundity, than those with low levels of inbreeding and higher levels of genetic heterozygosity.

Australian barramundi have low heterozygosity. Thirty-two monomorphic loci and 13 polymorphic loci have been identified, and total heterozygosity (H_T) is 0.026 (Keenan 1994). This level of heterozygosity is less than half the average heterozygosity levels reported by Ward, Woodwark & Skibinski (1994) for 57 marine and 49 freshwater fish species (0.064 and 0.062 respectively). Because the founding populations of barramundi were most probably derived from a small offshoot of the south-east Asian population, the comparatively low level of gene diversity found in Australian barramundi is not unexpected.

Restocking has the potential to deplete heterozygosity further, regardless of the source of stocked fish. If large numbers of full-sib fish are stocked into a population, this may result in a rapid and significant loss of heterozygosity, particularly if these fish interbreed. Simple genetic testing can be used to maximize heterozygosity in the translocated fish, a desirable feature for both aquaculture and restocking programmes (Keenan 1995).

Summary

The recent development of population structure and small genetic distances between barramundi populations is confirmed by the growth and survival responses of widely separated stocks compared under the same environmental conditions. Two scientific studies have been conducted in which growth and survival have been compared for juveniles from tropical and temperate populations, and a hybrid between these populations. They tested the hypothesis that stocks from tropical Australia would have different responses to low temperature than stocks from temperate Australia that were assumed to be adapted to lower temperatures. There was no significant difference in specific growth rate (Rodgers & Bloomfield 1993) or low and high temperature tolerance (Burke 1994) between the different populations of barramundi as postulated.

As barramundi is an aquacultured species that may escape from enclosures into the wild population, we need to estimate the possible impact of escapees into the natural population. Based on a low level of genetic differentiation, high levels of gene flow between populations and little evidence of either adaptation or 'outbreeding depression', translocation between populations should not pose a significant risk or problem. Present government policy in Queensland is to allow the translocation of barramundi for aquaculture purposes, but to limit

translocation to within populations for restocking purposes.

Translocation of barramundi does not appear to pose a threat to current thinking on conservation values. Moritz (1999) suggests that the goal of conservation should be to conserve ecological and evolutionary processes and to explore the role of the use of translocation in conservation management. He suggests that translocation can take place between management units (MUs) within an evolutionary significant unit (ESU), but that translocation between ESUs may cause significant genetic disruption. ESUs are defined as 'historically isolated and independently evolving sets of populations...and are diagnosed as sets of populations showing reciprocal monophyly of mtDNA combined with significant divergence of allele frequencies at nuclear loci'. Using this definition, the mitochondrial DNA lineages of Australian barramundi (Chenoweth *et al*. 1998a,b) represent many MUs within a single ESU, which may be isolated from other ESUs in south-east Asia. To many conservationists, translocation between populations within an ESU does not represent a problem. In fact, under certain conditions in which connectivity between populations has been fragmented, translocation to increase heterozygosity and limit inbreeding should be considered (Conant 1988; Knapp & Dyer 1998; Saccheri *et al*. 1998; Moritz, 1999).

As a final comment, it is appropriate to quote the conclusion of Shield (1993), who states 'We should not be led to our beliefs about inbreeding and outbreeding and our recommendations for managing populations by dogma, but rather should continue to ask the organisms what is going on with inbreeding and outbreeding in nature.'

Acknowledgements

The author would like to thank Wayne Knibb, Shane Lavery and Craig Moritz for valuable discussions on this topic. The detailed comments of David Ayre and an anonymous referee helped to motivate the author and focus the paper. The objectivity and patience of Alan Lymbery were much appreciated.

References

Allendorf F.W. & Leary R.F. (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* **2**, 170–184.

Barrett S.C.H. & Kohn J.R. (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: *Genetics and Conservation of Rare Plants* (ed. by D.A. Falk & K.E. Holsinger), pp. 3–30. Oxford University Press, New York.

Burke J.B. (1994) *Temperature and Salinity Requirements in the Early Life-history of Two Catadromous Fishes, Barramundi,* Lates calcarifer *(Bloch), and Australian Bass,* Macquaria novemaculeata *(Steindachner) in Eastern Australia.* Unpubl. Master's Thesis, Centre for Marine Science, University of New South Wales.

Chenoweth S.F., Hughes J.M., Keenan C.P. & Lavery S. (1998a) Concordance between dispersal and mitochondrial gene flow: isolation by distance in the tropical teleost *Lates calcarifer* (Australian barramundi). *Heredity* **80**, 187–197.

Chenoweth S.F., Hughes J.M., Keenan C.P. & Lavery S. (1998b) When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface. *Proceedings of the Royal Society of London, Series B Biological Sciences* **265**, 415–420.

Coltman D.W., Bowen W.D. & Wright J.M. (1998) Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal Society of London, Series B Biological Sciences* **265**, 803–809.

Conant S. (1988) Saving endangered species by translocation. *Bioscience* **38**, 254–257.

Davis T.L.O. (1985) Seasonal changes in gonad maturity, and abundance of larvae and early juveniles of barramundi, *Lates calcarifer* (Bloch), in Van Diemen Gulf and the Gulf of Carpentaria. *Australian Journal of Marine and Freshwater Research* **36**, 177–190.

Davis T.L.O. (1987) Biology of wildstock *Lates calcarifer* in Northern Australia. In: *Management of Wild and Cultured Sea Bass/Barramundi (*Lates calcarifer*)*. Proceedings of an International Workshop Held in Darwin, NT, Australia, 24–30 September 1986 (ed. by J.W. Copland & D.L. Grey), pp. 22–29. Proceedings no. 20, Australian Centre for International Agricultural Research, Canberra.

Dobzhansky T. (1936) Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* **21**, 113–135.

Dobzhansky T., Ayala F.J., Stebbins G.L. & Valentine J.W. (1977). *Evolution.* W.H. Freeman, San Francisco.

Doupé R.G. (1997) *Genealogical Studies of Selected Australian Barramundi (*Lates calcarifer*) using mtDNA: Implications for Stock Transfer to the Kimberley Region of Western Australia.* Unpubl. Honours Thesis, Faculty of Science, Technology and Engineering, Edith Cowan University, WA.

Doupé R.G., Horwitz P. & Lymbery A.J. (1999) Mitochondrial genealogy of Western Australian barramundi: applications of inbreeding coefficients and coalescent analysis for separating temporal population processes. *Journal of Fish Biology* **54**, 1197–1209.

Hartl D.L. (1980) *Principles of Population Genetics*. Sinauer Associates, Sunderland, MA.

Harvey B., Ross C., Greer D. & Carolsfeld J. (eds) (1998) *Action Before Extinction: an International Conference on Conservation of Fish Genetic Diversity*. World Fisheries Trust, Victoria, Canada.

Henderson I.F. & Holmes S. (1979) *Henderson's Dictionary of Biological Terms,* 9th edn. Longman, London.

IUCN (1995) *IUCN Red List Categories.* Prepared by the IUCN Species Survival Commission, as approved by the 40th meeting of the IUCN Council, Switzerland, 30 November 1994. http: //iucn.org/themes/ssc/iucnred-lists/definit.htm.

Keenan C.P. (1994) Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): an example of isolation by distance in one dimension. *Australian Journal of Marine and Freshwater Research* **45,** 1123–1148.

Keenan C.P. (1995) Genetic implications of fish stocking programs. In: *Fish Stocking in Queensland – Getting It Right* (ed. by P. Cadwallader & B. Kerby), pp. 64–71. Proceedings of the Symposium held in Townsville, Queensland, 11 November 1995. Queensland Fisheries Management Authority, Brisbane.

Keenan C.P. (1998) Stock structure across Northern Australia. In: *Taking Stock: Defining and Managing Shared Resources.* Australian Society for Fish Biology and the Fish and Aquatic Resource Management Association of Australia Joint Workshop Proceedings, Darwin 15–16 June 1997 (ed. by D.A. Hancock), pp. 31–39. Australian Society for Fish Biology, Sydney.

Keenan C.P. & Salini J. (1990) The genetic implications of mixing barramundi stocks in Australia. In: *Introduced and Translocated Fishes and Their Ecological Effects*. Australian Society for Fish Biology Workshop (ed. by D.A. Pollard), pp. 145–150. Proceedings no. 8, Bureau of Rural Resources, Canberra.

Keenan C.P., Davie P.J.F. & Mann D.L. (1998) A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). *Raffles Bulletin of Zoology* **46,** 217–245.

Kimura M. & Weiss G.H. (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* **49,** 561–574.

Knapp E.E. & Dyer A.R. (1998) When do genetic considerations require special approaches to ecological restoration. In: *Conservation Biology for the Coming Decade,* 2nd edn (ed. by P.L. Fielder & P.M. Kareiva), pp. 345–363. Chapman & Hall, New York.

Krueger C.C., Gharrett A.J., Dehring J.R. & Allendorf F.W. (1981) Genetic aspects of fisheries rehabilitation programs. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 1877–1881.

Leary R.F., Allendorf F.W., Phelps S.R. & Knudsen K.L. (1984) Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. *Proceedings of the Montana Academy of Science* **43,** 1–18.

Li S., Lu W., Peng C. & Zhao P. (1987) A genetic study of growth performance of silver carp from the Changjiang and Zhujiang Rivers. *Aquaculture* **65,** 93–104.

May B. & Krueger C.C. (1990) Use of allozyme data for population analysis. In: *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management* (ed. by D.H. Whitmore), pp. 157–171. CRC Press, Boca Raton.

Mitton J.B. (1993) Theory and data pertinent to the relationship between heterozygosity and fitness. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed. by N.W. Thornhill), pp. 17–41. University of Chicago Press, Chicago.

Moritz C. (1994) Defining 'evolutionarily significant units' for conservation. *TREE* **9,** 373–375.

Moritz C. (1999) Conservation units and translocations: strategies for conserving evolutionary processes. *Hereditas* **130,** 217–228.

Pullin R.S.V. (ed.) (1988) *Tilapia: Genetic Resources for Aquaculture ICLARM Conference Proceedings 18.* ICLARM, Manila, Philippines.

Quattro J.M. & Vrijenoek R.C. (1989) Fitness differences among remnant populations of the endangered Sonoran topminnow. *Science* **245,** 976–978.

Rodgers L.J. & Bloomfield J.P. (1993) Comparison of growth, condition and mortality between stocks of barramundi *Lates calcarifer*: 1. Cairns and Burrum River strains. In: *Larval and Juvenile Culture of Barramundi* Lates calcarifer *(Bloch)* (ed. by C.G. Barlow & M.A. Rimmer), Chap. 9, pp. 9.1–9. 19. Final Report, Project no. 89/67. Fishing Industry Research and Development Council, Canberra.

Rowland S.J. (1984) Hybridization between the estuarine fishes yellowfin bream, *Acanthopagrus australis* (Gunther), and black bream. *A. butcheri* (Munro) (Pisces: Sparidae). *Australian Journal of Marine and Freshwater Research* **35,** 427–440.

Rowland S.J. (1985) *Aspects of the Biology and Artificial Breeding of the Murray Cod* Maccullochella peeli, *and the Eastern Freshwater Cod,* M. ikei *sp. nov. (Pisces: Percichthyidae)*. Unpubl. PhD Thesis, Macquarie University.

Rowland S.J. (1988) Eastern freshwater cod (*Maccullochella ikei*): identification, status, conservation and potential threats. In: *Proceedings of a Fish Genetics Workshop* (ed. by S.J. Rowland & R. Barlow), pp. 18–23. New South Wales Agriculture and Fisheries, Sydney.

Saccheri I., Kuussaari M., Kankare M., Vikman P., Fortelius W. & Hanski I. (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature* **392,** 491–494.

Shaklee J.B., Salini J. & Garrett R.N. (1993) Electrophoretic characterization of multiple genetic stocks of barramun-

di perch in Queensland, Australia. *Transactions of the American Fisheries Society* **122,** 685–701.

Shields W.M. (1993) The natural and unnatural history of inbreeding and outbreeding. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed. by N.W. Thornhill), pp. 143–169. University of Chicago Press, Chicago.

Smith S.M., Maceina M.J. & Dunham R.A. (1994) Natural hybridization between black crappie and white crappie in Weiss Lake, Alabama. *Transactions of the American Fisheries Society* **123,** 71–79.

