CHAPTER 4

CURRENT STATUS AND OPTIONS FOR BIOTECHNOLOGIES IN AQUACULTURE AND FISHERIES IN DEVELOPING COUNTRIES

SUMMARY

The rapid growth of aquaculture has significantly benefited from both conventional technologies and biotechnologies and it is expected that advanced biotechnologies will further help the sector in meeting the global demand for aquatic food in the coming decades. While biotechnologies are being applied in fisheries management, their use is very limited compared with aquaculture. The four main areas where biotechnologies have been used in aquaculture and fisheries include genetic improvement and control of reproduction; biosecurity and disease control; environmental management and bioremediation; and biodiversity conservation and fisheries management.

One of the main reasons for the success of aquaculture is the diversity of species currently in culture (over 230) and the genetic diversity that can be exploited through captive breeding and domestication. However, the rearing of many newly cultured species is to a large extent based on juveniles and/or broodstock obtained from the wild. In order to establish practical breeding programmes to produce seed in hatcheries, it is necessary to have a detailed understanding of the complete production cycle. Such knowledge is also required to disseminate breeding improvements to the production sector. Improvements that allow the wider application of appropriate genetic and reproduction biotechnologies will undoubtedly increase aquaculture production, thus contributing to global food production. These biotechnologies include polyploidy, gynogenesis and androgenesis, the development of monosex populations and cryopreservation.

Disease outbreaks are a serious constraint to aquaculture development. Disease control and health management in aquaculture are different from the terrestrial livestock sector, particularly due to the fluid environment. Disease occurs in all systems, from extensive

to intensive, and losses are possible in all types of production systems. There is a need for better management of intensive systems, and biotechnologies are being used for this purpose. Immunoassay and DNA-based diagnostic methods are currently used to screen and/or confirm the diagnosis of many significant pathogens in aquaculture in developing countries. Also, one of the most important factors leading to reduced antibiotic use by the aquaculture sector is the availability of good prophylactic measures for diseases causing severe mortalities in cultured fish and shellfish. The use of vaccines provides good immunoprophylaxis for some of most important infectious diseases of finfish. As molecular-based vaccine production procedures rely heavily on biotechnological tools, vaccines are being produced mainly in developed countries.

Reducing the environmental impacts of aquaculture is a significant task. Aquaculture is often accused of being unsustainable and not environmentally friendly. Reducing the impacts of effluent discharge, improving water quality and responsible use of water are key areas to be considered in aquaculture development. Some biotechnologies are being used to address these areas, including bioremediation for the degradation of hazardous wastes and use of DNA-based methodologies for the early detection of toxin-producing algae.

In capture fisheries, the sustainable management and conservation of fisheries is a priority. Better understanding of the population structure of the fishery is therefore of paramount importance. Some biotechnologies have already been applied but there is ample scope for the greater use of biotechnologies in fisheries management worldwide. The use of molecular markers and the principles of population genetics have proved very effective for assessing the actual levels of genetic variability within single populations and for measuring the extent of differentiation between populations.

4.1 INTRODUCTION

Capture fisheries and aquaculture supplied the world with over 113 million tonnes of food fish in 2007, providing an apparent per capita supply of 17.1 kg (live weight equivalent), which is among the highest on record. Global production of fish from aquaculture has grown rapidly during the past four decades, contributing significant quantities to the world's supply of fish for human consumption. Aquaculture currently accounts for nearly half (44.3 percent) of the world's food fish (Figure 1). With its continued growth, it is expected that aquaculture will in the near future produce more fish for direct human consumption than capture fisheries (FAO, 2009).

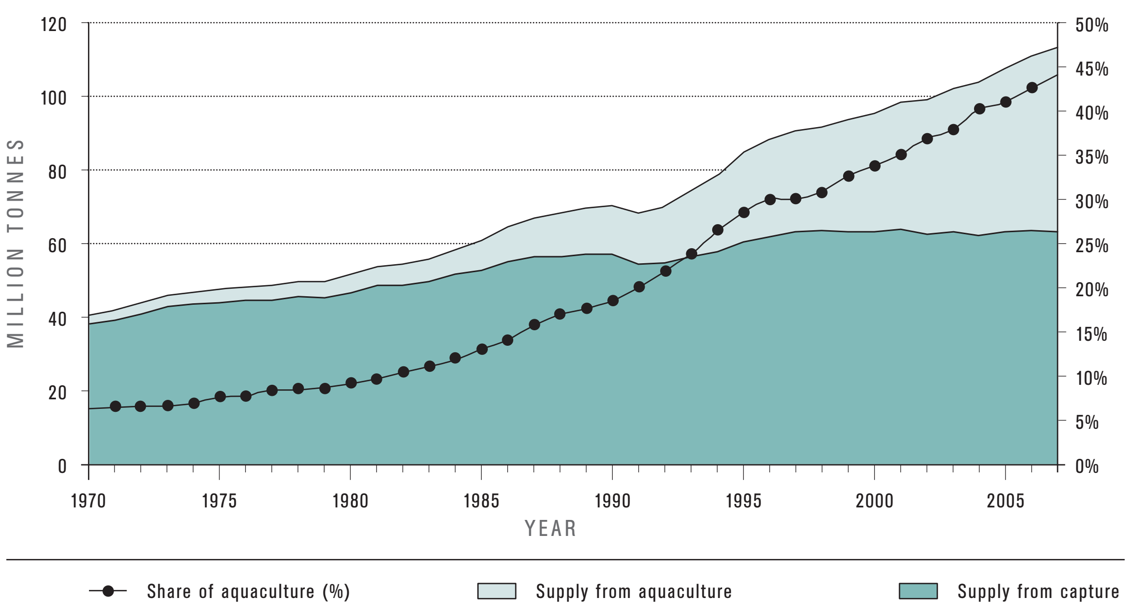
Started as primarily an Asian freshwater food production system, aquaculture has now spread to all continents, encompassing all aquatic environments and utilizing a range of aquatic species. From an activity that was principally small-scale, non-commercial and family-based, aquaculture now includes large-scale commercial or industrial production of high value species that are traded at national, regional and international levels. Although production remains predominantly Asian and is still largely based on small-scale operations, there is a wide consensus that aquaculture has the potential to meet the growing global demand for nutritious food fish and to contribute to the growth of national economies, while supporting sustainable livelihoods in many communities (Subasinghe, Soto and Jia, 2009).

In 2006, fish provided more than 2.9 billion people with at least 15 percent of their average per capita animal protein intake. The contribution of fish to the total world animal protein supplies grew from 14.9 percent in 1992 to a peak of 16.0 percent in 1996 before declining to about 15.3 percent in 2005. Notwithstanding the relatively low fish consumption in low income food deficit countries of 13.8 kg per capita in 2005, the contribution of fish to total animal protein intake was significant – at 18.5 percent – and is probably higher than indicated by official statistics in view of the under-recorded contribution of small-scale and subsistence fisheries and aquaculture (FAO, 2009).

Aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies modifications and intervention in the production cycle such as regular stocking, sorting, feeding and protection from predators in order to enhance production.

FIGURE 1

CONTRIBUTION OF FOOD FISH SUPPLY FROM CAPTURE FISHERIES AND AQUACULTURE



Source: FAO FishStat and FAO (2009)

It is important to note that aquaculture has a long tradition in the developing countries of the Asia-Pacific region, supplying most of the world's aquaculture production (over 90 percent), and making important contributions to the livelihoods and subsistence of small-scale farmers and coastal populations in many countries in the region. In Latin America, small-scale aquaculture has yet to be widely developed; however, there are several examples of newly established industries based on intensive aquaculture practices, especially using exotic species. Salmon farming in Chile is one of the best examples, but there are also expanding aquaculture industries for shrimp and tilapia culture in Ecuador, Costa Rica and Honduras. While Europe and North America import significant quantities of farmed aquatic animals, they also produce fish and shellfish both from freshwater and marine environments. Africa's contribution to global aquaculture is still small; however, the region is moving forward and increasing production.

Aquaculture covers a wide range of species and methods. It is practised from the cold waters of the far north and south, where fish like salmon, Arctic char and sturgeon are grown in ponds, flowing raceways and cages in the sea, and through the latitudes as far as the tropics, where carp and tilapia flourish in freshwater and shrimp and sea bass are farmed along the coasts. It ranges from the production of fish in naturally occurring ponds in rural areas to the intensive culture of ornamental fish in plastic tanks in the middle of a city. It is practised by the poorest farmers in developing countries as a livelihood and supply of much needed protein for their families, and by urban sports shop owners in Europe and North America producing baitfish for weekend anglers.

Aquaculture systems can range from an intensive indoor system monitored with high tech equipment through to the simple release of fry and fingerlings to the sea, but the aim remains the same: to improve production. Some of the simplest production systems are the small family ponds in tropical countries where carp are reared for domestic consumption. At the other end of the scale are high technology systems such as the intensive indoor closed units used in North America for the rearing of striped bass or the sea cages used in Chile and Europe for growing salmon and bream.

All products and systems are geared to produce animals for market and are much governed by market demand at all levels. Regardless of whether it is a high value commodity like shrimp, salmon or grouper, or a low-value commodity such as carp and Tra catfish, all products are destined for markets, be they local, regional or international. All production systems contribute to food security and human development although small-scale rural production systems provide more support to improving or maintaining livelihoods and generating employment and income for many around the world.

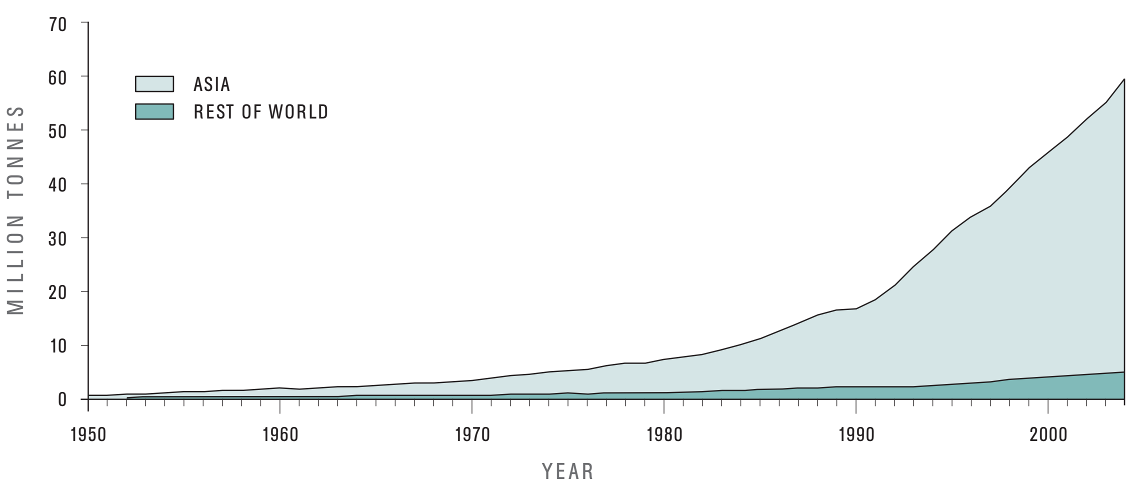
It is important to note that most of these small-scale aquaculture activities occur in developing countries, especially in regions or rural areas where food supply is at risk. For example, tilapia has become a globally important aquatic species that is produced in nearly 100 developing countries worldwide. According to FAO, about 80 percent of the world's farmed tilapia comes from small-holders in developing countries, and this species is particularly prominent in production systems in the Asia-Pacific, the region that provides most of the world's aquaculture supply (FAO, 2004a).

Another good example of extensive aquaculture is the production of major carps in India. In this case, the majority of the production takes place in rural areas with relatively few impacts on the environment, particularly by using multitrophic culture of species such as catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). It is true that some instances of uncontrolled aquaculture development have caused significant negative environmental and social impacts. However, except for a very few species, there are few negative environmental impacts associated with current production systems and practices. Moreover, most traditional and extensive systems produce fish with little or no negative environmental or social impact.

