8.2 Genetics and breeding in seed supply for inland aquaculture[1]

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Mair, G.C. 2007. Genetics and breeding in seed supply for inland aquaculture, pp. 519–547. In: M.G. Bondad-Reantaso (ed.). Assessment of freshwater fish seed resources for sustainable aquaculture. *FAO Fisheries Technical Paper.* No. 501. Rome, FAO. 2007. 628p.

ABSTRACT

It is estimated that no more than 20 percent of global aquaculture production utilizes genetically improved stocks. Furthermore, the vast majority (>95 percent) of freshwater aquaculture production comes from the developed world where resources are limited and breeding technologies less advanced. Thus, the proportion of production derived from genetically improved fish in freshwater aquaculture is likely to be considerably lower than the global average. The past 15 years have seen a rapid increase in the number and scope of breeding programmes for genetic improvement of aquaculture species and a number of important national and regional programmes for improving, commercializing and disseminating important aquaculture species are now underway or planned. There have been a number of important advances in research, most notably in the verification of the potential gains to be had from well-managed selection programmes with genetic gains for growth related traits of 7-10 percent readily achievable in well-managed programs. However, it is also evident that there has been genetic deterioration of existing domesticated and cultured stocks through poor genetic management during and subsequent to the domestication process. These effects need to be reversed and whilst progress has been made there remains a need to support implementation of best practices in basic genetic management when domesticating, translocating and maintaining discrete aquaculture stocks. Whilst the success with selective breeding in recent times has placed this technology at the core of efforts to improve aquaculture stocks, there has been some successes with other technologies such as sex control, hybridisation and polyploidy induction and these do have a role as components of integrated approaches to genetic improvement. Some extreme improvements in culture performance have been demonstrated using transgenesis but societal and regulatory concerns continue to preclude commercial application. Major research advances have been made in recent times with the application of genetic markers and the costs of widely applied and, to some degree standardized, techniques such as the application of microsatellite markers which have come down considerably. Genetic markers are thus now becoming valuable tools and will be increasingly applied to enhance traditional selection programmes and in more specific programmes of marker-assisted selection.

[1] This paper was peer-reviewed by Dr Devin Bartley of the Aquaculture Management and Conservation Service, Fisheries and Aquaculture Department, FAO, Rome, Italy.

Of the major freshwater aquaculture species, there have been very significant genetic gains through the application of genetics in tilapia and carps in particular. Efforts are now expanding to include shellfish species such as *Macrobrachium*.

This paper reviews core issues and progress in genetic management and improvement of species for inland aquaculture and highlights some of the technical, environmental and socio-economic issues still constraining the full implementation of genetic improvement programmes and the equitable dissemination of their outputs and benefits. A number of recommendations are made concerning the appropriate role of applied genetics in seed supply systems for the future development of inland aquaculture.

INTRODUCTION

With a few exceptions, aquaculture is a relatively recent development and contributor to global food security, having expanded at an average rate of 8.9 percent since 1970. During this time, aquaculture has increased from representing just 3.9 percent of global fisheries production volume up to almost 50 percent in 2004 (FAO, 2006). It is inevitable that aquaculture production will overtake capture fisheries production within the next 10-20 years. All future predictions call for further significant increases in aquaculture production to meet expanding demand from a growing population and increases in per capita consumption of seafood. In this context, the supply of quality seed has increased, and must continue to increase dramatically to sustain growth in aquaculture. Gjedrem (2002) estimated then that genetically improved stock accounted for no more than 10 percent of aquaculture production. Whilst this figure is undoubtedly rising, as the benefits of genetic improvement become apparent, it is unlikely to be more than 20 percent at present. It is almost certainly much lower in most developing countries. Relatively high adoption rates of genetically improved stocks exists in some developed countries such as Norway where Gjedrem (2000) estimates 65 percent of aquaculture production takes place using stocks resulting from modern and efficient breeding programs. Likewise, for species such as Atlantic salmon, over 97 percent of global production is from genetically improved lines.

The major factor in the relatively low impact of genetic improvement in aquaculture is the embryonic status of aquaculture production sectors. During the early stage of aquaculture development, rapid advances can be made in production volumes and efficiency with relatively simple and cost effective improvements or innovations in general husbandry, nutrition and health management. In contrast, genetic changes whilst capable of dramatic improvements inevitably require long-term strategic planning and investment in order to secure medium to long-term gains. A further reason for the 'slow start' in implementing genetically based technologies, was an early misconception that, despite positive evidence from livestock and crop sectors, selective breeding might prove ineffective in aquatic organisms. This view was perpetuated by the failure to bring about significant improvements during early attempts to consciously select for commercially important traits in carps and tilapia (e.g. Moav and Wohlfarth, 1976; Moav, 1979; Wohlfarth, 1983).

An era of substantial genetic improvement, contributing to a "blue revolution" in aquaculture, is undoubtedly upon us with some form of genetic improvement evident in the majority of important aquaculture species. This paper focuses on the role that genetics is playing, and will play, in developing the supply of quality seed for inland aquaculture. Given that FAO statistics (FAO, 2006) record that 97 percent of freshwater aquaculture production (27.8 million tonnes in 2005) comes from the developing world (95 percent from Asia) this paper will, by default, focus on seed supply for aquaculture in developing countries. The paper will review the current status of genetic improvement technologies focusing on finfish, molluscs and crustacea and will describe their applications to some important species in inland production

systems. One of the key objectives, in the context of current developments and status of genetic improvement, will be to identify and discuss the key issues facing the future application and uptake of genetics based technologies and genetically improved stocks in inland aquaculture. This will include a review of technological and environmental constraints to genetic advancement, identification of research priorities to address these along with issues related to human and physical resource capacity and mechanisms for disseminating improved seed focused on equitable uptake of benefits.

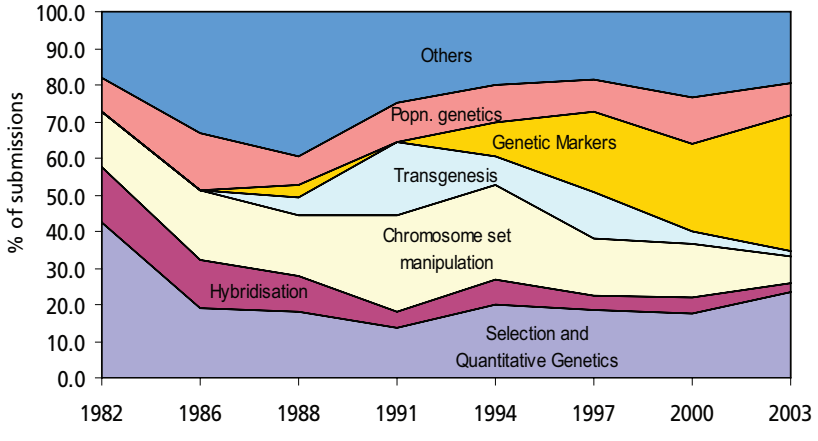
GENETIC TECHNOLOGIES IN AQUACULTURE

This section of the review focuses on the technology options for genetic improvement. Over recent decades there has been a very significant body of research on applying genetic technologies to aquacultured species and distinct trends are visible. An analysis of the main subject areas of papers published in the proceedings of triennial symposia on genetics in aquaculture from the first in 1982, up to 2003, shows the trends in the main focal areas of aquaculture genetics research (Figure 8.2.1). This indicates a decline in selective breeding research up to 1991 followed by a steady increase. This decline was mirrored by an increase in research on alternative or 'competing' technologies such as chromosome set manipulation and transgenesis. The basis of this was the prevailing belief, at this time, that these technologies were likely to bring about more rapid genetic and culture performance gains than selective breeding. Research in both these areas has now declined and some of the reasons behind this will be discussed later in this paper. Another evident trend, since the early 1990s, is the rapid increase in the volume of research focused on the applications of genetic markers.

