1   INTRODUCTION

The role of aquaculture in food production, economic development and food security is now well recognized. As the fastest growing food production sector, aquaculture holds promise to help provide a growing human population with food as many of the world's capture fisheries have reached their biological limits of production or have been depleted through over-fishing and habitat degradation. Less well recognized is aquaculture's role in conservation and the recovery of threatened and endangered species. In fact, aquaculture has often been implicated in contributing to the endangerment of aquatic biodiversity.

The aquaculture sector has made significant advances in increased production and environmental protection. However, the sector is now being criticized for degrading the aquatic habitat through release of effluents that include uneaten food, waste products, and pharmaceuticals, and through the escape of farmed fish. There is potential to improve the production, efficiency and environmental sustainability of the sector and the effective management of aquatic genetic resources can assist in addressing all of the above issues. Genetically improved fish (Chapters 4, 5 and 6) grow faster and use food more efficiently, which will produce less waste. Disease resistant fish require less pharmaceutical treatments. Some farmed fish can be made sterile to reduce the chance of them breeding with native species or establishing feral populations. Broodstock management (Chapters 3 and 8), genetic improvement programmes (Chapters 4, 5 and 6), and gene banking (Chapter 10) will help improve production and profitability, as well as assist in protection and conservation of wild resources (Chapter 9). Risk assessment (Chapter 7), adhering to international guidelines (Chapter 2) and a precautionary approach (Chapter 11) will help ensure wise decisions that will protect society and the environment, while at the same time allowing the sector to develop.

Fish genetic resources (FiGR) comprise all finfish and aquatic invertebrate genetic material that has actual or potential value for capture fisheries and aquaculture. This includes DNA, genes, gametes, individual organisms, wild, farmed and research populations, species and organisms that have been genetically altered (for example by selective breeding, hybridization, chromosome set manipulation and gene transfer). How these resources can be used to help aquaculture realize it full potential and conserve valuable wild genetic diversity is the subject of these guidelines.

The purpose of these guidelines is to provide a succinct set of instructions as a framework that can direct policy-makers and senior resource managers

towards improved management of fish genetic resources (FiGR). Throughout these guidelines, management is understood to include use and conservation. Management of genetic resources is approached from a holistic viewpoint that incorporates economics, conservation, risk analysis and uncertainty, as well as increased production and profitability.

1.1 Value of genetic diversity and the need for genetic resource management

Of the over 230 species of farmed aquatic animals and plants for which FAO has statistics, only a few have been the subject of deliberate genetic resource management programmes. Channel catfish, Nile tilapia, Atlantic salmon and many farmed carps are cases that demonstrate the significant gains in production possible from genetic improvement programmes. Only a few culture-based fisheries, usually salmonids, purposefully choose the stocks to release so that they either match or differ completely from the native fishes. One estimate made by a prominent geneticist indicated that the supply gap caused by decreasing output from capture fisheries and the increasing human population could be filled simply by incorporating genetic improvement programmes into already existing aquaculture systems (i.e. no additional farming systems, land or water usage would be required).

Management of FiGR is necessary for more than just increased production. Besides being essential for genetic improvement programmes in aquaculture, genetic resources are the necessary raw ingredients that allow species to adapt to short-term and long-term changes in their environment; they provide species, populations and individuals with the flexibility of dealing with and adapting to changes to their environment, changes both from humans and from natural causes. That is, genetic diversity is necessary for the continued evolution of species. Genetic diversity interacts with environmental variation to produce the variety of shapes, sizes, life-history characters, behaviour, and colours that make aquatic species so valuable and interesting. Some of these differences show up as different colours of fish or as different scale patterns, whereas other differences show up as different migration patterns or reproductive behaviour. Without genetic diversity, there would be no species diversity, no adaptation, no breeds, and no evolution; there eventually would be extinction as climate and habitats change as a result of natural or human actions.

The common carp has by far the longest history of domestication and genetic improvement for aquaculture. Farmed Atlantic salmon, channel catfish and

Nile tilapia have been genetically improved more recently. However, with the success of these breeding programmes (i.e. changing the genetic structure of a wild fish) and the inevitable use of these improved breeds in many farming systems, comes the problem of interaction between genetically improved aquaculture stocks and their wild relatives. These wild relatives often support viable fisheries and will provide new genetic material that can be useful to aquaculture. The aquaculture sector is in an advantageous position to minimize extinction of the wild relatives of farmed species, as was allowed to happen to many in the livestock and crop sectors.

Management of aquatic genetic resources must have defined objectives in order to plan programmes and to judge success and impact. These objectives will depend on the purpose of the aquaculture facility: whether it is maximizing production, maximizing efficiency, reducing inputs, releasing fish for culture-based fisheries, or helping restock threatened or endangered species. Each of these objectives will require different management programmes for aquatic genetic resources.

1.2 Relevant articles of the Code

These guidelines are organized by general subject areas that are important for genetic resource management, rather than by specific articles of the Code. This will allow decision makers and resource planners to find guidance on a specific area of genetics in aquaculture quickly. Given the importance of genetic resource management for a variety of aquaculture objectives, there are several articles of the Code that a particular chapter my help implement. These guidelines provide information on the following articles for the Code (relevant chapters are included).

ARTICLE 2 – OBJECTIVES OF THE CODE

2e *facilitate and promote technical, financial and other cooperation in conservation of fisheries (including aquaculture) resources and fisheries management and development* (Chapters 2, 5, 6, 7, 9, 10 and 11).

2g *promote protection of living aquatic resources and their environments and coastal areas* (Chapters 2, 5, 7, 9, 10 and11).

ARTICLE 6 – GENERAL PRINCIPLES

6.2 *Fisheries management should promote the maintenance of the quality, diversity and availability of fishery resources in sufficient quantities for present and future generations in the context of food security, poverty alleviation and sustainable development. Management measures should not only ensure the conservation target species but also of species belonging to the same ecosystem or associated with or dependent upon the target species* (Chapters 7, 9, 10 and 11).

6.8 *All critical fisheries habitats in marine and fresh water ecosystems, such as wetlands, mangroves, reefs, lagoons, nursery and spawning areas, should be protected and rehabilitated as far as possible and where necessary. Particular effort should be made to protect such habitats from destruction, degradation, pollution and other significant impacts resulting from human activities that threaten the health and viability of the fishery resources* (Chapters 9 and 10).

6.12 *States should, within their respective competences and in accordance with international law, cooperate at subregional, regional and global levels through fisheries management organizations, other international agreements or other arrangements to promote conservation and management, ensure responsible fishing and ensure effective conservation and protection of living aquatic resources throughout their range of distribution, taking into account the need for compatible measures in areas within and beyond national jurisdiction* (Chapter 2, 5 and 9).

ARTICLE 7 – FISHERIES MANAGEMENT

7.2.2.d *biodiversity of aquatic habitats and ecosystems is conserved and endangered species are protected* (Chapter 9 and 10);

7.4 Data gathering and management advice (Chapters 9 and 10)

7.5.1   *States should apply the precautionary approach widely to conservation, management and exploitation of living aquatic resources in order to protect them and preserve the aquatic environment. The absence of adequate scientific information should not be used as a reason for postponing or failing to take conservation and management measures* (Chapter 11).

7.6.8   *The efficacy of conservation and management measures and their possible interactions should be kept under continuous review. Such measures should, as appropriate, be revised or abolished in the light of new information* (Chapter 8, 9 and 11).

ARTICLE 9 – AQUACULTURE DEVELOPMENT

9.1.2   *States should promote responsible development and management of aquaculture, including an advance evaluation of the effects of aquaculture development on genetic diversity and ecosystem integrity, based on best available scientific information* (All chapters).

9.1.3   *States should produce and regularly update aquaculture development strategies and plans, as required, to ensure that aquaculture development is ecologically sustainable and to allow the rational use of resources by aquaculture and other activities* (Chapters 7, 8, 9 and 11).

9.3.1   *States should conserve genetic diversity and maintain integrity of aquatic communities and ecosystems by appropriate management. In particular, efforts should be undertaken to minimize the harmful effects of introducing non-native species or genetically altered stocks used for aquaculture including culture-based fisheries into waters, especially where there is a significant potential for the spread of such non-native species or genetically altered stocks into waters under the jurisdiction of other States, as well as waters under the jurisdiction of the State of origin. States should, whenever possible, promote steps to minimize adverse genetic, disease and other effects of escaped farmed fish on wild stocks.* (Chapters 2, 5, 8, 9 and 10).

9.3.3   *States should, in order to minimize risks of disease transfer and other adverse effects on wild and cultured stocks, encourage adoption of appropriate practices in the genetic improvement of broodstocks, the introduction of non-native species, and in the production, sale and transport*

of eggs, larvae or fry, broodstock or other live materials. States should facilitate the preparation and implementation of appropriate national codes of practice and procedures to this effect. (Chapters 3, 4, 5, 8 and 9).

9.3.5 *States should, where appropriate, promote research and, when feasible, the development of culture techniques for endangered species to protect, rehabilitate and enhance their stocks, taking into account the critical need to conserve genetic diversity of endangered species* (Chapters 3 and 9).

2 INTERNATIONAL SETTING

The Code of Conduct for Responsible Fisheries (CCRF) and the international community have recognized the vital role that genetic resources, including FiGR, play in sustainable development and conservation. As a result, international mechanisms, guidelines and codes of practice have been developed. The Convention on Biological Diversity (CBD)[1] arose from the Earth Summit in 1992 and has more signatories than any other piece of international legislation. It is a legally binding instrument that requires the conservation and sustainable use of biological diversity (including genetic diversity), and the fair and equitable sharing of benefits derived from that use. In recognizing the need for scientific and technological advice in order to implement the articles of the Convention, the CBD established a Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA). The CBD further established the Cartagena Protocol on Biosafety,[2] a set of binding international protocols on the international movement of living modified organisms, which would include genetically modified organisms (GMOs) (i.e. transgenic organisms). Similar to the CCRF, the CBD recognizes both the need to use and to conserve biodiversity.

The precautionary approach to development is an essential attribute of both the CBD and the CCRF. Aside from agreement to be cautious and use the best information available, there are a variety of opinions on what this approach means in practice; it forms the basis of Chapter 11.

The Convention on International Trade in Endangered Species of Fauna and Flora (CITES) is another significant instrument that impacts on the management of FiGR. CITES restricts the international trade in species that are threatened in the wild – the degree of threat or endangerment indicates how restrictive trade will be. Some aquatic species that are threatened in the wild are also farmed, e.g. sturgeons (Acipenseriformes), and arowana or dragon fish (*Scleropages formosus*). International trade in these species must ensure that the species being traded actually come from licensed farms and not from the wild, and that the trade of farmed species does not create a market for the endangered species in the wild. Genetic markers and genetic stock identification have been used to help differentiate species and stocks of wild and farmed species.

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[1]   www.biodiv.org
[2]   http://www.cbd.int/biosafety/default.shtml. As of August 2008, there were no aquatic GMOs being produced for human consumption.

The Ramsar Convention on Wetlands mandates countries to identify and protect wetlands, including coastal and inter-tidal areas that are of national importance. Primary criteria for establishing importance were the roles wetlands play in maintaining wild biodiversity, primarily waterfowl. However, Ramsar expanded the criteria to include historical use of wetlands as a fishery resource[3] and now allows aquaculture of native species as an acceptable activity in Ramsar sites. However, farming of native species could eventually lead to their domestication and genetic alteration through natural selection to farm environments and breed improvement programmes.

More specific guidelines have been developed by FAO and others that apply indirectly to the management of FiGR. Technical Guidelines on Aquaculture have been developed for general issues relating to FiGR.[4] FAO, the WorldFish Centre (WFC) and other partners established the Nairobi Declaration (Annex 1) on recommendations for importing genetically improved tilapia into Africa. These non-binding resolutions layout a framework that is elaborated here in these guidelines in regards to the responsible use of genetically improved fish in aquaculture.

Fish health concerns play a major role in the trade and movement of aquatic species. Dissemination of genetically improved stocks (Chapter 5) requires adherence to the World Organization for Animal Health (OIE) with regards to transboundary pathogens. Technical guidelines have been established[5] that are consistent with OIE and World Trade Organization (WTO) requirements.