There has been a steady increase in the growth of aquaculture in developing countries, the rate of growth being twice that of developed nations. The most recent figures for global aquaculture production show that more than 90 percent of total fish production comes from developing countries, particularly China which contributes about 70 percent of the total global fish and shellfish production (Subasinghe, Soto and Jia, 2009). Aquaculture is thus often one of the most important food production sectors in developing countries, and in many cases it is one of the most important sources of both food and income for rural populations (Figure 2).

FIGURE 2

GLOBAL AQUACULTURE PRODUCTION



Source: FAO FishStat and FAO (2009)

Aquaculture practice is an example of a strong continuum of production systems. From the simplest production system with absolutely no inputs and with minimal interventions, aquaculture ranges up to highly sophisticated, fully automated, industrial production systems comprising submerged offshore cages producing large quantities of fish from a single unit. Intensive or extensive aquaculture requires good quality seed for farming. Seed quality is not only dependent on good hatchery technology, but also on good broodstock with improved genetic quality. The genetic quality of the broodstock and seed used in aquaculture can be improved using biotechnological tools and procedures. There have been some interventions, and good results have been reported.

Modern aquaculture, through the intensification of culture systems and the diversification of both the species cultured and the culture methods employed, often creates an ideal environment for disease-causing organisms (pathogens) to flourish. The expanded and occasionally irresponsible global movement of live aquatic animals has been the cause of transboundary spread of many pathogens, which have sometimes resulted in serious damage to aquatic food productivity. Some of these pathogens have become endemic in culture systems and in the natural aquatic environment, thus making them difficult to eradicate. Since they have become endemic, recurrent pathogen incursions and disease outbreaks occur in farms making it difficult for the farmers to effectively manage farm health. Instead of implementing effective health management strategies and practices, many farmers opt to use antimicrobials as treatments. There is therefore a need to develop alternate methodologies and tools for maintaining aquatic animal health in aquaculture systems. Such tools and methodologies are generally the result of biotechnological research and several success stories exist. Similarly, biotechnological research has also helped in the improvement of feeds, feeding and nutrition as well as of water quality and the environmental impacts of aquaculture.

This paper is divided into two main Sections: “Stocktaking: Learning from the Past” and “Looking Forward: Preparing for the Future”. For the first one, Part 4.2 provides a brief overview of the main areas where biotechnologies are currently been applied; Part 4.3 documents the current status of application of biotechnologies in developing countries; and Part 4.4 presents two relevant case studies. For the second Section, Part 4.5 examines a couple of key issues for the future where biotechnologies could be useful; Part 4.6 identifies a number of specific options for developing countries to help them make informed decisions regarding adoption of biotechnologies; and Part 4.7 proposes a set of priorities for action for the international community (FAO, UN organizations, NGOs, donors and development agencies).

A. STOCKTAKING: LEARNING FROM THE PAST

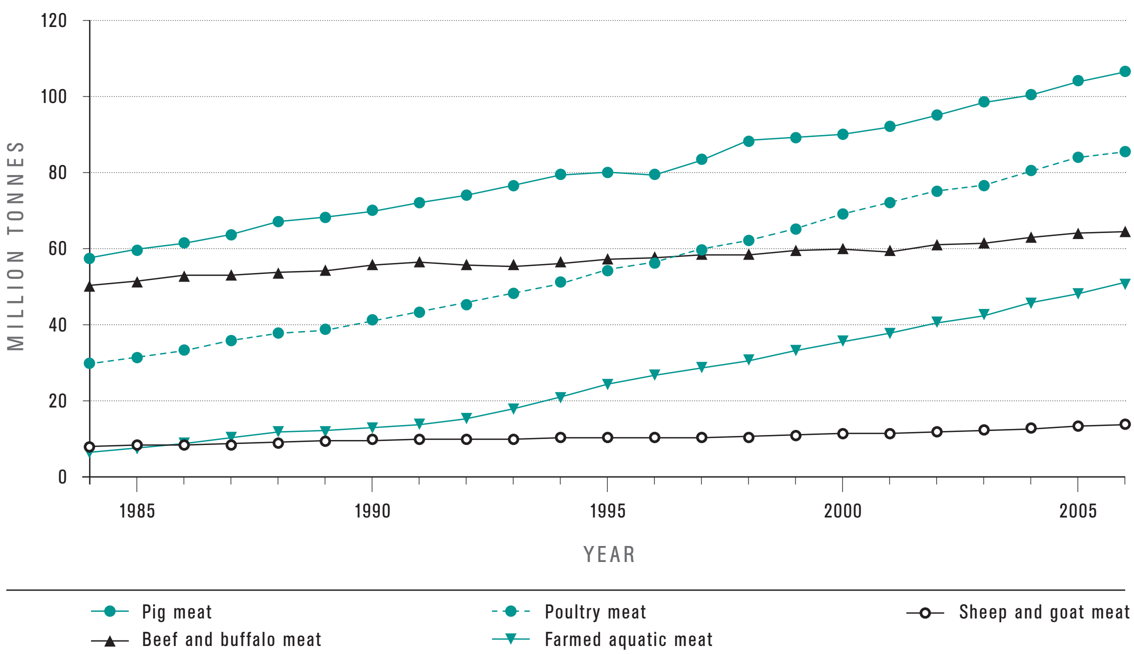
4.2 OVERVIEW OF MAIN AREAS WHERE BIOTECHNOLOGIES ARE BEING APPLIED IN AQUACULTURE AND FISHERIES IN DEVELOPING COUNTRIES

4.2.1 Genetic improvement and control of reproduction

Aquaculture is still the fastest growing food producing sector, compared with other food commodities (FAO, 2009) (Figure 3). One of the reasons for this is the diversity of species in culture at present (over 230), and the genetic diversity that can be exploited through captive breeding and domestication, enabling the development of improved culture methods for a diverse array of species to expand commercial aquaculture (Subasinghe, 2009). A lack of knowledge of the biology of many of these species and the cost of technology development are constraints that explain in part why biotechnologies are only now emerging as useful tools for increasing the productivity and sustainability of this sector. Aquaculture is a sector that is likely to benefit greatly from the application of appropriate genetic and reproduction biotechnologies to increase food production.

FIGURE 3

GROWTH IN PRODUCTION OF DIFFERENT FOOD COMMODITIES: 1984–2006



Source: data calculated from FAOSTAT Database (2008)

Despite the current trend towards the intensification of production systems, aquaculture has not made full use of conventional technologies such as genetic selection and breeding improvement programmes to increase production as have other food production sectors. The rearing of many newly cultured species is to a large extent based on juveniles and/or broodstock obtained from the wild. In order to establish practical breeding programmes to produce seed in hatcheries it is necessary to have a detailed understanding of the complete production cycle. Such knowledge is also required in order to disseminate breeding improvements to the production sector.

One of the best examples is the inability to fully domesticate *Penaeus monodon*, the black tiger prawn which is arguably the most valuable species produced globally. Although specific pathogen-free (SPF) hatchery stocks bred for improved growth have become available recently, production still depends on broodstock collected from the wild. As a result, production of this species has been replaced over the last few years by that from the white shrimp, *L. vannamei*. Improved SPF *L. vannamei* have been readily available for some time and now supply essentially all farmed white shrimp and more than 60 percent of all farmed penaeid shrimp world wide. The shrimp aquaculture sector therefore illustrates the benefits of genetic improvement for increasing production and the competitiveness of aquaculture industries.

The *P. monodon* example illustrates how a lack of knowledge concerning some phases of the life cycle such as reproduction or metamorphosis may be a limiting factor in developing domesticated stocks. Certain species of tuna, a marine resource that is being harvested under a quota system, are now produced in considerable quantities in captivity or culture. The aquaculture production of this valuable species will undoubtedly increase once the life cycle is closed and the hatchery production of tuna fry becomes a reality. This scenario is also applicable to the hatchery production of mollusc species. There is a huge demand for spats (fertilized shellfish larvae) but most spats are still coming from the wild.

The use of hormones for the control of reproduction has been primarily developed for inducing the final phase of ova production, i.e. for synchronizing ovulation and for enabling broodstock to produce fish in the first part of the season or when environmental conditions suppress the spawning timing of females. These procedures began with the pioneering work of Houssay (1930), who demonstrated that extracts of the hypophysis (pituitary gland) can have an effect on sexual maturation of fish and reptiles (Zohar and Mylonas, 2001). These results allowed the development of a relatively simple procedure consisting of injecting hypophyseal extracts purified by chromatography that contain products such as inductive hormones related to sexual maturation. Human chorionic gonadotrophin and the gonadotrophin-releasing hormone (GnRH) were also used to control the maturation of many fish species without limitations due to species-specific effects (Zohar and Mylonas, 2001). GnRHa, an analogous GnRH developed chemically, is more efficient in inducing maturation and is relatively inexpensive. It can be injected or administered by means of pellet implants which facilitate its practical use. The use of hormones such as GnRHa has allowed advancement of the date of egg-laying in several species of fish, mainly salmonids, although for relatively short periods of time (Valdebenito, 2008). Several other molecules are currently under development for use in molluscs (e.g. scallops, oysters and mussels), where synchronous reproduction is required for the hatchery rearing of larvae for aquaculture production in developing countries instead of using seed obtained from natural banks.

4.2.2 Biosecurity and disease control

Disease outbreaks are a serious constraint to the development of intensive aquaculture systems and can have a major impact on production due to mortality and decreased growth. It has been recognized that disease is the most significant factor impacting the intensive production of shrimp, salmon, carp and tilapia, with losses of 10-90 percent of total production (Peinado-Guevara and López-Meyer, 2006). Although many aquatic animal pathogens are well studied, unlike in terrestrial animals the spread of pathogens is easy through water and control is difficult due to high density culture in fluid environment. Disease occurs in all systems, from extensive to intensive, although heavy losses are always possible in intensive production systems (Bondad-Reantaso *et al.,* 2005).

Intensive and semi-intensive aquaculture can have important effects on the quality of the aquatic environment in which the animals are reared. Poor water quality resulting from increased waste products, inadequate farm management, increased stocking densities within farms and increased densities of aquaculture units per sector can increase the likelihood of disease outbreaks and other environmental problems such as eutrophication, episodic oxygen shortages, algal blooms etc., all of them potentially resulting in high mortalities. A more “systems-oriented approach” is therefore needed to provide suitable husbandry for effective growth and to control disease outbreaks effectively.

There is a greater need for management intervention in intensive systems. Here biotechnological tools can be a valuable part of management approaches. Their scope of application is broad – they can be used as sensors in the production environment, for waste management (through controlled microbial technologies), and for disease detection and control (molecular methods). Traditionally, disease control is often carried out only after mortality has been observed. In the past, the diagnosis of fish diseases has been achieved primarily using histopathological methods supported by parasitological, bacteriological and viral studies based on necropsy and *in vitro* cell culture. These are well-proven techniques. However, they require a high level of expertise and are often quite time-consuming, not being amenable to automation. For these reasons, although expert training is required, polymerase chain reaction (PCR) technology (described later) has become an important tool for pathogen assessment in developing countries, for example in the shrimp industries of Asia and Latin America.

4.2.3 Environmental management and bioremediation

Aquaculture has often been accused of being unsustainable and not environmentally friendly. Although in some cases, where aquaculture development has failed to live up to the global expectations of sustainable development, these allegations are not entirely unfounded, the majority of aquaculture is practised sustainably and with a high degree of environmental conscientiousness. Reducing the impact of effluent discharge, improving water quality and the responsible use of water are key areas to be considered during aquaculture development. A number of biotechnologies are being used to address these areas: bioremediation for the degradation of hazardous wastes; the use of vaccination and probiotics to reduce antimicrobial use; and the use of DNA-based methodologies for the early detection of toxin-producing algae.