Templeton A.R. (1986) Coadaptation and outbreeding depression. In: *Conservation Biology* (ed. by M.E. Soulé), pp. 105–116. Sinauer Associates, Sunderland MA.

Wajon S. (1983) *Hybridization between Murray Cod and Trout Cod in Cataract Dam, NSW.* Unpubl. Honours Thesis, University of New South Wales, Sydney.

Waldman B. & McKinnon J.S. (1993) Inbreeding and outbreeding in fishes, amphibians, and reptiles. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed. by N.W. Thornhill), pp. 250–282. University of Chicago Press, Chicago.

Ward R.D., Woodwark M. & Skibinski D.O.F. (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology* **44,** 213–232.

Waser N.M. (1993) Sex, mating systems, inbreeding, and outbreeding. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (ed. by N.W. Thornhill), pp. 1–13. University of Chicago Press, Chicago.

Wright S. (1943) Isolation by distance. *Genetics* **28,** 114–138.

Aquaculture Research, 2000, **31**, 133–143

Measuring and interpreting genetic structure to minimize the genetic risks of translocations

M S Johnson
Department of Zoology, University of Western Australia, Nedlands, Western Australia 6907, Australia

Correspondence: M S Johnson, Department of Zoology, University of Western Australia, Nedlands, Western Australia 6907, Australia

Abstract

Genetic subdivision of a species indicates the potential for local adaptation, and the genetic differences among populations are a key component of genetic diversity. Molecular genetic markers are generally used to assess the extent and pattern of subdivision. These traits provide an abundance of simple genetic markers, and they allow comparisons across studies. However, the connection of molecular genetic variation to local adaptation and, hence, to possible genetic problems of translocation, is weak. In the extreme case of no genetic subdivision, there is no reason to expect genetic problems with translocation. Where there is deep genetic structure, indicating substantial evolutionary independence of sets of populations, translocations may threaten basic components of genetic diversity. Between these extremes, however, predicting genetic problems of translocations is extremely difficult. The molecular markers used to measure genetic structure indicate where there has been opportunity for local adaptation, but they are not directly related to such adaptation. The relationship of the level of genetic divergence to genetic incompatibilities is very loose, although quantitative tests are scarce. However, studies of reproductive isolation between species illustrate the fundamental inadequacy of using measures of genetic divergence to predict interactions between populations. Although it is tempting to use simple measures as predictors, such use may provide a false sense of scientific rigour. There is no substitute for direct tests for variation in ecologically relevant traits and possible genetic incompatibilities among populations.

Introduction

In the vast majority of species, populations are not totally mixed in each generation, but are isolated to varying extents. This isolation reduces gene flow and, therefore, allows genetic differences to accumulate between populations, through random drift, natural selection and accumulation of novel mutations. The degree of isolation and consequent independence of populations are important in both ecological and evolutionary contexts, and are highly relevant for the management and conservation of natural populations. Indeed, among the first to recognize the importance of subdivided populations were fisheries biologists, concerned with the North Sea herring fishery, a century ago (see Sinclair 1988). In that case, morphological and biological differences between stocks from different areas indicated not only that there were independent populations, which should be managed as separate units, but that these populations were locally adapted. The importance of recognizing independent stocks has long been part of fisheries management, and fisheries biologists have been quick to use each advance in the use of genetic traits to identify such independence (for example, see Ward & Grewe 1995). However, until recently, the importance of the genetic composition of those stocks received little emphasis. With increasing threats to many natural populations and increasing manipulations through intended and accidental translocations, the focus is shifting towards greater recognition of the possible importance of genetic differences among populations.

The possible genetic consequences of mixing stocks have been well documented, especially for

aquatic animals (e.g. Hindar, Ryman & Utter 1991; Rhymer & Simberloff 1996). The focus of this paper is the predictability of those consequences from amounts and patterns of genetic subdivision. This will include a brief overview of how genetic structure is measured, examples of the diverse patterns of genetic subdivision, emphasizing Western Australian species, and an examination of the problems of moving from genetic structure to the likely consequences of translocation.

Measuring genetic structure

In principle, geographic variation in any heritable trait can indicate genetic subdivision and, historically, morphology, chromosomes and immunological differences have been used in the context of fisheries stocks. In practice, however, molecular genetic markers are overwhelmingly the basis for assessing genetic structure (see Avise 1994; Hillis, Moritz & Mable 1996). The general advantages of molecular genetic markers are their ready availability, genetic simplicity, comparability across taxa and ease of use with population genetic models. The most extensive data are from allozymes, allelic forms of enzymes detected by gel electrophoresis (see Richardson, Baverstock & Adams 1986; Avise 1994; Shaklee & Bentzen 1998). Allozyme electrophoresis provides an efficient and economical source of genetic markers, but is increasingly complemented or supplanted by the use of DNA markers. Where allozymes provide insufficient markers, microsatellite DNA provides a potential source of highly polymorphic nuclear genes for the study of fine-scale population structure (Queller, Strassmann & Hughes 1993). Of special interest in the study of population genetic structure is the increasing use of direct sequencing of DNA, especially mitochondrial DNA (mtDNA). There are three special advantages of mtDNA in detecting genetic structure. First, it tends to evolve more rapidly than nuclear genes, such as those represented by allozymes, and can therefore reveal the effects of more recent historical subdivision. Secondly, it is haploid and inherited almost entirely through the female line. As a result, the effective population size for mtDNA is only one quarter as great as that for diploid nuclear genes, which means that variation in mtDNA is more sensitive to the effects of isolation of populations. Finally, mtDNA is clonally inherited, without genetic recombination, so its lineages retain the phylogenetic information of accumulated mutations. It is the ability to examine the phylogenies of genes themselves that has led to the rapidly expanding field of phylogeography, in which population genetic structure is interpreted within a historical framework (Avise, Arnold, Ball, Bermingham, Lamb, Neigel, Reeb & Saunders 1987; Avise 1998).

At the most basic level, the data from molecular analyses of genetic structure are sets of allele frequencies for nuclear genes (such as those represented by allozymes or microsatellites) or haplotype frequencies for mtDNA. The most basic analysis of these frequencies is simply to determine whether they vary geographically, thereby providing evidence for genetic subdivision. Although often ignored, it is essential that apparent geographic variation is not an artifact of temporal variation, which is tested most directly with comparisons among cohorts (e.g. Smith 1979; Johnson & Black 1984). If distant sites differ, but adjacent ones do not, it is possible to use the pattern of genetic differences to estimate the geographical scale of mixing, or the genetic neighbourhood (e.g. Richardson *et al.* 1986; Brown 1991).

If there is geographic subdivision, two approaches are commonly taken for quantification. From an evolutionary perspective, it is useful to quantify comparisons in terms of average genetic similarity or distance between pairs of populations, most commonly Nei's (1978) measures. Such measurement should be based on a random set of genetic markers, not a selected set of polymorphisms, if the results are to be comparable with other studies. When the focus is specifically on population subdivision, especially related to gene flow, more appropriate measures are those related to Wright's standardized variance in allele frequencies, F_{ST}, which is the proportion of the total genetic diversity that results from differences among subpopulations (Wright 1969). Although generally measured for a set of populations, F_{ST} is also useful as a pairwise measure of genetic divergence between populations, more appropriate than Nei's D, when the underlying processes are on more ecological than evolutionary timescales (Slatkin 1993).

Because the genetic differences among populations result from both current patterns of subdivision and deeper historical divergence, the most powerful analyses of geographic variation include a phylogenetic perspective (for a review in the marine context, see Hilbish 1996). Nucleotide sequences of DNA provide data for analysis of gene phylogenies.

When viewed in a geographic context, such phylogenies can differentiate between the effects of present isolation and historical events (e.g. Templeton 1998).

Patterns of genetic subdivision in aquatic species

The extent and pattern of genetic subdivision of a species depend on its life history, which determines the inherent potential for dispersal, and its present and past environments. For example, in marine species, the longer the period of planktonic development, the greater is the potential for dispersal. Comparative studies both in California and on the Great Barrier Reef fit with the prediction of greater genetic subdivision in species of fish that have a shorter or no period of planktonic larval development (Waples 1987; Doherty, Planes & Mather 1995). Similarly, among intertidal snails, those with direct development of benthic eggs tend to show higher levels of genetic subdivision than do planktotrophs over the same geographic range (e.g. Berger 1973; Ward 1990; Parsons 1996). Nevertheless, even species with planktotrophic dispersal can show genetic subdivision over small distances, emphasizing the importance of larval behaviour and local patterns of entrainment (e.g. Burton & Feldman 1981).

Compared with the potential for dispersal over large distances in the ocean, isolation of populations is much more likely in freshwater species, which are more likely to have highly fragmented distributions. Reviews of genetic studies of fish clearly support this prediction: freshwater species show, on average, more than three times as much subdivision throughout their sampled ranges, which are often smaller than those of marine species (Gyllensten 1985; Ward, Woodwark & Skibinski 1994).

The marron *Cherax tenuimanus* (Smith), a commercially valuable species of crayfish in Western Australia, illustrates the extreme subdivision characteristic of many freshwater species. Populations from two rivers about 30 km apart were compared for 30 allozyme loci. At all three of the variable loci detected in this species, the two populations were fixed for different alleles (Austin & Knott 1996). Because of the relatively low level of polymorphism, this gave an average genetic identity of 0.90, but the level of subdivision was the maximum possible, with $F_{ST} = 1.00$. A preliminary study of two microsatellite DNA polymorphisms found further differences among populations of marron, confirming widespread genetic subdivision in this species (Imgrund, Goth & Wetherall 1997). This species is widely cultured in farm ponds, with the potential for escape into native populations. Such mixture of genetically divergent stocks was detected in the Margaret River, Western Australia, using the geographically variable allozyme loci in the early 1980s (C M Austin, pers. commun.), but the subsequent development of the hybrid population has not been examined. Three other species of *Cherax* native to south-western Australia also show high levels of genetic subdivision, with fixed allelic differences between at least some populations (Austin & Knott 1996).

Also in the south-west of Western Australia, rare endemic frogs of the genus *Geocrinia alba* Wardell-Johnson & Roberts show very high levels of genetic subdivision over short distances, which are relevant to possible translocations for the conservation of these species (Driscoll, Wardell-Johnson & Roberts 1994; Driscoll 1998). In *G. alba*, which has a range spanning less than 20 km, two genetically distinct groups were found. Although no fixed allelic differences were detected in a survey of 17 allozyme loci, high levels of subdivision were found both within creeks ($F_{ST} = 0.26$) and between creeks ($F_{ST} = 0.33$). Even in *G. vitellina* Wardell-Johnson & Roberts, with a geographic range spanning only 4 km, subdivision was high ($F_{ST} = 0.30$), emphasizing the fine scale over which geographic structure can occur.

In extreme contrast to such high levels of genetic subdivision in freshwater species, many marine species show little subdivision over large distances, as expected for species with long-lived planktotrophic larvae or pelagic adults. Relatively few species, however, show no evidence of any genetic subdivision. Among those few are three commercially significant species in Australia: southern bluefin tuna *Thunnus maccoyii* (Castelnau) from South Africa, Western Australia, South Australia and Tasmania (Grewe *et al.* 1997); the western rock lobster *Panulirus cygnus* George over a range of 700 km along the Western Australian coast (Thompson, Hanley & Johnson 1996); and the Australian salmon *Arripis truttaceus* (Cuvier) along the entire south coast of Australia (MacDonald 1980). These examples of absence of genetic structure are consistent with expectations based on the biology of these species, so they are likely to be real, and not artifacts of inadequate sampling.

Extensive gene flow, as evidenced by very low levels of genetic subdivision, is common among marine species along the Western Australian coast. In a summary of allozyme studies of 14 species of fish, bivalves, gastropods and urchins, sampled over ranges of 500–2080 km in northern Western Australia, average values of F_{ST} ranged from 0.003 to 0.058, with a mean of 0.021 (Johnson, Hebbert & Moran 1993).

In some marine species, there is a clear pattern of decreasing genetic similarity with increasing distance between populations. This pattern has been observed on a scale of 5000–10 000 km across the Pacific in skipjack tuna *Katsuwonus pelamis* (Linnaeus) (Richardson 1983) and in *Echinometra* spp. urchins (Palumbi, Grabowsky, Duda, Geyer & Tachino 1997). In coastal species, which have more continuous populations, isolation by distance has been found over 2500 km in the blacklip abalone *Haliotis rubra* Leach in southern Australia (Brown 1991) and over 300 km in the Western Australian littorine *Littoraria cingulata* (Philippi) (Johnson & Black 1998). Such a pattern indicates that subpopulations are not discrete, but are connected by a diffusion process of gene flow.