DOMESTICATION AND MANAGEMENT OF GENETIC VARIATION

Domestication and subsequent broodstock management practices have very important implications for the long-term quality of cultured stocks. A domesticated species is defined broadly by the Convention on Biological Diversity to be one *in which the evolutionary process has been influenced by humans to meet their needs*. In the context of aquaculture, domestication is the process whereby a population becomes adapted to man and the captive environment by some combination of genetic change over several generations and environmentally induced developmental events recurring during each generation. The consequence of domestication for the subsequent development of aquaculture based on these domesticated stocks can be profound. Due to the relative infancy of aquaculture as a major form of primary production, many cultured stocks

FIGURE 8.2.1
Trends in genetics research as reported in the triennial symposia of the International Association for Genetics in Aquaculture (IAGA)



are only relatively recently domesticated, still undergoing domestication or in some cases yet to be domesticated. Several genetic processes operate during domestication and the subsequent genetic management of domesticated stocks impacts both positively and negatively on culture performance and culture potential. These processes include domestication selection, indirect selection, inbreeding and genetic drift. Several studies have illustrated that domestication can lead to the loss of alleles and/or reductions in observed heterozygosity in domesticated stocks. For example, salmonids (Kim *et al.*, 2004); tilapias, *Oreochromis mossambicus* (Agustin, 1999) and *O. shiranus* (Ambali, Doyle and Cook, 1999); a siluriform catfish, *Heterobranchus longifilis* (Agnese, Oteme and Gilles, 1995); penaeid shrimp (Goyard *et al.*, 2003; Ramos-Paredes and Grijalva-Chon, 2003); and oysters, *Crassostrea gigas* and *C. angulata* (Sly and Hedgecock, 1988; Rebordinos, Garcia and Cantoral, 1999; Kittel and Chew, 2000).

The ideal population size is infinitely large, ensuring no loss of genetic variation. In reality hatchery managers must work with relatively small, finite populations. However, the total number of broodfish held at the farm is not the critical factor in broodstock management but rather the number that contribute genes to subsequent generations. Of critical importance thus is the concept of effective population size (N_e), which is a function of the total number of breeding individuals, the mating system employed, the sex ratio and the variance of family size in the grow-out of fish that are used to produce the next generation. Several publications (e.g. Tave, 1999) describe the principle and its application. Greater use of the principle would assist hatchery managers in determining strategies and best management practices to minimize inbreeding in hatcheries which are responsible for long-term genetic management of their stocks.

Whilst loss of genetic variation in cultured stocks is commonplace, the opposite can occur when several wild stocks are deliberately interbred during domestication to create a highly genetically variable base population. This is now being carried out in several species with the objective of maximizing genetic variation as the basis for selective breeding.

The impacts of domestication on culture performance may be more variable and less predictable than on genetic variation. Eknath *et al.* (1993) demonstrated in the Nile tilapia *O. niloticus* that newly introduced, wild caught specimens performed better in aquaculture systems than their "domesticated" counterparts despite the fact that the cultivated stocks should have undergone domestication selection for adaptation to these environments. In reality these cultivated stocks were probably inbred and/or introgressed with other species, due to the lack of any conscious genetic management, leading to their poor performance. Similar experiments with Indian carp, rohu (*Labeo rohita*) produced ambiguous results from consecutive trials of wild caught and domesticated stocks (Gjerde *et al.*, 2002; Reddy *et al.*, 2002). Conversely, Dunham *et al.* (2001) indicate that "domestication effects" can have substantial positive impacts upon growth rate in the channel catfish, *Ictalurus punctatus.* They cite 3-6 percent increases in growth per generation during early domestication, also noting that the oldest domesticated strain (of 89 years) had the fastest growth rate of all channel catfish strains prior to the initiation of selection programs.

It appears likely that the relative impact of positive (domestication selection) and negative factors (loss of genetic variation through founder effects, genetic drift, inbreeding and negative selection) on production performance will vary between species. Likewise, the degree of heritability of the key traits will impact performance as will the amount of genetic variation that might be lost. The results will also depend on the circumstances of domestication with regard to the attention given to both genetic factors and the nature of the production environment.

One further genetic factor linked to domestication is the potential for hybrid introgression, either between stocks and or more particularly between species. This issue is dealt with below (see section on hybridisation).

The major challenge in domestication is to ensure that new aquaculture species and stocks are domesticated and managed with a view to optimal retention of genetic diversity. Genes and genotypes that are favored in the wild may be lost from cultured populations as a result of domestication processes without evident negative consequences. However, genetic variation must not be reduced to a point where inbreeding, loss of adaptive capacity and other problems arise. In the domestication process, the potential impacts of the widespread culture of genetically variable composite or genetically changed strains on natural genetic diversity must be actively considered and this issue is covered further later in this paper. This issue is particularly important in the case of culture-based fisheries where hatchery reared stock are deliberately released into the environment. If domesticated stock, genetically changed by the domestication process, are released the genetic integrity of wild con-specific populations can be seriously compromised.

SELECTIVE BREEDING

Relatively little research or development had been conducted on selective breeding in fish prior to 1970. This changed with the initiation of several selection programmes including a major salmon breeding programme in Norway (reviewed by Gjedrem, 2000) and smaller programmes in various other species (reviewed by Hulata, 2001). The success of the GIFT (Genetically Improved Farmed Tilapia) project (Eknath and Acosta, 1998) based in the Philippines extended this belief in selection approaches to the developing world. It is now widely considered that selective breeding should be at the core of most genetic improvement programmes in fish. A number of conditions must be met prior to initiating a breeding program. Firstly, the lifecycle of the species in captivity must be closed. It is then necessary to identify the commercially valuable traits to target and establish that these traits have both genetic variability and moderate to high levels of heritability.

Given the long-term commitment implicit in initiating a selective breeding, it is imperative that the stock to be improved forms a commercially significant and sustainable industry. There is clearly little point in initiating genetic improvement of a species that might be a component of a "boom and bust" commercial development scenario, as appears to have happened for example with the introduction of the African catfish *Clarias gariepinus*, in several Asian countries. The majority of freshwater species used in aquaculture today has been or can be bred in captivity with major advances being made in induced breeding during the late 1950s. The most important freshwater species in terms of volume of production are the Chinese and Indian major carps which were effectively domesticated in the 1960s. However, the initiation of selective breeding has only recently occurred for some of these species.

Whilst this section deals primarily with the improvement in quantitative traits (i.e. those phenotypes that are quantitative in nature and continuous in distribution) there are examples of simpler breeding approaches to modify qualitative traits such as colour, body shape and sex. Such breeding programmes generally involve research to understand the genetic basis of inheritance of the trait followed by a planned programme to either remove or fix the trait within a breeding population. Breeding programmes based on qualitative traits are usually focused on ornamental species but there are some examples in cultured food fish such as in body and peritoneal lining colour in tilapia (Shirak *et al*., 2000), shell colour in scallops (Winkler *et al*., 2001) and shell colour in freshwater crayfish (Walker, Austin and Meewan, 2000).

In developing country inland aquaculture, the major quantitative trait targeted in selective breeding has been growth rate. The objective of these programmes is commonly the production of faster growth, larger size at harvest, shorter culture period or possibly higher stocking densities whilst maintaining harvest size. Growth rate is generally the key trait of highest economic value in the majority of inland

aquaculture systems. This is especially true in extensive and semi-intensive systems where fish are sold whole or gutted and chilled rather than processed. More intensive systems see traits such as food conversion efficiency and fillet yield become relatively more economically important, these particularly traits are however more difficult to select.

Traits such as growth rate in fish and shellfish have relatively high levels of genetic variability and heritability. Tave (1993), Dunham *et al.* (2001) and Lutz (2001) reviewed heritability for growth-related traits (commonly weight or length at a specific age) in fish and shellfish with estimates for freshwater species ranging from -0.2 up to 0.8 with an average heritability value of 0.2 which is considered moderate. Moderate to high heritabilities and/or response to selection have recently been observed for other traits such as carcass quality (Rye and Gjerde, 1996; Neira *et al.*, 2004; Bosworth and Wolters, 2005; Cerda, Neira and Baria, 2005; Quinton, McMillan and Glebe, 2005) and health related traits (Okamoto *et al.* 1993; Kolstad *et al.*, 2005; Gitterle *et al.*, 2006).

High levels of genetic variability in today's cultured fish stocks compared to other livestock breeds are likely to be due, in part, to their relatively recent domestication and thus shorter period under which inbreeding and genetic drift have reduced genetic variation within a breed. In addition, the majority of aquacultured organisms are highly fecund and cultured in larger numbers compared to terrestrial species, permitting much higher selection intensities. Selection intensity must always be balanced with maintenance of adequate N_e to ensure long-term retention of genetic variability. Many species have relatively short generation times permitting selection on multiple generations within relatively short time frames enabling rapid improvement. This is compensated to some degree in aquatic species by characteristics such as lower survival, difficulties in marking/tagging and difficulties associated with sampling and visibility of fish along with a lack of standardization of production systems. In principle, it should be possible to secure genetic gains, through selection, in most aquatic species faster than is possible in terrestrial species. This has been shown to be the case in well planned selection programmes to date. There are, nevertheless, differences in the properties of aquatic species which affect their potential for genetic degradation or improvement through domestication processes and deliberate genetic improvement and these are summarised in Figure 8.2.2.