4.2.4 Biodiversity conservation and fisheries management

In fisheries management, conservation is an important concept. Good fisheries management requires effective conservation measures, which require better understanding of the population structure of the fishery. One of the most important population parameters for assessing the fate of a population is the effective population size (N_e), which determines the amount of genetic variation, genetic drift and linkage disequilibrium in populations and can be calculated as half the reciprocal of the rate of inbreeding (e.g. Tenesa *et al.*, 2007). There is much concern in fisheries and aquaculture production about the potential loss of genetic variation that may result from the relatively high rates of inbreeding expected in these populations. This is because many fish and shellfish species produce thousands or even millions of fertile eggs from a single female. Due to differences in the biological and environmental factors affecting the survival of individual families, many species show a relatively large variance in family size, further decreasing the N_e (Falconer and MacKay, 1996). Fisheries resource managers have focused on the actual number of individuals in a population (census numbers) (Grant, 2007), which may be many times higher than the N_e (Hauser *et al.*, 2002; FAO, 2006). Therefore, it is difficult or even impossible in some cases to infer the N_e using the census number. Inadequate procedures for stock enhancement can yield a very small effective population size due to the high prolificacy of fish and shellfish species. Thus, a very small number of breeders could be used for restocking purposes, and bottlenecks can affect the fitness of the population in future generations. A range of biotechnology-based approaches are being used to conserve wild fish populations such as the use of molecular markers: to estimate N_e in wild populations; to study gene flow between farmed and wild fish populations; and to monitor and understand changes in wild fish population sizes (FAO, 2006; Hansen, 2008).

4.3 CURRENT STATUS OF APPLICATION OF BIOTECHNOLOGIES IN DEVELOPING COUNTRIES

In fisheries and aquaculture, although perhaps not as much as in livestock and crop production, some biotechnologies have been used in developing countries. As mentioned earlier, use of biotechnologies in fisheries is very limited whilst in aquaculture biotechnologies are represented in a few fields such as genetic improvement, disease control, feeds and nutrition and environmental improvement.

4.3.1 Genetic improvement and control of reproduction

4.3.1.1 Polyploidy

Many fish and shellfish species are relatively tolerant to chromosomal manipulation in the early stages of their development. The use of genetic manipulation including polyploidy (i.e. increasing the number of sets of chromosomes) to improve aquaculture production has been examined. However, there has been little discussion of the use of these technologies in practical management programmes in developing countries or on how they can be used efficiently within the context of breeding programmes. Furthermore, the potential value of this technology under practical conditions for enhancing the performance of commercial populations in developing countries is not clear.

The induction of polyploidy has been considered by many researchers (Purdom, 1983; Thorgaard, 1986) because of the advantages related to triploid sterility. For example, triploids (with three sets of chromosomes) may be useful for conservation programmes where sterility can prevent introgression of genes from escaped individuals of commercial stocks into natural populations (Galbreath and Thorgaard, 1994), or in commercial operations where sterile fish are desirable to prevent side effects such as deterioration of carcass quality due to maturation or the occurrence of high mortalities in stocks when males mature early or that occur prior to maturation, especially in populations of Pacific salmon (Purdom, 1983; McGeachy, Benfey and Friars, 1995).

Triploidy leads to the production of nearly completely sterile populations, as has been observed in rainbow trout populations with spontaneously occurring triploids (Thorgaard and Gall, 1979). However, the degree of reproductive disruption varies depending on the species and the sex. Gametogenesis is severely disrupted in triploid females of salmon while, in contrast, triploid males usually display secondary sexual dimorphism (i.e. darkened skin colour and modified body conformation), courtship behaviour and develop an endocrine profile similar to that of diploid males. Spermatogenesis, however, appears to be somewhat reduced in comparison with diploid males (Benfey *et al.*, 1986). Although triploid males are to a great extent sterile, fertilization has been reported to occur. In the salmon aquaculture industry, sexual maturity and the associated gonadal development is generally an economic drawback as metabolic energy is diverted from somatic cell growth to reproduction, resulting in the deterioration of flesh quality and appearance. In this situation, the advantages of triploidy occur primarily after the onset of maturation when triploid female fish may show an extension of growth (Thorgaard, 1986) and the inhibition of maturation prevents the normal degradation in carcass quality that is observed during the spawning season (Asknes, Gjerde and Roald, 1986). Furthermore, female salmon triploids show a significantly higher dress-out percentage (Thorgaard and Gall, 1979) and higher pigment (canthaxanthin) retention (Choubert and Blanc, 1989), but concomitantly, there is an increase of fat deposition surrounding the viscera.

In developing countries, the practical implementation of triploidy in fish production has not been very successful. Most of the research on the application of this biotechnology has been experimental, without extensive testing under practical conditions that consider the wide range of environments in which aquaculture takes place. In species such as tilapia and carp, testing of triploidy is a very important issue considering that there is intraspecies variation in the rate of triploidization due to the size and quality of the eggs. For this reason, it is not possible to ensure 100 percent triploidy when applying this technique on a commercial scale. Also, an increased mortality rate at the beginning of the life cycle and the detrimental effect of triploidy on growth and fitness could be significant constraints to the commercial production of triploids in some species (Basant *et al.*, 2004). The lack of knowledge about the effects of competition between triploids and diploids in large extensive conditions in species such as tilapia could also be a disadvantage, since triploids sometimes lack robustness compared with normal diploids, but this expression varies among species (Benfey, 1999). In many cases, the variation in performance between diploid and triploid stocks has not been fully estimated, and thus it may not be possible to accurately predict the relative performance of triploids in commercial conditions, which may be a problem in conventional breeding programmes of many fish and shellfish species (Pechsiri and Yakupitiyage, 2005).

In developing countries, for various reasons, these techniques are not currently used for commercial purposes. Tilapia, for example, cannot be easily reproduced using external fertilization which is a prerequisite for shock treatment. Furthermore, when a very small number of eggs are obtained per spawn, it is not possible to ensure a constant rate of triploidy per spawning. In rainbow trout, it is only profitable to use triploid females since males show some degree of reproductive onset. For developing such female triploid populations, neomales (i.e. morphologically male but genetically female) are required, which in some instances are difficult to stock up to a commercial scale. In Indian carps, sterility aiming at faster growth and thus enhanced production may not be cost-effective since harvesting after one year of age is not profitable (males mature at one year of age and females when approaching two years).

In southern India, precocious maturation is a potential constraint on yields of cultured common carp as both males and females can attain sexual maturity well before reaching a marketable size. However, triploid fish did not show any improvement over diploid individuals except for higher dress-out percentages (Basavaraju *et al.*, 2002).

Despite the plethora of research conducted on triploidization and chromosomal biotechnologies, there remains a gap between research findings and the practical implementation of triploidy. Several reasons explain this fact. The usefulness of applying chromosomal biotechnologies such as triploidy for aquaculture production seems to be very species-specific, and therefore in some cases (such as in salmon, tilapia and carp), the advantages due to delayed maturation or increased growth are unclear. Furthermore, the results of using these techniques to increase growth rate or delay reproduction are not seen as sufficiently beneficial for the technique to be implemented on a large scale (P. Routray, Central Institute for Freshwater Aquaculture, personal communication, 2009).

For the technology to be practical, it should be possible to produce all-triploid populations without the need to test the triploidy status of each batch of embryos produced. Because triploidy induction using thermal shock is not 100 percent effective, this is a serious drawback to the large-scale commercial application of the technique. Crossing between tetraploids (with four sets of chromosomes) and diploids is a way to produce 100 percent triploids; however, in most species tetraploid production is not straightforward. Furthermore, the genetic lag between the tetraploid population and the diploid breeding programme can seriously affect the efficiency of the production system. For all these reasons, this technology has not been used extensively in developing countries for production purposes.

4.3.1.2 Gynogenesis/androgenesis

Gynogenesis is the production of an embryo from an egg after penetration by a spermatozoon that does not contribute genetic material. Androgenesis is the production of an embryo from an egg whose DNA was inactivated and which was fertilized using normal sperm. In both cases, the diploidy is restored using heat/cold shocks. In gynogenesis, if diploidy is restored soon after fertilization, the procedure is called meiotic gynogenesis due to the fact that the second polar body is retained, and this procedure is similar to what is expected under autofertilization in terms of inbreeding. If shocks are applied later or in androgenesis where the ova were DNA-irradiated for DNA inactivation, the same chromosome is duplicated and thus the embryo is a double haploid individual which is completely inbred for every locus.

Several papers have discussed the usefulness of this type of reproduction for genetic analysis in carp, tilapia and rainbow trout breeding programmes. In some cases, the use of gynogenetic individuals has been suggested for capitalizing on non-additive genetic effects to increase additive genetic variance and for product uniformity (Bijma, van Arendonk and Bovenhuis, 1997). However, the production of gynogenetic lines is not without problems. After a first round of gynogenesis from an outbred population, deleterious and/or lethal effects can be fully expressed in the double haploid progeny, which may be a problem when implementing a breeding programme from this source. Furthermore, phenotypes cannot actually be a direct reflection of the same trait measured on normal progeny due to developmental instability. Therefore, the utility of this type of reproduction for practical use in breeding programmes is seen as risky in most cases. Nonetheless, they can be used effectively for developing powerful quantitative trait locus (QTL) mapping experiments using the surviving clonal lines of this sort obtained from an outbred population, but this requires having available the gynogenetic lines that are needed for further assessment (FAO, 2007a).

4.3.1.3 Controlling time of reproduction in fish and shellfish

So far, the application of hormonal treatment has been quite successful especially for controlling reproduction in broodstock. This is particularly the case in salmon and trout farming in Chile where either implants or injection of the hormonal compound are used extensively in salmon farming for synchronizing reproduction. Since hormone application is not done in the commercial fish, but rather in the broodstock which are discarded for human consumption, these procedures are not subjected to a negative consumer preference. In carp breeding, the use of hormones has made it possible to artificially manipulate the number of times and the timing of spawning of major Indian carps and African catfish (Routray *et al.*, 2007).

4.3.1.4 Development of monosex populations

One of the major constraints in practical programmes in developing countries is the fact that mixed sexed populations can behave poorly in production conditions (FAO, 2003). This is primarily due to the negative side-effects of early reproductive onset that decrease the growth rate through a series of physiological mechanisms. The faster growth rate of the other sex is probably caused by its later maturation. The negative relationship between growth rate and gonadal development has been found in many species. One explanation of this finding is the appearance and accumulation of sex hormones that act as growth inhibitory agents (Hulata, Wohlfarth and Moav, 1985).

The advantages of monosex culture depend on the species involved (FAO, 1995). This is because one sex may be superior in growth or have a more desirable meat quality, or to prevent reproduction during grow-out or the appearance of sexual/territorial behaviour (aggressiveness) that occurs when a mixed sexed group triggers the reproductive season. For example, female sturgeon are more valuable than males because they produce caviar; female salmon are more valuable because sexually precocious males die before they can be harvested, and salmon roe has an economic value; and male tilapia are more desirable than females because they grow twice as fast and because reproduction is not significant in males during grow-out.

The sex of fish can easily be manipulated using hormonal treatments. In many fish and shellfish species, sex is not permanently defined genetically and can be altered by a number of factors including hormonal treatment during the early stages of development. Gonadal development starts from primordial germ cells, with females starting differentiation prior to males (Phelps, 2001). The point in time when differentiation occurs depends on the species involved. In tilapia and trout, this mechanism is triggered early in life, while in grass carp and paddlefish it is the opposite (Phelps, 2001). Considering this pattern of development, treatment with the steroid methyl testosterone can be used to develop all-male tilapia populations (Mair, 1999) and androgens (male sex hormones) can be used in trout and carp monosex culture.

There has been concern about the use of hormones in animal production including in aquaculture systems, arising from the risk of presence of residues in final products. Although there is little evidence regarding hormonal residues in fish whose sex has been reversed early in life, consumer acceptance may be compromised as a result of the perception of hormonal treatment itself (FAO, 2003). For this reason, it appears that other biotechnologies have had more use in those developing countries whose production goes mainly to export markets.