The spatial scale of mixing under a model of isolation by distance is referred to as the neighbourhood (Wright 1969), within which spatial genetic divergence cannot accumulate from one generation to the next. Genetic markers can be used to estimate the extent of the neighbourhood. Using this approach, Richardson (1983) estimated the scale of mixing in southern skipjack tuna to be about 1100 km, which corresponds well with estimates from tagging studies. In blacklip abalone, the neighbourhood was estimated as about 500 km in length (Brown 1991). In contrast, in the direct-developing intertidal snail *Bembicium vittatum* Philippi, the estimated neighbourhood is only 150–300 m, emphasizing the fine scale of genetic subdivision possible in species without planktonic larvae (Johnson & Black 1995).

The example of *L. cingulata* illustrates another important point: that a species may show a combination of patterns of geographic variation. This species has extensive populations in Shark Bay and between North-west Cape and Cape Leveque, Western Australia, with a gap of about 300 km between the two sets of populations. Within regions, there is isolation by distance up to 300 km, with no increase in mean genetic divergence beyond that distance; within the northern region, the average F_{ST} between populations separated by more than 300 km was 0.028. In contrast, over the same distances, comparisons between southern (Shark Bay) and northern populations gave a mean F_{ST} of 0.055, indicating a twofold increase in genetic divergence associated with the isolation of the Shark Bay populations (Johnson & Black 1998).

Even more localized effects of isolation are evident in some species that otherwise have extensive genetic connections. Snapper *Pagrus auratus* (Forster) shows little geographic variation of allozyme frequencies over 6000 km in southern Australia, with a pattern of isolation by distance and an estimated neighbourhood size of about 1000 km (MacDonald 1980 in Richardson *et al.* 1986). Nevertheless, there are genetically distinct populations in Shark Bay, Western Australia, and in Spencer Gulf, South Australia (MacDonald 1980; Johnson, Creagh & Moran 1986). In the eastern gulf of Shark Bay, there are low-frequency alleles not found in the ocean populations, indicating significant isolation for sufficient time to accumulate novel mutations.

Other examples also indicate that embayments and estuaries can increase genetic subdivision by harbouring isolated populations. In comparisons of the extent of genetic subdivision among marine and estuarine populations of four species of fish in Western Australia, subdivision was consistently higher between adjacent marine and estuarine populations than between marine populations separated by similar distances (Watts 1991). In terms of F_{ST}, the increase was nearly 10-fold in the mouth-brooding cardinal fish *Apogon ruepellii* Günther, about threefold in the atherinids *Leptatherina presbyteroides* (Richardson) and *Craterocephalus capreoli* Rendahl, and very slight for the more mobile mullet *Mugil cephalus* Linnaeus. Similarly, genetic divergence, as measured by F_{ST}, was 40% greater among estuarine populations than among marine populations of the cobbler *Cnidoglanis macrocephalus* (Valenciennes), commercially the most significant estuarine fish in Western Australia (Ayvazian, Johnson & McGlashan 1994). In addition to showing that estuarine populations are often self-replenishing, these comparisons emphasize the potential for local adaptation of these isolated populations.

Similarly, anadromous fishes, which migrate to freshwater habitats for breeding, tend to be genetically subdivided, with average F_{ST}s between those of marine and freshwater species (Gyllensten 1985;

Ward *et al*. 1994). The most extensive examples are from the salmonids of the northern hemisphere. In contrast, relatively few catadromous species have been examined genetically, but subdivision in these seems to be relatively moderate. Studies of Australian bass *Macquaria novemaculeata* (Steincahner) over distances of more than 1500 km showed only moderate divergence among rivers, with mean F_{ST} of 0.02–0.04 (Chenoweth & Hughes 1997; Jerry 1997). An important species for aquaculture is the barramundi *Lates calcarifer* (Bloch), which lives as adults in rivers of northern Australia, spawning in the estuaries. In northern Queensland and the Northern Territory, studies of allozymes revealed 16 genetically separate populations of *L. calcarifer* associated with different rivers (Keenan 1994). Nevertheless, the differences were relatively moderate variations in allele frequencies. In the same area, sequences of the control region of mtDNA revealed additional subdivision (Chenoweth, Hughes, Keenan & Lavery 1998). Although the overall pattern of subdivision in *L. calcarifer* is one of isolation by distance, there was no sharing of mtDNA haplotypes between the Coral Sea, the Gulf of Carpentaria and the Western Arafura Sea, indicating a major regional component to the genetic structure.

Species often have layers of genetic subdivision, which reflect more than just spatial scale. For example, the pearl oyster *Pinctada maxima* (Jameson) shows little evidence of subdivision between Western Australian populations 800 km apart, but substantial differences between populations in the Northern Territory and north-eastern Queensland over a similar distance (Johnson & Joll 1993). The genetic difference between north-eastern Queensland is consistent with the pattern found in *L. calcarifer* and in green turtles *Chelonia mydas* (Linnaeus) (Norman, Moritz & Limpus 1994) but, in all cases, the geographic sampling is not adequate to identify regional discontinuities. Comparisons among species can reveal common patterns that apparently reflect shared historical exchanges or restrictions to gene flow. For example, in a comparison of geographic variation of allozymes in three species of giant clam, three species of sponge and a starfish, Benzie (1994) found less genetic connection between the Great Barrier Reef and the Solomons or Vanuatu than expected, based on the strong flow between them via the South Equatorial Current. He proposed that contrasting historical patterns of gene flow were the likely cause. The clearest example of different species showing deep genetic structure associated with a biogeographic boundary is the divergence of mtDNA lineages in several species, between Atlantic and Gulf of Mexico populations in eastern Florida (Avise 1992).

Predicting genetic risks of translocation

What do these patterns of genetic subdivision mean in terms of potential genetic risks of translocation? Can the genetic structure of a species be the basis of prediction of outcomes of translocation? In discussing these issues, my focus will be on translocation of animals between natural populations, either purposely or accidentally. Genetic changes in captivity, before release into the wild, raise an additional set of important questions (Hindar, Ryman & Utter 1991). Selective changes to hatchery populations, whether intentional or inadvertent, are likely to make hatchery stock less suited to natural conditions. Similarly, losses of genetic diversity and inbreeding can have serious consequences for the release of animals. These genetic problems are beyond the scope of this overview, but are additional problems that should be considered before any release of hatchery stock into natural populations.

If no genetic differences are found among geographic samples, it means one of two things. First, there may be no genetic subdivision, in which case there should be no genetic issues with translocation, except for problems resulting from genetic changes during the aquaculture phase. By the same token, in species that are naturally genetically mixed, there is no genetic basis for choosing broodstock from one place or another. The second possible explanation for finding no genetic differences among populations is simply that the genetic study was not sensitive enough. There are many examples in which increased numbers of gene loci or a different type of genetic marker have revealed structure that had not been recognized in a preliminary study. Adequacy of geographic sampling is also important. For example, a study of the copepod *Tigriopus californicus* (Baker) purportedly demonstrated genetic discontinuity of populations across the biogeographic boundary at Point Conception in California (Burton & Lee 1994), but higher density of sampling revealed that there are several genetic discontinuities, none of which correspond with Point Conception (Burton 1998). Any interpretations of the lack of genetic structure,

even within a portion of the geographic range, should be based on supporting ecological evidence, and not just on apparent genetic homogeneity. For example, in the cases of southern blue fin tuna, western rock lobster and Australian salmon mentioned earlier, the lack of geographic variation supported the predictions of complete mixing based on very long larval life or extensive migration.

At the other end of the spectrum, species may show deep genetic structure, representing separate evolution of sets of populations over long periods of time, such as with the divergence between Atlantic and Gulf of Mexico populations in the south-eastern United States. In such cases, the genetic differences between populations represent a significant component of the genetic diversity of the species, and there is a *prima facie* case that translocation could jeopardize that diversity. The importance of deep genetic structure for conservation genetics has been recognized in the concept of an evolutionarily significant unit, or ESU, which recognizes the biological uniqueness that results from genetic divergence over long periods (Ryder 1986; Dizon, Lockyer, Perrin, Demaster & Sisson 1992). Moritz (1994) suggested that ESUs could be recognized as having distinct lineages of mtDNA, along with supporting evidence of divergence for nuclear genes. The rationale for this perspective is that, in order for clonal lineages, as opposed to simple differences in frequencies of haplotypes, to characterize geographic sets of populations, there must have been evolutionary independence over significant amounts of time.

The perspective of ESUs has played a fundamental role in the development of policy for the translocation of salmonid fishes in the United States. The US Endangered Species Act defines 'species' to include 'any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature' (Waples 1991). Potential problems of translocation are strikingly evident in salmonids, in which large numbers of genetically isolated populations occur, and for which releases from hatcheries are common. Faced with managing such translocations under the Endangered Species Act, Waples (1991) interpreted the Act in terms ESUs, which must both be reproductively isolated from other conspecific sets of populations and represent 'an important component in the evolutionary legacy of the species.' The finding of distinct lineages of mtDNA, as suggested by Moritz (1994), would clearly indicate the former and provide a *prima facie* case for the latter. The presence of unique alleles at nuclear loci, such as those represented by allozymes, would provide similar evidence. In these clear cases, genetic mixing caused by translocation would reduce this evolutionary diversity, and thus would a priori be a genetic threat in the context of conservation genetics. This perspective is based on the philosophical position that important components of the evolutionary legacy of a species should be preserved, regardless of whether there are fundamental genetic incompatibilities between the divergent stocks.

Thus, at the extremes of either no subdivision or deep genetic subdivision, surveys of genetic structure can provide a basis for decisions in terms of genetic risks of translocation. It is the large continuum of intermediate levels of genetic divergence that is the problem. In the case of salmonids, for example, there are so many locally isolated populations, representing varying degrees of divergence, that it would be both ridiculous and impossible to attempt to impose a blanket policy of preservation of the minutiae of genetic structure (e.g. Bentsen 1991). Thus, it is necessary to judge which translocations are more likely to cause genetic problems. In this context, it would be extremely useful if there were a simple predictor of such problems. Unfortunately, measuring geographic variation of molecular genetic markers is inadequate for this task, both in practice and in principle.

Even the recognition of ESUs is not always straightforward. In Australian barramundi, for example, the phylogenetic analysis of mtDNA lineages revealed clades that characterize the regions of the Coral Sea, Gulf of Carpentaria and the Western Arafura Sea, indicating significant evolutionary independence (Chenoweth *et al.* 1998). However, other clades of just as recent origin have representative haplotypes in more than one area. A similar pattern has been found in a study of Western Australian populations (R Doupé, unpubl.). This combination of patterns indicates a high degree of historical isolation, combined with occasional gene flow among regions, and makes it clear that there is not a definitive boundary characterizing ESUs.

More fundamentally, even ESUs recognized purely on the basis of genetic structure do not necessarily differ in ecologically relevant traits. The same applies, of course, to less distinct patterns of geographic variation. The basic problem is that the

molecular markers used in population genetics have little or no direct connection to variation in traits that are relevant to local adaptation or to genetic incompatibilities between populations. While molecular measures of divergence provide evidence about the opportunity for adaptive divergence, they do not allow reliable inferences about the adaptive divergence that has actually occurred. Within the broader context of conservation genetics, the expectation of this disconnection from general principles has been emphasized by Lynch (1996), who also pointed out the scarcity of empirical tests of a relationship between estimates of molecular variation and genetic variance for traits likely to affect survival and reproduction. Although not involving specific tests, the extensive studies of genetic divergence in salmonids, for example, have revealed a poor relationship between overall genetic similarity and similarity in the quantitative traits that are more likely to be the basis for adaptive divergence (Taylor 1991).

Altukhov & Salmenkova (1987) specifically raised the question of predictability of success of translocation from estimates of genetic divergence between populations of chum salmon *Oncorhynchus keta* (Walbaum). They compared the rate of return for spawners from three pairs of populations with estimates of genetic distance for allozymes, and the results are shown in Fig. 1. Altukhov & Salmenkova (1987) concluded that 'An inverse proportionality between genetic distance and rate of return is apparent from these data.' Although this is a tantalizing indication, which has been accepted by some (e.g. Sherwin 1992), it is no more than a preliminary result. With only three data points, even the trend cannot be regarded as statistically significant. One important point to make from these results, however, is that relatively low success of transplanted individuals can occur, even when the genetic differences are very small. That point emphasizes the difficulty of predicting performance from the level of genetic divergence. Unfortunately, I have not found any follow-up to Altukhov & Salmenkova's (1987) preliminary test, or any other specific test of the predictability of genetic risks of translocation based on measures of genetic structure.