Recently, FAO has made progress with regard to aquatic genetic resources. The lack of coherent fish genetic resources management and of policies is in fact becoming a problem in the recent rapid expansion of aquaculture. A transition to more responsible, sustainable and productive aquaculture has been called for by Members of FAO and the international community. Its success will depend in large measure upon effective management of fish genetic resources.

At its Eleventh Session, the FAO's intergovernmental Commission on Genetic Resources for Food and Agriculture recognized the importance

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[3]   http://www.ramsar.org/res/key_res_vi.2.htm
[4]   FAO. 1997. Aquaculture development. FAO Technical Guidelines for Responsible Fisheries. No. 5. Rome, FAO.
[5]   FAO. 2007. Aquaculture development. 2. Health management for responsible movement of live aquatic animals. FAO Technical Guidelines for Responsible Fisheries. No. 5. Suppl. 2. Rome, FAO. ftp://ftp.fao.org/docrep/fao/010/a1108e/a1108e00.pdf

and vulnerability of aquatic genetic resources, their roles in an ecosystem approach for food and agriculture, and for their contributions to meeting the challenges presented by climate change. It agreed that its 10-year Multi-year Programme of Work should include coverage of aquatic genetic resources for the development of sustainable and responsible fisheries and aquaculture in cooperation with other forums and organizations, such as COFI or UNCLOS.[6]

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[6]   FAO/CGRFA. 2007. Report of the Eleventh Regular Session of the Commission on Genetic Resources for Food and Agriculture. CGRFA-11/07/REPORT. ftp://ftp.fao.org/ag/cgrfa/cgrfa11/r11repe.pdf

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Box 1. Terminology

The terminology used to describe organisms that are genetically different from wild types is extremely important because it has legal and policy implications and will influence how well the general public accepts the product or process. Therefore, farmed fish that have been genetically altered in some way by humans must be described clearly and accurately. Unfortunately, a variety of terms have been used to describe genetically altered fish. The usage is not standardized and can lead to consumer confusion and regulatory problems, such as when applying for farming licenses or trade permits. Aquaculturists and government regulators must be aware of these implications.

Fish produced through aquaculture have the potential to become genetically different from their wild ancestors through selection to hatchery and farm environments (Chapters 3 and 9), and/or through purposeful genetic improvement programmes (Chapter 4). In aquaculture, fish farmers seek to farm the best and most profitable fish available and to project an image to consumers that the product is both healthy and natural; consumers are increasingly seeking these qualities from their food. This interface is usually managed through labelling and marketing. The guidelines in this book do not address consumer labelling issues other than in a very general manner (Chapter 12). However, for government oversight of farmed fish and their marketing, it will be crucial to understand the genetic technologies being used and the changes those technologies impart on the farmed organism.

A general term for all human-induced changes to an organism is *genetically altered.* This term should be used as a neutral statement of fact without judging whether the alteration is good or bad, whether it is a result of modern biotechnology or traditional methods, or whether the alteration is deliberate or accidental. This is meant to be a very general term, reflecting the possibility that a genetically altered organism could have environmental or population risks independent of how it became altered (Chapter 7).

The following terms are important to use correctly as they are associated with consumer perception and government oversight. Additional terms can be found in the FAO glossaries.[1]

Genetically modified organism (GMO): An organism in which the genetic material has been altered by humans through gene or cell technologies. A genetically modified fish is usually a transgenic fish (i.e. a fish with a gene inserted from another organism in a manner that is not possible through natural processes). At present, there are no genetically modified fish available to the consumer. There are currently several restrictions on the international movement of GMOs. This class of organisms is regulated by the Cartagena Protocol of the CBD[2]. Additionally, many consumer groups are currently against the use of GMOs, including *genetically modified* fish. Thus, a fish farmer wishing to import a fish genetically *improved* through selective breeding, should not use the term *genetically modified,* but instead use *genetically improved through selective breeding (or through traditional breeding).*

Hybrid: Offspring of the mating between parents of different species or varieties. Offspring of matings between parents of the same species are **intra-specific hybrids,** whereas offspring of matings between parents of different species are **inter-specific hybrids.** The distinction is important because some areas have laws against mating different species, or importing inter-specific hybrids, whereas matings or importation of the same species may not regulated.

Living modified organism (LMO): "Living organism that possesses a novel combination of genetic material obtained through the use of modern

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[1]   http://www.fao.org/fi/glossary/default.asp and http://www.fao.org/biotech/index_glossary.asp
[2]   http://www.cbd.int/biosafety/default.shtml

biotechnology". Synonym of GMO used primarily by the Convention on Biological Diversity.

Polyploids: Plants or animals having more than 2 sets of chromosomes (called diploids and designated as 2N). Organisms having 3 sets are called triploids (3N), those with 4 sets are tetraploids (4N). The distinction is important because diploids and tetraploids are usually fertile whereas triploids are usually sterile. It is possible to mate tetraploids with diploids to get triploids.

Traditional breeding: refers to selective breeding programmes that do not use modern gene manipulation technologies (Chapter 4). Traditional breeding has been practiced and refined for millennia in terrestrial agriculture.

An international group of experts stated that it is more important to understand what actual changes the genetic alteration has caused to the farmed fish, rather than the techniques used to produce that change.[3] That is, addressing questions such as, does the fish consume more food or have better conversion efficiency, does it have wider environmental tolerances, is it fertile, is it more nutritious, can it become invasive, or does it produce new substances that the un-altered fish does not produce are more important in risk assessment (Chapter 7) than what technology was used to create the organism. Current policy, farm practices and public perception do not necessarily recognize this fact; it is recommended that more informative descriptions should be used to describe the actual changes to an organism as a result of genetic technologies.

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[3]   Page 253, in Pullin, R.S.V., Bartley, D.M., Kooiman, J. (eds). 1999. Towards Policies for Conservation and Sustainable Use of Aquatic Genetic Resources. ICLARM Conference Proceedings No. 59. Manila, Philippines. " … in the formulation of biosafety policy and regulation for living modified organisms, the characteristics of the organisms and of potentially accessible environments are more important considerations that the processes used to produce those organisms.

3 BROODSTOCK MANAGEMENT: INBREEDING, GENETIC DRIFT AND DOMESTICATION [7]

3.1 Introduction

Aquaculture is not only a critical sector of food production, it is also a necessary component of recreational and commercial fisheries and a required management tool for conservation programmes. As is the case for all types of animal husbandry, aquaculture means that humans must intervene in and manage a species' life cycle. The moment this occurs, we usually produce irreversible changes in the population's gene pool. These changes can be desired, which occurs when selective breeding programmes are conducted to improve growth (Chapter 4) or when domestication creates fish that are better adapted to the hatchery environment. Unfortunately, we also produce undesired, damaging changes in the genome through inbreeding and genetic drift (section 3.3), which lower viability and growth and increase developmental instability. While domestication is beneficial in food fish culture, it is harmful for fish that are stocked in the wild, because fish that are well adapted for a hatchery may be maladapted in the wild (Chapter 8). In this chapter, broodstock management involves the control of inbreeding and genetic drift and the process of domestication.

3.2 Inbreeding

Inbreeding is the mating of relatives. Inbreeding is one of the three traditional breeding programmes, and it has been used to develop new breeds and can be used in combination with cross-breeding to produce uniform, outstanding individuals (Chapter 4). Even though planned and directed inbreeding can be beneficial, unintentional and unplanned inbreeding will cause problems.

Genetically, inbreeding increases homozygosity in the offspring (i.e. genetic similarity) which means it also decreases heterozygosity (i.e. genetic diversity). Homozygosity is also produced when non-relatives mate and, genetically, the two forms of homozygosity are identical. Even though the two forms of homozygosity are identical, a distinction is made because of the way homozygosity is produced and its consequences.

Relatives are more alike genetically than non-relatives. Consequently, when relatives mate, they produce offspring that are more homozygous than is the

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[7]   Contributed by Douglas Tave.

case when non-relatives mate; the closer the relationship between mates, the more homozygous the offspring.

This is of concern, because all animals contain a small number of harmful or deleterious recessive alleles.[8] In most cases an individual is not affected and survives because it has only one copy of the harmful allele, inherited from one of its parents (it is heterozygous); two copies of the allele are needed (one from both parents) to produce the harmful or lethal effect (it is homozygous). Because relatives are more alike than non-relatives, they tend to share the same deleterious recessive alleles. Two non–relatives might only share one or two in common, while relatives usually share more in common; the closer the relationship, the more that are shared. When relatives mate, the pairing and subsequent expression of these deleterious recessive alleles in their offspring produces inbreeding depression–lower growth rate, viability, and fecundity and an increase in the number of abnormalities. Studies in fish have shown that inbred fish have these classical clinical signs of inbreeding depression,[9] as well as a decreased return rate when stocked in the wild.[10]

The negative effects of inbreeding usually do not occur immediately. Inbreeding depression is often delayed (i.e. they might not occur until several generations after inbreeding has begun). How quickly inbreeding depression occurs depends on the amount of inbreeding that has been produced and the trait.

The ideas that were described above can give farmers the erroneous idea that inbreeding is a major reason behind many of their production problems, so they come to the erroneous conclusion that inbreeding has occurred and their stock is no longer of good quality when they observe a deformed individual or yield has declined. Deformities and a decrease in yield are often due to non-genetic factors such as developmental errors, toxins, nutritional deficiencies, or weather and may not be due to inbreeding.

Because inbreeding is the mating of relatives, if individuals can be given unique tags, it is rather easy to prevent inbreeding or to minimize it by

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[8] An allele is an alternate form of a gene.
[9] e.g. Kincaid, H.L. 1976. Effects of inbreeding on rainbow trout populations. Transactions of the American Fisheries Society, 105:273-280; Kincaid, H.L. 1976. Inbreeding in rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada, 33:2420-2426; Su, G.-S.; Liljedahl, L.-E, Gall, G.A.E., 1996. Effects of inbreeding on growth and reproduction traits in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 142:139-148.
[10] Ryman, N. 1970. A genetic analysis of recapture frequencies of released young salmon (*Salmo salar*) L. Hereditas, 5:159-160.

preventing parent-offspring, brother-sister, and half-sib matings. If the closest mating allowed is between second cousins, inbreeding will never become a problem.

When conducting selective breeding programmes, inbreeding is inevitable, because when you only allow the best to reproduce, you often mate relatives. Minimizing inbreeding during a selective breeding programme is important, because you do not want to use the genetic gain produced via selection simply to counteract inbreeding depression. To prevent this, a number of breeding programmes have been designed to minimize inbreeding during a selective breeding programme.[11] While it is important to prevent the systematic mating of close relatives in selective breeding programmes, incidental (random) matings of close relatives (e.g., brother-sister matings) in large-scale breeding programmes is not as big a problem as it is in small populations, because it is likely that the offspring produced by these matings will be mated to non-relatives in the following generation, which will produce fish with no inbreeding.[12]

While it is easy to give livestock individual marks and thus prevent relatives from mating, it is rather difficult for fish. Therefore, aquaculturists must manage the population as a whole to minimize the accumulation of inbreeding. To do this, aquaculturists must manage the effective breeding number (N_e).[13]

One way N_e is determined is by the number of males and number of females that reproduce and leave viable offspring:

$$N_e = \frac{4 \text{ (number of females) (number of males)}}{\text{(number of females)} + \text{(number of males)}}$$

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[11] Dupont-Nivet, M.; Vandeputte, M.; Haffray, P.; Chevassus, B. 2006. Effect of different mating designs on inbreeding, genetic variance and response to selection when applying individual selection in fish breeding programs. Aquaculture, 252:161-170; Gallardo, J.A.; Lhorente, J.P.; García, X., Neira, R. 2004. Effects of nonrandom mating schemes to delay the inbreeding accumulation in cultured populations of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences, 61:547-553; 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; Inbreeding and Brood Stock Management. Fisheries Technical Paper. No 392. Rome, FAO.

[12] Dupont-Nivet, M.; Vandeputte, M. 2005. Does avoiding full sibs matings preserves genetic variability in a selection scheme? Case of single pair matings. Aquaculture, 247:12.