A variation on this scheme is to produce all-male progeny in one more generation. This requires feeding young fish with estrogens (female sex hormones), resulting in a population of all-female fish (Fitzsimmons, 2001). These morphologically female but genetically male fish (neofemales) are then raised to maturity when they are mated to normal male fish. After maturation, the all-male fry produced are tested in order to identify the "super males" (YY), which are then crossed to normal females (XX), thus generating all true male (XY) progeny. The importance of this method is that male fry for commercial production can be produced that have never been treated with hormones. However, one of the disadvantages is that this technique requires more than a single generation to obtain the all-male fry, i.e. this procedure cannot be used without extensive progeny testing to determine which "female" fish will produce all-male progeny, thus requiring a reasonable time span for developing the neomales.

Although tilapia breeding programmes using YY super males are possible, this procedure is not necessarily required because the application of direct hormonal treatment of undifferentiated fry to produce monosex populations is still a major breakthrough.

However, the great expansion of tilapia aquaculture in Asia has been due to mixed-sex tilapia culture which addresses the high demand for relatively small fish (i.e. fish less than 300 g) that can be obtained by rearing the highly selected genetically improved farmed tilapia and other strains.

4.3.1.5 Cryopreservation

The aim of the cryopreservation of gametes is related to:

- disseminating semen from males obtained from selection programmes showing significant response;
- "refreshing" commercial populations in order to avoid the negative impact of bottlenecks;
- directly assessing the rates of genetic gain in ongoing breeding programmes;
- making semen available across the reproduction window when asynchrony of reproduction exists between males and females (usually males mature earlier than females).

Sperm cryopreservation has been successfully implemented for a number of cultured finfish and shellfish species, and modest success has been achieved in the cryopreservation of shellfish embryos and early larvae. Cryopreservation of finfish ova and embryos has not been successful, which is a major difference with respect to terrestrial animals. This is mainly due to the size of the ova which are usually large and have thick chorionic membranes that do not facilitate the inclusion of cryoprotectors.

The use of cryopreserved gametes for commercial purposes is still very limited in developing countries. One explanation is that this biotechnology may require specialized labour and automated procedures to decrease variability in success rates among batches of sperm. Furthermore, it is still uncertain whether this method is economically advantageous compared with disseminating improved broodstock using larval material. In spite of this, the technology has been used for disseminating improved "Jayanti" rohu in India and for the dissemination of improved semen in Sri Lanka (P. Routray, personal communication). In rainbow trout, cryopreservation has been used for storing semen from neomales, but the problem of highly variable fertilization success remains.

4.3.1.6 Genomics

Genomics is the study of the genomes of organisms. It includes the intensive efforts to determine the entire DNA sequence of organisms via fine-scale genetic mapping.

Genome sequencing

One of the major constraints in the rearing of many different aquaculture species is the lack of adequate genomic information. This is because sequencing all the species currently used in aquaculture would be costly. Productive species currently being sequenced are the tilapia and the Atlantic salmon (*Salmo salar*). A multinational initiative for Atlantic salmon aims to sequence the genome using the Sanger method to obtain a coverage of more than six-fold. The project is a partnership between Canada, Norway and Chile, countries that are interested in applying this sequence data for studies related to enhancing conservation and production. The project's output will be delivered to the public domain and provide the required genomic resources for developing single nucleotide polymorphism (SNP) chips that will help implement marker-assisted selection (MAS) programmes in Chilean salmon aquaculture.

Functional genomics

The recent availability of massive amounts of information from functional genomics such as microarrays that are used to assess gene expression or sequence polymorphisms has contributed significantly to the genomic biotechnology in aquaculture. Two colour microarrays have been developed for salmonid species that are publicly available and are currently used to assess disease resistance traits in salmon and for candidate gene discovery. In shrimp, several platforms have been devised in China, Australia, Taiwan Province of China, Singapore and also the United States (Wilson and de la Vega, 2005).

The main use of this resource has been to study differential expression of the transcriptome after viral or bacterial acute infection, but also as bioindicators for assessing chronic disease response. Microarrays are being applied to the fields of ecotoxicology and nutrigenomics. For example, gene expression analysis has been used for assessing the effect of pre-challenging white spot syndrome virus (WSSV) on different genes in order to investigate the immunological mechanisms behind the genetic resistance and to assess potential genes explaining disease resistance at the experimental level in the culture of Pacific whiteleg shrimp (*Litopenaeus vannamei*) in Colombia. In Chile, the salmon microarray available for the consortium for genomics research on all salmon project (cGRASP[1]) in Canada has been used in collaboration with the University of Victoria for assessing disease resistance of piscirickettsia and infectious pancreatic necrosis virus (IPNV) in Atlantic salmon.

4.3.1.7 Genetic modification

A genetically modified organism (GMO) is one whose genetic material has been altered through genetic engineering techniques with DNA molecules from different sources that are combined into one molecule to create a new set of genes. Typically, it involves introduction of a single gene from an unrelated species. After about two decades of very intensive research, the

1 http://web.uvic.ca/grasp/

technology has reached the stage where it is possible to produce GM carp, tilapia and salmon. However, no aquatic GMOs have yet been approved for commercial release for food and agriculture purposes in any country. There are potential concerns about the environmental impact of raising such fish (e.g. effects of possible interbreeding with native populations) and the greater amount of feed required for sustaining the increased growth rates, as well as problems with consumer acceptance, which may be one of the most important reasons that transgenic technology has not developed beyond the experimental phase. Many developing countries have yet to develop a clear policy on the use of transgenic fish.

4.3.1.8 Molecular markers

Marker systems

Molecular markers are identifiable DNA sequences found at specific locations of the genome, transmitted by standard Mendelian laws of inheritance from one generation to the next. They rely on a DNA assay and a range of different kinds of molecular marker systems exist, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites. The technology has improved in the past decade and faster, cheaper systems like SNPs are increasingly being used. The different marker systems may vary in aspects such as their technical requirements, the amount of time, money and labour needed and the number of genetic markers that can be detected throughout the genome (reviewed in detail in FAO, 2007b). RAPDs and AFLPs have been used extensively in aquaculture due to their relatively easy development, i.e. they do not require construction of genomic libraries. Microsatellite markers are used increasingly in aquaculture species (see the review by Liu and Cordes, 2004), due to their higher polymorphic information content, codominant mode of expression, Mendelian inheritance, abundance and broad distribution throughout the genome (Wright and Bentzen, 1994).

Molecular markers are being applied in developing countries in both aquaculture and fisheries management. Here, an overview is provided on their use for parentage analysis and genetic selection in aquaculture and for fisheries management and stock enhancement.

Parentage analysis

Molecular markers can be used successfully to trace alleles inherited by progeny from a group of candidate parents, thus providing a means of parentage analysis. In many fish and shellfish species, reproduction cannot be fully controlled and thus natural mating is the only way to produce offspring for the next generation of a breeding programme. For example, tilapia and carp breeding typically involves mass spawning where males and females are stocked in large "hapas" suspended in ponds, where a relatively large number of parents spawn simultaneously. Since constrained rates of inbreeding are required for sustained rates of genetic gain, in uncontrolled mating schemes it is not always possible to control the genetic contributions of broodstock or, therefore, the rates of inbreeding in a breeding programme using pedigree information. Small sample sizes together with sperm competition (Withler and Beacham, 1994), mating preference (as in Artemia) and other biological factors after fertilization can increase the variance of family size, thereby decreasing the N_e to unsustainable levels (Brown, Woolliams and McAndrew, 2005).

When it is possible to control matings, one of the most important constraints still facing effective breeding programmes of species such as salmon, carp and trout is that newborn individuals are too small to be tagged individually using the traditional marking systems for livestock. The application of sustainable breeding programmes requires tagging a constant number of individuals from each family with passive integrated transponders (PIT tags) when they become sufficiently large after a period of individual family rearing, in order to manage the rates of inbreeding. However, this system of early management creates common environmental effects for full-sib families (Martinez, Neira and Gall, 1999). To address these issues, mixtures of equal-aged progeny from different families can be reared communally to preclude the development of such family-specific environmental effects, and genetic markers can be used subsequently to assign individuals to families after evaluation of individual performance (Doyle and Herbinger, 1994). Thus, the impact of early common environmental effects is considerably reduced if markers are used for parentage analysis when selecting individuals for early growth rate traits (Herbinger *et al.*, 1999; Norris, Bradley and Cunningham, 2000). Several multinational salmon companies are using this system of tagging but there is still no information regarding its economic value compared with conventional tagging systems such as PIT tags. This may be important in species such as carp and tilapia where the costs of genotyping can greatly outperform the use of tanks and individual tagging systems. Furthermore, it is expected that rates of genetic gain for economic traits will not be significantly affected when common environmental effects are present.

Even though there is a plethora of information in the scientific literature on the use of markers for parentage analysis in fish and shellfish, this procedure has not been fully used in species such as tilapia in developing countries where basic conventional breeding programmes have proved very successful (Ponzoni, Nguyen and Khaw, 2006). The sample size (i.e. the numbers of individuals and markers required for accurately reconstructing the pedigree of a population) is a practical issue since not all individuals in a population can be genotyped for all markers available. The issue of sample size may also arise in species where physical tagging is not possible or not economically sound (e.g. shrimp or marine species), or when disease challenges (e.g. with infectious pancreatic necrosis) are carried out very early stages in the life cycle.

For most breeding programmes, physical tagging will prove efficient both in economic and biological terms to achieve acceptable rates of genetic gain while minimizing rates of inbreeding. Genetic marker technology can still be costly in developing countries for routine assignment of parentage, although these costs can be reduced using multiplex PCR technology in which more than one marker can be genotyped simultaneously in a single gel lane or capillary (Paterson, Piertney and Knox, 2004). This is especially the case when only DNA markers are used without physical tagging, since individuals must be re-typed when records for multiple traits are included in the selection criteria (Gjerde, Villanueva and Bentsen, 2002). When it is possible to isolate families, multistage selection offers the possibility of first selecting individuals on a within-family basis directly from tanks or hapas (for traits influenced by common environmental effects) and then selecting at a second stage for traits measured at harvest. This alternative would maintain the rates of gain while decreasing the costs associated with tagging, or even increase rates of gain, when recording traits such as body weight from tanks (within families) that can be carried out relatively inexpensively (Martinez *et al.*, 2006).

Marker-assisted selection

Molecular markers can also be used in genetic improvement through MAS, where markers physically located beside (or even within) genes of interest (such as those affecting growth rates in salmon) are used to select favourable variants of the genes (FAO, 2007b). MAS is made possible by the development of molecular marker maps, where many markers of known location are interspersed at relatively short intervals throughout the genome and the subsequent testing for statistical associations between marker variants and the traits of interest. In this way, genes (called QTLs) thought to control quantitative traits (traits of agronomic importance controlled by many genes and many non-genetic factors, such as growth rate in fish) can be detected.

MAS can enhance rates of genetic gain compared with conventional breeding for traits that are difficult or expensive to measure or when the heritability is relatively low. So far, many QTLs have been identified in different experiments involving trout, salmon, carp and tilapia, but the main problem with the actual use in MAS is to have enough replications or powerful experiments to validate that the QTLs detected in a given experiment are actually real, and are segregating across populations or crosses. Furthermore, many of the QTLs detected were discovered using dominant markers such as RAPDs which are very difficult to replicate in different laboratories, basically due to the use of insufficient sample sizes and failure to account for the presence of false positives. This outcome is explained by the fact that there is a lack of complete genome sequences for many of the species currently used in aquaculture in developing countries such as tilapia, carp and shrimp. This is an important practical issue, because without information from physical maps it may be difficult to characterize the actual genes explaining the genetic variation explained by the QTLs. This situation reflects the relatively high level of financial resources needed both to carry out a genome sequence project for many species used in aquaculture and to actually implement a MAS programme. This is a very important issue in developing countries where smallholders are less likely to have the financial revenue to allow breeding programmes that incorporate the use of molecular information. Although MAS is potentially useful for many cultured species, conventional breeding programmes may be more profitable in the short to medium term in developing countries in low-input environments.