In the absence of such tests, a reasonable analogy can be made with studies of molecular genetic divergence and the evolution of reproductive isolation between species. In a summary of hundreds of allozyme comparisons among populations within

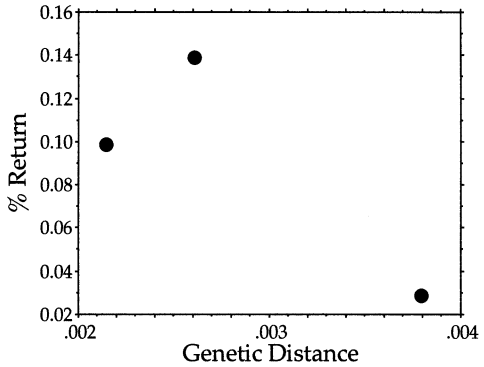


Figure 1 Rates of return of transplanted chum salmon and the genetic distances between source and recipient populations. Data from Altukhov & Salmenkova (1987).

and between species, Thorpe (1982) showed that the large majority of populations of the same species have genetic identities of 0.90 or above, while the large majority of comparisons between species showed identities of 0.80 or less. The frequency distributions of the intraspecific and interspecific comparisons overlap at about 0.85. Superficially, this pattern might suggest the usefulness of a genetic yardstick, whereby populations of unknown reproductive relationships are classified as the same or different species, based on their genetic identities. The problem is that, while there is a general relationship between reproductive relationships and overall genetic similarity, there are many exceptions. One of the first examples, which is relevant to aquaculture, was the finding of a genetic identity of 0.975, based on 54 allozyme loci, between two reproductively isolated and co-occurring populations of brown trout *Salmo trutta* Linnaeus (Ryman, Allendorf & Ståhl 1979). Thus, high genetic similarity need not mean reproductive compatibility.

One problem with the overall pattern evident in Thorpe's (1982) summary is that the reproductive relationships were inferred from taxonomy, not from direct evidence. Direct tests of reproductive isolation are relatively scarce, and there are even fewer attempts to test the relationship with measures of genetic divergence. The most extensive data are for species of *Drosophila* flies, for which many tests of reproductive isolation have been made in the laboratory, and which have been extensively compared with molecular markers. Summarizing the results from these studies, Coyne & Orr (1989, 1997) showed that there is indeed an overall

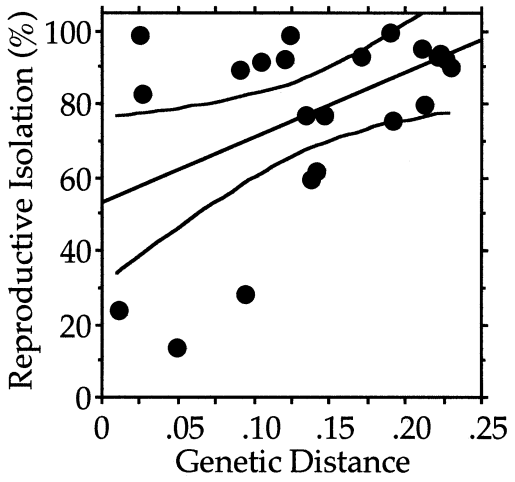


Figure 2 Regression of degree of intrinsic reproductive isolation against genetic distance in pairs of species of *Drosophila*, showing 95% confidence limits of the mean. Note that, for genetic distances <0.15, almost all observations lie outside the confidence limits, undermining the usefulness of the regression as a predictor. Data from Coyne & Orr (1989, 1997).

increase in intrinsic reproductive isolation with increasing genetic distance, as measured by allozymes. In Fig. 2, I have plotted the results tabulated by Coyne and Orr, for comparisons with genetic distances less than 0.25. The graph shows a clear trend, in which reproductive isolation is greater at higher levels of divergence; the regression is statistically significant. However, there is a large variance in that relationship, especially at low levels of divergence; for example, two of the four comparisons involving genetic distances less than 0.05 (i.e. genetic identities >0.95) show very high inherent genetic incompatibility. The problem with using this overall association for predicting genetic incompatibility from genetic distance is especially clear when the 95% confidence limits for the regression line are shown (Fig. 2). Taking into account this error of estimation from the regression line, the mean reproductive isolation for a genetic distance of 0.025, for example, could be anywhere in the range of 35–75%. However, at genetic distances less than 0.15, nearly all the points are outside the 95% confidence interval of the mean. That wide variation means that the problem is not just a statistical one, which might be solved with larger samples. Instead, it is inherently biological: reproductive isolation is not a unitary genetic trait, but can be caused by a wide array of simple or complex genetic differences. The same point applies to local adaptation of populations. This biological complexity means that, although there is an underlying average association of reproductive isolation with overall genetic divergence, the quantitative measures of genetic divergence can never be reliable estimators of genetic incompatibility or local adaptation.

Faced with this poor predictability, what can be done? One tempting approach would be to use measures of molecular genetic divergence as the best available. Even if surrounded by the essential caveats, however, this is a dangerous route. The caveats are likely to be ignored, and the prediction given a façade of scientific rigour. Caughley (1994, p. 239) highlighted the 'derangement' of the view that scientific rigour 'might have a place within the pages of a scientific journal, but can be dispensed with by practical people solving practical problems'. Those who need to predict the risk of a specific translocation must face the fact that there is no substitute for direct tests of genetic incompatibility or local adaptation.

Furthermore, those tests must be experimental, and not simply observations of natural populations, because there are complex interactions between genetics and environment in determining the phenotypic characteristics. For example, in the western rock lobster in Western Australia, there are differences in physiology, morphology and size at maturity between northern and southern populations, but the evidence that this species is genetically mixed indicates that these phenotypic differences are direct responses to the environment and are not the result of genetic differences (Thompson *et al.* 1996). As a contrasting example, Parsons (1997) examined growth rates between populations of the intertidal gastropod *Bembicium vittatum* in the Houtman Abrolhos Islands and Albany, Western Australia, which are about 900 km apart. Although the populations had very similar growth rates in their native environments, the southern population (Albany) was shown to have an inherently much higher growth rate, when the two were grown in a common environment. Thus, the striking genetic difference represented local genetic adaptation that was not evident simply from comparisons of field populations. These examples underscore the fundamental point, that there is no substitute for direct tests of relevant genetic variation.

The fundamental need for direct tests of differences in ecologically or reproductively relevant

traits does not mean that molecular surveys are irrelevant. As the overview of patterns of genetic subdivision indicated, such studies are very useful in determining past and present connections among populations. As tests of subdivision, they can reveal whether there is even a potential genetic risk of translocation. As tests of deep genetic structure, they can reveal cases in which translocation could put at risk basic components of genetic diversity of a species. Finding patterns between these extremes points to where direct tests of relevant genetic differences are required, and can suggest which populations might be useful candidates for such tests.

Acknowledgments

Unpublished information was kindly provided by C M Austin and R Doupé. Members of the genetics discussion group in the Department of Zoology made useful comments on the manuscript.

References

Altukhov Y.P. & Salmenkova E.A. (1987) Stock transfer relative to natural organization, management, and conservation of fish populations. In: *Population Genetics and Fishery Management* (ed. by N. Ryman & F. Utter), pp. 333–344. University of Washington Press, Seattle.

Austin C.M. & Knott B. (1996) Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in south-western Australia: electrophoretic, morphological and habitat variation. *Australian Journal of Zoology* **44,** 223–258.

Avise J.C. (1992) Molecular population structure and biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63,** 62–76.

Avise J.C. (1994) *Molecular Markers, Natural History and Evolution.* Chapman & Hall, New York.

Avise J.C. (1998) The history and purview of phylogeography: a personal reflection. *Molecular Ecology* **7,** 371–379.

Avise J.C., Arnold J., Ball R.M. Jr, Bermingham E., Lamb. T., Neigel J.E., Reeb C.A. & Saunders N.C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18,** 489–522.

Ayvazian S.G., Johnson M.S. & McGlashan D.J. (1994) High levels of genetic subdivision of marine and estuarine populations of the estuarine catfish *Cnidoglanis macrocephalus* (Plotosidae) in southwestern Australia. *Marine Biology* **118,** 25–31.

Bentsen H.B. (1991) Quantitative genetics and management of wild populations. *Aquaculture* **98,** 263–266.

Benzie J.A.H. (1994) Patterns of gene flow in the Great Barrier Reef and Coral Sea. In: *Genetics and Evolution of Aquatic Organisms* (ed. by A.R. Beaumont), pp. 67–79. Chapman & Hall, London.

Berger E. (1973) Gene-enzyme variation in three sympatric species of *Littorina*. *Biological Bulletin* **145,** 83–90.

Brown L.D. (1991) Genetic variation and population structure in the blacklip abalone, *Haliotis rubra*. *Australian Journal of Marine and Freshwater Research* **42,** 77–90.

Burton R.S. (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution* **52,** 734–745.

Burton R.S. & Feldman M.W. (1981) Population genetics of *Tigriopus californicus*: II. Differentiation among neighboring populations. *Evolution* **35,** 1192–1205.

Burton R.S. & Lee B.-N. (1994) Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proceedings of the National Academy of Sciences USA* **91,** 5197–5201.

Caughley G. (1994) Directions in conservation biology. *Journal of Animal Ecology* **63,** 215–244.

Chenoweth S.F. & Hughes J.M. (1997) Genetic population structure of the catadromous Perciform: *Macquaria novemaculeata* (Percicthyidae). *Journal of Fish Biology* **50,** 721–733.

Chenoweth S.F., Hughes J.M., Keenan C.P. & Lavery S. (1998) Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). *Heredity* **80,** 187–197.

Coyne J.A. & Orr H.A. (1989) Patterns of speciation in *Drosophila*. *Evolution* **43,** 362–381.

Coyne J.A. & Orr H.A. (1997) 'Patterns of speciation in *Drosophila*' revisited. *Evolution* **43,** 295–303.

Dizon A.E., Lockyer C., Perrin W.F., Demaster D.P. & Sisson J. (1992) Rethinking the stock concept: a phylogenetic approach. *Conservation Biology* **6,** 24–36.

Doherty P.J., Planes S. & Mather P. (1995) Gene flow and larval duration in seven species of fish from the Great Barrier Reef. *Ecology* **76,** 2373–2391.

Driscoll D.A. (1998) Genetic structure, metapopulation processes and evolution influence the conservation strategies for two endangered frog species. *Biological Conservation* **83,** 43–54.

Driscoll D., Wardell-Johnson G. & Roberts J.D. (1994) Genetic structuring and distribution patterns in rare southwestern Australian frogs: implications for translocation programs. In: *Reintroduction Biology of Australian and New Zealand Fauna* (ed. by M. Serena), pp. 85–90. Surrey Beatty & Sons, Chipping Norton.

Grewe P.M., Elliott N.G., Innes B.H. & Ward R.D. (1997) Genetic population structure of southern bluefin tuna (*Thunnus maccoyii*). *Marine Biology* **127,** 555–561.

Gyllensten U. (1985) The genetic structure of fish:

differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology* **26,** 691–699.

Hilbish T.J. (1996) Population genetics of marine species: the interaction of natural selection and historically differentiated populations. *Journal of Experimental Marine Biology and Ecology* **200,** 67–83.

Hillis D.M., Moritz C. & Mable B.K., eds. (1996) *Molecular Systematics,* 2nd edn. Sinauer Associates, Sunderland, MA.

Hindar K., Ryman N. & Utter F. (1991) Genetic effects of cultured fish on natural fish populations. *Canadian Journal of Fisheries and Aquatic Science* **48,** 945–957.

Imgrund J., Goth D. & Wetherall J. (1997) Genetic analysis of the freshwater crayfish *Cherax tenuimanus. Electrophoresis* **18,** 1660–1665.

Jerry D.R. (1997) Population genetic structure of the catadromous Australian bass from throughout its range. *Journal of Fish Biology* **51,** 909–920.

Johnson M.S. & Black R. (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* **38,** 1371–1383.