[13] Hallerman, E. 2003a. Inbreeding. Pages 215-237 *in* E.M. Hallerman, ed. Population genetics: Principles and Applications for Fisheries Scientists. Bethesda, MD, American Fisheries Society; Tave, D. 1993. Genetics for Fish Hatchery Mangers, 2nd ed. New York, Van Nostrand Reinhold; See footnote 14.

Thus, N_e is determined by the number of males that leave viable offspring, the number of females that leave viable offspring, and by the sex ratio of the brood fish. This means that N_e is maximized by increasing both the number of males and females that are spawned and by bringing the sex ratio as close to 1:1 as possible. Skewed sex ratios, which are often used in aquaculture, make N_e far smaller than the number of brood fish that are spawned. N_e is most strongly influenced by the least represented sex (e.g. when few males are used, N_e approximates the number of males rather than the total).
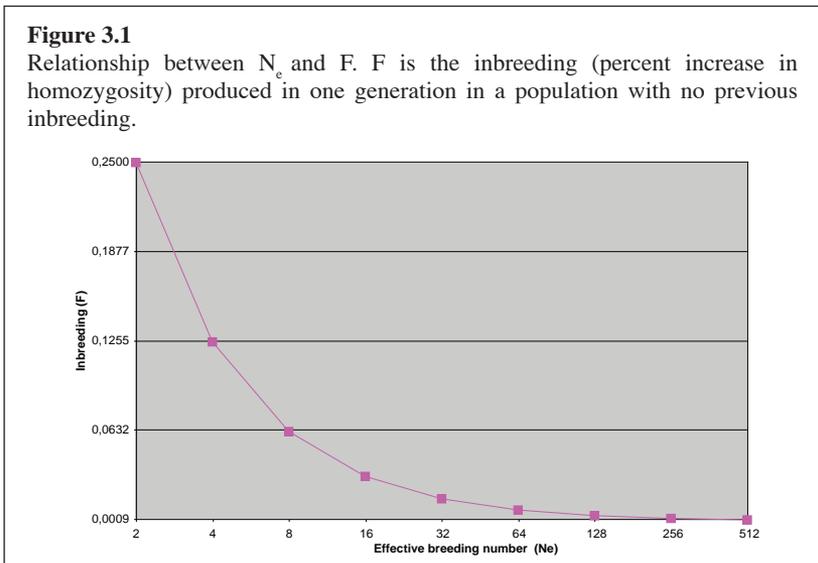
The reason aquaculturists and fisheries biologists need to manage N_e is that it is inversely related to inbreeding:

$$F = 1/2N_e$$

where F is the amount of inbreeding produced (0-100%) in a single generation; F is the percent increase in homozygosity. This formula shows that as N_e decreases, F increases (Figure 3.1); N_e s <50 produce large amounts of inbreeding per generation.

In a closed population, once inbreeding has occurred, it lowers the N_e of the next generation:

$$N_{eF} = N_e /1 + F$$

Figure 3.1
Relationship between N_e and F. F is the inbreeding (percent increase in homozygosity) produced in one generation in a population with no previous inbreeding.

where N_{eF} is the effective breeding number in a closed population with F >0%. For practical purposes, the total F that is produced over a series of generations can be calculated by summing the F that is produced in each generation, without considering previous inbreeding.

How big should N_e be to minimize inbreeding? Unfortunately, there is no universal value of F that aquaculturists or fisheries biologists want to avoid, which means there is no universal N_e. N_e s ranging from 30-500 have been recommended, 50 being the most common.[14] To calculate a desired N_e, one must determine what level of genetic risk is acceptable; in this case, it is the maximum amount of inbreeding that is desired after a given number of generations.[15] In addition, the concern about inbreeding, whether N_e needs to be managed, and how large N_e must be depends on the purpose/goal of the hatchery or fish farm, if fish are spawned, and how broodstock will be managed.

Figure 3.2 shows the constant N_e s that are needed to produce F of 1%, 5%, and 10% for 1-50 generations. The common recommendation of $N_e = 50$ is effective in minimizing inbreeding for a single generation (F = 1%), but it does a marginal job after 10 generations (F = 10%).

Figure 3.2
N_e needed per generation to produce F of 1%, 5%, and 10% after 1-50 generations.

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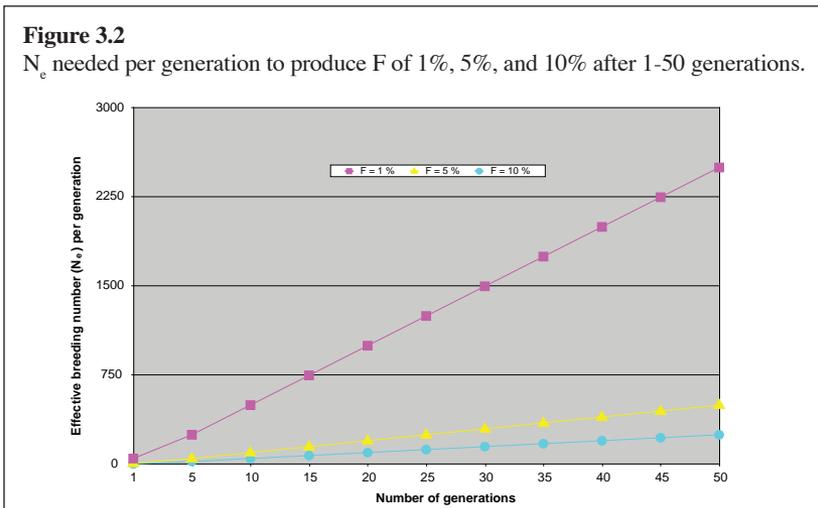
14   See footnote 11 and 13.
15   See footnote 11 and 13.

Farmers who acquire brood fish from a breeding center (Chapter 5), spawn them once and then acquire new brood fish, or farmers who simply acquire genetically improved fingerlings for grow-out from "multiplier" hatcheries every growing season (Chapter 6) do not have to worry about inbreeding or managing N_e. The breeding centers or multiplier hatcheries must manage their stocks to minimize inbreeding, but these farmers do not need to worry about inbreeding.

It may be difficult for subsistence and small-scale farmers who maintain and spawn their own broodstock to manage inbreeding, but they should be encouraged to try, because improving their animal husbandry skills will lead to increased productivity. If they maintain a constant $N_e = 50$ for 5 generations, they will keep $F \leq 5\%$. This recommendation produces good short-term (5 generations) management and this recommendation is not excessive, so many small-scale farmers could incorporate it into yearly work plans.

Large commercial farmers and those who produce fingerlings or conduct selective breeding programmes should try and keep $F = 5\text{-}10\%$, with $F = 5\%$ being the desired goal for 10-20 generations, so that selection and domestication aren't being used simply to counteract inbreeding depression. Those who raise fish for fisheries or conservation programs should try and keep $F = 1\text{-}5\%$, with $F = 1\%$ being the desired goal for a minimum of 20 generations, since the major management effort in these enterprises is to prevent changes in the gene pool over a long period.

A critical concept in Figure 3.2 is that the N_e's are constant N_e's. N_e can be larger than the desired number, but if it is smaller for just one generation, the inbreeding goal will not be achieved. This is because the mean N_e over a series of t generations is not the arithmetic mean, but the harmonic mean:

$$N_{e\ mean} = 1/t(1/N_{e1} + 1/N_{e2} + \ldots 1/N_{et})$$

This formula shows that the generation with the smallest N_e has a disproportional impact on mean N_e.

There are a number of management techniques that can be used to increase N_e. The most obvious way to increase N_e is to increase the number of fish that are spawned and produce viable offspring and to spawn a 1:1 sex ratio. One way to increase the number of brood fish that are spawned in order to satisfy production quotas is to keep only a small portion of each family. These simple ideas are contrary to standard fish culture management; aquaculturists tend to spawn as few fish as possible due to the fecundity of fish, and often use a highly skewed sex ratio because this enables them to maintain fewer brood fish.

A second technique is to switch from random mating (the normal practice at most hatcheries) to pedigreed mating.[16] In pedigreed mating, each female leaves one daughter and each male leaves one son as broodstock for the following generation (it can be more than one as long as all leave the same number). Pedigreed mating dramatically increases N_e, and when the sex ratio is 1:1, N_e is twice the number that spawn. However, to do this, each family has to be raised in a separate culture unit until fish can be marked to ensure that each parent leaves an offspring of the correct sex.

A third technique is to equalize the number of offspring from each mating, because unequal reproductive success lowers N_e.[17] However, to do this, each family must be raised in a separate culture unit until family size can be equalized.

A fourth technique is to modify stripping practices.[18] If fish are stripped, milt should not be pooled or added in a sequential manner. These practices cause gametic competition and one male can fertilize most of the eggs, producing an N_e much smaller than expected.

A fifth technique is to stretch generations. The desired N_e s in Figure 3.2 are given for generations, not years. A generation is the time interval for the replacement of parents with their offspring. If the goal is to keep inbreeding below a given value for 20 years and the normal procedure is to use a 2-year generation interval, 10 generations will be produced during the 20-year plan. But if the generation interval could be stretched to 3 years, only 7 generations would be produced during the 20 years, which means a smaller N_e could be used to achieve the desired goal.

Sixth, change the population from a closed to an open population. The above discussion assumed that the population is closed. If 10-25% new brood fish are imported each generation, the amount of inbreeding that is produced

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[16]   Tave, D. 1984. Effective breeding efficiency: An index to quantify the effects that different breeding programs and sex ratios have on inbreeding and genetic drift. Progressive Fish-Culturist, 46:262-268.

[17]   Fiumera, A.C.; Porter, B.A.; Looney, G.; Asmussen, M.A.; Avise, J.C. 2004. Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conservation Biology, 18:94-101.

[18]   Withler, R.E. 1988. Genetic consequences of fertilizing Chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture, 68:15-25; Withler, R.E. 1990. Genetic consequences of salmonid egg fertilization techniques. Aquaculture, 85:326.

can be drastically reduced.[19] In fisheries and conservation management, one approach is to capture and spawn wild brood fish or collect wild-spawned eggs and culture them. Care must be taken if brood fish are collected to avoid broodstock mining (i.e. reducing the number of fish that will spawn naturally in the wild to a dangerous level).

Seventh, a fish farmer can maintain two unrelated populations and produce hybrids. Hybrids have inbreeding of zero; hybridization is often used in plant and animal breeding programmes to eliminate or to counteract inbreeding. If multiple unrelated lines are maintained, a rotational mating programme can be used to prevent inbreeding for a number of generations.[20]

Finally, a factorial mating pattern can be used to increase N_e, which will minimize inbreeding.[21]

3.3 Genetic drift

Genetic drift is random changes in gene frequency—changes that are not due to selection, migration, or mutation. The causes of the random changes can be natural, such as a landslide that divides a population or a storm that kills a large percentage of a population or destroys portions of its habitat, or it can be man-made, which occurs when fish culturists acquire or spawn their fish.

Under normal conditions, the number of fish that reproduce and leave viable offspring is far less than the number of adults; this is especially true in aquaculture. When this subsample spawns, there is a chance that the frequencies of one or more genes will be different in the offspring than they were in the parental generation, and the fewer that are spawned, the more likely that changes will occur. The ultimate effect of genetic drift is the loss of alleles, and the lower the gene frequency the more likely the allele will be lost via genetic drift. Aquaculturists also cause genetic drift when they choose which fish they will buy for their foundation population. The acquisition of fish is critical, and small samples often produce what is called the founder effect—a condition where genetic drift creates a population in which the gene

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[19]   Bartley, D.M.; Kent, D.B.; Drawbridge, M.A. 1995. Conservation of genetic diversity in a white seabass hatchery enhancement program in southern California. American Fisheries Society Symposium, 15:249-258.

[20]   Kincaid, H.L. 1977. Rotational line crossing: An approach to the reduction of inbreeding ccumulation in trout brood stocks. Progressive Fish-Culturist, 39:179-181; See footnote 11.

[21]   Busack, C.; Knudsen, C.M. 2007. Using factorial mating designs to increase the effective number of breeders in fish hatcheries. Aquaculture, 273:24-32.

frequencies are markedly different from those of the population from which they originated. The founder stock determines the maximum genetic variance that will exist in a closed population.