It is beyond the scope of this review to discuss selection methods and breeding programme design in detail. Decisions on designs invariably involve a series of compromises of breeding objectives given the resources available and the characteristics

FIGURE 8.2.2
Matrix showing the implications for effective population size (N_e) and selection intensity of differing properties of cultured aquatic species on the rate of genetic change (which can be negative in the case of poor genetic management or positive in the case of well managed genetic improvement)

		Generation time	
		Long	**Short**
Fecundity	*High*	*Few broodfish required so N_e is often low but long generation time slow down rate of genetic change. e.g. major carps, abalone, sturgeon*	*Few broodfish required so N_e is often low and rapid genetic deterioration can occur. Genetic gains from genetic improvement breeding programmes can be rapid. e.g. common carp (especially in the tropics)*
	Low	*Rare. Required to retain large numbers of broodfish so N_e is high with slow rate of genetic change so little genetic deterioration but difficult to improve. e.g. dragon fish (arowana), gouramis*	*Required to retain large numbers of broodfish so N_e is high but poor genetic management can rapidly lead to deteriorating culture performance. However breeding programmes can produce rapid gains. e.g. tilapia*

of the species in question. Ideal designs should maximize the chance of correctly ranking the performance of individuals or their relatives whilst minimizing loss of genetic variation. Ideal designs are rarely possible and are most commonly restricted by the limitations on marking/tagging or physical separation of families and individuals.

The basis of selective breeding is to identify traits which are significantly influenced by genetic factors and to choose individuals possessing a majority of positive (desirable) genes responsible for that trait, to be parents in the next generation so that their progeny, as a group, have the highest possible additive genetic merit for the trait or traits in question. A major rational for applying selective breeding is that it is a continuous and long-term approach to genetic improvement with gains being secured with each generation of selection. Implementation of genetic improvement, thus, represents a long- term and sustained approach to improving the culture performance of stocks. It is not always obvious which phenotypic characters are genetically determined rather than primarily influenced by the environment and what component of the genetic determination results from additive genetic variance, i.e. the type of genetic variance that can be selected. It is thus usually necessary to attempt to quantify this through the estimation of heritability, a measure of the degree to which a trait is transmitted from parents to offspring. Due to high fecundity the selection methods usually applied are individual (mass), family or combined selection. Decisions on the correct approach to adopt also depend on the long term objective of the breeding program, the resources available, the nature of the targeted trait itself and its degree of heritability.

Individual or mass selection is based on the performance of the individual itself and is often the simplest and cheapest method to apply. However, problems may occur if there are significant environmental variations (e.g. age or culture system differences) affecting individual performance; these must be controlled as far as possible. Furthermore, given that pedigreed mating is rarely possible in mass selection, it is very difficult to control inbreeding. This has led to problems in several breeding programmes as exemplified in a mass selection programme for common carp *Cyprinus carpio* in Vietnam in which the realized heritabilities for a growth trait dropped to almost zero in just four generations of mass selection (Dan, Thien and Tuan, 2005; Tran and Nguyen, 1993). There are alternative approaches to mass selection designed to obviate this problem such as the PROSPER approach (Procedure Optimisee de Selection individuelle Par Epreuves Repetees = enhanced individual selection procedure through recurrent challenging) in which two lines are mass selected and production stock is generated by crossing between the two lines (Chevassus *et al.,* 2004).

Mass selection is also limited to traits that can be measured in live breeding individuals and cannot thus be used effectively for important traits such as carcass quality or disease resistance. Mass selection is also inefficient for traits with low heritability.

Family selection involves family groups being ranked and either retained or discarded according to their mean performance. Individuals from each selected family are then used as breeders for the next generation. The selection differential is thus a function of differences among families, not among individuals. Family selection is most efficient where heritability for the target trait is low and when environmental deviations contribute a large part of phenotypic variation. The efficiency of family selection is compromised by significant environmental differences between families and thus culture systems for all families should be standardized, e.g. by marking families and stocking them in common environments. A major advantage of family selection is that breeding values can be estimated for traits that cannot be measured directly on the breeding individuals such as carcass quality traits and disease resistance. The successful Norwegian salmon breeding programme (Gjedrem, 2000) and the GIFT programme in tilapia (Eknath and Acosta, 1998) have combined family and individual selection to maximize efficiency of their breeding designs. Family deviations and the

mean individual phenotypic values are taken into account in the estimation of breeding values when selecting breeders for the next generation.

Within family selection involves individuals within a family being selected based on the deviation of each individual from the mean value of its family. This method is inefficient compared to other mating schemes but can be relatively easy to manage and apply and the use of rotational mating schemes between families minimizes inbreeding over the long term. Genetic gains from within-family selection have been demonstrated in rainbow trout (Gall and Huang, 1988) and tilapia (Bolivar and Newkirk, 2002).

With the recent initiation of numerous selective breeding programmes some impressive results are starting to emerge in terms of response to selection. Dunham *et al.* (2001) review the progress made in a number of significant breeding programmes and tabulated selection response in 13 key aquaculture species (predominantly freshwater). This indicated genetic gains in growth related traits from 4.4 percent per generation in shrimp (Fjalestad *et al.,* 1997) to 20 percent in a single generation of selection in channel catfish (Bondari, 1983) with an average response to selection of 11.6 percent. Similarly Gjedrem (2000) tabulated genetic gains from programs, primarily in coldwater fish, averaging 13.5 to 15 percent per generation. These summaries may serve to highlight the more successful breeding programmes from which results have been published and it has been observed that gains estimated under experimental conditions are not always fully realized in commercial systems. Nevertheless, it is very evident that in most stocks of aquatic organisms genetic gains in growth-related traits of 7-10 percent per generation are readily achievable. Clearly the economic value of such significant gains, over several generations, are very significant and Gjedrem's estimate of a 15:1 return on investment in the Norwegian salmon breeding programme is not unrealistic (Gjedrem, 2000).

One of the major selective breeding innovations in recent years has been the integration of genetic markers as tools to enhance traditional selective breeding and as markers of quantitative traits for selection. These advances will be reviewed briefly later in this paper.

Significant challenges in the development of selective breeding in inland aquaculture include the identification of resources (funding, expertise and facilities) for initiation and long term sustainability of appropriate breeding programmes which address the real needs of the industries which they support.

HYBRIDISATION AND CROSSBREEDING

Hybridisation is breeding individuals from two separate species whilst crossbreeding is mating two different varieties/strains within a species. Both these crosses are commonly made with the objective of exploiting non-additive genetic variance through identification of significant positive heterosis, also known as "hybrid vigour", for commercially important traits. Positive heterosis occurs when the hybrid or crossbred performs better than the average of the two parental species or stocks. In practical terms, heterosis only becomes really significant when the hybrid or crossbred performs better than either parental species or stocks.

Both crossbreeding and hybridisation are relatively simple techniques to master and can have an immediate impact on performance within one generation. However, this benefit is finite and only present in the F_1 hybrid, unless the parental lines are then selected over generations for their general or specific combining ability, resulting in highly complex and relatively slow breeding programs. Crossbreeding is thus usually looked upon as a supplement to a programme for additive genetic improvement, as mentioned above, to negate the effects of inbreeding in mass selected lines by producing progeny crossed between two such lines (e.g. Chevassus *et al.* 2004; Camara *et al*., 2006). Substantial evidence for heterosis for growth (Hedgecock, McGoldrick and Bayne, 1995) suggests a role for crossbreeding in commercial improvement of oysters.

Due to its relative simplicity there has been considerable research effort to evaluate hybrid crosses between multiple species with hundreds of hybrid crosses being attempted in the past three decades. Considering this large research effort, particularly with cyprinids in Asian aquaculture, there are relatively few hybrids in commercial production. Bartley, Rana and Immink (2000) presented a comprehensive review of hybridisation in aquaculture concluding that hybrid application is under reported and that there remains a significant potential role for hybrids under specific circumstances. In inland aquaculture, there is significant production of a small number of hybrids including hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) in Thailand and Southeast Asia, hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) in China and Israel and hybrid striped bass (*Morone chrysops* x *M. saxatilis*) in North America. The significance of these hybrids is however more related to a specific combination of desirable traits between the two parental species, including the growth rates, appearance and flesh quality of the hybrid catfish and the high proportions of males in the hybrid tilapia, rather than heterosis for any specific trait. One of the major challenges of applying commercial-scale hybridisation is the risk of introgression of pure species parental stocks through contamination by hybrids. Once this occurs the system tends to break down and performance of the supposed F_1 hybrids will become inconsistent and unpredictable.