Johnson M.S. & Black R. (1995) Neighbourhood size and the importance of barriers to gene flow in an intertidal snail. *Heredity* **75,** 142–154.

Johnson M.S. & Black R. (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littoraria cingulata. Marine Biology* **132,** 295–303.

Johnson M.S., Creagh S. & Moran M. (1986) Genetic subdivision of stocks of snapper, *Chrysophrys unicolor*, in Shark Bay, Western Australia. *Australian Journal of Marine and Freshwater Research* **37,** 337–345.

Johnson M.S., Hebbert D.R. & Moran M.J. (1993) Genetic analysis of populations of north-western Australian fish species. *Australian Journal of Marine and Freshwater Research* **44,** 673–685.

Johnson M.S. & Joll L.M. (1993) Genetic subdivision of the pearl oyster *Pinctada maxima* (Jameson, 1901) (Mollusca: Pteriidae) in northern Australia. *Australian Journal of Marine and Freshwater Research* **44,** 519–526.

Keenan C.P. (1994) Recent evolution of population structure in Australian barramundi, *Lates calcarifer* (Bloch): an example of isolation by distance in one dimension. *Australian Journal of Marine and Freshwater Research* **45,** 1123–1148.

Lynch M. (1996) A quantitative-genetic perspective on conservation issues. In: *Conservation Genetics: Case Histories from Nature* (ed. by J.C. Avise & J.L. Hamrick), pp. 471–501. Chapman & Hall, New York.

MacDonald C.M. (1980) *Population Structure, Biochemical Adaptation and Systematics in Temperate Marine Fishes of the Genera* Arripis *and* Chrysophrys *(Pisces: Perciformes).* PhD Thesis, Australian National University.

Moritz C. (1994) Application of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* **3,** 401–411.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89,** 583–590.

Norman J., Moritz C. & Limpus C.J. (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. *Molecular Ecology* **3,** 363–373.

Palumbi S.R., Grabowsky G., Duda T., Geyer L. & Tachino N. (1997) Speciation and population genetic structure in tropical Pacific sea urchins. *Evolution* **51,** 1506–1517.

Parsons K.E. (1996) The genetic effects of larval dispersal depend on spatial scale and habitat characteristics. *Marine Biology* **126,** 403–414.

Parsons K.E. (1997) Contrasting patterns of heritable geographic variation in shell morphology and growth potential in the marine gastropod *Bembicium vittatum*: evidence from field experiments. *Evolution* **51,** 784–796.

Queller D.C., Strassmann J.E. & Hughes C.R. (1993) Microsatellites and kinship. *Trends in Ecology and Evolution* **8,** 285–288.

Rhymer J.M. & Simberloff D. (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27,** 83–109.

Richardson B.J. (1983) Distribution of protein variation in skipjack tuna (*Katsuwonus pelamis*) from the Central and South-western Pacific. *Australian Journal of Marine and Freshwater Research* **34,** 231–251.

Richardson B.J., Baverstock P.R. & Adams M. (1986) *Allozyme Electrophoresis.* Academic Press, Sydney.

Ryder O.A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution.* **1,** 9–10.

Ryman N., Allendorf F.W. & Ståhl G. (1979) Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). *Genetics* **92,** 247–262.

Shaklee J.B. & Bentzen P. (1998) Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* **62,** 589–621.

Sherwin W.B. (1992) Conservation of genetic resources. In: *Population Genetics and its Applications to Fisheries Management and Aquaculture* (ed. by P.I. Dixon), pp. 171–190. Centre for Marine Science, University of New South Wales, Kensington.

Sinclair M. (1988) *Marine Populations: an Essay on Population Regulation and Speciation.* University of Washington Press, Seattle.

Slatkin M. (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47,** 264–279.

Smith P.J. (1979) Esterase gene frequencies and temperature relationships in the New Zealand snapper *Chrysophrys auratus. Marine Biology* **53,** 305–310.

Taylor E.B. (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* **98,** 185–207.

Templeton A.R. (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* **7,** 381–397.

Thompson A.P., Hanley J.R. & Johnson M.S. (1996) Genetic structure of the western rock lobster, *Panulirus cygnus*, with the benefit of hindsight. *Marine and Freshwater Research* **47,** 889–896.

Thorpe J.P. (1982) The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics* **13,** 139–168.

Waples R.S. (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* **41,** 385–400.

Waples R.S. (1991) *Definition of 'Species' under the Endangered Species Act: Application to Pacific Salmon.* NOAA Technical Memorandum NMFS F/NWC-194. NOAA, Seattle.

Ward R.D. (1990) Biochemical genetic variation in the genus *Littorina* (Prosobranchia: Mollusca). *Hydrobiologia* **193,** 53–69.

Ward R.D. & Grewe P.M. (1995) Appraisal of molecular genetic techniques in fisheries. In: *Molecular Genetics in Fisheries* (ed. by G.R. Carvalho & T.J. Pitcher), pp. 29–53. Chapman & Hall, London.

Ward R.D., Woodwark M. & Skibinski D.O.F. (1994) A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. *Journal of Fish Biology* **44,** 213–232.

Watts R.J. (1991) *The Effects of Estuaries and Islands on the Genetic Structure of Marine Inshore Fishes.* PhD Thesis, University of Western Australia.

Wright S. (1969) *Evolution and Genetics of Populations,* Vol. 2, *The Theory of Gene Frequencies.* University of Chicago Press, Chicago.

Aquaculture Research, 2000, **31**, 145–149

Workshop summary

Genetic improvement in the Australian aquaculture industry

A J Lymbery
Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

Correspondence: A J Lymbery, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

Abstract

Most aquaculture industries in Australia are at an early stage of development and would benefit from the introduction of genetic improvement programmes. Size at harvest is perceived by industry participants, managers and researchers as the trait that will most influence profitability. Although most current genetic improvement programmes in aquaculture use mass selection, inbreeding is widely regarded as an important problem, which could be overcome by the use of family data in selection decisions. The major research priority is the development of genetic markers to enable accurate pedigree determination. The major constraint upon the implementation of genetic improvement programmes by aquaculture industries is lack of available funds and resources. Industry ownership and national co-ordination of research and development is seen as the best way of addressing this constraint.

Introduction

Aquaculture has been the world's fastest growing food production system for the past decade, with an average compound growth rate of 9.6% per year since 1984, compared with a growth of 3.1% for terrestrial livestock meat production and 1.6% for capture fisheries production over the same period (Tacon 1998). Australia has mirrored this global trend. Since 1985, the gross value of aquaculture production in Australia has grown from $49 million to around $400 million, with a current annual growth rate of about 30% (Brown, Van Landeghem & Scheule 1997).

This spectacular growth has been fuelled by a steadily increasing demand for seafood and a levelling of production from wild fisheries throughout the world. Since 1984, aquaculture's contribution to total seafood production has increased from 11.5% to 23% by weight (Tacon 1998). Aquaculture is seen by many as offering the greatest potential of any primary production system to meet the future food demands of a growing world population.

A major constraint to the ability of aquaculture to fill the gap between supply from wild fisheries and demand from world population growth is the relatively poor production efficiency of aquaculture species (FAO 1995). Most aquaculture production is carried out using wild stock recently captured from natural environments. Aquaculture species have hardly benefited from modern developments in animal breeding, despite their typically high reproductive capacity and therefore high potential for genetic improvement.

This paper reports the main findings from a workshop that brought researchers and industry representatives together to consider the current state and future potential for genetic improvement programmes in the Australian aquaculture industry.

Methodology

Sixty-one delegates attended the workshop. By occupation, 17 were from the commercial aqua-

culture industry, 16 held fisheries management positions and 28 were aquaculture researchers.

More than 60 aquaculture species are farmed in Australia, although 80% of the total value of production comes from less than 10 species. Four taxonomic groups were considered in the workshop: edible molluscs, freshwater crustaceans, marine crustaceans and finfish. Delegates were split into four groups, depending on their major commercial or research interest, and each group worked through a series of structured questions for their species:

- What is the breeding objective?
- What are the selection criteria?
- What are the best methods of genetic improvement?
- What are the R & D priorities?
- What are the major risks in implementing genetic improvement programmes?

The species considered by the mollusc group were Pacific oysters *Crassostrea gigas*, Sydney rock oysters *Saccostrea commercialis* and abalone *Haliotis* spp. The freshwater crustacean group considered marron *Cherax tenuimanus*, yabbies *C. albidus* and *C. destructor* and redclaw *C. quadricarinatus*. The marine crustacean group dealt with prawns *Penaeus monodon*, *P. esculentus* and *P. japonicus* and mud crabs *Scylla serrata*. The finfish group considered Atlantic salmon *Salmo salar*, rainbow trout *Oncorhyncus mykiss*, barramundi *Lates calcarifer*, silver perch *Bidyanus bidyanus*, snapper *Pagrus auratus* and black bream *Acanthopagrus butcheri*.

Breeding objectives and selection criteria

The first step in a genetic improvement programme is to determine which traits should be improved. These traits constitute the breeding objective. In a commercial enterprise, defining the breeding objective means asking economic questions about the biology of production and product quality. Selection criteria are the measures of the traits in the breeding objective. If traits in the breeding objective are the ends for genetic improvement, then selection criteria are the means by which improvement is achieved (Ponzoni & Newman 1989).

The relative immaturity of most aquaculture industries in Australia means that market signals are unreliable, and it is often difficult to detail accurately the major sources of income and expense in commercial operations. As a consequence, although groups identified the biological traits influencing returns and costs, they did not attempt to derive economic values for each trait. Table 1 shows the traits considered to be important components of the breeding objective by different groups.

Table 1 Biological traits included in the breeding objective for different aquaculture species groups (edible molluscs, freshwater crustaceans, marine crustaceans, finfish)

Trait	Ranking
Size at harvest	4
Survival to harvest	4
Meat yield at market	3
Feed efficiency	3
Size uniformity	2
Disease resistance	2
Taste	2
Flesh colour	2
Reproductive output	1
Temperature tolerance	1
Survival to (live) market	1
Shell shape	1
Claw size	1
Peelability	1

Ranking refers to the number of groups (out of four) that placed that trait in the breeding objective.

All groups considered size at harvest to be the major determinant of profitability. For most species, the recommended selection criterion for this trait was body weight or body size at harvest. Earlier predictors of harvest size were considered desirable, but information on genetic correlations among growth traits is often lacking for aquaculture species (Gjerde 1986; Shultz 1986; Benzie, Kenway & Trott 1997). Survival to harvest, while not considered as important as size, was included in the breeding objectives of all groups. Survival rate typically has a low heritability and is therefore difficult to improve through selection (Gjedrem 1985). Disease resistance, as a separate trait to survival, was considered very important in some species, but useable selection criteria have not yet been widely developed (Beardmore & McConnell 1998).

Other traits of general importance were meat yield and feed conversion efficiency. Meat yield is typically highly correlated genetically with body size (Gjerde & Gjedrem 1984), and may be most effectively improved indirectly through selection

for size at harvest. Although feed conversion efficiency is often considered to be economically important in aquaculture enterprises, measurement difficulties restrict its use in practice (Kinghorn 1983; Gjedrem 1983). Specific quality and appearance traits (e.g. colour, claw size, shell shape) were considered important for some species, although it was recognized that there was little information with which to quantify economic value. Also lacking were many of the genetic parameter estimates that would allow effective selection criteria to be defined for these traits (Gjedrem 1997).

Selection methods

Each group considered the relative importance of different strategies for genetic improvement of the traits identified in the breeding objective (Table 2). The general consensus across all industries was that traditional genetic improvement programmes, using selection on estimated breeding values, need to be implemented as soon as possible, or continued where they are already in place. Mass selection, in which breeding stock is chosen on the basis of individual performance, is most common in aquaculture species. The emphasis on developing family selection, in which the performance of relatives is taken into account when choosing breeding stock, reflects concerns over inbreeding that may arise through mass selection in many species with high reproductive potential (Engström, McMillan, McKay & Quinton 1996; Gjerde, Gjøen & Villanueva 1996; Davis & Hetzel 1999). A combination of mass selection and family selection will almost always provide the most efficient method of genetic improvement, but requires individual identification and pedigree determination (Gjedrem 1983; Gall 1990).

Although selection among stocks was not rated as a high priority, there was support for the development of selection lines from different stocks to provide the potential for cross-breeding. There are conflicting data on the importance of heterosis effects and other benefits from cross-breeding in aquaculture species (e.g. Gjedrem 1985; Sheridan 1997; Knibb, Gorshkova & Gorshkov 1998; Knibb 1999).