The loss of genetic variance makes a wild population more vulnerable to extinction, because it has lost the genetic variability that might have enabled it to adapt to changes in the environment. A number of hatchery stocks have been evaluated, and no matter how much effort was expended in preventing genetic drift, it occurred and reduced genetic variance.[22] The loss of genetic variance via genetic drift was shown to prevent selection for increased growth rate,[23] and has been shown to increase the number of fish with developmental disorders.[24]

The relationship between N_e and genetic drift is:

$$\sigma^2_{\Delta q} = pq/2N_e$$

where $\sigma^2_{\Delta q}$ is the variance of the change in gene frequency (the way genetic drift is measured), and p and q are the frequencies of alleles *p* and *q* for a given gene.

As was the case with inbreeding, genetic drift is inversely related to N_e; the smaller N_e, the more likely that genetic drift will change gene frequencies. The effect that a reduction in N_e can have on gene frequencies via genetic drift is immediate.

Because it is difficult to prevent genetic drift in managed populations, genetic drift must be partitioned into acceptable and unacceptable changes for management purposes. A change in the frequency of an allele from, say, 0.4 to 0.38 might not be critical so that can be classified as acceptable, but

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[22] Allendorf, F.W.; Phelps. S.R. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Transactions of the American Fisheries Society, 109:537-543; Hallerman, E.M.; Dunham, R.A.; Smitherman, R.O. 1986. Selection or drift—isozyme allele frequency changes among channel catfish selected for rapid growth. Transactions of the American Fisheries Society, 115:60-68; Vuorinen, J. 1984. Reduction of genetic variability in a hatchery stock of brown trout, *Salmo trutta*. Journal of Fish Biology, 24:339-348.

[23] Tave, D.; Smitherman, R.O. 1980. Predicted response to selection for early growth in *Tilapia nilotica*. Transactions of the American Fisheries Society, 109-439-445; Teichert-Coddington, D.R.; Smitherman, R.O. 1988. Lack of response by *Tilapia nilotica* to mass selection for rapid early growth. Transactions of the American Fisheries Society, 117:297-300.

[24] Leary, R.F.; Allendorf, F.W.; Knudsen; K.L. 1985. Developmental instability as an indicator of reduced genetic variation in hatchery trout. Transactions of the American Fisheries Society 114:230-235.

the change in the frequency of an allele to 0.0 is critical and needs to be prevented, which means that is classified as unacceptable. Consequently, N_e must be managed to minimize the loss of alleles; since rare alleles are more likely to be lost than common ones, preventing the loss of rare alleles via genetic drift should be the management goal.

The probability of losing an allele of frequency q via genetic drift in a single generation is:

$$P = (1 - q)^{2N_e}$$

The probabilities of losing an allele (f = 0.001-0.5) for a single generation are shown in Figure 3.3. Figure 3.3 shows that small N_e s are needed to prevent the loss of common alleles (f $\geq$ 0.2), while large N_e s are needed for rare alleles (f $\leq$ 0.01).

When managing a population's N_e to minimize genetic drift, one must determine what genetic risk is acceptable; in this case, it is the desired guarantee of keeping an allele (1.0 - P) of a specific frequency after a given number of generations.[25] Geneticists and population biologists consider that an allele whose f = 0.01 contributes to polymorphism, so the goal of fisheries

Figure 3.3
Probabilities of losing an allele (f = 0.001-0.5) for various N_e s. These probabilities are for a single event (spawning season or acquisition of broodstock).
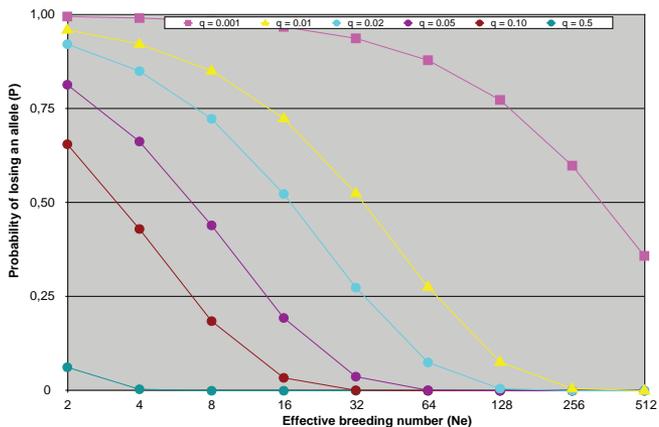
management and conservation programs should be to save alleles whose f = 0.01 (if this is done, more common alleles will also be saved). Saving rare alleles is not as important for food fish farming. If rare alleles improve viability, growth, and other culture traits, domestication will increase their frequency. Because of this, the genetic risk for food fish farmers can be to save alleles whose f = 0.05.

Constant N_e s needed to produce a 95% guarantee of saving alleles (f = 0.005-0.1) for 1-50 generations are shown in Figure 4. The methodology used to calculate these N_e s is described in an FAO book on managing inbreeding and genetic drift in hatchery populations.[26] It is easy to prevent the loss of an allele whose f ≥0.05, but it can be difficult when f ≤0.005.

As was the case for management of inbreeding, farmers who do not spawn fish or who only spawn fish once and then acquire new stock do not need to manage their population to minimize genetic drift. Even though most subsistence or small-scale farmers will not understand genetic drift or its consequences, many can easily incorporate management that will minimize its effects. If they maintain a constant N_e = 45 for 5 generations, they will produce a 95% guarantee of saving an allele whose f = 0.05. This recommendation is not excessive and produces good short-term (5 generations) genetic management.

For large commercial farmers and those who produce fingerlings or conduct selective breeding programmes, the goal of saving alleles whose f = 0.05 is easily achievable; N_e = 59 will produce a 95% guarantee for 20 generations. The goal of saving alleles whose f = 0.01 should be achievable for fisheries/conservation programs; N_e = 297 will produce a 95% guarantee for 20 generations. The values in Figure 4 are for a single allele. If there are 100 such alleles, a 95% guarantee means that 95 will be saved, while 5 will be lost. The management techniques that were described in the inbreeding section can also be used to increase N_e in order to minimize genetic drift.

Recommended N_e s to minimize genetic drift have ranged from 500-5 000, with 500 being the most common.[27] Figure 4 shows that the common recommendation of N_e = 500 will do a good job of minimizing genetic drift;

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[26]   See footnote 11.
[27]   Lande, R. 1995. Mutation and conservation. Conservation Biology 9:782-791; Hallerman, E. 2003b. Random genetic drift. Pages 197-214 *in* E.M. Hallerman, ed. Population genetics: Principles and Applications for Fisheries Scientists. Bethesda, MD, American Fisheries Society; National Research Council. 2002. Science and the Endangered Species Act. Washington, DC. National Academy Press; See footnote 11 and 13.

Figure 3.4

N_e needed per generation for 1-50 generations to produce 95% guarantees of saving alleles whose f = 0.1-0.005.
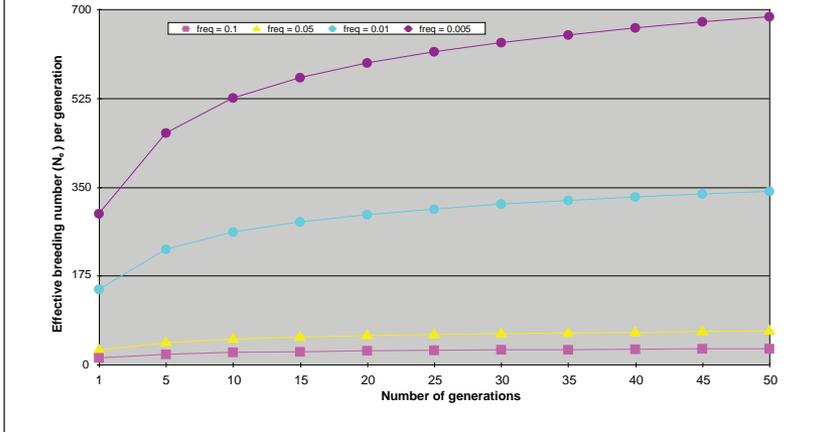

it will produce a 95% guarantee of saving an allele whose f = 0.01 for >50 generations. However, depending on the genetic goal (risk), the desired N_e can be less than 500, which is often the case for food fish farming.

Since both inbreeding and genetic drift are inversely related to N_e, it should be managed to minimize both. The information in Figures 3.2 and 3.4 can be combined to create a constant N_e to achieve both goals; to achieve both goals, the larger N_e must be used. Figures 3.5 and 3.6 list constant N_e s needed for food fish and for fisheries/conservation aquaculture, based on different levels of genetic risk.

Figure 3.5 shows that the N_e needed to minimize the damaging effects of inbreeding and genetic drift are not excessive and can be incorporated into most food fish operations. Even though genetic management is often considered either to be of little value or as inappropriate technology for subsistence or small-scale farmers, those who maintain and spawn their own broodstock can easily incorporate "moderate risk" (Figure 3.5) short-term (5 generations) management within the framework of routine work plans. $N_e = 50$ will keep F ≤5% and will also produce a 95% guarantee of keeping an allele whose f = 0.05 for 5 generations. In this case, the N_e needed to manage inbreeding is larger than the N_e needed to manage genetic drift, so

Figure 3.5

N_e needed per generation to minimize inbreeding and genetic drift in hatchery populations on food fish farms. N_e s are for three options (level of genetic risk): high risk--F $\leq$10% and a 95% guarantee of keeping an allele whose f = 0.05; moderate (acceptable) risk--F $\leq$5% and a 95% guarantee of keeping an allele whose f = 0.05; low risk--F $\leq$5% and a 95% guarantee of keeping an allele whose f = 0.05.
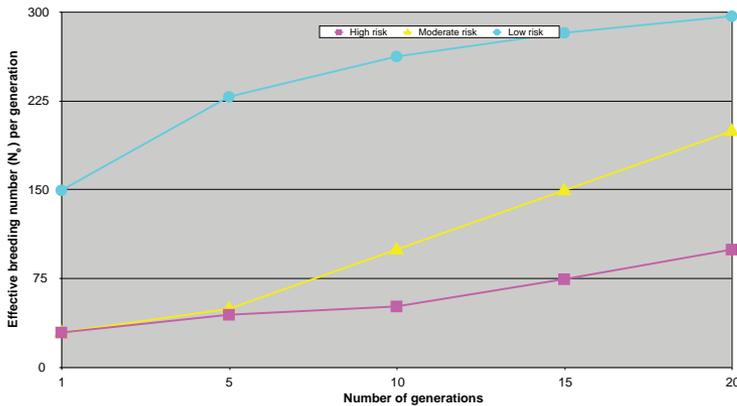

the inbreeding N_e is the one that is used. Because of this, extension agents only need to explain genetic management in terms of minimizing inbreeding, a concept that's easily understood, since most societies have taboos against consanguineous (blood-related) marriages.

If large commercial farmers, those who produce fingerlings, or those who conduct selective breeding programmes maintain a constant N_e = 100 (this includes the foundation stock), they can minimize genetic problems for 10 generations (F $\leq$5% and a 95% guarantee of keeping an allele whose f = 0.05 ("moderate risk" in Figure 3.5). However, if they acquire their stock from a farm where N_e was lower than 100, they will import fish that might already have accumulated a high level of inbreeding or suffered decreased genetic diversity and poor performance due to genetic drift.