Planned hybridisation is based on the exploitation of the desired, defined and predictable traits of the F_1 hybrids between the two parental species. Given the relative ease of hybridisation between closely related fish species, hybridisation can be haphazard, as has been seen, for example, in the production of major carps in some countries. This can occur in captivity, either through deliberate or accidental hybridisation. Hybrids may then be used as broodstock in backcrosses or in the production of F_2 crosses. Over generations there is a general mixing and segregation of genes from the original parental species, known as introgression. With this independent segregation of the genes the phenotypes resulting are highly variable and some of the fish carrying the introgressed genes cannot be easily distinguished from the original pure species. Introgression is common in tilapia and other interbreeding species groups, where hybrids can be easily produced either artificially or naturally. Hybrid introgression is thought to have occurred in some major carp populations, e.g. in Chinese carp in Bangladesh (Mia *et al*., 2005; Simonsen *et al*., 2005) where hybrids were originally produced, either out of scientific interest or through reasons of shortage of broodstock. Hybrid introgression in the Chinese or Indian major carps, which form the basis of successful polyculture systems, is very likely to have negative consequences as a result of loss of the distinct feeding strategies of the pure species.

Long-term consequences of *ad hoc* hybridisation events can be avoided if systems are in place that exclude hybrids from use as future broodstock. However, typically such hybrids are rarely recorded or traceable and the evidence of the occurrence of hybrids among both production stock and broodstock in Bangladesh (Mia *et al*., 2005, Simonsen *et al*., 2005) would indicate that it is not an uncommon occurrence.

The major challenge in planned hybridisation is to ensure that it is used appropriately, that there are real economic benefits to a hybridisation programme and that it is managed such that unwanted or uncontrolled introgression does not occur in hatchery or wild stocks.

SEX CONTROL

There is a strong commercial incentive to culture single sex (monosex) populations in species in which there is significant sexual dimorphism for commercially important traits and where species become sexually mature within the culture environments before attaining harvest size. There may also be applications of monosex stocks for biological containment although this is less effective than using sterile stocks. These

factors combined can profoundly affect the profitability of culture in some species, most notably in the tilapias.

Monosex populations can be generated through manual sexing, hybridisation, direct and indirect use of hormonal sex reversal. Manual sexing tends to be labour-intensive and inefficient and hybrid crosses only apply to specific combinations of species, again notably in the tilapias (important hybrid crosses including tilapia are summarized by Bartley, Rana and Immink, 2000). The most generally applicable biotechnological methods for producing monosex stocks are through direct sex reversal using hormones or indirectly through genetic breeding programs. Direct sex reversal can generally be applied regardless of the sex determining system and has been successfully achieved in a range of species (Piferrer, 2001). The indirect approach, however, relies on an understanding of the genetic mechanisms of sex determination in a species, the main factor for success being that it is a monogenic system such as male heterogamety (XX female; XY male) as in salmonids or female heterogamety (WZ female; ZZ male) as in some tilapias and crustacea.

The potential benefits of monosex female stocks in salmonid aquaculture have been long established and relate to the enhanced availability of female broodstock and to avoid precocious maturation of males which results in reduced growth, poorer survival and loss of flesh quality post-maturation (Hunter and Donaldson, 1983). In salmonids, sex differentiation occurs at the time of hatch and first feeding (Piferrer, 2001) and direct feminization can be achieved by exposing the fish to exogenous estrogens at this time, either by oral administration or immersion treatments. Sex reversed "neofemales" have normal female phenotypes.

Indirect feminization in species with heterogametic males is achieved by fertilizing normal ova with sperm derived from masculinized (XX) homogametic 'neomales'. This model has been applied for large scale production of several species, most significantly in salmonids (see review by Donaldson and Devlin, 1996) but also in silver barb *Barbodes gonionotus* (Pongthana *et al.,* 1999). Whilst relatively few all female salmon or silver barb are produced worldwide, a significant proportion of commercial production of trout is all female, particularly in Europe, and all-female Chinook salmon are produced in New Zealand.

Monosex male stocks can also be produced, again by either direct or indirect masculinization. Sex reversal to male has been achieved in a range of finfish through application of exogenous androgens. Breeding programmes for all-male production depend on the sex determining mechanism for the target species. In female heterogametic species, the breeding programme is similar to that described above for salmonids involving feminisation to produce ZZ neofemales, and has been developed in the tilapia *O. aureus* (Melard, 1995) and in the giant freshwater prawn *Macrobrachium rosenbergii* (Sagi and Aflalo, 2005). In male heterogametic species, it is also possible to produce genetically all-male progeny through the generation of novel YY "supermales" and such a breeding programme has been developed commercially with Nile tilapia *O. niloticus* resulting in the commercial scale production of genetically male tilapia (GMT) (Mair *et al*., 1997).

Sex control programmes are likely to be of relevance only to some species for which significant economic benefits would accrue from culture of monosex stocks. Direct induction of sex change is always likely to meet resistance from consumers unless achieved using ecologically and ethically sound approaches (such as manipulation of environmental sex determination rather than by hormone treatment). Indirect approaches such as breeding programmes for monosex production are likely to meet with broader acceptance but face the major challenge that they are based on comprehensive understanding of the genetic mechanisms of sex determination which can require considerable research effort.

CHROMOSOME SET MANIPULATION

In fish it is possible to manipulate whole sets of chromosomes through disruption of the process of cell division in the newly fertilized egg. These techniques are generally not possible in higher organisms. There are four types of manipulation which have been commonly applied in fish and shellfish, i.e. gynogenesis, androgenesis, triploidy and tetraploidy.

Androgenesis and gynogenesis are forms of induced uni-parental inheritance in which respectively the female or male genetic contribution is de-activated and the chromosome complement from the male or female is doubled. In androgenesis, the maternal DNA is denatured prior to fertilization, usually via gamma, X-ray or UV irradiation. In finfish the fertilized eggs are then subjected to some form of physical shocks (cold, heat or pressure) at a fixed intervals postfertilization (depending on the species) to disrupt mitosis and restore diploidy resulting in homozygous androgenetic progeny. In gynogenesis, the paternal DNA in the sperm is denatured, commonly through exposure to ultraviolet light, but the sperm remains motile and can activate egg development. Physical shocks (in finfish) or sometimes chemical shocks (common in molluscs) are administered to restore diploidy. Early shocks can block extrusion of the 2nd polar body in meiosis restoring the diploid chromosome complement but retaining some heterozygosity. Later shocks can disrupt metaphase of first mitosis retaining two identical chromosome complements within a single nucleus. Such 'mitotic' gynogens, like androgenetic progeny, are completely homozygous. These homozygous fish can be used as the basis for producing isogenic clonal lines.

Polyploids are produced in a similar way with application of physical or chemical shocks to normal fertilized eggs with disruption of meiosis resulting in triploids with two maternal and one paternal chromosome set. Disruption of mitosis produces tetraploids with a duplicate diploid chromosome complement.

Gynogenesis and to a lesser extent androgenesis has been applied to a wide range of finfish and shellfish species and have a number of research and practical applications such as for the elucidation of the genetic basis of sex determination (Flynn *et al.*, 2006; Pongthana *et al*., 1995; Tariq Ezaz *et al*., 2004) and the rapid induction of inbreeding (Komen *et al*., 1992; Nagy and Csanyi, 1985). Generally, induction and survival rates for these techniques are very low, typically being less than 5 percent of all eggs treated and for homozygous mitotic gynogens and androgens, less than 1 percent, due to the deleterious impact of the physical shocks and the expression of deleterious recessive genes.

Androgenesis could also be used in principle, to recover genotypes from cryopreserved sperm. Clonal lines have value for research, for example as isogenic controls in a selective breeding programme or in eliminating genetic variation in experimentation on the effects of environmental factors or in immunogenetic research. Clonal lines have been produced in a number of inland aquaculture species including Nile tilapia *O. niloticus* (Hussain, Penman and McAndrew, 1998), crucian carp *Carassius auratus gibelio* (Yang *et al.,* 2001), common carp *Cyprinus carpio* (Ben-Dom *et al.,* 2001), rainbow trout *Oncorhynchus mykiss* (Young, Wheeler and Thorgaard, 1995) and the Japanese ayu *Plecoglossus altivelis* (Han, Taniguchi and Tsujimura, 1991).

The main commercial application of chromosome set manipulation has been associated with the sterility of induced triploids which have been produced in many fish species and several bivalve molluscs. Sterile fish have attractions for aquaculture. Firstly, they may put relatively more energy into somatic growth and secondly they provide potential biological containment benefits facilitating the culture of exotic genotypes and possibly in the future, the culture of growth-enhanced transgenic fish. However, generally triploid finfish do not grow faster than their diploid counterparts (Tiwary, Kirubagaran and Ray, 2004), although this may occur post-maturation when the triploids may also have higher dress-out proportions. All induced triploid female

finfish produced to-date have been shown to be fully sterile; triploid males show more gonad development than females, are generally sterile but rare incidences of fertility of triploid male finfish cannot be completely discounted. Conversely, many studies have shown that triploid bivalves, although not fully sterile in several species, show better performance than control diploids.