The application of molecular genetic technologies was considered a secondary priority (except that molecular markers are needed so that animals can be identified in selection programmes, see below), although it was emphasized by the mollusc and marine crustacean groups that quantitative trait locus (QTL) information would greatly assist the incorporation of traits such as disease resistance into the breeding objective. Chromosome manipulations (especially the production of different ploidy levels) and transgenesis were considered important for the protection of intellectual property in improved strains, rather than as priorities for the genetic improvement of production traits.

Table 2 Relative importance of different strategies for genetic improvement in aquaculture species, as ranked by groups at the workshop

Strategy	Ranking
Family selection within stocks	4
Mass selection within stocks	3
Cross-breeding	3
Selection among stocks	2
Marker-assisted selection	2
Transgenesis	2
Chromosome manipulation	2
Sex ratio control	1

Ranking refers to the number of groups (out of four) that considered the strategy to be a priority.

Table 3 Research priorities for genetic improvement in aquaculture species, as determined by groups at the workshop

R & D issue	Ranking
Establish genetic markers for pedigree analysis	3
Assess wild genetic resources	2
Determine genetic basis of disease resistance	2
Improve hatchery production	2
Estimate genetic parameters	1
Estimate genetic gains from selection	1
Estimate net economic values	1
Estimate G × E effects	1
Determine genetic basis of behavioural traits	1
Obtain objective data on risks from translocation	1
Improve methods for cryopreserving genetic material	1
Improve methods for ploidy manipulation	1
Develop transgenics	1

Ranking refers to the number of groups (out of four) that considered the strategy to be a priority.

R & D priorities

Table 3 lists the areas considered to be research priorities. The principal research requirement, identified by all except one of the groups, was the establishment of genetic markers for individual identification and pedigree verification of group-reared progeny. This would allow the more widespread use of family data in selection decisions, without the costly maintenance of separate family lines until individuals can be physically marked (see Davis & Hetzel 1999).

The recommendation to assess wild genetic resources reflects the finding that wild stocks from different geographic areas often show differences in performance traits (Kinghorn 1983; Fetzner, Sheehan & Seeb 1997; Knibb *et al.* 1998). Concerns over hatchery production and disease resistance arose from the recognition that disease still poses a significant problem for aquaculture, especially in intensive culture systems (Beardmore & McConnell 1998; Nell, Smith & McPhee 1999).

The estimation of genetic parameters and the comparison of predicted and observed gains from selection received surprisingly low priorities, given the perceived lack of information with which to develop selection criteria for breeding objectives in aquaculture species (Kinghorn 1983). Also low on the priority list, but clearly recognized as important in other parts of the workshop, was the need to determine economic weights for traits in the breeding objectives. This may reflect the early developmental stage of many Australian aquaculture industries, and a perceived need to develop basic infrastructure and selection technologies before genetic improvement programmes can be put in place effectively.

Industry constraints

There were two major areas of concern, which were consistently identified over groups. The first relates to lack of industry will and funds to implement genetic improvement programmes. Many aquaculture industries are small and immature, and uncertainty over production costs and market opportunities constrains investment in long-term genetic improvement programmes. This is exacerbated by the large range of aquaculture species that can potentially be farmed in particular production systems. Encouraging industry ownership of genetic improvement programmes from an early stage and ensuring that the infrastructure requirements and strategic directions of R & D effort are co-ordinated nationally were seen as important strategies to overcome a lack of industry investment. However, there remains an unresolved tension between the need to begin genetic improvement programmes at an early stage of industry development, if the full benefits are to be realized, and the commercial reality that traits can often be improved and problems eliminated more rapidly and cheaply in the short term through changes in management (Shultz 1986). One method of breaking this nexus is to begin genetic improvement programmes with government support, but with a clear direction to transfer the programme to industry as soon as commercial benefits can be demonstrated (e.g. Gjedrem 1999). The obvious weakness of this strategy is that the number of publicly funded genetic improvement programmes is limited, and some method of choosing candidate industries ('picking winners') must be found.

The second major area of concern relates to the hazards, real and perceived, of translocating aquaculture species. Translocation is the assisted movement of an organism beyond its accepted distribution. Disease risks arise principally from the introduction of exotic pathogens into natural water bodies. These risks can be reduced through disease certification of translocated stock, treatment and disposal of transport medium, appropriate quarantine procedures, disease zoning policies and procedures for surveillance and monitoring. Genetic risks arise when organisms are moved from one locality to another within their natural range, and donor and recipient populations differ genetically in traits that affect their adaptation to the local environment. The risks can be reduced by minimizing escapes of translocated stock through licensing provisions, industry self-regulation and monitoring procedures (see Doupé & Lymbery 1999).

Acknowledgments

The workshop was undertaken as part of a symposium sponsored by the Fisheries Research and Development Corporation and the Aquaculture Development Council of Western Australia, and hosted by the Aquaculture Council of Western Australia, Agriculture Western Australia, Edith Cowan University, Fisheries Western Australia and the South Metropolitan College of TAFE.

References

Beardmore J.A. & McConnell S.J. (1998) Genetics and disease resistance in aquaculture. *Journal of Applied Ichthyology* **14**, 167–171.

Benzie J.A.H., Kenway M. & Trott L. (1997) Estimates for the heritability of size in juvenile *Penaeus monodon* prawns from half-sib matings. *Aquaculture* **152**, 49–53.

Brown D., Van Landeghem K. & Schuele M. (1997) *Australian Aquaculture: Industry Profiles for Selected Species*. ABARE report 97.3. Australian Bureau of Agricultural and Resource Economics, Canberra.

Davis G.P. & Hetzel D.J.S. (1999) Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research* **31**, 3–10.

Doupé R. & Lymbery A.J. (1999) Managing translocations of aquatic species. *Aquaculture Research* **31**, 151–156.

Engström G., McMillan I., McKay L. & Quinton M. (1996) Comparison of mating systems in a fish population: a simulation study. *Journal of Animal Breeding and Genetics* **113**, 559–566.

FAO (1995) *Review of the State of World Fishery Resources: Aquaculture*. Fisheries Circular no. 886. Food and Agriculture Organization of the United Nations, Rome.

Fetzner Jr J.W., Sheehan R.J. & Seeb L.W. (1997) Genetic implications of broodstock selection for crayfish aquaculture in the midwestern United States. *Aquaculture* **154**, 39–55.

Gall G.A.E. (1990) Basis for evaluating breeding plans. *Aquaculture* **85**, 125–142.

Gjedrem T. (1983) Genetic variation in quantitative traits and selective breeding in fish and shellfish. *Aquaculture* **33**, 51–72.

Gjedrem T. (1985) Improvement of productivity through breeding schemes. *Geojournal* **10**, 233–241.

Gjedrem T. (1997) Flesh quality improvement in fish through breeding. *Aquaculture International* **5**, 197–206.

Gjedrem T. (1999) Genetic improvement of cold water fish species. *Aquaculture Research* **31**, 25–33.

Gjerde B. (1986) Growth and reproduction in fish and shellfish. *Aquaculture* **57**, 37–55.

Gjerde B. & Gjedrem T. (1984) Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* **36**, 97–110.

Gjerde B., Gjøen H.M. & Villanueva B. (1996) Optimum designs for fish breeding programmes with constrained inbreeding. Mass selection for a normally distributed trait. *Livestock Production Science* **47**, 59–72.

Kinghorn B.P. (1983) A review of quantitative genetics in fish breeding. *Aquaculture* **31**, 283–304.

Knibb W. (1999) Genetic improvement of marine fish – which method for industry? *Aquaculture Research* **31**, 11–23.

Knibb W., Gorshkova G. & Gorshkov S. (1998) Genetic improvement of cultured marine finfish: case studies. In: *Tropical Mariculture* (ed. by S. De Silva), pp. 111–149. Academic Press, London.

Nell J.A., Smith I.R. & McPhee C.C. (1999) The Sydney rock oyster *Saccostrea commercialis* breeding program: progress and goals. *Aquaculture Research* **31**, 45–49.

Ponzoni R.W. & Newman S. (1989) Developing breeding objectives for Australian beef cattle production. *Animal Production* **49**, 35–47.

Shultz F.T. (1986) Developing a commercial breeding program. *Aquaculture* **57**, 65–76.

Sheridan A.K. (1997) Genetic improvement of oyster production – a critique. *Aquaculture* **153**, 165–179.

Tacon A.G.J. (1998) Global challenges exist in aquaculture. *Feedstuffs* **August 31**, 12–17.

Aquaculture Research, 2000, **31**, 151–156

Workshop summary

Managing translocations of aquatic species

R G Doupé[1] & A J Lymbery[2]

[1]Centre for Ecosystem Management, Edith Cowan University, Joondalup, WA 6027, Australia

[2]Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

Correspondence: A J Lymbery, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia

Abstract

The translocation of aquatic organisms is an issue of increasing concern. In assessing the genetic risks from translocations, a primary distinction should be made between translocation for the purpose of aquaculture and translocation for the purpose of stock enhancement. When translocation is for aquaculture, the question of interest is: what is the maximum level of escapes that should be permitted, beyond which there is an unacceptable probability of adverse genetic effects upon the natural population? Risk minimization should concentrate on management solutions that reduce escapes from aquaculture facilities. When translocation is for stock enhancement, the question of interest is: what is the maximum level of genetic differences between hatchery and wild stock that should be permitted, beyond which there is an unacceptable probability of adverse genetic effects upon the natural population? Risk minimization should concentrate on hatchery management procedures that reduce genetic differences in fitness traits between hatchery stock and wild stock from the proposed recipient population. Where translocation poses a significant risk of adverse genetic changes, then a monitoring programme should be put in place, linked to a policy that prescribes management actions for the range of possible outcomes from the monitoring. The main limitation to our ability to develop an effective risk assessment and monitoring process is our lack of understanding of how the interaction between genetically different stocks affects the genetic basis of quantitative fitness traits that adapt organisms to their local environment.

Introduction

Translocation is the transfer of an organism, by human agency, from one place to another (Hodder & Bullock 1997). Translocation therefore includes the introduction of exotic species into areas in which they did not previously exist, and the movement of individuals or populations from one locality to another within the natural distributional range of the species. The intentional translocation of aquatic species has occurred for a number of reasons, principally for commercial aquaculture ventures and for (re)stocking for recreational fishing or conservation purposes (Cross 1999).

In recent years, there has been increasing interest and concern over the possible consequences of the translocation of aquatic organisms (Ferguson 1990; Horwitz 1990; Allendorf 1991; Gaffney & Allen 1992). At national and international levels, guidelines and codes of practice have been developed to minimize any adverse effects of translocations (e.g. ICES 1994; FAO 1996; SCFA 1998). However, this interest and activity has produced neither an accepted view of the degree of risk posed by translocations, nor a standardized approach to assessing the risk. For example, uncertainty over public perceptions of translocation and legislative responses to such perceptions are regarded as major constraints to the development of aquaculture industries in Australia (Lymbery 1999). Similarly, despite the long history of marine stock enhancement in Europe and the USA, the biological and economic feasibility of enhancement is still a controversial topic (Grimes 1998).

The potential risks associated with translocation arise principally from the introduction of pathogens

and parasites to new areas, adverse environmental impacts of exotic species through direct competition or habitat disturbance and genetic shifts in natural populations through hybridization with translocated species or genetically different strains. In this paper, we report the main findings from a workshop in which researchers, fishery managers and representatives of Australian aquaculture industries considered the genetic risks of the intentional translocation of aquatic organisms and the way in which genetic risks could be minimized.

Methodology

Sixty-one delegates attended the workshop. By occupation, 17 were from the commercial aquaculture industry, 16 held fisheries management positions and 28 were aquaculture researchers. Delegates were split into six groups, containing a mix of occupations, and each group was presented with a fictional, although realistic, translocation scenario. Each group then considered a number of questions relating to their scenario:

1. What is the purpose of the movement?
2. What are the genetic risks associated with the movement?
3. What more do we need to know to assess the risks?
4. What can be done to minimize the risks?

The following is our interpretation of the approaches to risk assessment and risk minimization that arose from a consideration of these questions.