The development of molecular markers and linkage maps can greatly help scientists to understand the different factors that influence the expression of quantitative traits. A number of genetic linkage maps have been published in aquaculture, some of the most comprehensive being for rainbow trout (Young *et al.*, 1998; Sakamoto *et al.*, 2000; Nichols *et al.*, 2003), channel catfish (Waldbieser *et al.*, 2001), tilapias (Kocher *et al.*, 1998; Lee *et al.*, 2005), Japanese flounder (Coimbra *et al.*, 2003) and mussels (Lallias *et al.*, 2007). In shrimp, recent mapping has demonstrated the nature of sex control in shrimp as WZ/ZZ like chickens and unknown until now. Still, in important species such as Indian major carps and Chinese carps, they have not been developed. There are a number of ways in which this information can be used, the difference between them being the level of resolution with which these factors can be mapped. For example, QTLs with major effects on quantitative traits are mapped using markers to track the inheritance of chromosomal regions in families or in inbred line crosses using the extent of linkage disequilibrium generated in the population.

In practice, the identification of genes influencing specific traits is achieved using a combination of genetic mapping (linkage and fine mapping) to localize the QTL to a small region on the chromosome under analysis, and candidate gene or positional cloning approaches are used to identify the genes within the QTL region. According to the literature survey, it appears that very little information has come from developing countries on such research issues.

In some cases, it is possible to use sufficient biochemical or physiological information to investigate the association between the quantitative genetic variation and the level of marker polymorphisms within specific genes. Nevertheless, this approach requires a great amount of detailed information in order to choose which gene explains the greatest effect and to have sufficient power to detect the association. This information is starting to appear in the aquaculture literature from multinational projects such as cGRASP, but it is still scarce for other fish species of interest in developing countries.

So far, QTL mapping in aquaculture using commercial populations has been carried out mainly in developed countries, mostly with single-marker analysis (microsatellites and AFLPs) and using relatively sparse linkage maps when interval mapping is used. In tilapia, the F_2 design and a four-way cross between different species of *Oreochromis* have been used for detecting QTLs affecting cold tolerance and body weight (Cnaani *et al.*, 2003). In outbred populations of salmonids, QTLs that influence body weight have been mapped (Reid *et al.*, 2005).

Studies seeking linkage of markers to traits amenable to MAS, such as disease resistance, have begun to appear in the literature over the past few years. For example, QTLs for resistance have been mapped for IPNV in salmonids (Ozaki *et al.*, 2001; Houston *et al.*, 2008), infectious salmonid anaemia (Moen *et al*., 2007), infectious haematopoietic necrosis virus (Rodriguez *et al.*, 2004; Khoo *et al.*, 2004) and stress and immune response (Cnaani *et al.*, 2004) and cold tolerance in tilapia (Moen *et al.*, 2003). Also, Somorjai, Danzmann and Ferguson (2003) reported evidence of QTLs for upper thermal tolerance in salmonids, with differing effects in different species and genetic backgrounds. To date, there are no examples of the application of these QTLs in practical fish and shellfish breeding programmes in developed or developing countries.

4.3.2 Biosecurity and disease control

Like other farming systems, the aquaculture industry has been overwhelmed by a fair share of transboundary aquatic animal diseases caused by viruses, bacteria, fungi, parasites and other undiagnosed and emerging pathogens. Disease has thus become a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries. As a result, there will be increasing demand for improved aquatic animal biosecurity, particularly addressing the emerging health problems based on risk analysis. Epidemiological studies generate the data required for risk analysis; biosecurity measures require good information for accurate assessment and this leads to appropriate risk management. Thus, biosecurity, risk analysis and epidemiology are highly interrelated. All are aimed at making good use of scientific research for disease prevention, control and management.

Of equal importance is the need for fundamental information that characterizes diseases in aquaculture. Import risk assessment will of necessity set the risk as "high" when there are little data on modes of transmission, host susceptibility, tolerance to abiotic factors (e.g. temperature, salinity) and immune response elicited, for a particular pathogen under consideration. The clear, unambiguous and rapid detection and identification of potential pathogens using morphological and molecular diagnostic tools are of paramount importance prior to making decisions on the disease status of any aquaculture zone.

Although conventional disease control strategies focus largely on diagnosis and therapy, the prevention of disease through vaccination, immunostimulation, the use of probiotics and bioremediation in culture environments, nutritional improvements etc., has also been practised. Significant advances in these areas have been achieved using biotechnological approaches.

Given the taxonomic diversity of aquaculture species, there is also a need to develop better information on the response of these species to disease in order to develop management strategies for them. Biotechnology approaches are sometimes the only means by which tools for this can be developed.

4.3.2.1 Pathogen screening and disease diagnostics

The control of disease outbreaks relies heavily on having rapid and accurate diagnostic tools available in order to detect and identify the pathogen causing mortality. DNA and RNA methods have been used extensively for detecting a number of viral and bacterial pathogens in aquaculture worldwide. The techniques rely upon the fact that each pathogen species carries a unique DNA or RNA sequence that can be used for identification. The techniques offer high sensitivity and specificity, and the commercial development of PCR primers and diagnostic kits allows rapid screening for a number of serious viral and bacterial infections and has direct application. Molecular-based techniques such as PCR also have applications in situations where the animal shows no antibody response after infection. For example, as molluscs do not produce antibodies, antibody-based diagnostic tests have limited application to pathogen detection in these species.

Considering the difficulties that developing countries may face in using advanced molecular diagnostics, and the importance of gradually improving national diagnostic capacities in developing countries, FAO recommended a three-level diagnostic process (FAO/NACA, 2000). This involves: field observations and necropsy (Level I); laboratory observations, bacteriology and histopathology (Level II); and electron microscopy, molecular biology and immunology (Level III). In countries where Level II and Level III diagnostic capabilities are not found, initial disease screening is carried out using Level I gross clinical examination. Accompanied by histopathology, this has been the traditional method of detecting pathogens in both developed and developing countries. There is a clear need to improve national diagnostic capacities to reach Level II and Level III diagnostic procedures, including molecular diagnostics.

These tools include both immunoassay- and DNA-based diagnostic methods, e.g. fluorescent antibody tests, enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), *in situ* hybridization (ISH), dot blot hybridization and PCR amplification techniques. They are currently used to screen and/or confirm the diagnosis of many significant pathogens of cultured finfish such as channel catfish virus, infectious haematopoietic necrosis virus, IPNV, viral haemorrhagic septicaemia virus, viral nervous necrosis virus and bacterial kidney disease, as well as shrimp diseases such as WSSV, yellow head virus (YHV), infectious hypodermal and haematopoietic necrosis virus (IHHNV) and Taura syndrome virus (TSV) (FAO, 2000). Similar tools are under development for molluscan pathogens (*Haplosporidium* sp., *Bonamia ostreae*, *Marteilia refringens* and Herpes virus). Immunoassays and nucleic acid assays provide quick results with high sensitivity and specificity at relatively low cost, and are particularly valuable for infections that are difficult to detect (e.g. subclinical infections) using standard histology and tissue culture procedures. Molecular tools are also useful for research into the pathology and immunology of specific infections. They can be used with non-lethal sampling and are valuable for monitoring challenge experiments under controlled laboratory conditions. Further development of these technologies is likely to speed up the detection (field monitoring and laboratory examination) and diagnosis of disease, which is crucial for early and effective control of emergent disease situations.

Antibody-based techniques

A variety of antibody-based tests and molecular tests have been developed to detect mainly bacterial and viral fish pathogens, although tests have also recently been reported for parasites and fungal agents. The antibody-based tests include slide agglutination, co-agglutination/latex agglutination, immunodiffusion, direct and indirect fluorescent antibody tests, immunohistochemistry and ELISA, dot blot/dip-stick and Western blot. The antibody-based test selected for the identification of pathogens depends on a variety of factors since each method has its merits and disadvantages. Although such methods are useful for the detection of pathogens in pure culture or/and in infected fish tissue, their sensitivity thresholds limit their use in environmental samples, especially where pathogen levels are extremely low. DNA detection methods, however, such as PCR and ISH are ideally suited.

DNA-based techniques

Molecular technologies are also widely used for the detection of fish pathogens (Adams and Thompson, 2006 and 2008). They have been successfully utilized for the detection and identification of low levels of aquatic pathogens. Such methods are also particularly useful for micro-organisms that are difficult to culture, may exist in a dormant state, are involved in zoonosis, or in the elucidation of pathogen life cycles. In addition, molecular methods can be used for the identification of pathogens to the species level (Puttinaowarat, Thompson and Adams, 2000) and in epidemiology for the identification of individual strains and differentiating closely related strains (Cowley *et al.*, 1999). Because of the general unavailability of the traditional pathogen isolation methods and immunodiagnostics for molluscs and crustaceans, molecular techniques have increasingly been used (Lightner, 1996; Lightner and Redman, 1998; Berthe, Burreson and Hine, 1999).

DNA-based methods such as PCR are extremely sensitive. However, false positive and false negative results can cause problems due to contamination or inhibition (Morris,

Morris and Adams, 2002). Real-time PCR (closed tube to reduce contamination) and nucleic acid sequence-based amplification are alternatives that reduce these risks and offer high sample throughput (Overturf, LaPatra and Powell, 2001; Starkey *et al.*, 2004). Some of the most common PCR-based technologies used for the detection of pathogens are nested PCR, RAPDs, reverse transcriptase PCR (RT-PCR), reverse cross blot PCR and RT-PCR enzyme hybridization assay (Puttinaowarat, Thompson and Adams, 2000; Wilson and Carson, 2003; Cunningham, 2004). ISH is also widely used in the detection of shrimp viruses (Lightner, 1996; Lightner and Redman, 1998; Tang and Lightner, 1999; Tang *et al.*, 2005) and in the confirmation of mollusc parasites (Stokes and Burreson, 1995; Le Roux *et al.*, 1999; Cochennec *et al.*, 2000; Carnegie *et al.*, 2003). Colony hybridization has also been used successfully for the rapid identification of *Vibrio anguillarum* in fish (Aoki *et al.*, 1989) and has the advantage of detecting both pathogenic and environmental strains (Powell and Loutit, 2004).

In recent years, the use of PCR-related tools has gained wide acceptance in developing countries. The advent of PCR has lead to important advances in the development of routine diagnostic tests, and it has been possible to develop probes aimed at the detection of pathogen genetic material in host tissue, as well as for assessing genetic variability within and between fish and shellfish populations. Both DNA- and RNA-based methods have been devised to detect pathogen genetic material. Depending on the pathogen, conventional PCR can be replaced by the more sensitive nested PCR method, in which primers within the region amplified in a first step are used for further amplification of DNA. RNA quantification can be carried out using RT-PCR of the viral nucleic acids present in sample tissues. As with the immunological methods described earlier, it should be noted that PCR does not demonstrate the presence of disease nor of a viable pathogen, but only that pathogen genetic material was present in the sample being examined. Despite this limitation and other problems related to ease of contamination, false positives, the limited number of primers available etc., when properly applied, PCR offers a relatively rapid and inexpensive way for the routine screening of large numbers of aquatic animals for commercial aquaculture and for testing of imported stocks during quarantine. For example, PCR is very important in the routine screening of massive numbers of penaeid shrimp larvae for serious viral pathogens such as WSSV, TSV etc. in Asian and Latin American countries.

DNA probes and epidemiology

DNA probes have particular value in the fields of epidemiology, routine disease surveillance and monitoring, treatment and eradication programmes in aquaculture and efforts to prevent the spread of pathogens to new geographical areas. These biotechnologies also have important application in risk management for aquatic animal diseases including inspection and certification of production and facilities and consignments for freedom from specific pathogens; achieving recognition of a country as having disease-free status; and implementing disease zoning programmes and effective quarantine measures etc. (Bernoth, 2008).