The N_e s in Figure 3.6 are considerable larger, because managing a population's gene pool over a long period of time ($\geq$10 generations, with 20 generations being the desired minimum) should be the primary goal for fisheries/conservation-based aquaculture programs, and little genetic

Figure 3.6

N_e needed per generation to minimize inbreeding and genetic drift in hatchery populations that are used for fisheries or conservation management projects. N_e s are for four options (level of genetic risk): moderate risk--F $\leq 5\%$ and a 99% guarantee of keeping an allele whose f = 0.01; low risk--F $\leq 5\%$ and a 99% guarantee of keeping an allele whose f = 0.005; little risk--F $\leq 1\%$ and a 99% guarantee of keeping an allele whose f = 0.005; no risk--F $\leq 1\%$ and a 99% guarantee of keeping an allele whose f = 0.001.
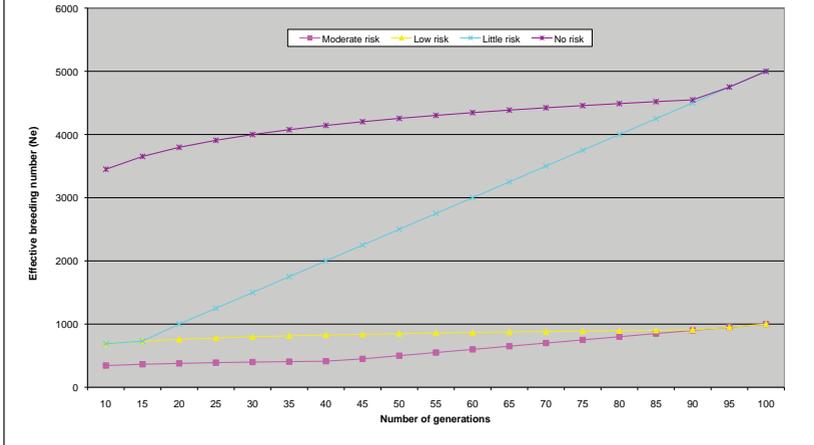
risk should be accepted. The constant N_e s needed for 20 generations are 378-1 000, depending on the genetic risk. Although the "no risk" option in Figure 3.6 is the most desired from a purely genetic view, it is unlikely that it can be incorporated from a management perspective. Consequently, the "low risk" or "little risk" options in Figure 3.6 are those that should be incorporated into this type of work. The "little risk" option in Figure 3.6 combines F $\leq 1\%$ with a 99% guarantee of keeping an allele whose f = 0.005 rather than f = 0.01, because by generation 20 the N_e s are identical. If the combination of F $\leq 1\%$ and f = 0.01 for less than 20 generations is desired, the N_e s in Figures 3.2 and 3.4 can be used to produce the desired N_e.

It has been suggested that an $N_e \geq 1,000$ will make a population "genetically secure." [28] An $N_e = 1,000$ will achieve "moderate" and "low risk" goals for 100 generations and will achieve the "little risk" goal for 20 generations (Figure 3.6).

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[28]   National Research Council. 2002.

The discussion about calculating N_e assumes that the population is fairly stable, which is often the case at a hatchery. When this is the case, the N_e for inbreeding and genetic drift is calculated as described earlier. However, when the population is small, fluctuates wildly over generations, or is declining, the N_e for inbreeding and genetic drift are different, because the effect of N_e on the population can be immediate (genetic drift) or delayed (inbreeding). When this is the case, N_e for genetic drift is called variance effective number (N_{ev}) [29] and is:

$$N_{ev} = \frac{4N - 2}{V_k + 2}$$

where N is the number of fish in the parental generation and V_k is the variance of offspring production.

N_{ev} is far more important for fisheries and conservation management than it is for food fish culture, because genetic drift can have a more damaging effect on the ability of the species to survive in the wild. If the only genetic goal of fisheries/conservation programs is to minimize genetic drift, N_{ev} should be managed; N_{ev} is estimated for each generation, independently from those of the previous generations. In this case, the common recommendation of N_e (N_{ev}) = 500 will do a good job of minimizing genetic drift from parents to their offspring for >50 generations (Figure 3.4). However, if N_{ev} of the population was small in previous generations, an N_{ev} = 500 will only keep genetic drift-produced problems from getting worse; it will not reverse genetic damage that has already occurred.

One reason to know the N_{ev} of hatchery-produced fish that are stocked for fisheries or reclamation projects and the N_{ev} of the wild population into which they are stocked is that the stocking program can actually decrease the overall N_{ev}.[30] For example, if the N_{ev} of the stocked fish is small but if they contribute a disproportionate number of offspring to the next generation, the N_{ev} of the population in the wild can decline. Another way N_{ev} can decline is if hatchery fish produce large families, while wild parents produce small families; this

[29] Waples, R. S. 2002. Definition and estimation of effective population size in the conservation of endangered species. Pages 147-168 in S.R. Beissinger; D.R. McCullough, eds. Population Viability Analaysis. Chicago. The Unviersity of Chicago Press.

[30] Ryman, N; Laikre. L. 1991. Effects of supportive breeding on the genetically effective population size. Conservation Biology, 5:325-329; Waples, R.S.; Do, C. 1994. Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. Canadian Journal of Fisheries and Aquatic Sciences, 51 (Supplement 1):310-329.

increases the variance of offspring production, which can actually produce an overall N_{ev} that is smaller than the combined N_{ev} s.

3.4 Domestication

Domestication is a form of selection that makes an organism more adapted to the culture environment and to all aspects of the management that is used to raise it (i.e. it changes a population's gene pool by selecting for alleles that are able to exploit culture conditions and by eliminating alleles that are less fit in the hatchery). Domestication is a combination of intentional and unintentional selection. Domestication changes the gene pool, so these changes are transmitted to subsequent generations and, over time, the population becomes markedly different. In agriculture, all of the important plants and animals that are raised for food have become domesticated; aquaculturists, on other hand, raise animals and plants that are not domesticated. Domestication is a term that is difficult to quantify, because there is no absolute line that divides wild from domesticated; instead it is continuous process (Domestication has been defined as defined as continuous controlled reproduction for more than 3 generations).[31] We can see an end product (domesticated) and a beginning (wild), but every aquacultured organism is somewhere along the continuum; most are nearer the beginning than the end. Domestication begins when fish culturists take control of the fish's life cycle and determines the conditions under which the fish will be raised (the kind of feed that is used; the stocking rate; water quality management, etc.) and, most especially, which fish will be spawned.

Domestication can change a population in subtle ways due to unintentional selection as the fish adapts to the way a farmer or hatchery manger operates the facility. For example, the way a farmer spawns his fish can produce a stock that responds more readily to hormone injections, or the spawning season can shift if a hatchery manager only spawns fish at the beginning of the spawning season. Choosing brood fish from the first seine haul could select for fish that are easy to harvest, while choosing the fish that remain in the pond after several seine hauls could select for fish that are escape artists. Raising fish under intensive culture condition selects for fish that tolerate degraded, stressful living conditions. Discarding brood fish that thrash about and that are difficult to handle can produce more docile fish.

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[31]   Bilio, M. Controlled reproduction and domestication in aquaculture. The current state of the art. Part II. Aquaculture Europe, 32 (3): 5-23.

Simple behavioral modification may or may not be a component of domestication. For example, fish learn when they will be fed and respond to the sound of a feed truck or the noise the feeder makes. This behavior is desirable, because it ensures that the feed will be eaten quickly and less will be wasted. If such behavior has a genetic basis, then it is a part of domestication, but if this learned behavior does not have a genetic basis then it will not be transmitted to the next generation, and it is not domestication.

Domestication is beneficial in food fish culture, because it produces more docile fish, those that thrive on artificial feed which increases growth rate, and those that tolerate crowding, handling, and degraded water quality conditions that might induce stress and subsequent disease. Domestication has been shown to increase growth rate of farmed fish by 2-6% per generation.[32]

However, aquaculture also cultures fish that will be stocked in the wild to support culture-based fisheries (Chapter 8) and in endangered species recovery programmes (Chapter 9), and for this type of management, domestication is detrimental because it can produce unwanted changes in the genome.[33] Unfortunately, the simple act of spawning fish, the way they are spawned, or raising them creates domestication selection, which could produce fish that are less fit in the wild. For example, survival in the wild is tenuous and most fish die when they are fry. Aquaculturists create environments that maximize survival, so genotypes that would have been maladaptive in the wild are not lethal at a hatchery. Improving early survival in a hatchery, a routine practice in aquaculture, is a form of domestication selection that has been shown to have an indirect effect of selecting for smaller egg size which, in turn, decreases viability in the wild. Even if the only form of fish culture is the collection of wild eggs, culturing the fry/fingerlings for a brief period, and then stocking them, domestication selection will occur if survival and reproductive success of the stocked fish is different in the wild than that of their wild counterparts.

Management practices that minimize domestication for fish that will be stocked in the wild are listed in Chapter 8.

[32] Dunham, R.A.; Smitherman, R.O. 1983. Response to selection and realized heritability for body weight in three strains of channel catfish, *Ictalurus punctatus*, grown in earthen ponds. Aquaculture, 33:89-96.
[33] Araki, H.; Cooper, B.; Blouin, M.S. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science, 318:100-103; Heath, D.D.; Heath, J.W.; Bryden, C.A.; Johnson, R.M.; Fox, C.W. 2003. Rapid evolution of egg size in captive salmon. Science, 299:1738-1740.

3.5 Constraints and opportunities

A major constraint to improve management in this area is the simple fact that most aquaculturists are poorly trained in genetics and feel that this type of management is not necessary—often because they do not understand it. A second constraint is that many aquaculturists are unaware of the long-term benefits to be derived from incorporating genetic management programmes (Chapters 4 and 6), and routinely raise fish that are inbred or have reduced genetic diversity and do not perform as well as genetically undamaged stock would. They also do not realize that improvements in other aspects of fish husbandry (e.g. better feed) may only offset decreases in genetic potential. There are increased costs associated with minimizing inbreeding and genetic drift, but these will be offset by improved production (Chapter 6). A second constraint occurs when hatchery managers and resource managers are erroneously rewarded for producing lots of fish, but fish that do not necessarily perform well because proper genetic management and evaluation aren't done. Fish culturists and resource managers must understand that producing fewer fish that perform better actually improves productivity and resource management. A third constraint is financial in that many hatcheries cannot be expanded, rebuilt, and extra labour cannot be hired, so it is not possible to spawn the required number of fish or minimize domestication for fisheries/conservation programmes. Finally, incorporating genetic management into fish culture must go hand in hand with good aquaculture husbandry and developments in nutrition or fish health.

Fortunately, many leaders and policy-makers are beginning to understand that management of genetic resources is not an abstract concept, but one that can improve food security and ecological stability.

4   GENETIC IMPROVEMENT METHODOLOGIES IN AQUACULTURE [34]

4.1 Introduction

The FAO Code of Conduct for Responsible Fisheries contains several articles that address issues specific to the application of genetic improvement methodologies in aquaculture (Articles 9.1.2, 9.1.3, 9.3.1 and 9.3.3). These articles refer to the risks associated with the development of aquaculture, and specifically of the development and spread of genetically altered stocks and to the genetic and environmental integrity of natural ecosystems. This chapter focuses on reviewing the methodologies used in the genetic improvement of aquaculture species and the extent of their current and likely future application in aquaculture. These methodologies are considered with a view to their short and long term benefit to the development of aquaculture but with references made to the risks posed by their adoption and application. These risks are described in more detail in Chapters 7 and 9. Guidelines are presented for consideration in the application of the various genetic improvement technologies in aquaculture.

As has been indicated in other chapters most forms of human intervention in more than one generation of the life cycle of cultured species will genetically alter the stock through changes in gene frequencies. Chapter 3 has outlined the processes whereby this genetic alteration can occur, namely through inbreeding and genetic drift and the change of gene and allele frequencies (and associated phenotypic traits) through unconscious selection. These genetic changes are generally the result of ignorance of the genetic consequences of management of captive stocks through successive generations. This chapter focuses on the additional consequences associated with the deliberate genetic change of cultured stocks through the application of a range of genetic improvement technologies.

4.2 Genetic improvement in aquaculture

The modern era of genetic improvement in aquaculture has been underway since the mid 1970s with the initiation of selective breeding programmes in Norwegian salmon. It is only over the past two decades that there has been widespread acceptance that genetic improvement has an important role to play in aquaculture development and that very significant genetic gains

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[34]   Contributed by Graham C. Mair

can be achieved through the appropriate application of well planned genetic breeding programmes in aquatic species. As a result there are now many such breeding programmes being implemented worldwide.

There is also a changing perception of the role of genetics in aquaculture and the appropriate timing of genetic interventions. It is now more widely understood that it is important to manage genetic variation from the time that stocks are first domesticated (or introduced) in order to avoid deterioration of stocks through inbreeding, genetic drift and unconscious selection (see Chapter 3), to take advantage of domestication selection and to maximize potential for subsequent genetic improvement (Figure 4.1).