The purpose of translocation

Although hybridization between different aquatic species does occur (see Keenan 1999), most genetic issues in translocation arise when indigenous species are moved from one locality to another within their natural range. Potential risks from such movement occur when donor and recipient populations differ genetically in traits that affect their adaptation to the local environment or their future adaptive potential (Gaffney & Allen 1992). The likelihood of such genetic differences will depend to a large extent upon the reasons for the translocation (Cross 1999).

Translocation for aquaculture

Aquaculture broodstock are invariably subject to artificial selection, whether intentional or unintentional. The results of such selection should be enhanced survival and performance in captivity, but are also likely to have correlated genetic effects on survival and performance in the wild. The extent of escape of cultured stock is therefore the critical issue when translocation occurs for aquaculture. If artificial selection reduces reproductive success in the wild, only continuous and relatively large-scale escape of cultured stock will impact significantly upon the genetic composition of natural populations, because natural selection will continually remove cultured genotypes from the population. Conversely, if artificial selection increases reproductive success in the wild, then even very low levels of escape will impact upon the genetic composition of natural populations (Knibb 1997).

Translocation for stock enhancement

Stock enhancement programmes may have different aims (e.g. conservation or recreational fishing), but they all involve release of stock into the natural environment, and significant interaction with natural populations is usually inevitable. The critical issue in stock enhancement, then, is the extent of genetic differentiation between cultured stock and wild stock. The greater the genetic difference, the greater the likelihood that releases will alter the genetic diversity or genetic composition of the natural population (Tave 1986; Allendorf & Ryman 1987; Utter, Hindar & Ryman 1993; Sheridan 1995).

What is the genetic risk?

When translocation is for the purpose of aquaculture, the question of interest is: what is the maximum level of escapes that should be permitted, beyond which there is an unacceptable probability of adverse genetic effects upon the natural population? When translocation is for the purpose of stock enhancement, the question of interest is: what is the maximum level of genetic differences between hatchery and wild stock that should be permitted, beyond which there is an unacceptable probability of adverse genetic effects upon the natural population?

Answering these questions, even on a case-by-case basis, will be very difficult for a number of reasons. The first problem is defining an unacceptable probability of genetic changes, which requires a detailing of societal and environmental costs and

benefits, many of which are measured in different currencies (Waples 1999). How, for example, do we equate societal benefits, such as increased employment, increased production and export growth, with environmental costs, such as reduced size or increased probability of extinction of natural populations?

Even when a probability level is set, determining the scale at which releases (in the case of aquaculture) or divergence (in the case of stock enhancement) will have a genetic impact is not straightforward. The genetic changes we are interested in are those that affect quantitative, ecological traits, so they will be influenced by the heritability of the traits, genetic correlations between traits and the intensity of selection (Bulmer 1971). Estimates of heritabilities and genetic correlations are available for economically important traits for captive populations of some aquaculture species (e.g. see Davis & Hetzel 1999) but, for the most part, there are few reliable estimates of these parameters for natural populations (Barton & Turelli 1989).

It is often assumed, on the basis of classical mutation and selection experiments, that most genetic changes in hatcheries will reduce fitness in the wild and therefore be selected against (Hindar, Ryman & Utter 1991; Knibb 1997; Skibinski 1998). The long-term demographic consequences of these genetic changes will therefore depend on the relative rates of selection intensity, removing the inferior genotypes from the wild, and escape/release rates, adding the inferior genotypes to the wild (Yokota & Watanabe 1997). However, empirical studies suggest that the situation in nature may sometimes be more complex. Several studies have shown that genetic changes in hatcheries will actually increase fitness in the wild for some traits or at some life history stages, and decrease fitness for other traits or at other life history stages (Einum & Fleming 1997; Kallio-Nyberg & Koljonen 1997; McGinnity, Stone, Taggart, Cooke, Cotter, Hynes, McCamley, Cross & Ferguson 1997). Negative correlations in fitness between traits or for the same traits over time will make the long-term effects of hatchery escapes or releases on population survival very difficult to predict.

The role of genetic monitoring

Monitoring the genetic diversity and genetic composition of both hatchery stock and natural (recipient) populations is often regarded as a priority, and a number of studies have reviewed the suitability of different genetic markers, such as allozymes, restriction fragment length polymorphisms (RFLPs), minisatellites, microsatellites and random amplified polymorphic DNAs (RAPDs), for monitoring (Ferguson, Taggart, Prodöhl, McMeel, Thompson, Stone, McGinnity & Hynes 1995; Ferguson & Danzmann 1998; Skibinski 1998). Properly designed and conducted monitoring studies using these markers can track temporal changes in genetic diversity and gene frequencies, and provide estimates of important genetic parameters, such as inbreeding rate, effective population size and gene flow. They do, however, have an important technical limitation: they will almost never be directly monitoring the genes we are most interested in.

Most genetic markers are neutral, single-gene loci, but the traits in which we are usually interested are polygenic, quantitative traits affecting fitness. There will often be no strong connection between marker gene diversity and quantitative genetic diversity within populations. For example, monitoring of hatchery populations often aims to detect declines in genetic variation caused by reductions in effective population size (Allendorf & Ryman 1987). However, the recovery of genetic variation after a population bottleneck may be orders of magnitude faster for quantitative traits than for neutral marker genes (Lande & Barrowclough 1987). Indeed, theoretical and empirical studies have shown that inbreeding as a result of restricted population size may actually increase additive genetic variance for quantitative traits (Robertson 1952; Bryant & Meffert 1993). Similarly, using genetic markers to monitor changes in genetic diversity and genetic composition of wild populations subject to translocation risk may have little predictive value in inferring genetic changes in quantitative fitness traits.

Just as marker gene diversity and quantitative genetic diversity may vary independently within populations, empirical studies have also shown no strong correlations between these parameters among populations (Taylor 1991; Spitze 1993; Podolsky & Holtsford 1995; Bonnin, Prosperi & Olivieri 1996). This means that estimates of genetic distances among populations, using allozymes, microsatellites or mitochondrial DNA sequences, may not provide an accurate indication of whether these populations differ genetically in quantitative

fitness traits, and are therefore adapted to local conditions (Johnson 1999).

Monitoring directly for genetic changes in quantitative traits will almost never be feasible. Traditional breeding experiments to separate genetic and environmental effects are too expensive and time consuming. The identification of genetic markers for quantitative traits (quantitative trait loci or QTLs, see Davis & Hetzel 1999) may provide an alternative to monitoring with neutral markers, but the range of traits for which QTL markers are available is very small, even for the most intensively studied species, such as salmonids. In addition, unless the QTL markers explain a very large proportion of variance, they may be no better correlated with variation in quantitative traits than are neutral markers (Latta 1998).

A strategy for assessing genetic risks

1. Any risk assessment process must make a fundamental distinction between translocation for the purposes of aquaculture and translocation for the purposes of stock enhancement.

2. There need be no restrictions on the geographic origin of aquaculture broodstock, because selection will rapidly cause genetic divergence between cultured and natural populations. Whether broodstock are drawn from the area in which the aquaculture facility is located, or from another part of the species range, is not a relevant question in assessing the genetic risks from translocation in this situation. When translocation occurs for the purposes of aquaculture, therefore, risk minimization should concentrate on management solutions that minimize escape from aquaculture facilities. Such solutions include (but are not limited to):

- Setting maximum escape levels for preventing genetic changes to natural populations. These will be impossible to specify precisely on a case-by-case basis, because of the need to estimate genetic parameters for quantitative fitness traits and (positive or negative) selection intensities, but detailed modelling studies could set guidelines for appropriate parameter ranges.
- Maintaining legislative control over system design (e.g. closed vs. open) in different environmental situations (e.g. sea ranching vs. land based).
- Encouraging the development of self-regulating industrial codes of practice, coupled with audited quality assurance processes, to ensure compliance with stated environmental objectives (Gavine, Rennis & Windmill 1996; Doupé, Alder & Lymbery 1999).
- Using hormonal or genetic manipulation to develop sterile strains of aquaculture species (Cross 1999).

3. When translocation occurs for the purposes of stock enhancement, risk minimization should concentrate on hatchery management procedures that minimize genetic differences in fitness traits between hatchery stock and wild stock from the recipient population. Such procedures may include:

- Choosing broodstock with similar adaptive potential. Using as broodstock animals from the same population into which releases are to be made is often considered optimal (Cross 1999). However, this may not always be possible or desirable, for example if natural population size is already greatly reduced, and an alternative source of broodstock may be required. The best predictor of similarity in adaptive potential may be whether populations belong to the same evolutionarily significant unit (ESU; Ryder 1986). An ESU is a group of populations with a common evolutionary history, often inferred from mitochondrial DNA lineages (Moritz 1994). However, we need to be aware that, while a common evolutionary history may suggest similar adaptive potential, it provides no direct evidence about genetic differences or similarities in ecologically relevant traits.
- Using large broodstock numbers to prevent loss of genetic diversity through inbreeding and genetic drift (Tave 1986; Allendorf & Ryman 1987). In using this strategy, hatchery managers need to be aware that, in rapidly growing or declining populations, there may be quite different relationships between effective population size and the rate of inbreeding, and between effective population size and the rate of genetic drift (Ryman 1994).
- Avoiding intentional artificial selection (Allendorf & Ryman 1987). Unintentional artificial (domestication) selection is probably unavoidable. It has been suggested that domestication selection could be minimized by introducing new broodstock from the wild every generation (Cross 1999). The effectiveness of this strategy is unknown, however, because genetic changes can occur in a single generation, and these changes will not necessarily be eliminated in the wild.

4. Where translocation poses a significant risk of adverse genetic changes, then a monitoring programme should be put in place. This may be aimed at the natural population or both the natural

population and the hatchery stock. Cost of implementation and considerations of statistical power to detect genetic changes will require the use of neutral markers, such as microsatellites. We need to be aware, however, that such a programme will not monitor the genes in which we are most interested. Even if we can reasonably infer from a monitoring programme that escapes from an aquaculture facility or releases from a hatchery are altering the diversity of genes influencing important quantitative traits, this does not of itself provide a basis for action. To be effective, a monitoring programme needs to be linked to a policy that describes the range of possible outcomes from the monitoring and prescribes management actions that will be triggered by each outcome (Waples 1999).

Acknowledgments

The workshop was undertaken as part of a symposium sponsored by the Fisheries Research and Development Corporation and the Aquaculture Development Council of Western Australia, and hosted by the Aquaculture Council of Western Australia, Agriculture Western Australia, Edith Cowan University, Fisheries Western Australia and the South Metropolitan College of TAFE. Thanks to Pierre Horwitz and Wayne Knibb for comments on the manuscript.

References

Allendorf F.W. (1991) Ecological and genetic effects of fish introductions: synthesis and recommendations. *Canadian Journal of Fish and Aquatic Sciences* **48** (Suppl. 1), 178–181.

Allendorf F.W. & Ryman N. (1987) Genetic management of hatchery stocks. In: *Population Genetics and Fish Management* (ed. by N. Ryman & F.M. Utter), pp. 141–159. University of Washington Press, Seattle.

Barton N.H. & Turelli M. (1989) Evolutionary quantitative genetics: how little do we know? *Annual Review of Genetics* **23**, 337–370.

Bonnin I., Prosperi J.M. & Olivieri I. (1996) Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosae): a comparative analysis of population structure. *Genetics* **143**, 1795–1805.

Bryant E.H. & Meffert L.M. (1993) The effect of serial founder-flush cycles on quantitative genetic variation in the housefly. *Heredity* **70**, 122–129.

Bulmer M.G. (1971) Stable equilibria under the two-island model. *Heredity* **27**, 321–330.

Cross T.F. (1999) Genetic implications of translocation and stocking of fish species, with particular reference to Western Australia. *Aquaculture Research* **31**, 83–94.

Davis G.P. & Hetzel D.J.S. (1999) Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research* **31**, 3–10.

Doupé R.G., Alder J. & Lymbery A.J. (1999) Environmental and product quality in finfish aquaculture development: an example from inland Western Australia. *Aquaculture Research* **30**, 1–8.

Einum S. & Fleming I.A. (1997) Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. *Journal of Fish Biology* **50**, 634–651.

FAO (1996) *Precautionary Approach to Capture Fisheries and Species Introductions.* FAO Technical Guidelines for Responsible Fisheries No. 2. Food and Agriculture Organization of the United Nations, Rome.

Ferguson M.M. (1990) The genetic impact of introduced fishes on native species. *Canadian Journal of Zoology* **68**, 1053–1057.