Triploids can also be produced from diploid x tetraploid matings in species for which tetraploids have been produced and shown to be viable, most notably in oysters (Guo and Allen, 1994) and this is likely to represent a more commercially viable and reliable means of mass producing triploids, as demonstrated in oysters (Eudeline, 2001). However, viable fertile tetraploids have been produced in few commercially important fish species, predominantly salmonids but also in the Korean mud loach *Misgurnus mizolepis* (Nam *et al.*, 2001) and the blunt snout bream *Megalobrama amblycephala* (Zou *et al.*, 2004). Sterile triploids are likely to grow in importance for intellectual property protection of broodstock and for biological containment. For this latter application, it will be necessary to quickly and cost effectively verify rates of triploidy induction and assess the potential for fertility of triploid fish and the associated risk.

Whilst much research on chromosome set manipulation has been carried out on finfish, probably the most significant applications are in bivalve shellfish where triploids are cultured widely, for example approximately 50 percent of cultured oysters produced in the US and France are triploids produced from diploid x tetraploid matings (Boudry, pers. comm.). Crustacea have not proved amenable to chromosome manipulation research due to the challenge of obtaining ovulated eggs for artificial fertilization. However, manipulations have been possible in some species including reports of triploidy having been successfully induced (Norris *et al.*, 2005).

One further form of chromosome set manipulation is nuclear transplantation in which cell nuclei from various donor cells can be transplanted into recipient eggs. This research is outside the scope of this review being mainly carried out for research purposes (Liu *et al.*, 2002) with, as yet, no commercial application.

Probably the mass production of sterile triploids is the most important application of chromosome set manipulation in aquaculture. The main challenges for these technologies are to reliably and verifiably produce 100 percent sterile fish in a range of commercially important species, particularly those where the resulting options for biological containment and/or IP projection have significant value.

GENETIC ENGINEERING AND TRANSGENESIS

Main form of genetic engineering of practical interest to aquaculture at present is transgenesis. Transgenesis is a technology wherein a novel isolated gene sequence is used to transform an organism. This transgene, which is a construct of a functional gene and a promoter gene that acts as a switch to activate the functional gene, can be from a different species than that of the recipient or a gene cloned from the same species. Indeed there has been a trend away from early research which used foreign gene constructs including human growth hormone genes (Dunham *et al.*, 1987) to use of all-fish and con-specific constructs (Wu, Sun and Zhu, 2003) in response to concerns over ethics, human health and environmental impacts of transgenic fish (Maclean and Laight, 2000). Organisms resulting from successful transgenesis are classed as genetically modified organisms (GMO) and thus subject to societal and regulatory concerns over GMOs. Transgenesis has been a major area of research in fish genetics since the early 1990s and research in this area is more advanced in fish than in other livestock as the reproductive biology of fish makes them more amenable to induction compared to higher organisms such as poultry or mammals. Figure 1 clearly indicates a peak in transgenic fish research in the 1990s with a decline at the turn of the decade, most likely associated with difficulty in securing funding to develop transgenic lines which were unlikely to be approved for use in commercial aquaculture in the near future.

The induction of transgenesis involves a number of steps: identification of the target gene and construct development; introduction of the gene into newly fertilized eggs, usually by micro-injection or electroporation; determination of incorporation of the transgene into the host genome; determination of transgene expression; determination of inheritance of the transgene and quantification of the effect of the transgene on target and non-target traits. The main target trait of transgenic research in fish to-date has been the enhancement of growth rate in aquaculture through the introduction of growth hormone constructs. This research was relatively successful with the above-mentioned steps have being completed and a number of transgenic lines produced with sometimes dramatically improved growth performance. Such transgenic lines have been produced in a number of species important in aquaculture including Atlantic salmon (Du *et al.*, 1992), Coho salmon (Devlin *et al.*, 1994) tilapia (Martinez *et al.*, 1996; Rahman and Maclean 1999), channel catfish (Dunham *et al.*, 1987) and common carp (Wu, Sun and Zhu, 2002). FAO (2000) produced a timely and useful review of the transgenic fish that had been produced and were being evaluated for use in aquaculture (Table 8.2.1). Whilst early research focused on growth related traits more recent research has looked at disease resistance (Zhong, Wang and Zhu, 2002; Weifeng *et al.*, 2004) and product quality (Yoshizaki *et al.*, 2005) issues. Clearly transgenesis has the potential for integration with selective breeding programs, particularly for the improvement of traits which are difficult to improve through quantitative approaches.

TABLE 8.2.1
Summary of transgenic fish being evaluated for aquaculture production indicating the nature of the transgene, the target trait and the location of the research (adapted from FAO, 2000)

Species	Novel gene	Desired effect and comments	Location
Atlantic salmon	AFP AFP salmon GH	Cold tolerance Increased growth and feed efficiency	United States, Canada United States, Canada
Coho salmon	Chinook salmon GH + AFP	After 1 year, 10- to 30-fold growth increase	Canada
Chinook salmon	AFP salmon GH	Increased growth and feed efficiency	New Zealand
Rainbow trout	AFP salmon GH	Increased growth and feed efficiency	United States, Canada
Cutthroat trout	Chinook salmon GH + AFP	Increased growth	Canada
Tilapia	AFP salmon GH	Increased growth and feed efficiency; stable inheritance	Canada, United Kingdom
Tilapia	Tilapia GH	Increased growth and stable inheritance	Cuba
Tilapia	Modified tilapia insulin-producing gene	Production of human insulin for diabetics	Canada
Salmon	Rainbow trout lysosome gene and flounder pleurocidin gene	Disease resistance, still in development	United States, Canada
Striped bass	Insect genes	Disease resistance, still in early stages of research	United States
Mud loach	Mud loach GH + mud loach and mouse promoter genes	Increased growth and feed efficiency; 2- to 30-fold increase in growth; inheritable transgene	China, Korea, Rep.
Channel catfish	GH	33% growth improvement in culture conditions	United States
Common carp	Salmon and human GH	150% growth improvement in culture conditions; improved disease resistance; tolerance of low oxygen level	China, United States
Indian Major carps	Human GH	Increased growth	India
Goldfish	GH AFP	Increased growth	China
Abalone	Coho salmon GH + various promoters	Increased growth	United States
Oysters	Coho salmon GH + various promoters	Increased growth	United States

Note: The development of transgenic organisms requires the insertion of the gene of interest and a promoter, which is the switch that controls expression of the gene.
AFP = anti-freeze protein gene (Arctic flatfish). GH = growth hormone gene.

Transgenic fish can also be useful as models for studies of gene regulation and gene expression and have potential as biofactories to produce valuable pharmaceuticals (Hwang *et al*., 2004).

Although enhanced performance under culture conditions has been clearly demonstrated for a number of species there are no transgenic food fish currently under commercial production. The only example on the market at present is the GloFish®, a fluorescent transgenic zebrafish which has been approved for sale and is only sold in the United States (Blake, 2005).

Whilst there are some technical reasons behind the lack of commercialization of transgenic fish the main reason is the concern over the ethical, fish health, human food safety and environmental risks associated with the culture of transgenic fish, the full analysis of which is outside the scope of this review (see Maclean and Laight, 2000 and FAO, 2000 for discussion of these issues). Some research has been carried out in attempts to allay consumer fears over these issues (Guillen *et al*., 1999; Wu, Sun and Zhu, 2003). However, there remains an absence, in many circumstances, of clear guidelines for appropriate risk assessment. There is a very important test case with a North American company AquaBounty Technologies having applied to the US Food and Drug Administration (FDA) for permission to distribute their AquAdvantage™ growth enhanced transgenic Atlantic salmon. Whilst this is a lengthy process there seems little doubt that conditional approval by the FDA will rapidly lead to approvals for other transgenic fish in other locations.

With solutions now developed for many of the technical constraints to successful application of transgenesis in fish, the main challenges lie in full assessment of environmental, ethical and consumer health risks which currently limit the commercialisation of this technology.

GENETIC MARKERS AND THEIR APPLICATIONS

A genetic marker is a polymorphic genetic property for which there is an experimental procedure allowing identification of genotypes. Prior to the advances in molecular genetics since the 1980s, isozymes and other proteins were the markers of choice. Nowadays there are a variety of DNA markers, such as mitochondrial DNA (mtDNA) polymorphisms, restriction fragment length polymorphisms (RFLP); random amplified polymorphic DNA (RAPD), repeat sequent markers (mainly microsatellites), amplified fragment length polymorphisms (AFLPs) and single nucleotide polymorphisms (SNPs). The most used markers in aquaculture genetics are microsatellites although AFLPs and SNPs are finding increasing application. The nature of these markers and their potential applications are reviewed in full by Lui and Cordes (2004).