The Manual of Diagnostic Tests for Aquatic Animals, regularly published by the World Organisation for Animal Health (OIE), validates the use of traditional diagnostic methods such as evaluation of clinical signs, necropsy, histopathology, parasitology, bacteriology, virology, mycology etc., as well as immunological tests such as ELISA for the presumptive and confirmatory identification of OIE-listed diseases. The introduction to the Manual notes that "For the most part, molecular methods for fish diseases are recommended for either direct detection of the pathogen in clinically diseased fish or for the confirmatory identification of a disease agent isolated using the traditional method. With one or two exceptions, molecular techniques are currently not acceptable as screening methods to demonstrate the absence of a specific disease agent in a fish population for the purpose of health certification in connection with international trade of live fish and/or their products. There is a need for more validation of molecular methods for this purpose before they can be recommended in the Aquatic Manual" (OIE, 2009; see also Adams and Thompson, 2008). This highlights the importance of further validating these diagnostic tools for serious and emerging diseases across a range of different laboratories worldwide.

4.3.2.2 Vaccines

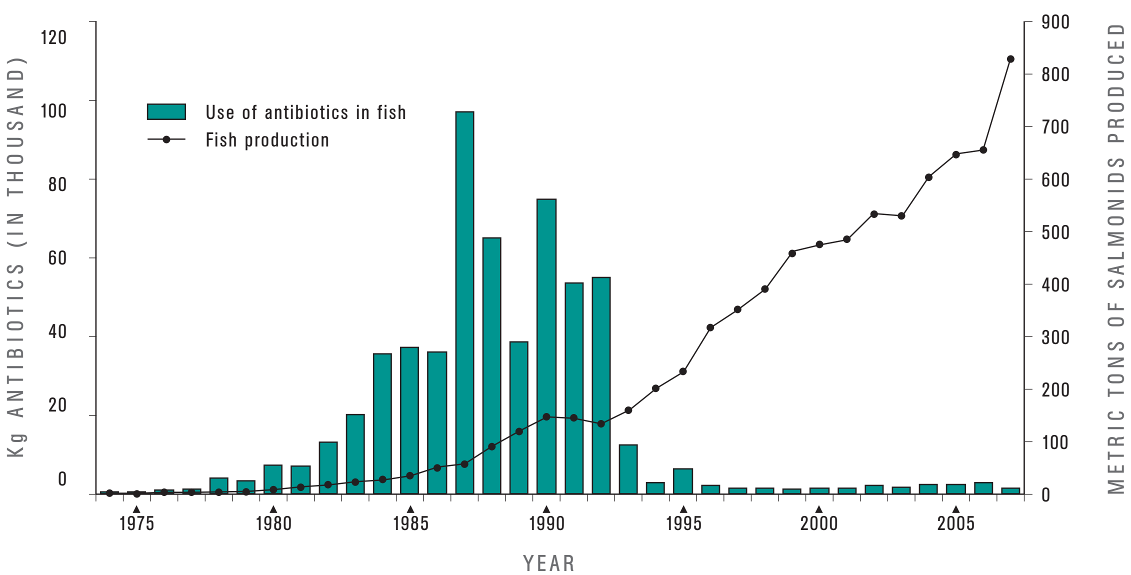
Adams *et al*. (2008) reviewed the vaccine technologies in aquaculture. Vaccination is the action in which a host organism is exposed to organic (biological) molecules that allow the host to mount a specific immune reaction through which it has a better capability to fight subsequent infections of a specific pathogen compared with genetically similar non-vaccinated hosts. It has also been shown to be cost-effective and has led to the reduction in use of antibiotics. In Norway, for example, antibiotic use has decreased from 47 tons to approximately one ton annually (Markestad and Grave, 1997 and Figure 4).

A wide range of commercial vaccines is available against bacterial and viral pathogens and many new vaccines are under development. Most target salmon and trout, and there are expanding opportunities for marine fish (Thompson and Adams, 2004). Traditionally, the organic molecules used for vaccination are directly derived from the pathogen in question. The most straightforward approach is to culture the pathogen after it has been inactivated and presented to the host. So far, vaccines containing more than ten bacterial pathogens and five viral pathogens have been produced based on such inactivated antigens (Sommerset *et al.*, 2005). Alternatively, the pathogen is not inactivated but chemically or genetically weakened so as to survive only for a limited period in the host where it induces a specific immune response without causing disease and mortality. Such vaccines are generally described as “live” vaccines, and there is concern that the attenuated strain may back-mutate and revert to the virulent wild type (Benmansour and de Kinkelin, 1997). Due to environmental and control concerns in most countries, only two live bacterial (*Edwardsiella ictaluri* and *Flexibacter columnarae* for Channel catfish in the United States) and one live viral vaccine (koi herpesvirus for carp in Israel) are commercially available at present.

One of the most important factors leading to reduced antibiotic use by the aquaculture sector is the availability of good prophylactic measures for diseases causing severe mortalities in cultured fish and shellfish. The use of vaccines provides good immunoprophylaxis for some of the most important infectious diseases of finfish. In developed countries, their use has proved very effective at decreasing the unsustainable use of antibiotics. For example, in Norway antibiotic use in salmon farming has become almost negligible, at less than 1 gram per tonne of production, due mainly to the availability of vaccines for furunculosis and cold water vibriosis (Figure 4) (Smith, 2008). At almost similar production levels, Chilean salmon farming shows much more antibiotic use due to the emergence of *Piscirickettsia salmonis*, a pathogen causing severe losses of stock prior to harvest. Thus, there have been recent attempts to develop immunoprophylactic measures.

FIGURE 4

USE OF ANTIBIOTICS VS. PRODUCTION OF FISH IN NORWAY



Source: T. Hastein, personal communication

As molecular-based vaccine production procedures rely heavily on biotechnological tools, vaccines are produced mainly in developed countries. A DNA vaccine is a circular DNA plasmid that contains a gene for a protective antigenic protein from a pathogen of interest (Kurath, 2008). Considerable industrial research has been conducted towards developing DNA vaccines for species such as salmonids against pathogens (generally viruses) for which traditional methods have not been successful. As many strains and varieties of a single pathogen are generally present in the tropics, unlike in temperate pathogens, monovalent vaccines are not practical under tropical conditions. Such difficulties, together with the lack of adequate biotechnological knowledge and financial resources, have led to fewer advances in vaccine development in the tropics, and for tropical species. Commercial vaccines using inactivated bacterial pathogens are available for some species: channel catfish, European seabass and seabream, Japanese amberjack and yellowtail, tilapia, Atlantic cod, salmon and trout (Sommerset *et al.*, 2005). Fewer commercially available viral vaccines have been produced, and no commercially available parasite vaccines exist.

4.3.3 Environmental management and bioremediation

Aquaculture, like any other live production system, produces effluents rich in nutrients. Some aquatic production systems also produce effluents with harmful substances such as residues and metabolites of antibacterials and therapeutics. Developing systems that produce effluents with acceptable standards and improving the quality of the aquatic environment where effluent discharges are unacceptably high is a challenge. Biotechnological interventions such as bioremediation, the use of probiotics, and vaccination offer significant promise for addressing these important issues.

Bioremediation is a promising biotechnological approach for the degradation of hazardous waste to environmentally safe levels using aquatic micro-organisms or other filtering macro-organisms. Although this procedure has been used in various situations such as sewage treatment (e.g. FAO, 2008), application to shrimp and other aquaculture wastes is fairly novel. There are many commercial products on the market, mainly bacterial preparations, but the mode of action and efficacy of many of these have yet to be scientifically measured. In addition to microbes, bivalves, seaweeds, holothurians (sea cucumbers) etc., have been tested to assess their ability to reduce organic loading or reduce the excess nutrients produced during culture production. Various bioremediation preparations have also been developed with a view to removing nitrogenous and other organic waste in water and bottom sludge and thus reduce chemically-induced physiological stress, e.g. in pond-reared shrimp. More products will undoubtedly emerge with continued research in this field, but controlled field trials are urgently needed to determine the effectiveness and cost-benefit of these products under culture conditions.

Probiotics are generally administered as live microbial feed supplements which affect the host animal by improving the intestinal microbial balance to optimize the presence of non-toxic species. A stable gut microflora helps the host resist pathogenic invasions, particularly via the gastro-intestinal tract. Antibiotics reduce specific or broad-spectrum gut microflora and probiotics may have post-antibiotic treatment potential for restoring the microbial balance. Probiotics are widely used in animal husbandry but their use in aquaculture is still relatively new. However, there are increasing reports of potential probiotics for shrimp aquaculture which has been plagued by opportunistic bacteria such as the luminescent *Vibrio harveyi*, and in some cases probiotics have been reported to significantly reduce antibiotic use in shrimp hatcheries. Suppression of proliferation of certain pathogenic bacteria (e.g. *Vibrio* spp.) in shrimp hatcheries has been achieved by introducing (inoculating) non-pathogenic strains or species of bacteria that compete for microbial metabolite resources. This procedure shows promise to be effective and economical. However, further refinement of the administration and concentration loads needed for effective pathogen suppression is required. Effective and economically viable probiotics also require greater research into optimal strains of probiotic micro-organisms and stringent evaluation under field conditions.

As discussed earlier, the control of disease using vaccines is a reputed technology. There are interesting examples of reducing antibacterial use in aquaculture through the use of vaccination particularly in temperate species such as salmon and trout. Reduction of the use of antibacterials not only diminishes the risk of rejection of aquatic products at international trading borders due to the presence/detection of residues above acceptable levels, it also helps in reducing the contamination of natural water bodies with harmful residues and the development of antimicrobial-resistant bacteria.

The proliferation of red tides with the blooming of harmful algae has been increasingly reported in many parts of Latin America, where the toxins represent a threat to food safety as well as a cause of fish and shellfish losses from the associated mortalities. Red tides can produce significant economic losses to fisheries and aquaculture due to bans on the marketing of fish and shellfish from the affected geographical area and to the toxic effects on fish. In Central America and the Caribbean, la “ciguatera” is the most important cause of toxic poisoning, resulting from consumption of tropical fish. In Latin America, blooms of *Alexandrium* spp. are one of the major causes of large economic losses due to the banning of commercial sales of mussels. In Chile, preventive closures cause about US$100 million in annual losses to the artisanal bivalve fishery. Furthermore, these closures have a direct negative impact on local employment in the shellfish production sector, which is labour intensive, thus having a detrimental effect on livelihoods. While it is not known if climate change is increasing the number of episodes of algal blooms, it is recognized that red tide episodes have recently become more common (Jessup *et al.*, 2009). Warm episodic currents also play a key role in causing large economic losses through mass mortalities of fish (Kedong *et al*., 1999).

To date, the detection of toxins due to algal blooms is carried out using mouse bioassays and high performance liquid chromatography, but new methodologies are being developed for detection of *Alexandrium catenella* (Uribe and Espejo, 2003). Expressed sequence tag (EST) libraries are now publicly available (Uribe *et al.*, 2008), so that it may be possible to develop molecular diagnostic techniques. To improve the prevention of impacts on aquaculture, PCR techniques and EST libraries can be used also to assist the early detection of toxin-producing algae in vast marine areas.

4.3.4 Biodiversity conservation and fisheries management

Restocking procedures are common in many developing countries, but the potential of restocking and stock enhancement stems primarily from the development of the technologies used to produce hatchery-reared juveniles (Bell *et al.*, 2006). The production of large numbers of juveniles and their subsequent release into the wild can affect a fishery resource in at least two ways (Bell *et al.*, 2006): 1) when stocking is done to restore a spawning biomass there is some scope for interbreeding between the natural population and the introgressed population and 2) there may be enough individuals used to restore the carrying capacity of the fishery.

From a genetic point of view, the main consequence of restocking may be the hybridization of non-native individuals with natural stocks, which can have important impacts on natural biodiversity. Fish are very prolific, and under many hatchery production systems a relatively small number of parents can provide sufficient numbers of juveniles for release, in which case the genetic variability of the fishery may be reduced. This situation can easily lead to genetic bottlenecks, the forthcoming generations of population being subjected to relatively high rates of inbreeding thus inadvertently reducing the genetic variability of the population (Povh *et al.*, 2008). This can have large effects on the sensitivity of individuals to environmental variations and could possibly cause the extinction of a population or species in a particular environment (Guttman and Berg, 1998). In addition, inbreeding can affect growth and reproduction.