With many aquatic organisms used in aquaculture there are actually more options for genetic improvement than can be readily applicable in higher

Figure 4.1
Hypothetical illustration of the different scenarios that can arise with poor vs. good genetic management. Ignorance of genetic management issues has on numerous occasions resulted in a decline in performance of cultured stocks. The objective of a developing aquaculture sector should be to effectively manage genetic diversity in domesticated stocks enabling the benefits from domestication selection to be realised. Management and retention of genetic diversity provides the raw material for the success of selective breeding at such time as an enterprise or production sector reaches a level of maturity, value and/or economic sustainability as to trigger investment in planned genetic improvement.
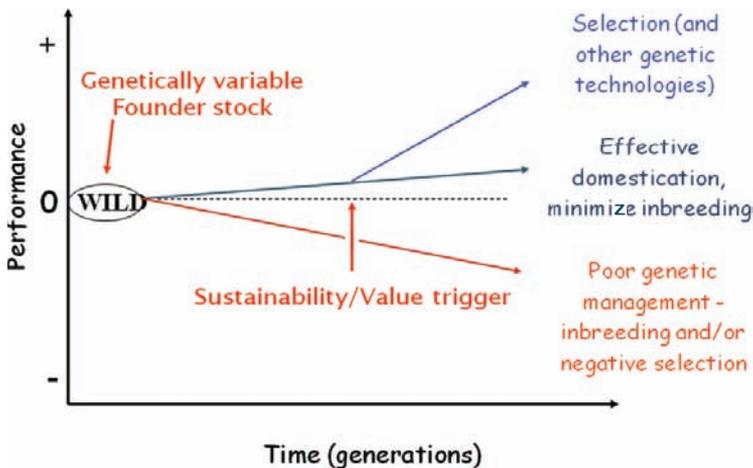
organisms as a result of their particular biological features and properties. Thus techniques such as chromosome set manipulation, sex control and transgenesis can be applied enabling many interesting improvement options to be explored.

While significant progress is being made and genetically improved stocks are becoming more readily available in aquaculture, the majority of cultured fish are still very similar to wild genotypes. There is a long way to go before aquaculture will attain the status seen today for livestock and crops where production is based almost exclusively on genetically improved varieties and the culture of wild germplasm is not practised.

4.3 Approaches to genetic improvement

This section briefly describes the various genetic improvement methodologies applicable to cultured aquatic species, summarises the progress being made in modern day aquaculture and identifies the key issues for optimising benefits to aquaculture and minimizing risks to the genetic and environmental integrity of wild stocks.

4.3.1 Selective breeding

The basis of selective breeding is to select individuals that possess a high additive genetic value for a desired phenotype (trait) as parents such that they can pass on their superior genes to progeny in following generations. In this way it should be possible to shift the mean value of the target trait for the cultured population in the desired direction in each successive generation. In selective breeding programmes it is necessary to minimize loss of genetic variation (e.g. that might arise through inbreeding) during this process to ensure that genetic gains are achieved and sustained for many generations.

Given the long term commitment implicit in initiating selective breeding, it is imperative that the stock to be improved is part of a commercially significant and sustainable aquaculture sector. It is therefore important to review the development and future potential for the sector in question prior to investing in any long term genetic improvement strategy.

A number of other conditions must be met prior to initiating a breeding programme. Firstly, the lifecycle of the species in captivity must be closed. It is then necessary to identify the **commercially valuable** trait or traits to target through a process of estimation of economic weights of traits that can

realistically be modified through genetic improvement. These traits should be variable within the population and preferably quantifiable in reproductively viable animals (traits such as fillet yield which are best measured in animals which have been sacrificed, present greater challenges for inclusion in selective breeding programmes). Ideally traits for selection should have moderate to high levels of heritability, and thus be likely to readily respond to even basic selective breeding. Heritability is a measure of how much of the variability in a particular trait is determined by genetics and varies from 0 (no genetic influence) to 1 (where genetics controls the entire trait). In more formal terms heritability is the proportion of phenotypic variance of a trait contributed by additive genetic variance and reflects the potential for generating a response to selection for that trait. Heritabilities of 0.15-0.5 indicate a trait will respond well to selection although there are procedures and statistical analyses that can effectively select for traits of low heritability.[35] It is also important to determine phenotypic and genetic correlations between traits particularly when developing selection indices in which two or more traits may be combined into a single index value.

A vital first step in initiating a selective breeding programme is the identification or development of an appropriate founder stock. To maximize the potential for long term genetic gain and enhancement of culture performance this founder stock should be highly genetically variable and should be based primarily on the best performing stocks available (where performance data is known or can be obtained). In reality this will likely involve the creation of a composite founder stock using source germplasm of varying origin, very often derived from different genetically discrete populations (domesticated and/or wild) as was the case in at the commencement of a major tilapia breeding programme known as GIFT.[36]

It is thus important to understand that well constructed founder stocks for selective breeding programmes will be genetically distinct from any individual wild population and thus represent a potential threat to the genetic integrity of those populations as, over time, gene frequencies in the wild could be changed by significant introgression of wild with genetically altered cultured

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[35]   Gjedrem, T. 2005. Selection and Breeding Programmes in Aquaculture. Springer, Netherlands. Van Vleck, L.D. (1993) « Selection Index and Introduction to Mixed Model Methods », CRC Press Inc., Florida, USA

[36]   Eknath, A.E., Bentsen, H.B., Ponzoni, R.W., Rye, M., Nguyen, N.H., Thodesen, J. & Gjerde, B. 2007. Genetic improvement of farmed tilapias: Composition and genetic parameters of a synthetic base population of *Oreochromis niloticus* for selective breeding. Aquaculture 273: 1-14.

stocks. As selective breeding progresses the genetic identity of selected stock is likely to diverge progressively further from any wild stock (See Chapters 3, 4, 8 and 9). One means to address this risk is to develop selected lines only from indigenous stocks (i.e. founders are taken from within discrete local populations) and to then limit their culture to sites within the natural distribution of that population. This option is however likely to be cost prohibitive. Selective breeding focuses on the improvement in quantitative traits (i.e. those phenotypes that are quantitative in nature and continuous in distribution) and growth rate is commonly the first trait considered for improvement. Qualitative traits (such as colour, body or fin shape or sex) are usually controlled by one or two gene loci and can be influenced through gaining an understanding of their inheritance. This is commonplace in ornamental aquaculture sectors but has little relevance to aquaculture for food production.

Growth rate is often the key trait of highest economic value in the majority of aquaculture sectors and this is especially true in extensive and semi-intensive systems where fish are sold whole or gutted and chilled rather than processed. Successful selection for growth can enable fish to be harvested at a larger and more valuable size, to shorten the culture period or possibly to stock animals at higher densities whilst maintaining harvest size. More intensive production systems often give relatively greater priority to traits such as food conversion and fillet yield due to their relatively greater economic importance, although these particular traits are more difficult to select.

It is beyond the scope of this chapter to review the different selection methods and breeding programme designs in any detail.[37] The decision on the method of selection to be used depends on a range of factors including the heritability of the target trait (if known), the type of trait, its ease of measurement in reproductively viable organisms and the biology and reproduction of the species (including fecundity). Table 4.1 summarises the key features of different approaches to selective breeding. The aim of any selection programme is to select the best individuals to be used to create the next generation without losing genetic variation. Breeding programme design invariably involves a series of compromises on what traits or combination of traits are selected for. Ideal designs will include fully pedigreed matings but are rarely feasible and are limited by physical and human resources, numbers of animals, marking/tagging systems (or the capacity to physically separate

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[37] Detailed manuals exist for example Tave, D. 1995. Selective breeding programmes for medium-sized fish farms. FAO Fisheries Technical Paper T352. FAO, Rome.

Table 4.1 Summary of main properties of the different options for selective breeding design. [a]

Type of Selection	Description	Advantages	Disadvantages
Individual or mass selection	All individuals are pooled and measured. Selection of those to use as future broodstock is based only on the phenotypic value for the target trait in the individual.	Easy and requires few records Individual identification and pedigree records are not required Relatively low cost Can make gains for traits with high heritability	Limited to traits with high heritability Can only be applied to traits that are measured on the breeding candidate Difficult to control inbreeding Important to control key environmental effects such as age, size at stocking, water quality, feeding etc. Potential for highly variable progeny contribution to future generations promoting genetic drift
Within cohort selection	A variation in which broodstock are arbitrarily divided into cohorts with individual selection carried out within cohorts. Inbreeding is avoided by rotational mating of selected individuals with those from other cohorts	Controls inbreeding Pair mating not required Individual identification and pedigree records not required Eliminates environmental effects on individual performance within cohorts Requires limited technical expertise	Works best with traits of high heritability Can only be applied to traits that are measured on the breeding candidate Still requires control of age within cohorts
Within family selection	Multiple families are maintained with individuals selected independently within each family based on the deviation of their trait value from the family mean.	Can control inbreeding by rotational mating between families Relatively easy to manage Useful where environmental variance is common to members of a family Easy to control environmental variance for each family Allows for high selection intensities	Does not fully exploit heritability and thus response to selection is reduced Does not take account of variance in performance between families Can only be applied for traits that can be measured on the breeding candidate Families must be maintained separately or all individuals marked by family

[a] A useful summary of the key features of these approaches to selective breeding are presented in Ponzoni, R.W., Nguyen, N.H & Khaw, H.L. 2006. Importance and implementation of simple and advanced selective breeding programs for aquaculture species in developing countries. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, 13-18 August 2006, Belo Horizonte, MG, Brazil. (Web reference: http://www.wcgalp8.org.br/wcgalp8/articles/paper/9_683-1814.pdf)

Table 4.1 (continued) Summary of main properties of the different options for selective breeding design

Type of Selection	Description	Advantages	Disadvantages
Combined selection	Selection of individuals is based on individual information and information from relatives. Mean values for each family is determined and whole families are retained or culled depending on this mean.	Fully exploits additive genetic variance Inbreeding can be controlled Good for traits with low heritability Good for traits that can't be measured in breeding individuals (e.g. fillet yield) or that introduce risk such as disease challenge tests, as siblings can be selected Models developed to take account of all systematic effects on the target trait	Requires pair mating and family identification Generally expensive requiring large facilities and labour intensive. Costs are reduced if families are marked and stocked in common environment. Requires high levels of genetics expertise Common environment effects should be minimized and family size maintained Requires detailed record keeping including pedigrees Generally need to rear individual families up to tagging leading to tank effects during separate rearing

Adding value to selection	Description	Advantages	Disadvantages
Pooled families and genotyping	Where genetic markers exist that can enable pedigree assignment, individuals to be selected can be pooled at a very early stage and their family of origin determined at harvest.	Permits families to be reared in a common environment throughout the production cycle Defers some costs until harvest reducing risk Less resources required	Differential survival in pooled batches, especially at young ages can lead to uneven family contribution. Family data only obtained at harvest Need to identify selected fish whilst genetic marker analysis is being conducted.
Selection indices	A selection index can be generated based on several commercially important traits weighted by their relative importance. Used as standard in combined selection.	Optimizes gains for multiple trait selection	Requires economic weights for the traits in the breeding objective

families and individuals) and the properties of the species (such as fecundity, generation time and ease of controlled breeding).

With the recent initiation of numerous selective breeding programmes some impressive results are starting to emerge in terms of response to selection with gains in key traits of 13 to 15% per generation being possible in well managed and well resourced breeding programmes. These gains are greater than commonly achieved in livestock breeding programmes and may be a function of the high levels of genetic variability found in cultured aquatic species and the high fecundity of many species enabling higher selection intensities than is possible in less fecund livestock. Clearly the economic value of such significant gains, over several generations, are very significant and Gjedrem's[38] estimate of a 15:1 return on investment in the Norwegian salmon breeding programme is not unrealistic (see also Chapter 6).