There is a long history of application of genetic markers in fisheries and aquaculture but Figure 8.2.3 clearly illustrates a significant increase in the research application of genetic markers in aquaculture in the past decade, coinciding with the development and standardisation of DNA based marker technologies and the reduction in the cost of their application. DNA markers that can be amplified by polymerase chain reaction (PCR) have the additional advantage over protein based markers that samples (typically of fin tissue) can be readily taken in the field or farm without killing the fish and easily and cheaply preserved for the medium to long term.

Polymorphic DNA markers have now been developed from DNA libraries for the majority of the important aquaculture species including the carps, tilapias, shrimps, salmonids and catfish. These markers have a number of important applications which are being increasingly utilized in aquaculture (mainly in research but there is now some commercial use) and in some fishery management applications; the following paragraphs summarize these applications in regards to aquaculture.

Species and strain identification

Several polymorphic DNA markers can be used for identification of species or stocks which can be applied to differentiate between cultured stocks or cultured from wild stocks. Such techniques can be applied from a biosecurity perspective to identify sources of escapes from aquaculture, to determine the impact of introductions and/or escapes of cultured stocks on wild stocks (see review by Utter, 2003) and to trace the origin of non-native farmed stocks. They can also be used for traceability and intellectual property protection in the case of distinct genetically improved strains. Population genetic markers can also be used for the identification of genetically differentiated wild stocks of cultured species which can then guide the collections from the wild to form base populations for aquaculture and breeding programmes. The same markers can also be used for characterizing genetic diversity in founder broodstock. The markers most commonly used for stock identification today are primarily microsatellites and SNPs.

Detection of hybrid introgression

In species for which diagnostic markers have been developed, these markers can be readily used for detection of hybridization in cultured or wild stocks. As outlined above, this can have significant consequences if it occurs in hatchery stocks. Padhi and Mandal (1997) used RFLP to identify F_1 hybrids between catla, rohu and mrigal in fry from a mixed species spawning pool in a West Bengal hatchery indicating that 'incidental' hybridisation can occur in such systems making it likely that hybrids would eventually enter the broodstock. Allozymes were the tools of choice by Simonsen *et al.* (2005) in their identification of hybridisation in between Chinese carp species in Bangladesh, with mitochondrial markers used to identify how such hybrids might have been produced. These findings were confirmed to some extent by Mia *et al.* (2005) in their investigations into the extent of uncontrolled hybridisation in Bangladeshi carp hatcheries using microsatellite markers.

Parentage assignment

Due to their highly polymorphic nature and the ability to combine (through multiplexing) several markers in a single reaction, microsatellites are generally the markers of choice for parentage assignment. These enable progeny to be identified to parental pairings after they have been reared in communal groups. One application of this technique is to determine the consequences of spawning or mating designs on genetic contributions and effective population sizes. For example in pooled spawnings it is often known which females have contributed eggs but often impossible to know how many and which males have contributed to matings. With parentage assignment this can be determined and even compared between different mating systems. It has been shown that relatively small proportions of males contribute to progeny in pooled matings in Barramundi *Lates calciferer* (Frost, Evans and Jerry, 2006) and tilapia (Fessehaye *et al.*, 2006) leading to low effective population sizes.

Parentage assignment can be particularly usefully applied in enhancing selective breeding programmes based a family designs which often requires families to be reared separately for several months until they are big enough to be tagged. This may then increase the probability of confounding environmental effects on the trait in question. Parentage assignment allows progeny to be pooled at a very early stage (preferably at the same age to minimise interaction effects between families) and to be identified to family at harvest. One of the draw backs of this method is that contributions from different families can vary substantially due to differential larval survival and size-based grading, especially when pooled very early, which may result in numbers being highly skewed to just a few families reducing effective population sizes. One way to partially circumvent this problem, even in mass selection, is to use parentage assignment to identify selected progeny and then

'walk-back' from those with the highest breeding value for the trait(s) being selected to those with lower values until enough pairs have been selected from enough families to meet the targeted effective population size (Sonnesen, 2005).

Genetic mapping, QTL and marker assisted selection

Genetic markers can be used to construct genetic maps in which linked markers are assigned, using pedigreed matings, to linkage groups resulting in the assignment of chromosomal location, order, and distance between markers. Genetic markers (not necessarily genes themselves) that are closely linked to genes that contribute to quantitative traits are known as quantitative trait loci (QTLs). Gene mapping programmes are now going on for several important aquaculture species including the Pacific oyster, Atlantic salmon, Arctic char, Japanese flounder, rainbow trout, channel catfish, Nile tilapia, and European sea bass (Garber and Sullivan, 2006). Once linkage maps have been developed they can be screened to identify QTLs of interest.

QTL mapping is based on the premise that the QTL and the marker are closely associated on the same chromosome and that they co-segregate. The QTL effect can then be quantified by correlating the inheritance of marker alleles with individual performance for the targeted trait. QTL analyses rely on high levels of heterozygosity which can be achieved by looking for QTL markers in F_1 crosses between species or strains.

A number of QTL for important traits have been identified in fish such as temperature tolerance, growth and disease resistance (e.g. Cnaani *et al*., 2003 in tilapia broodstock).

Marker assisted selection (MAS) is the use of genetic markers linked to QTL in genetic improvement programs. In effect QTL refers only to major genes, as only these will be large enough to be detected and mapped. However, it is likely that most of the traits of interest to aquaculture will be polygenic traits, controlled by a large number of loci. Thus MAS using single QTL markers will be unlikely to generate major responses to selection and should best be combined with traditional selective breeding. Maximum benefits of MAS will be for traits that cannot be measured directly on breeding individuals and for traits that have low heritability. Whilst there are a number of research efforts developing and evaluating QTL there is as yet, no commercial stocks utilising MAS.

The potential benefits of genetic markers in the majority of applications is not contested although the real potential for incorporation of marker assisted selection into breeding programmes and the production and economic gains that will result remain to be verified and this currently represents a major research challenge.

DISSEMINATION AND UPTAKE OF GENETIC IMPROVEMENTS

One of the major challenges in the supply of genetically improved quality seed is to effectively and equitably disseminate the benefits of research and development. In the case of selective breeding, the majority of work carried out to date in developing countries has been supported by international donors and implemented by state and international agencies. There are exceptions where multi-national companies get involved in proprietary breeding programmes such as in Chile with Atlantic salmon. Clearly, genetic improvement programmes must have long term objectives and thus cannot depend on international donor funding indefinitely so it is of critical importance that strategies are developed for the long term sustainability of genetic improvement programs. The main strategy to achieve this would be through a commercialization process where revenue generated from dissemination of improved fish is used to fund the cost of on-going genetic improvement. This can be achieved through the development of a quasi private body such as a foundation or the transfer of the control of the breeding and dissemination to a private sector breeding company.

Taking a look back at similar progressions with genetic improvement of livestock it is possible to see a pattern of transition of roles and responsibilities of the government and private sectors with regard to seed supply and genetic improvement of seed quality as illustrated in Figure 8.2.3.

It is already possible to see differing phases of this development for different fish species in different locations. For example Indian major carp production in Karnataka, Southern India is in Phase I where virtually all seed is supplied by state run hatcheries although there is some progression to Phase II with the initiation of genetic management of stocks in some state hatcheries. Tilapia seed production in the Philippines and to some extent internationally, is in transition from Phase II to Phase III with commercial or quasi commercial entities adopting the outputs from projects on selective breeding and sex control. Currently these commercial entities work alongside and even compete to some extent, with state controlled supply of improved seed which is perhaps to be expected during this stage of transition. Managing this transition represents a major challenge in ensuring equitable uptake of genetic improvements and should be the subject of coordinated policy among the major stakeholders.