The mating of wild fish with those released by restocking programmes can promote the loss of genes important for local adaptation (Vasemägi, Nilsson and Primmer, 2005; Sønstebø, Bergstrøm and Huen, 2006) in a genetic mechanism called outbreeding depression. While this concern has been effectively studied in terrestrial animals and in salmon populations in developed countries, this is not the case in other fisheries from developing countries.

Therefore, careful restocking procedures need to be developed in order to reduce the potential for the introgressed population to reduce the genetic variability and therefore the sustainability of the resource. Assessing the genetic diversity of managed stocks or highly selected populations is an important issue when pedigree information is lacking or in situations where some kind of quality assurance is needed.

The use of molecular markers and the principles of population genetics have proved very effective in assessing the actual levels of genetic variability within single populations and in measuring the extent of differentiation between populations. For example, the Centro Nacional de Pesquisa de Peixes Tropicais in Brazil has studied the use of RAPD markers for the Amazonian fish "matrinxa" (*Brycon cephalus*) and has shown a relatively large reduction in genetic variability in fish used for restocking purposes compared with the native Amazonian river population (Povh *et al.*, 2008).

In developing countries, the markers have been used mainly for assessing genetic variation in tilapia and carp populations in Thailand, the Philippines and India. Markers have been used for characterizing stocks and comparing levels of genetic variability in *Oreochromis* species. Agustin (1999) used markers to assess genetic differences between indigenous samples from Africa and populations from Asia, concluding that the low performance of *O. mossambicus* stocks can be explained by the effect of large bottlenecks in the populations used for aquaculture in Asia. Molecular markers have also been used to assess population differentiation of Nile tilapia (*O. niloticus*) for both domesticated and feral populations (Agnèse *et al.*, 1997). In both cases, moderate to great genetic differentiation was found between strains and the use of markers successfully correlated with the actual biogeographical data.

The escape of farmed fish from aquaculture may influence the genetic variability of native populations. The possible genetic impacts resulting from introductions and invasive alien species include: interbreeding between alien and native genotypes causing, in some cases, reduced reproductive efficiency and generating nonviable offspring; decreased fitness from loss of co-adapted gene complexes; and indirect genetic impacts resulting from other ecological interactions (FAO, 2005a).

Climate change and related climatic events such as the El Niño-Southern Oscillation (ENSO) can have serious impacts on the distribution of fishery resources between countries. Based on census numbers, mackerel fisheries were apparently depleted in Chilean coastal waters during the occurrence of ENSO episodes. However, markers have shown little differentiation with other populations in the Pacific Ocean (such those observed in New Zealand), and so it is likely that the drop in numbers is related to migration of the mackerel populations to colder waters in the Pacific rather than to fishery depletion (IFOP, 1996).

4.3.5 Concluding remarks

Compared with livestock and crop production, aquaculture is a novel production system in many developing and developed countries. As shown above, biotechnologies are being applied in fisheries management but their use is very limited compared with aquaculture. The use of successful and effective biotechnologies in aquaculture is very much confined to genetic manipulations and improvements, and to health management.

The success or failure in using biotechnologies in developing countries depends to a large extent on: 1) the markets for each of the products within the production sectors, and 2) the investment and acquisition capacity for the fisheries and aquaculture sectors. In the case of aquaculture, the latter is very important considering that the largest proportion of world production comes from developing countries and from small farmers (specifically in Asia). Most biotechnological interventions have been developed for improved production and the better management of aquaculture. Most have been targeted towards high value commercial aquaculture species generally produced for international markets. Although many small-scale farmers are producing for export markets, the significant uptake of many biotechnological interventions and innovations has generally been restricted to commercial or industrial aquaculture operations. This is certainly due to the cost of the technologies as well as the organized nature of industrial aquaculture.

Recently, however, as a result of better organization in the small-scale farming sector, certain biotechnologies have been effectively taken up by the small farmers in many parts of the developing world. They include DNA probes for detecting pathogens in some species (mainly PCR detection of major viral pathogens of shrimp), the use of SPF shrimp broodstock or postlarvae, the use of certain DNA vaccines, the all-male (genetically male) tilapia and, in some cases, markers for pedigree evaluation in salmon worldwide. In fact, almost everywhere in the world, shrimp farmers, whether small or large, currently use only PCR-tested postlarvae for stocking. For example, in India there are more than 90 laboratories providing PCR services for the shrimp sector – mainly for the screening of seed and broodstock. In Vietnam, there are over 40 laboratories. This pattern holds true in many countries of the region as the cost of using such biotechnologies has declined over the years and the benefits have increased tremendously.

As mentioned above, the majority of aquaculture produce comes from the small-scale farming sector, in many instances comprising low-input extensive production systems. Although there is scope for biotechnologies, and although they are already being employed by small-scale farmers, classical environmental improvements and better management practices such as conventional genetic selection of broodstock, conventional health management through the avoidance of pathogens etc., can also contribute significantly towards improving small-scale aquaculture production and sustainability.

4.4 CASE STUDIES

Biotechnologies are used in aquaculture for reducing losses due to diseases and improving production through genetic manipulation. These technologies are regularly used in almost all countries at different rates and levels based on the intensity and commerciality of the production system. Here, two case studies are presented, outlining specific successful applications of biotechnological tools in aquaculture in developing countries.

4.4.1 PCR-based pathogen detection in shrimp aquaculture in India

At present, shrimp is the most valuable aquaculture commodity sector in the world. This sector has been continuously facing the challenge of new diseases, particularly viral pathogens. Some 20 years ago, there was hardly any accurate molecular-based pathogen detection system available in any part of the world. Now, as a result of advanced molecular research and biotechnology, there are many DNA-based detection technologies such as PCR methodologies available for all the major shrimp viruses. A number of PCR, nested-PCR and hybridization tests have been developed for virus detection. The tests use a range of different PCR primers and hybridization probes targeted to different and poorly defined sites in the virus genome. Several RT-PCR tests are also available. The application of PCR detection of viruses of broodstock and postlarvae in both *Penaeus monodon* and *Penaeus vannameii* is now practised in all countries producing commercial shrimp at all levels (Lo, Chang and Chen, 1998; Karunasagar and Karunasagar, 1999; Peinado-Guevara and López-Meyer, 2006). Recently, lateral flow chromatographic immunodiagnostic strips similar to common drug store pregnancy tests have begun to appear for some shrimp diseases. Using these, unskilled farm personnel can easily diagnose shrimp disease outbreaks at the farm. The strips are relatively cheap and quick. Other methods comparable to PCR and RT-PCR are now available or are being developed for single and dual or multiple viral detection but they currently require advanced equipment and personnel.

This rapid detection technology has given a new dimension to the shrimp industry and losses due to viral diseases have been reduced tremendously by the use of PCR-tested postlarvae for stocking. Recent successes in farmer group or cluster formation and management in shrimp aquaculture, particularly in India and Indonesia, are to a large extent based on good health management which includes the use of PCR tested postlarvae for stocking in ponds. This demonstrates a scenario in which a successful biotechnology has not only contributed towards realizing its scientific objective, but also towards improving the overall governance of the sector (Subasinghe, Soto and Jia, 2009).

To consider a specific case study, the use of PCR detection technology was the key basic step towards developing an effective better management practice (BMP) for small-scale shrimp aquaculture in Andhra Pradesh. In India, aquaculture is mainly carried out by small- and marginal-scale farmers located in the remote villages of the country. They are largely unorganized, scattered and poorly educated. The farmers mostly opt for traditional methods for operating their farms and do not have access to technological innovations or scientific applications. A joint MPEDA-NACA (Marine Products Export Development Authority – Network of Aquaculture Centres in Asia-Pacific) project assisted by FAO was initiated in 2002 to support shrimp farmers in disease control and coastal management, leading to the participatory development of BMPs that provided significant improvements in profits and reduced shrimp disease risks for farmers. One of the key interventions that the farmers adopted in applying BMPs in their quest to reduce losses due to disease was the use of PCR-screened postlarvae for stocking.

The project supported farmers in the implementation of BMPs through the formation of self-help groups around local "clusters". An economic analysis of 15 farmer groups in Andhra Pradesh clearly demonstrated that farmers adopting BMPs including the use of PCR-screened postlarvae for stocking had higher profitability, lower production costs and were able to produce quality and traceable shrimp without using any banned chemicals.

The project has been highly successful in forming a self-help movement of farmers across India through a grassroots approach. From a mere five farmers who first adopted the cluster-farm approach and BMPs in 2002, the programme had swelled to more than 1 000 farmers in 30 aquaculture societies in five coastal states by 2007. Beginning in 2007, the MPEDA-NACA project became the National Centre for Sustainable Aquaculture (NaCSA). NaCSA is an outreach organization of MPEDA established to service the small-scale aquaculture sector and provide technical support to farmer groups. It aims to empower and build the capacity of small-scale farmers to produce quality shrimps in a sustainable and more profitable manner.

Perhaps one of the keys to the above success is the ability to reduce losses due to disease in production systems, and to a large extent this has been possible through the use of PCR technology for screening and detecting major viral pathogens in broodstock and postlarvae.

4.4.2 Specific pathogen-free stocks in shrimp aquaculture

Only a few species have so far been domesticated in the aquaculture sector. One group of species on which most research has been focused on the domestication and development of SPF strains is the penaeid shrimp. SPF shrimp are produced in SPF facilities using many biotechnological tools, particularly DNA-based pathogen detection and diagnostic techniques. The primary goal of SPF facilities is to produce strains of shrimp that are disease-free, domesticated and genetically improved for aquaculture. SPF lines are available for *P. vannamei*, *P. stylirostris* and *P. monodon*. The SPF status should signify that the shrimp have passed through a rigorous quarantine and disease-screening process that has found them to be free from specified pathogens of concern to culturists. This characteristic means that countries or regions which still do not have this species can be reasonably sure that importation of SPF animals will not result in the introduction of the specified pathogens from which the animal is declared free. This does not, however, guarantee against the animal being infected with unknown pathogens or known pathogens for which the animal was not screened.

Genuine SPF shrimp are produced in biosecure facilities that have been repeatedly examined and found free of specified pathogens using intensive surveillance protocols, and originate from broodstock developed with strict founder population development protocols. These founder populations are generated by extensive quarantine procedures that result in SPF F_1 generations derived from wild parents. Only stocks raised and held under these conditions can be considered truly SPF. There is not yet an internationally agreed protocol for the development of SPF shrimp, and certainly some variation exists in the quality of different SPF stocks. Once the animals are removed from the SPF production facilities, they should no longer be referred to as SPF even though they may remain pathogen-free. Once outside the SPF facility, the shrimp may be designated as High Health (since they are now subject to a greater risk of infection), but only if they are placed into a well-established facility with a history of disease surveillance and biosecurity protocols. If the shrimp are put elsewhere, for example into a non-biosecure maturation unit, hatchery or farm, they can no longer be called SPF or High Health as they are now exposed to a high risk of infection (FAO, 2005b).

One potential drawback of SPF animals is that they are only SPF for the specific diseases for which they have been checked. Typically this will consist of the viral pathogens which are known to cause major losses to the shrimp culture industry, including WSSV, YHV, TSV, IHHNV, *Baculovirus penaei* virus and Hepatopancreatic parvovirus as well as microsporidians, haplosporidians, gregarines, nematodes and cestodes. Despite this screening, new, hidden or "cryptic" viruses may be present, but because they are as yet unrecognized they may escape detection. Thus, it is believed that SPF shrimp shipped from Hawaii resulted in the contamination of shrimp in Brazil and Colombia with TSV. This was because, at the time, TSV was not known to have a viral cause and therefore went unchecked in SPF protocols.