Given the relative infancy of selective breeding in aquaculture the selected stocks that are being developed are not yet highly phenotypically divergent from wild types in most cases and are still able to survive in the wild and to interbreed with wild relatives if they escape or are deliberately introduced into the environment from which they were derived. Over time with continued selection for commercially important traits this level of phenotypic divergence will increase to the extent that selected lines will be so specifically adapted as to be dependent on the culture environment and unable to thrive or reproduce away from it as is the case nowadays for many of our livestock and crop species. It is difficult to estimate how long this transition will take but it is likely to be many decades before the majority of aquaculture stocks become this divergent from wild types. During this transition phase selectively bred stocks represent a risk to the genetic integrity of wild stocks and steps should be taken to assess, manage and minimize this risk (Chapters 7&9).

4.3.2 Hybridization and cross-breeding

Hybridization is breeding individuals from two separate species while cross-breeding is the mating of two different varieties/stocks 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

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[38]   Gjedrem, T. 2000. Genetic improvement of cold-water fish species. Aquaculture Research 31: 25-33.

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. When evaluating heterosis it is important to evaluate reciprocal crosses as heterosis can vary depending on the maternal or paternal parent species/stock.

Both cross-breeding and hybridization are relatively simple techniques to master and can have an immediate impact on performance within one generation. However, this benefit is finite and only optimized in the specifically targeted hybrid cross between the original parental lines, unless the parental lines are then selected over generations for their general or specific combining ability, resulting in complex and relatively slow breeding programmes. Some heterosis can be retained in later generations if the numbers of populations crossed is high. Cross-breeding is thus usually looked upon as a potential supplement to a programme for additive genetic improvement, as mentioned above. For example it is possible to negate the effects of in-breeding that might occur in individual mass selected lines by generating production stock by crossing between two such lines. Evidence of significant heterosis for commercially important traits is relatively rare although substantial evidence for heterosis for growth suggests a role for cross-breeding in commercial improvement of oysters.[39]

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. While the use of hybrids in aquaculture may be under reported to some extent this relatively poor rate of return on hybridization research would indicate that commercial benefits of most hybrids are limited or nonexistent. There are a few examples where there has been commercial uptake of hybrids such as hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) in China and Israel, hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) in Thailand and Southeast Asia, and hybrid striped bass (*Morone chrysops* x *M. saxatilis*). The success of these hybrids is due to "complementary effect" (i.e. specific marketable properties of the combination of the parental species such as high % males in the tilapia hybrids and good product quality attributes in the hybrid catfish) rather than positive heterosis for quantitative traits.

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[39] Hedgecock, D., McGoldrick, D.J. & Bayne, B.L. 1995. Hybrid vigor in Pacific oysters: An experimental approach using crosses among inbred lines. Aquaculture 137: 285-298.

Where hybrids are produced commercially a major challenge is the risk of introgression of pure species parental stocks through contamination by hybrids (i.e. backcrossing of hybrids with parents). This should be avoided as once introgression occurs the system breaks down and performance of the *supposed* F_1 hybrids (which may actually be a mix of F_2 hybrids and backcrosses) will become inconsistent and unpredictable.

Given the relative ease of hybridization between closely related fish species, hybridization can be haphazard, as has been seen for example in the production of major carps in some countries. F_1 hybrids may be used either accidentally or deliberately as broodstock in backcrosses or in the production of F_2 crosses. Over generations this will lead to a general mixing and segregation of genes from the original parental species, known as introgression. With this independent segregation of the genes the resulting phenotypes are highly variable and some of the fish carrying the introgressed genes cannot be easily distinguished from the original pure species. Introgression is now relatively commonplace in tilapia and other interbreeding species groups, where hybrids can be easily produced either artificially or naturally. Where this is occurring in Chinese and Indian major carp (e.g. in Bangladesh[40] where hybrids were originally produced, either out of scientific interest or through reasons of shortage of broodstock of some species) hybrid introgression is very likely to have negative consequences for the widespread carp polyculture systems as a result of loss of the distinct feeding strategies of the pure species, with a resulting decrease in feeding efficiency and production. Where unplanned hybridization events are unavoidable the long term consequences of such ad hoc hybridization events can be minimized if systems are in place that exclude hybrids from use as future broodstock.

It is thus recommended that hybridization in aquaculture should be avoided unless it is part of a systematic strategy to exploit heterosis or complementary effects for commercially important traits. The major challenge in planned cross-breeding and hybridization is to ensure that it is used appropriately, that there are real economic benefits to a hybridization programme and that it is

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[40] Mia, M.Y., Taggart, J.B., Gilmour, A.E., Gheyas, A.A., Das, T.K., Kohinoor, A.H.M., Rahman, M.A., Sattar, M.A., Hussain, M.G., Mazid, M.A., Penman, D.J. & McAndrew, B.J. 2005. Detection of hybridization between Chinese carp species (*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci. *Aquaculture* 247: 267-273.
Simonsen, V., Hansen, M.M., Mensberg, K-L.D., Sarder, R.I. & Alam, S. 2005. Widespread hybridization among species of Indian major carps in hatcheries, but not in the wild. *J. Fish Biol.* 67: 794-808.

managed such that unwanted or uncontrolled introgression does not occur in hatchery or wild stocks.

4.3.3 Chromosome set manipulation

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

Androgenesis and gynogenesis are forms of induced uni-parental inheritance or parthenogenesis in which respectively the female or male genetic contribution is de-activated by some form of irradiation of gametes and the chromosome complement from the male or female is then doubled. The doubling of the haploid complement is achieved by application of physical (in finfish) or sometimes chemical (mainly in molluscs) shocks to restore diploidy. The resulting gynogenetic or androgenetic diploid progeny are highly or fully inbred depending on how diploidy is restored. Homozygous (i.e. 100% inbred) individuals can be used as the basis for producing isogenic clonal lines in which all fish are genetically identical. Gynogenesis and to a lesser extent androgenesis have 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, the rapid induction of inbreeding, genetic mapping and QTL (quantitative trait loci) analysis. Androgenesis can also be used in principle for recovering genotypes from cryopreserved sperm where eggs from the same species are not available. There have however been very few commercial applications of these technologies.

Polyploids are produced in a similar way with application of physical or chemical shocks to normal fertilized eggs with disruption of the second meiosis resulting in triploids with two maternal and one paternal chromosome set. Disruption of mitosis produces tetraploids with a duplicate diploid chromosome complement. Triploids have been produced in a wide range of fish and mollusc species and can be achieved on a commercial scale for some species provided that large scale artificial fertilization of eggs is possible. Viable tetraploids have been produced in only a few commercially important species, predominantly salmonids and oysters.

The main application of chromosome set manipulation in aquaculture 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, triploid finfish generally do not grow faster than their diploid counterparts[41] 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.

The most significant commercial application of chromosome set manipulations are in bivalve shellfish where triploids are cultured widely, for example approximately 50 percent of cultured oysters produced in the United States of America and France are triploids. 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 in Penaeid shrimp. Triploids can also be produced from diploid x tetraploid matings in the few species in which tetraploids have been produced and shown to be viable, most notably in oysters[42] and this is likely to represent a more commercially viable and reliable means of mass producing triploids.

Sterile triploids are likely to grow in importance in aquaculture for protection of breeders rights 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. These factors are vital in determining the risk that escapes or releases from culture will be reproductively viable. 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 intellectual property protection have significant value.

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[41] Tiwary, B.K., Kirubagaran, R.& Ray, A.K. 2004. The biology of triploid fish. *Reviews in Fish Biology and Fisheries* 14, 391-402.

[42] Guo, X. & Allen, S.K. 1994. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs from triploids. *Molecular Marine Biology and Biotechnology* 3: 42-50.

4.3.4 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 or near monosex populations can be generated through manual sexing, hybridization, selection and direct and indirect use of hormonal sex reversal. Manual sexing is labour intensive and inefficient and hybrid crosses only apply to specific combinations of species, again notably in the tilapias. It has also recently been shown that there is a genetic basis to temperature dependent sex differentiation in tilapia such that the percentage of males resulting from high temperature treatment of fry can be increased by selective breeding[43]. The most applicable methods for producing monosex stocks are through direct sex reversal using hormones or indirectly through genetic breeding programmes. Direct sex reversal can generally be applied regardless of the sex determining system and has been successfully achieved in a range of species by immersion of eggs and fry in hormone solutions or by feeding with hormone treated diets.[44] The indirect approach through the application of breeding programs, however, requires 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 and some tilapia or female heterogamety (WZ female; ZZ male) as in some tilapias and crustacea. Figure 4.2 illustrates alternative breeding programmes to produce all male progeny in species with female heterogamety and all female progeny in species with male hetorerogemety.
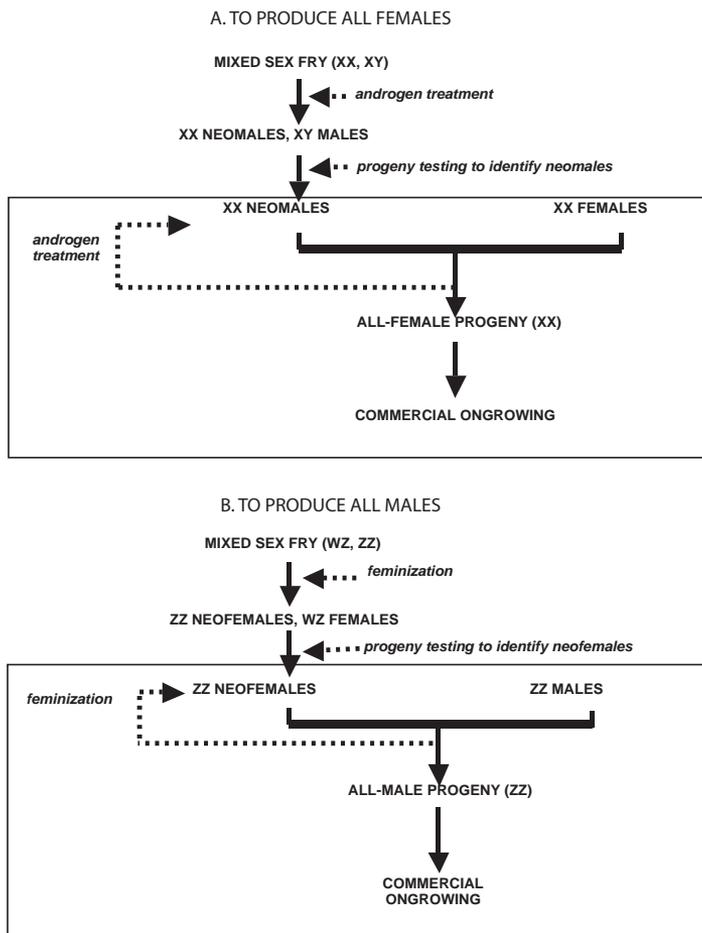
The potential benefits of monosex female stocks in salmonid aquaculture have been long established and relate to the enhanced availability of female broodstock and to avoiding precocious maturation of males which results in

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[43]   Wessels, S. and G. Hörstgen-Schwark. 2007. Selection experiments to increase the proportion of males in Nile tilapia (*Oreochromis niloticus*) by means of temperature treatment Aquaculture 272, Supplement 1: S80-S87.

[44]   Piferrer, F. 2001. Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture* 197: 229-281.

Figure 4.2
Illustrations of breeding programmes for production of monosex females (A) for species with male heterogamety and monosex males (B) in species with female heterogamety. Components within the boxes are those steps that can be repeated in cycles as part of commercial production in order to maintain supply of sex manipulated broodstock.

A. TO PRODUCE ALL FEMALES

MIXED SEX FRY (XX, XY)

androgen treatment

XX NEOMALES, XY MALES

progeny testing to identify neomales

XX NEOMALES   XX FEMALES

androgen treatment

ALL-FEMALE PROGENY (XX)

COMMERCIAL ONGROWING

B. TO PRODUCE ALL MALES

MIXED SEX FRY (WZ, ZZ)

feminization

ZZ NEOFEMALES, WZ FEMALES

progeny testing to identify neofemales

ZZ NEOFEMALES   ZZ MALES

feminization

ALL-MALE PROGENY (ZZ)

COMMERCIAL ONGROWING

reduced growth, poorer survival and loss of flesh quality post-maturation. Use of all female progeny produced using sex reversed (neomale) broodstock is not universal but there are sectors in some states in which significant proportions of production are monosex.