One of the other challenges in dissemination of the benefits of genetic improvement is in the maintenance of quality and of genetic gains through the dissemination process. Effective dissemination in most cases will involve the establishment of a breeding nucleus where the genetic improvement (or effective genetic management of introduced improved stock) takes place. The nucleus would be at the centre of a network of multiplier hatcheries mass producing quality seed and/or further broodstock, depending on the scale of the dissemination strategy. Where there is a regular supply of future broodstock from the breeding nucleus then the responsibility of maintenance of genetic gains lies only with the nucleus and not with the multipliers. However, where one-off introductions or very irregular introductions are made then it is beholden to the multipliers to also manage stocks to maintain genetic gains. In the absence of a coordinated dissemination strategy, incorporating a genetic management plan, developed in consultation with key stakeholders, genetic gains from introduced stocks can be readily lost. There are undocumented cases of both successful and unsuccessful introductions of genetically improved stocks such as in the dissemination of GIFT and GMT producing broodstock tilapia, predominantly in Asia. In successful cases introduced broodstock were successfully managed and distributed to avoid significant genetic deterioration and even in some cases used as founders to initiate local breeding programmes to further improve the stocks. In unsuccessful introductions

FIGURE 8.2.3

Illustration of the potential evolution of seed supply systems and the respective roles of state and private sectors as aquaculture develops. Similar evolution has been seen in other agricultural production sectors

Phase I	**Phase II**	**Phase III**
Government agencies play a major role in providing quantities of seed required for aquaculture whilst private sector hatcheries develop.	Government agencies take responsibility for quality of germplasm used in aquaculture including conduct of genetic improvement programmes anddissemination of improved breeds. Private sector hatcheries are primary seed producers and become multipliers for improved breeds.	Private sector become developers and primary multipliers for improved breeds. Government sector responsible for ensuring rural and small-scale farmers can access benefits of improved breeds.

stocks are either poorly managed resulting in inbreeding or introgression with inferior local stocks or simply not distributed effectively. It is important to learn lessons from introductions that have taken place and to ensure that adequate advanced planning has taken place.

KEY ISSUES IN THE FUTURE APPLICATIONS OF GENETICS

This section of the paper briefly highlights some of the key issues, currently facing the development and application of genetics based technologies in aquaculture in addition to technical challenges identified in earlier sections. These issues will ultimately impact upon the benefit to be derived from these technologies.

CENTRALIZATION OF GENETIC IMPROVEMENT

Given the benefits of concentrating resources for genetic improvement at a single location it is evident that significant and sustainable genetic improvement is more readily achieved through centralization. Some of the most successful breeding programmes to date such as the Norwegian salmon breeding programme and the GIFT tilapia project have been highly centralized with genetic improvement, at least in the first instance, being carried out in a single breeding nucleus facility with dissemination achieved through sale or distribution of improved broodstock to multiplier hatcheries. Such centralization is not always possible where international or commercial cooperation is lacking or where effective seed supply systems are decentralized. Such a system exists in the North East of Bangladesh where a decentralized rice-field based seed supply system has developed to effect a sustainable supply of seed (Haque and Barman, 2004). Maintenance of genetic quality of such a stock is a major challenge in the development and sustainability of such as system. On the other hand, the benefits of centralization as part of a national strategy for genetic management and improvement of aquaculture stocks can be seen in the development of a series of National Broodstock Centres in Viet Nam (Mair and Dan, 2001). These centres are strategically located in the key aquaculture producing regions of the country. Each is responsible for the long term genetic management or genetic improvement of species important to its respective region. The development of these centres is as a result of a coordinated national policy on supply of quality seed and should enable the development and distribution, through networks of multiplier hatcheries, of genetically superior strains nationwide.

HUMAN RESOURCE CAPACITY BUILDING

Often one of the key constraints to implementation of effective genetic management or genetic improvement programmes is the lack of adequate knowledge of the key principles and, particularly in the case of implementation of effective selective breeding, the lack of trained and skilled personnel. Many of the principles for effective genetic management are not complex but require hatchery operators to have a clear understanding of them in order to effectively implement best management protocols. Whilst some well written summaries of the key principles and effective management practices have been produced (Basavaraju, Mair and Penman, 2004; Tave, 1993; Tave, 1999) there remains a need for widely distributed, accessible and highly readable information resources covering these topics.

Implementation of modern and effective breeding programmes requires specialist expertise in quantitative genetics for which extensive specialised training in needed. In general, and especially across the developing world, there is shortage of people adequately training in the application of quantitative genetics to the genetic improvement of aquaculture species and thus a need for key personnel to receive training in this discipline. There is a similar deficit of people adequately trained in the principles and practice of ecological and economic risk assessment of introduced and genetically improved fish stocks

AQUACULTURE GENETICS AND CONSERVATION

Balancing the need for genetic change (i.e. improvement) in aquaculture stocks with the desire to conserve genetic diversity in wild stocks represents a central dilemma in aquaculture genetics. For the sustainable development of aquaculture and the preservation of the potential for long term genetic improvement (through selective breeding) of a domesticated and cultured stock it is highly desirable to maximize the genetic variability of the stock at the outset of the domestication process. However, in so doing, it is likely that representatives of distinct wild populations will be mixed in the creation of composite populations, as was performed for example in the initiation of the GIFT project (Eknath and Acosta, 1998) for improvement of tilapia, based in the Philippines.

However, it becomes inevitable in most cases that this genetically variable stock, further modified by genetic improvement and genetic processes that occur during domestication, when widely adopted in aquaculture, will re-enter the natural environment through deliberate stocking (for example in cultured based fisheries) or escapes. This then creates a risk to the integrity of wild populations and may result in a breakdown in the population of wild stocks and the loss of unique reservoirs of genetic diversity for the species.

There exists therefore a dilemma for authorities wishing to promote aquaculture development. Policies based on long term strategies for genetic management and improvement of cultured stocks to support the sustainable growth of aquaculture risk compromising the genetic diversity of local indigenous population. It thus becomes imperative that good risk assessment is carried out when developing aquaculture and the genetic management and improvement options within the industry.

Another important aspect of conservation is gene banking, the protection of genetic diversity through preservation of diverse genotypes. At the moment the options for gene banking in fish are limited to live gene banks or cryopreserved sperm gene banks. Live gene banks are difficult to maintain and require the application of best practices in domestication and genetic management as outlined in previous sections of this paper. There are relatively few live gene banks being maintained for fish due to the challenges of maintaining adequate effective population size. However, some do exist for example for sturgeons in Russia (Chebanov Galich and Chmir, 2004) and for common carp in Hungary (Bakos and Gorda, 2001)

The technology for sperm cryopreservation has been developed for a range of aquatic species (Chao and Liao, 2001; Tiersch and Mazik, 2000) but has not been successfully extended to eggs or embryos other than for a few bivalve molluscs (Chao *et al.,* 1997). Generally it is not a major technical challenge to cryopreserve sperm provided that milt quality is good prior to freezing, the correct cryoprotectant is used and freezing protocols stay within accepted parameters. Generally the quality of thawed sperm following cryopreservation is lower than that of freshly collected sperm but, given the high fecundity of many fish species, adequate to obtain significant numbers of fertilized eggs. Whilst this lower fertility from cryopreserved sperm might be a constraint to the routine use of cryopreservation of sperm in commercial hatcheries this should not represent a major problem in gene banking where the primary objective is to preserve genotypes rather than to produce large numbers of seed.

Whilst cryopreservation is in widespread use for some livestock, including in developing countries, it is rarely used for gene banking in fish. The main reason for this may be the lower priority of gene banking for conservation relative to the commercial incentive for genetic improvement.

Cryopreservation has a range of applications in addition to conservation including enhancing breeding programmes through maintenance of larger effective population sizes, maintenance of specific genotypes (such as sex manipulated broodstock), preservation and biosecure dissemination of improved breeds, and as controls to

quantify genetic gains in selection programs. It seems likely, as breed development becomes more sophisticated and moves more into the realm of the private sector, that cryopreservation will become a more common biotechnology in aquaculture in coming years.

STRAIN NOMENCLATURE AND CERTIFICATION

One of the problems facing farmers today is that there are a lot of so called "strains" available for culture but there is no consistently applied definition of what is a strain. The term "strain" should be reserved for domesticated sub-populations within a species which exhibit one or more distinctive and heritable physiological and/or morphological traits. However, the term is more often used to refer to stock at a particular location or of a particular origin, which very often will not have any distinctive recognizable or quantifiably distinct trait. The situation can be further confused by claims and counter claims for particular qualities or properties of these "strains", many of which are difficult or impossible to verify. This situation is particularly prevalent in species such as tilapia, which have been the subject of genetic improvement efforts and in which translocation of stocks is common place.

This scenario generates a strong need for a certification system which would only certify true strains and would incorporate some form of verification and/or monitoring of the properties of the strain. Such a certification system would assure genetic quality of certified seed but could also integrate assurance of appropriate husbandry, handling and point of sale quality measures. The author is not aware of such a certification system currently being applied to seed for inland aquaculture and it would be appropriate to look to the livestock sector for examples of certification systems that could be adapted to aquaculture seed. Certification systems could be applied nationally but ideally would also be applicable for the relatively small but growing international trade in fish seed.