Ferguson M.M. & Danzmann R.G. (1998) Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1553–1563.

Ferguson A., Taggart J.B., Prodöhl P.A., McMeel O., Thompson C., Stone C., McGinnity P. & Hynes R.A. (1995) The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo. Journal of Fish Biology* **47** (Suppl. A), 103–126.

Gaffney P.M. & Allen Jr S.K. (1992) Genetic aspects of introduction and transfer of molluscs. *Journal of Shellfish Research* **11**, 535–538.

Gavine F.M., Rennis D.S. & Windmill D. (1996) Implementing environmental management systems in the finfish aquaculture industry. *Journal of the Chartered Institution of Water and Environmental Management* **10**, 341–347.

Grimes C.B. (1998) Marine stock enhancement: sound management or techno-arrogance? *Fisheries* **23** (9), 18–23.

Hindar K., Ryman N. & Utter F. (1991) Genetic effects of cultured fish on natural fish populations. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 945–957.

Hodder K.H. & Bullock J.M. (1997) Translocations of native species in the UK: implications for biodiversity. *Journal of Applied Ecology* **34**, 647–565.

Horwitz P. (1990) The translocation of freshwater crayfish in Australia: potential impact, the need for control and global relevance. *Biological Conservation* **54**, 291–305.

ICES (1994) *ICES Code of Practice on the Introductions and Transfers of Marine Organisms.* Cooperative Research Report no. 204. International Council for the Exploration of the Seas, Brussels.

Johnson M.S. (1999) Measuring and interpreting genetic structure to minimize genetic risks of translocations. *Aquaculture Research* **31,** 133–143.

Kallio-Nyberg I. & Koljonen M.-L. (1997) The genetic consequences of hatchery-rearing on life-history traits of the Atlantic salmon (*Salmo salar* L.): a comparative analysis of sea-ranched salmon with wild and reared parents. *Aquaculture* **153,** 207–224.

Keenan C.P. (1999) Should we allow human-induced migration of the Indo-West pacific fish, barramundi (*Lates calcarifer* Bloch) within Australia? *Aquaculture Research* **31,** 121–131.

Knibb W. (1997) Risk from genetically engineered and modified marine fish. *Transgenic Research* **6,** 59–67.

Lande R. & Barrowclough G.F. (1987) Effective population size, genetic variation, and their use in population management. In: *Viable Populations for Conservation* (ed. by M.E. Soulé), pp. 87–123. Cambridge University Press, Cambridge.

Latta R.G. (1998) Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. *American Naturalist* **151,** 283–292.

Lymbery A.J. (1999) Genetic improvement in the Australian aquaculture industry: Workshop summary. *Aquaculture Research* **31,** 145–149.

McGinnity P., Stone C., Taggart J.B., Cooke D., Cotter D., Hynes R., McCamley C., Cross T. & Ferguson A. (1997) Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES Journal of Marine Science* **54,** 998–1008.

Moritz C. (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* **3,** 401–411.

Podolsky R.H. & Holtsford T.P. (1995) Population structure of morphological traits in *Clarkia dudleyana*. I. Comparison of F_{st} between allozymes and morphological traits. *Genetics* **140,** 733–744.

Robertson A. (1952) The effect of inbreeding on variation due to recessive genes. *Genetics* **37,** 189–207.

Ryder O.A. (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* **1,** 9–10.

Ryman N. (1994) Supportive breeding and effective population size: differences between inbreeding and variance effective numbers. *Conservation Biology* **8,** 888–890.

SCFA (1998) *Consultation Draft. National Translocation Policy for Live Aquatic Organisms*. Standing Committee on Fisheries and Aquaculture, Canberra.

Sheridan A.K. (1995) The genetic impacts of human activities on wild fish populations. *Reviews in Fisheries Science* **3,** 91–108.

Skibinski D.O.F. (1998) Genetical aspects of fisheries enhancement. *FAO Fisheries Technical Paper* **374,** 205–222.

Spitze K. (1993) Population structure in *Daphnia obtusa*: quantitative genetic and allozyme variation. *Genetics* **135,** 367–374.

Tave D. (1986) *Genetics for Fish Hatchery Managers*. AVI Publishing Company, Westport, CT.

Taylor E.B. (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture* **98,** 185–207.

Utter F., Hindar K. & Ryman N. (1993) Genetic effects of aquaculture on natural salmonid populations. In: *Salmon Aquaculture* (ed. by K. Heen, R.L. Monahan & F. Utter), pp. 144–165. Fishing News Books, Oxford.

Waples R.S. (1999) Dispelling some myths about hatcheries. *Fisheries* **24** (2), 12–21.

Yokota M. & Watanabe S. (1997) One-way gene flow by stocking and its effects on a fish population. *Fisheries Science* **63,** 539–542.

Aquaculture Research

Instructions for Authors

Submission. One original and three copies of each typescript should be submitted to either of the Editors:

Dr Ronald W Hardy, Director, UI Hagerman Fish Culture Experiment Station, 3059 F National Fish Hatchery Road, Hagerman, Idaho 83332, USA

Dr S J de Groot, PO Box 97, 2080AB, Santpoort-Zuid, The Netherlands

A disk should accompany the final version of the manuscript. Authors are requested to use an IBM-compatible system (see below for more details).

Papers are accepted on the understanding that they have not been and will not be published elsewhere. Copyright of accepted manuscripts and all published material becomes the property of the Journal.

Preparation of the manuscript. All sections of the typescript should be on one side of A4 paper, double-spaced and with 30mm margins. Articles are accepted for publication only at the discretion of the Editor(s). Authors will receive prompt of receipt of their paper and a decision will be reached within 3 months of receipt. A manuscript should consist of the following sections:

Title page. This should include:
- the full title of the paper
- the full names of all the authors
- the name(s) and address(es) of the institution(s) at which the work was carried out (the present addresses of the authors, if different from the above, should appear in a footnote)
- the name, address, and telephone and fax numbers of the author to whom all correspondence and proofs should be sent
- a suggested running title of not more than 50 characters, including spaces.

• ***Main text.*** Generally, all papers should be divided into the following sections and appear in the order: (1) Abstract or Summary, not exceeding 150-200 words, (2) Introduction, (3) Materials and Methods, (4) Results, (5) Discussion, (6) Acknowledgments, (7) References, (8) Figure legends, (9) Tables, (10) Figures.

The Results and Discussion sections may be combined and may contain subheadings. The Materials and Methods section should be sufficiently detailed to enable the experiments to be reproduced. Trade names should be capitalized and the manufacturer's name and address given.

All pages must be numbered consecutively from the title page, and include the acknowledgments, references and figure legends, which should be submitted on separate sheets following the main text. The preferred position of tables and figures in the text should be indicated in the left-hand margin.

• ***Units and Spellings.*** Système International (SI) units should be used. The salinity of sea water should be given as g L^{-1}. Use the form g mL^{-1} not g/mL. Avoid the use of g per 100g, for example in food composition, use g kg^{-1}. If other units are used, these should be defined on first appearance in terms of SI units, e.g. mmHg. Spelling should conform to that used in the Concise Oxford Dictionary published by Oxford University Press. Abbreviations of chemical and other names should be defined when first mentioned in the text unless they are commonly used and internationally known and accepted.

• ***Scientific names and statistics.*** Complete scientific names, including the authority with correct taxonomic disposition, should be given when organisms are first mentioned in the text and in tables, figures and key words together with authorities in brackets e.g. rainbow trout, *Oncorynchus mykiss* (Walbaum) but Atlantic salmon *Salmo salar* L. without brackets. For further information see American Fisheries Society Special Publication No 12 *A List of Common and Scientific Names of Fishes from the United States and Canada*.

Carry out and describe all appropriate statistical analyses.

• ***References (Harvard style).*** References should be cited in the text by author and date, e.g. Lie & Hemre (1990). Joint authors should be referred to in full at first mention and thereafter by *et al.* if there are more than two, e.g. Hemre *et al.* (1990).

More than one paper from the same author(s) in the same year must be identified by the letters a, b, c, etc., placed after the year of publication. Listings of references in the text should be chronological. At the end of the paper, references should be listed alphabetically according to the first named author. The full titles of papers, chapters and books should be given, with the first and last page numbers.

Chapman D.W. (1971) Production. In: *Methods for the Assessment of Fish Production in Freshwater* (ed. by W.E. Ricker), pp. 199–214. Blackwell Scientific Publications Ltd, Oxford.

Utting S.D. (1986) A preliminary study on growth of *Crassostrea gigas* larvae and spat in relation to dietary protein. *Aquaculture* **56**, 123–138.

Authors are responsible for the accuracy of their references. References should only be cited as in 'press' if they have been accepted for publication. Manuscripts in preparation, unpublished reports and reports not readily available should not be cited. Personal communications should be cited as such in the text.

It is the authors' responsibility to obtain permission from colleagues to include their work as a personal communication. A letter of permission should accompany the manuscript.

• ***Illustrations and Tables.*** These should be referred to in the text as figures using Arabic numbers, e.g. Fig. 1, Fig. 2, etc., in order of appearance. Three copies of each figure should be submitted and each figure should be marked lightly on the back with its appropriate number, together with the name(s) of the author(s) and the title of the paper. Where there is doubt as to the orientation of an illustration the top should be marked with an arrow.

Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Labelling, including scale bars if necessary, should be clearly indicated on an overlay or photocopy. Magnifications should be included in the legend. Colour illustrations are acceptable when found necessary by the Editor; however, the author may be asked to contribute towards the cost of printing. Illustrations should not be larger than 200 × 124mm.

Line drawings should be on separate sheets of white paper in black indelible ink (dot matrix illustrations are not permitted); lettering should be on an overlay or photocopy and should be no less than 4 mm high for a 50% reduction. Please note, each figure should have a separate legend; these should be grouped on a separate page at the end of the manuscript. All symbols and abbreviations should be clearly explained.

Tables should be self-explanatory and include only essential data. Each table must be typewritten on a separate sheet and should be numbered consecutively with Arabic numerals, e.g. Table 1, and given a short caption. No vertical rules should be used. Units should appear in parentheses in the column headings and not in the body of the table. All abbreviations should be defined in a footnote.

All tables and figures that are reproduced from a previously published source must be accompanied by a letter of permission from the Publisher or copyright owner.

Please send us digital versions of your figures. These should be supplied as eps or tiff files only.

Avoid using tints if possible; if they are essential to the understanding of the figure, try to make them coarse.
Always enclose a hard copy of digitally supplied figures.

• ***Acknowledgments.*** These should be brief and must include references to sources of financial and logistical support.

• ***Page Proofs and Reprints.*** Proofs will be sent to the author's address on the title page and must be returned to the Publisher within 3 days of receipt. Only typographical errors can be corrected at this stage. Major alterations to the text cannot be accepted.

• ***Offprints.*** Twenty-five offprints of each paper, including Short Communications (see below), will be provided free of charge and additional copies may be ordered when proofs are returned. A scale of charges will be sent with the proofs.

• ***Short Communications.*** These should differ from full papers on the basis of scope or completeness, rather than quality of research. They may report significant new data arising from problems with narrow, well-defined limits, or important findings that warrant rapid publication before broader studies are complete. Their text should neither exceed 1500 words (approximately six pages of typescript) nor be divided up into conventional sections. When submitting Short Communications, authors should make it clear that their work is to be treated as such.

• ***Disks.*** The Journal encourages submission of accepted manuscripts on disk. These should be IBM-compatible. An accurate hardcopy must accompany each disk, together with details of the type of computer used, the software employed and the disk system if known. Do not justify. Particular attention should be taken to ensure that any articles submitted in this form adhere exactly to the journal style in all respects. Further details can be obtained from the Publisher; the Editor(s) will supply 'disk submission' forms on acceptance of a manuscript. Disks will not be returned to the author.

Aquaculture Research

Volume 31, Number 1, January 2000

Introduction

Contents

Workshop summaries

Aquaculture Research is now covered in *Aquatic Science and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Contents, Elsevier BIOBASE/Current Awareness in Biological Sciences, Ecological Abstracts, FISHLIT, Index Veterinarius, Nutrition Abstracts and Review Series B, Oceanic Abstracts, SciSearch* and *The Veterinary Bulletin*

Blackwell Science

1355-557X(200001)31:1;1-4

Information on this journal can be accessed at http://www.blackwell-science.com/are

Typeset by Information Press, Oxford, UK, and printed by The Devonshire Press Limited, Torquay, UK