Monosex male stocks have considerable commercial benefit in a number of species, most notably in tilapia due to problems of both precocious maturation and unwanted reproduction within the production system exhibited by this species. These can also be produced by either direct or indirect masculinization. Sex reversal to male has been achieved in a range of finfish through application of exogenous androgens such as through the administration of diets treated with methyltestosterone during the early life of the animal and is commonplace in tilapia hatcheries worldwide. Breeding programmes for all-male production are relatively easily achieved in female heterogametic species such as blue tilapia (*Oreochromis aureus*) and the giant freshwater prawn (*Macrobrachium rosenbergii*). 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.*[45]

Sex control programmes are only of relevance to some species for which significant economic benefits will accrue from culture of monosex stocks. Direct induction of sex change by use of hormones is likely to meet social resistance from potential consumers of the treated fish even though studies have shown that excess exogenous hormone disappears from the tissues of the fish shortly after cessation of treatment. More acceptable would be the use of 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.

Sex control can be used as a form of biological containment on the basis that any introductions or escapes from aquaculture would not be able to breed with each other and thus be unable to form sustainable feral populations. Cultured stock would need to be guaranteed 100% monosex for this technology to be

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[45] Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A. & Beardmore, J.A. 1997. Genetic manipulation of sex ratio for the large scale production of all-male tilapia *Oreochromis niloticus* L. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 396-404.

an effective form of containment and it could only be applicable where there are no reproductively compatible species in the receiving environment.

4.3.5 Transgenesis

Transgenesis is a genetic engineering technology wherein an isolated gene sequence from one organism is inserted into another organism to confer a new or modified trait. This isolated gene sequence is called a construct and is composed of a functional gene and a promoter gene that acts as a switch to activate the functional gene. Organisms resulting from successful transgenesis are classed as genetically modified organisms (GMO) and thus subject to societal and regulatory concerns. Early research used foreign gene constructs from other species, including terrestrial species. When planning transgenic research it is very important to be fully aware of the risks and concerns over ethics, human health and environmental impacts of transgenic fish and to understand the policy environment in which the research would be conducted and under which any products of the research would be regulated. A recommended response to the risks and concerns over transgenic fish is to focus research and development where appropriate on the production of autotransgenics in which the gene sequence introduced is derived from the same species.

Transgenesis has been a major area of research in fish genetics since the early 1990s. Research in this area is more advanced than in other livestock due to the relative ease of manipulation of reproductive biology in aquatic species. The induction of transgenesis should involve a number of steps: identification of the appropriate 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 final step in this sequence is of critical importance as it will be necessary to fully characterise the properties of the transgenic fish in order to be able to assess the potential risks associated with its culture. 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 gene constructs. Research has also targeted other traits such as disease and control of reproduction and the focus of transgenic research should be on traits which are difficult to improve through quantitative approaches. Transgenic fish can also be considered as useful models for studies of gene regulation and gene expression and have potential as biofactories to produce valuable pharmaceuticals.

The aforementioned development steps have been successfully completed in a number of finfish species and transgenic lines produced with sometimes dramatic increases in growth performance.[46] Clearly transgenesis has the potential to bring about fairly rapid changes in commercially important traits but awareness of associated risk is critical to planning and implementing such research (see Chapter 7).

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 of America. At the time of writing there is an important test case in which the US Food and Drug Administration (FDA) is evaluating an application for a licence to commercialise a transgenic salmon species for use in aquaculture. While this is a lengthy process any conditional approval by the FDA (likely to be limited to closed, land based production systems) will represent a model for consideration of approvals for commercialisation of other lines of transgenic fish in other states.

Whilst there are some technical reasons behind the lack of commercialisation of transgenic fish the main reason is the concern over the ethical, animal welfare, human food safety and environmental risks associated with the culture of transgenic fish. Policy-makers, resource managers and those contemplating the use of GMOs should become very familiar with issues of environmental risk assessment and management with transgenic fish, both in research and potentially in commercial production.[47]

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.

4.3.6 Genetics markers and marker assisted selection

A genetic marker is a variation in a gene or sequence of DNA that can be identified by molecular techniques and used to allow identification of

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[46] FAO. 2000. *The State of World Fisheries and Aquaculture* 2000. Rome. 142pp
[47] Kapuscinski, A.R., Hayes, K.R., Li, S. & Dana, G. (eds). (E. M. Hallerman and P.J. Schei, series editors). 2007. Environmental Risk Assessment of Genetically Modified Organisms, Vol 3: Methodologies for Transgenic Fish. CABI Publishers. 310 pp.

genotypes and thus identify individuals or groups of interest. Prior to the advances in molecular genetics, 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. Table 4.2 summarises the potential applications for genetic markers in and around aquaculture and lists the preferred markers for different priorities.

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 (mostly in research but there is increasing commercial application) as more enterprises invest in genetic programmes and the cost of genetic marker analysis falls.

The most common application of markers in genetic programmes currently is in parentage assignment in which the efficiency of selective breeding programmes can be enhanced by using genetic markers to identify selected fish to families and thus to individual parents. The removal of the need to keep families separate (either throughout the performance evaluation or at least until they can be physically marked) should reduce the problem of environmental effects on family performance and enable more families to be evaluated which can produce higher selection intensities and increase the response to selection.

In an ideal breeding programme, genetic markers can be used to: (1) characterise potential founder stock(s) to inform the development of a genetically variable base population; (2) to understand natural population structure to inform both the formation of founders stocks and the assessment of the risks posed by culture of genetically altered stocks; (3) to enhance the efficiency of selective breeding through pedigree assignment and (4) to characterize the longer term impact of domestication and genetic management (or mis management) of captive stocks (e.g. to determine loss of genetic variation where effective population size is sub optimal).

Genetic markers can also be used to construct genetic maps in which linked markers are assigned to linkage groups and ultimately to individual

Table 4.2 Description of practical applications of genetic marker technologies in aquaculture.[a]

Technique	Description	Applications	Remarks
Strain/stock identification	A suite of genetic markers can be used to identify markers that are diagnostic for species or stocks. Preferred markers: Microsatellites, SNPs, AFLP, Isozymes and RAPD.	• Discriminating different cultured stocks including protection of breeders rights on improved stocks • Identifying intentional or unintentional hybrid introgression • Identification or confirmation of escapes from aquaculture • Identification of recapture rates in enhanced fisheries	Markers (of different types) diagnostic for species have been developed for many commercially important species and can be readily accessed. Markers for different stocks or strains generally need to be developed. Such markers may become increasingly important for traceability of aquaculture stocks.
Quantification or characterization of genetic variation	Genetic markers can be used to quantify and characterise levels of genetic variation such as number of alleles per locus, proportion of polymorphic alleles and average heterozygosity. Preferred markers: AFLP and microsatellites.	• Determining likelihood and severity of inbreeding • Estimation of current and historic effective population sizes (N_e) • Comparing the merits of candidate founder stocks for base populations for selective breeding • Confirmation of homozygosity in double haploids	In genetic programs it is useful to have a standard set of genetic markers to determine baseline levels of variability (along with other applications) in founder stocks so that longer term impacts of domestication and genetic management can be quantified.
Determination of genetic relationships among stocks	Markers can be used to construct genetic relationships between multiple stocks such as phylogenetic trees. Preferred markers: mtDNA, microsatellites, AFLPs	• Identifying the origin (e.g. source population) of cultured stocks • Determining the genetic structure of wild populations	It is very useful to understand the genetic structure of wild fish in order to assess the risks of genetic contamination from aquaculture based on genetically altered stocks and to inform develop policies on translocation. Also useful information when forming genetically variable founder stocks

[a] Adapted from Liu, Z.J. & Cordes, J.F. 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238: 1-37.

Table 4.2 (continued) Description of practical applications of genetic marker technologies in aquaculture

Technique	Description	Applications	Remarks
Parentage assignment (pedigree determination)	The use of a suite of genetic markers to determine the probability that an individual progeny is derived from a cross of specific two parents. Preferred markers: Microsatellites and SNPs	• Determine the numbers of broodstock contributing to progeny in pooled spawning and thus estimate N_e • Identify family of origin in selecting future broodstock to minimize inbreeding	Cost of application of marker systems for parentage assignment is reducing (especially for SNPs) and they are increasingly used for identification of families in breeding programmes. Can be a problem in how to identify fish whilst they are genotyped for assignment
Marker Assisted Selection	The selection of a specific marker known to be associated with a commercially important trait (known as a quantitative trait locus – QTL) rather than the traits itself. Requires genetic maps to be constructed. Preferred markers: SNPs, microsatellites and others	• Currently no commercial application in aquaculture • Few species have been adequately mapped • Potential to be developed for traits that are difficult to improve using traditional approaches	MAS is being used for genetic improvement for a small number of traits in livestock breeding but is not yet adequately developed or proven for aquaculture.

chromosomes. Genetic markers closely linked to genes that contribute to quantitative traits are known as quantitative trait loci (QTLs). Gene mapping programmes are now on going for several important aquaculture species including the Pacific oyster, salmonids, channel catfish, Nile tilapia, and European sea bass.[48] Linkage maps once developed, can be screened to identify QTLs of interest.

The QTL effect can then be quantified by correlating the inheritance of marker alleles with individual performance for the targeted trait. A number of QTLs for important traits have been identified in fish such as temperature tolerance, growth and disease resistance (e.g. cold tolerance in tilapia[49]).

Marker assisted selection (MAS) is the incorporation of genetic markers linked to QTLs into genetic improvement programmes and has the potential to enhance selection, particularly for traits which might have low heritability or which cannot be measured directly on the breeding individuals. Whilst there are a number of research efforts developing and evaluating QTL there are 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.

4.4 The current status of genetic improvement and future scenarios

It is difficult to estimate the proportion of global aquaculture production which is currently of domesticated stock but best estimates would indicate approximately 35% of aquaculture production is of undomesticated, essentially wild stock which are thus not adapted to captive environments. This compares with other forms of agricultural production which is almost exclusively of domesticated and genetically improved germplasm. The benefits of domestication in terms of adaption to captive environments is

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[48]   Garber, A.F. and Sullivan, C.V. 2006. Selective breeding for the hybrid striped bass (*Morone chrysops*, Rafinesque x *M. saxatilis*, Walbaum) industry: status and perspectives. *Aquaculture Research* 37: 319-338.

[49]   Cnaani, A., Hallerman, E.M., Ron, M., Weller, J.I., Indelman, M., Kashi, Y., Gall, G.A. and Hulata, G., 2003. Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid. *Aquaculture,* 223(1-4): 117-128.

such that it is recommended that any significant and potentially long term sustainable aquaculture industry should initiate domestication programmes in which the genetic diversity of stocks is effectively managed.

The proportion of global aquaculture production which is based on genetically improved stock (mostly selectively bred but also including monosex and triploid stock) is estimated to be between 10 and 20% so clearly there is scope for very significant increases in production and production efficiency through the widespread implementation of effective genetic improvement programmes with a focus on selective breeding.

Among the challenges facing the future development of aquaculture genetics is the relative lack of resources to support the development of breeding programmes. The resources in deficit include physical, economic and human resources. Well run breeding programmes generating strong genetic gains utilise facilities enabling the raising of multiple families of fish. Given their long term nature, they require sustained funding as it can often be many years before returns on investment in genetic programmes are fully realised through increased income from seed production or through improved production efficiencies (Chapter 6). A further limitation lies in human resources particularly in the area of quantitative genetics which generally requires specialised training to a relatively high level.

There seems little doubt that the consistently rising demand for aquaculture produce will continue to drive the search for improved production efficiencies and genetic improvement is becoming a major component of these efforts. Genetic improvement programmes are set to transform aquaculture stocks over the coming decades with selective breeding at the core of these programmes but with other technologies adding value to these efforts where clear benefits are apparent. Where states are promoting and/or investing in the expansion of aquaculture it important to be aware of the basic principles of genetic management, the most cost effective approaches to genetic improvement and the environmental and ecological risks associated with the widespread uptake of improved stock by producers. Alongside these technological factors the provision of adequate resources to support the implementation of long term genetic improvement strategies is also critical.