SUMMARY AND RECOMMENDATIONS

It is estimated that no more than 20 percent of global aquaculture production utilizes seed from directed genetic improvement programmes; many more species will be domesticated, or are now domesticated to some extent, through the process of controlled breeding and cultivation. Furthermore, the vast majority (>95 percent) of freshwater aquaculture production comes from the developing world where resources are limited and breeding technologies often less advanced, indicating that the proportion of improved fish in freshwater aquaculture is likely to be considerably lower than the global average. Given that major rapid advances in production possible from implementation of outcomes from R&D in husbandry and nutrition and, in some cases, health management, are likely to have already been made, the era of genetic improvement is truly upon us. The past 15 years has seen a rapid increase in the number and scope of breeding programmes for genetic improvement of aquaculture species and a number of important national and regional programmes for developing, mass-producing and disseminating important aquaculture species are now underway or in train.

There have been a number of important advances in research, most notably in the verification of the potential gains to be had from well managed selection programmes with genetic gains for growth related traits of 7-10 percent readily achievable. However, with these potential benefits in mind it is also evident that there has been genetic deterioration of existing domesticated and cultured stocks through poor genetic management during and subsequent to the domestication process. These effects need to be reversed and basic genetic management needs to be considered when domesticating and translocating aquaculture stocks.

Whilst the success with selective breeding in recent times has placed this technology at the core of efforts to improve aquaculture stocks there have been successes with other technologies and these do have a role as components of integrated approaches to genetic improvement. These include sex control techniques in species which exhibit precocious sexual maturation and/or sexual dimorphism for commercially important traits; chromosome set manipulation, particularly the induction of sterility through triploidy; crossbreeding or hybridisation where heterosis for commercially important traits are evident or where hybrids have a particularly marketable combination of traits. Some extreme improvements in culture performance have been demonstrated using transgenesis but biosafety and societal and regulatory concerns have restricted them to research use for the time being and until such time as some transgenic fish are licensed for commercial aquaculture it is difficult to see a significant role for this technology in aquaculture. Major research advances have been made in recent time with the application of genetic markers and the costs of widely applied and to some degree standardized techniques such as the application of microsatellite markers have come down considerably. Genetic markers are thus now becoming valuable tools and will be increasingly applied to enhance traditional selection programmes and possibly in the future to more specific programmes incorporating marker assisted selection.

Of the major freshwater aquaculture species there have been very significant genetic gains through selective breeding and to some extent also sex control in tilapia, with improved strains exhibiting performance gains of up to 100 percent compared to unimproved stocks. Past research on selective breeding of carp, which was focused in Eastern Europe, has impacted little on current production but several genetic improvement programmes are underway in Asia for a number of species. There have been relatively fewer advances to date in catfish with the exception of the prevalence of hybrids in the production of Clarias catfish in some countries. Similarly there have been few attempts to genetically improve crustaceans but *Macrobrachium* species are becoming a major focus for genetic improvement in Asia.

There are some major technical, environmental and socio-economic issues facing the full implementation of genetic improvement programmes in freshwater aquaculture. One of the fundamental dilemmas is the need for creation of highly genetically variable base populations to provide adequate additive genetic variation from which to select. This is an important first step in initiating a selective breeding programme but is often achieved by interbreeding two of more genetically distinct stocks. When these composite 'synthetic' stocks are adopted widely in indigenous species aquaculture there is significant risk of contamination of natural genetic diversity.

Efforts to disseminate the products of breeding programmes has to date met with variable results. The success of dissemination appears to depend upon well planned and structured strategies including good genetic management or further improvement of stocks, accompanied by awareness programmes and/or appropriate and enforceable seed certification systems. It is also important to consider mechanisms for economic sustainability of the publicly supported breeding programmes and the potential for inequitable distribution of the benefits of improved strains associated with commercialization. There are additional challenges where seed supply systems are decentralized as can occur in rural areas. A number of recommendations are made concerning the appropriate role of genetics in seed supply systems for freshwater aquaculture.

RECOMMENDATIONS

This section outlines a number of general and specific recommendations applicable on a global or regional scale, which if implemented, would further enhance the contribution of genetics based technologies and genetic improvement to seed quality and the sustainable growth of aquaculture.

General

- Good genetic management of stocks should be an essential component of all aquaculture production systems and should be an active consideration when new species are domesticated and when cultured stocks are translocated.
- There is a strong need for the development of national dissemination strategies with a focus on the advancement of plans for the effective, equitable and sustainable dissemination of improved fish stocks. This should include designation of areas where certain fish or even the aquaculture sector should not be allowed.
- There is a need to strengthen awareness of and institutional capacity to deal with ecological risks associated with introductions of alien and/or genetically improved fish.
- There are several genetic technologies available to the sector ranging from traditional animal breeding to advanced genetic engineering; risk assessment should be based on the changes the technology imparts on the organism and not on the technology itself. Where the changes are unknown the precautionary approach should be applied (see Pullin *et al*., 1999).
- It is important to conserve genetic diversity in wild relatives of cultured species
- There is a need to improve information systems related to genetic resources for aquaculture, e.g. FishBase strains registry and FAO aquaculture fact sheets.

Technical and environmental

- Selective breeding should be at the core of the majority of genetic improvement programmes for species for which sustainable aquaculture industries have developed. Selective breeding should focus on traits identified as economically important to the sector. Estimation of genetic parameters including correlations between traits is an important pre-requisite to initiation of breeding programmes and genetic gains should be measured against good genetic controls. In the longer term multiple trait selection not limited to production related traits (e.g. product quality) should be encouraged. Active consideration should be given to integration of other genetic technologies into selection programs.
- Efforts should be made to domesticate cultured organisms for which aquaculture remains dependent on wild caught seed or broodstock.
- Genetic markers, such as microsatellites and SNPs, are becoming increasingly useful and affordable tools for such tasks as characterising founder populations, assessing genetic impacts of aquaculture, parentage assignment and estimation of effective population sizes and their appropriate development and application should be encouraged.
- Hybridisation should be limited to cases where heterosis for commercially important traits has been clearly demonstrated or where the hybrid has a highly marketable combination of traits. The potential risk that hybrid production imposes on the genetic integrity of wild stocks should be actively assessed.
- Chromosome set manipulation is a useful research tool and triploidy is, in some species, an efficient and rapid means to improve production and/or a valuable mode of biological containment to reduce the potential environmental impact of aquaculture. Further research is required to assure reliable induction of sterility through triploidy in a range of species.
- Marker- (or gene) assisted selection has potential for improving traits such as disease resistance and carcass quality that are difficult to select by traditional means and the development of such approaches should be considered where these are economically important traits.
- Functional and comparative genomics and bioinformatics are rapidly developing fields and are likely to have important applications to breeding programmes in the near future and developments should be closely monitored.

- The potential for environmental and perceived human health risks contribute to producer and consumer resistance to commercialization of transgenic fish but the demands of food security may influence risk: benefit assessments in the developing world. Risk assessment and biosafety issues should be foremost in any consideration of development and adoption of transgenic fish for aquaculture.
- It is important to maintain and conserve genetic diversity of farmed species and genetic monitoring programmes are recommended utilizing genetic markers. Where appropriate cryoconservation of gametes should be applied to create international repositories for conservation of germplasm from both geographically diverse unselected lines and improved lines.

Economic and social

- Centralized coordinated dissemination programmes where resources can be shared between National Agricultural Research Systems (NARS) and farmers are more likely to lead to good genetic management and effective application, uptake and dissemination of the benefits of genetic technologies. However there may be some cases where dissemination through decentralized systems may be appropriate.
- Coordination between research institutions and development/extension agencies needs to be improved in many countries for effective implementation of dissemination strategies. International networking can assist in circumventing limited resources.
- Development of dissemination programmes should involve full consultation with stakeholders in aquaculture including policy makers, development or extension agencies, research institutions NGOs, seed producers and fish farmers at all levels.
- Dissemination strategies, where possible, should involve private sector hatcheries and NGOs as primary multipliers.
- Evaluation and verification of new or further improved breeds under different production systems is desirable and should involve extension agencies and the private sector as appropriate. An independent process can form the basis for certification of these breeds. Certification systems can incorporate both genetic and non-genetic factors contributing to seed quality.
- Strong awareness building including the development of brand names, through various media, is important to the successful uptake of genetically improved stocks.
- Government and NGO agencies should adopt a long term commitment to ensure the equitable dissemination of improved stocks to ensure that small scale, poor and geographically isolated rural farmers are not marginalized from the benefits of improved stocks. Such commitments should take into account the commercialization strategies of private sector producers/suppliers of quality seed.
- Institutional capacity could be strengthened in many of the developing countries with significant freshwater aquaculture sectors including the construction of appropriate facilities and the development of technical expertise in quantitative and molecular genetics. Expertise in environmental and socio-economic risk assessment associated with improved fish stocks is much needed.

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