In any case, the use of SPF stocks is only one part of a complete plan for minimizing disease risks in shrimp culture. The development of SPF strains is really designed to ensure that postlarvae stocked into grow-out ponds are free of disease, which is one, if not the most serious, source of contamination. Other areas of this strategy that must be implemented include ensuring that broodstock, eggs, nauplius, larvae and juveniles derived from SPF stock remain SPF.

Creating an enabling public sector environment is essential to improve governance at all levels of aquaculture development. There have been many regulatory rebounds in the aquaculture sector, in particular in shrimp farming in some countries. Uncontrolled and unregulated development of the sector has outstripped the carrying capacity in some locations, causing significant production losses mainly due to disease and resulting in the complete abandonment of farms. Significant improvements have been made in mitigating such catastrophic problems, and the negative environmental and social impacts of shrimp farming throughout the world have been significantly reduced. The use of wild-caught postlarvae in shrimp culture, which has a significant impact on aquatic biodiversity, has almost stopped or is little practised. The recent development of SPF broodstocks of some species of shrimp has reduced reliance on wild-caught postlarvae to a minimum.

SPF shrimp if produced and maintained under good biosecurity have proved successful. The success of SPF stocks may be more pronounced in large-scale industrial shrimp culture facilities where maintaining stringent biosecurity is possible. The use of this successful biotechnological approach in the rather disorganized small-scale shrimp aquaculture production sector poses another challenge (FAO, 2004b).

B. LOOKING FORWARD: PREPARING FOR THE FUTURE

4.5 KEY ISSUES WHERE BIOTECHNOLOGIES COULD BE USEFUL

Environmental sustainability

Aquaculture is the fastest growing food producing sector in the world. It is poised to expand, diversify and intensify over the coming decades to bridge the increasing global gap between the supply and demand of aquatic food. Responsible production through sustainable practices is the key to achieving this massive task. In the effort to maximize the contribution from aquaculture it is inevitable that many constraints and hurdles need to be overcome. The biggest hurdle is to maintain environmental sustainability,

Conventional methods of controlling diseases such as chemotherapeutants are ineffective for many new pathogens (notably viruses). Molecular techniques have therefore received increasing attention for pathogen screening and identification. In addition, these biotechnologies are providing significant insights into pathogenesis (disease development) and show strong potential for disease control and prevention programmes (e.g. DNA vaccines), as well as for treatments of diseases. The increased sensitivity and specificity conferred by DNA- or RNA-based probes has provided significant inroads for the early detection of diseases and identification of subclinical carriers of infections. This has had a direct effect on enhancing preventative management and control of disease in cultured species. Concomitant with this has been a decrease in the need for reactive treatments using traditional methodologies such as antibiotics or culling and disinfection. This has been particularly successful for shrimp broodstock selection and has broken the infection cycle perpetuated for years by accidental broodstock transmission of viral pathogens to developing offspring.

Biotechnologies can provide much assistance to improve aquatic animal health management in aquaculture in developing countries, in particular through the development of sensitive and accurate molecular diagnostic methods and tools as well as vaccines for tropical diseases. Bioremediation and probiotics also provide some further opportunities.

Climate change

In the future, one of the greatest constraints could be the impact of climate change on aquaculture. Climate change threatens fisheries and aquaculture through higher temperatures and changes in weather patterns, water quality and supply. Important differences in the magnitude and types of impacts on aquaculture are predicted for different regions. The ability to adapt will confer a major advantage and should be developed by countries and regions. There is a need for the aquaculture sector to join other economic sectors in preparing to address the potential impact of global warming. One of the practical responses to climate change for aquaculture could be to strengthen the adaptive capacity and resilience of the sector, particularly those of small farmers and aquatic resources users. Increased resilience is a desirable feature of any sector. It can mitigate the future impact of unforeseen events (e.g. economic change, disease epidemics, tsunamis, etc.), including those related to climate. There is some knowledge and experience from aquaculture itself, and from the broader area of agriculture and natural resource management, which could be used. Aquaculture, and particularly mariculture, could in fact provide adaptation opportunities to produce good quality protein when freshwater may become scarce. On the other hand, freshwater aquaculture can produce protein with higher water saving than other animal production sectors. Certain biotechnologies, particularly those dealing with genetic improvement, health and environmental mitigation should be of significant value for the discovery of adaptive technologies and interventions to counter the ever-present menace of climate change.

4.6 IDENTIFYING OPTIONS FOR DEVELOPING COUNTRIES

To bridge the future gap between demand and supply of aquatic food, production needs to be almost double in less than three decades. In the quest to meet this unprecedented demand, the aquaculture sector will face serious constraints. Four major constraints are inevitable: 1) disease prevention and health management, 2) genetic improvement and domestication, 3) environmental management and 4) food safety. These constraints are not new. They have been constantly addressed during the development of aquaculture over the past two decades, including through the use of biotechnologies.

Over the years, aquaculture biotechnologies and other technological innovations have had a positive impact on aquaculture diversification, investment potential, and international technology exchange. The development of biotechnologies in aquaculture should therefore provide a means of producing healthy and fast-growing animals by environmentally friendly means. However, this development will largely depend on the desire and willingness of producers to work hand-in-hand with scientists, and on the international donor community's readiness to assist developing counties in the related research, capacity building and infrastructure development. Improved exchange of information and discussion between scientists, researchers and producers from different regions about their problems and achievements will undoubtedly help this important sector to develop with a view to increasing sustainable global aquatic animal production.

Based on the overview and analysis contained in this Chapter, a number of specific options can be identified for developing countries to help them make informed decisions regarding the adoption of biotechnologies in the future, such as when – and if – they should deploy one or more biotechnologies and, if they decide to do so, how they can ensure the successful application of the chosen biotechnologies to enhance food security in the future.

- Few biotechnological advancements and tools are currently in use in small-scale aquaculture operations aiming at rural development, poverty alleviation and food security. However, there is a need to identify these, their application and socio-economic impact in developing countries. Developing countries should therefore collect information on the aquatic animal biotechnologies that may be used and analyse their national-level adoption and socio-economic impacts. Such information should be used to advise policy-makers on the cost/benefit implications of such applications. Increased efforts should be made to develop aquatic biosecurity policies within national research and development (R&D) programmes or national aquatic production programmes.
- The use of biotechnologies in aquaculture worldwide has increased incrementally over the past two decades. Several aquaculture biotechnologies have been used for improving aquatic food production in both developed and developing countries and have significant potential for future improvement. Since most aquaculture biotechnologies are still too technical and costly for small-scale farmers, efforts should be made to develop low-cost simple technologies that are easy to introduce to less advanced aquaculture farmers. Developing countries should give priority to developing aquaculture biotechnologies which are appropriate and conducive for both industrial and small-scale farmers.
- Major biotechnological achievements and advances in fisheries and aquaculture have been mainly restricted to aquaculture and to the fields of genetics, health and the environment. Genetic improvements using genetic manipulation (diploidy, triploidy) and hormonal therapy etc. have shown promise for producing fish and shellfish with improved and desirable production qualities. Disease prevention and health management in aquaculture have benefited significantly from advances in biotechnologies. Many reliable and accurate rapid diagnostic techniques have been developed which can be used by small-scale farmers. There are several efficient vaccines now available for certain aquaculture species which have significantly reduced the use of antibacterials in their culture. However, more research is required to develop vaccines for tropical species, particularly the major species of global production. Some environmental remediation tools and technologies have been developed using several biotechnologies. They are being applied in some production systems but their broad adoption across different production systems and practices is yet to be established.

  The potential contribution of biotechnologies for genetic improvement to improve production of culture aquatic species should be recognized. National research and development plans should include appropriate research in these areas. In aquatic animal health research, the development of molecular diagnostics, vaccines and probiotics should be prioritized and national research institutions should also carry out research using appropriate biotechnologies that can help the development of sustainable aquaculture in this area. National governments embarking on aquaculture development should also recognize that there is ample evidence for positive aquatic environmental impacts using various biotechnological interventions, and therefore the use of biotechnology for improving the aquatic environment should be considered.

- Until recently, perhaps because the application of biotechnologies in fisheries and aquaculture has been mainly restricted to the commercial and industrial aquaculture of temperate species, there has been little evidence in many developing countries of national-level efforts to prioritize the development and application of biotechnologies in aquaculture. Even when efforts were made to develop such technologies in the public sector of developing countries, they were not always directed towards or made available to improve small-farmer livelihoods. There is a need to create national policy environments in developing countries, including suitable investment and funding opportunities, to allow the development and application of appropriate biotechnologies in support of aquaculture development. National governments should pay special attention to the small-scale aquaculture sector. Preferential treatment of the sector towards capacity building in appropriate biotechnologies should also be considered.
- The funding required in developing countries for aquatic biotechnological research and applications should be found through national budgets or through extra budgetary resources. An integral part of funding should be directed towards investment in capacity building in the relevant fields of the aquaculture sector. A suitable investment environment and funding opportunities should be created to allow the development and application of appropriate biotechnologies in support of aquaculture development. The appropriate involvement of the relevant stakeholders in decision-making processes should be assured.
- The establishment of efficient institutional structures and enforceable legal frameworks are important for the responsible use of biotechnologies in aquaculture at the national level. Such institutional arrangements should also strengthen research and extension needs and enhance relevant human and infrastructural capacities. National legal frameworks in aquaculture biotechnologies should be developed within an integrated national biotechnology framework, which also complies with the legal or voluntary requirements of international treaties and agreements that the country has ratified.
- National biotechnology programmes in developing countries should include a special committee to oversee the aquatic biotechnology programme and research. Such committees should be formed in all countries and regional cooperation should be sought.

- Information gathering and dissemination on aquatic biotechnologies should be encouraged within and between countries in a given region, and developing countries should consider setting up dedicated websites for this purpose.
- Aquaculture products are facing increasing competition in accessing international markets. One of the key criteria is food safety and compliance with international food safety standards. Many such standards can be met through better farming that uses both simple and advanced biotechnological interventions. The aquaculture industry should therefore consider the importance of such biotechnological interventions in improving and maintaining food safety of cultured aquatic products. National governments in developing countries should consider R&D interventions on food safety within the broader framework of biotechnology.

4.7 IDENTIFYING PRIORITIES FOR ACTION FOR THE INTERNATIONAL COMMUNITY

The international community, including FAO and other UN organizations, NGOs, donors and development agencies, can play a key role in supporting developing countries by providing a framework for international cooperation and funding support for the generation, adaptation and adoption of appropriate biotechnologies. Here, a set of Priorities for Action is identified that can assist the international community in playing this role.

- Relevant international institutions, donors and development partners should recognize that biotechnological interventions can contribute to sustainable aquaculture development worldwide.
- Relevant international agencies should assist developing countries to collect, collate and analyse information about the biotechnologies in use in fisheries and aquaculture, and their contributions to national food security, poverty alleviation and social development.
- Relevant international agencies should make efforts to maintain databases and information systems to assist countries access information for national biotechnology development programmes relating to fisheries and aquaculture.
- Donors and international funding agencies supporting sustainable aquaculture development for food security and poverty alleviation should dedicate an appropriate share of their assistance projects to promoting and strengthening aquatic biotechnology R&D in developing countries. International research efforts should focus on developing interventions that are accessible to small-scale farmers.
- When supporting the application of biotechnologies in fisheries and aquaculture, the international community should consider that technical assistance in biotechnology R&D should not be done at the expense of funding for other key research fields and that it should support effective and intimate links to strong breeding and extension programmes.

- The international community assisting developing countries towards aquaculture sustainability should consider biotechnological advancement as an important area to be supported, and should assist developing countries in strengthening capacities for biotechnology policy development and long-term planning.
- The international community should assist developing countries to develop the capacities of their national agricultural research systems, which include aquaculture, to involve relevant stakeholders in decision-making processes.
- The international community should assist developing countries in establishing adequate institutional capacities for the development and enforcement of regulations related to use of biotechnologies in fisheries and aquaculture.

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