Novel and emerging technologies: can they contribute to improving aquaculture sustainability?

Expert Panel Review 1.2

Craig L. Browdy[1 (*)], Gideon Hulata[2], Zhanjiang Liu[3], Geoff L. Allan[4], Christina Sommerville[5], Thales Passos de Andrade[6], Rui Pereira[7], Charles Yarish[8], Muki Shpigel[9], Thierry Chopin[10], Shawn Robinson[11], Yoram Avnimelech[12] & Alessandro Lovatelli[13]

[1] Novus International Inc., 5 Tomotley Ct. Charleston SC 29407 USA. Craig.Browdy@novusint.com;
[2] Agricultural Research Organization, Volcani Center, POB 6, Bet Dagan 50250 Israel. vlaqua@volcani.agri.gov.il;
[3] Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849 USA. E-mail: liuzhan@auburn.edu;
[4] New South Wales Department of Primary Industries, Locked Bag 1, Nelson Bay, NSW 2315 Australia. E-mail: geoff.allan@dpi.nsw.gov.au;
[5] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK. chris.sommerville@stir.ac.uk;
[6] Dept. of Fish Engineering, State University of Maranhão, São Luis, Brazil. thalespda@hotmail.com;
[7] CIIMAR/CIMAR Centre for Marine and Environmental Research, Porto, Portugal & Algaplus, Production of Seaweed and Seaweed Derived Products, Ltd., Aveiro, Portugal. E-mail: rgpereira@netcabo.pt;
[8] Dept. of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT, USA. E-mail: charles.yarish@uconn.edu;
[9] The National Center for Mariculture, Israel Oceanographic and Limnological Research, P.O. Box 1212, Eilat 88112, Israel. E-mail: mshpigel@ocean.org.il;
[10] Canadian Integrated Multi-Trophic Aquaculture Network (CIMTAN), University of New Brunswick, P. O. Box 5050, Saint John, New Brunswick E2L 4L5 Canada. E-mail: tchopin@unbsj.ca;
[11] Department of Fisheries and Oceans, Biological Station, St. Andrews, New Brunswick, Canada. E-mail: Shawn.Robinosn@dfo-mpo.gc.ca;
[12] Department of Civil & Environmental Engineering Technion Israel Institute of Technology, Haifa, 32000 Israel. E-mail: agyoram@tx.technion.ac.il;
[13] Department of Fisheries and Aquaculture, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, Rome, Italy. E-mail: Alessandro.Lovatelli@fao.org

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Abstract

Aquaculture continues to be the fastest-growing food production sector with great potential to meet projected protein needs. The scientific and business communities are responding to the challenges and opportunities inherent in the

* Corresponding author: Craig.Browdy@novusint.com

growing aquaculture sector with research efforts generating novel technologies that mirror the diversity of the industry.

In genetics and breeding, the pace of advancement and innovation has been increasing exponentially. The number of breeding programmes, diversity of species, target traits and efficiency and sophistication of techniques applied continues to expand and advance. However, the pace of scientific development has at times outdistanced our ability to analyze risks and benefits, develop appropriate culture and containment technologies, educate and communicate, and reach policy and regulatory consensus. Now, more than ever, efforts must be made for society to accurately analyze and understand risks, to capture opportunities to raise healthier aquatic organisms faster with less environmental impact, while improving economic stability and providing associated social benefits.

Disease outbreaks continue to constrain aquaculture sustainability. Improvements in aquatic animal and plant health are coming from new technologies, improved management strategies and better understanding of the genetic and physiological basis of immunity. Vaccine development is benefiting from better specific antigen determination, more efficacious adjuvants and enhanced vaccine delivery. Traditional diagnostic technologies and newer methods have greatly improved speed, specificity and sensitivity. Research on improving oral delivery and disease management strategies that focus on prevention offer opportunities for improved control of pathogens and parasites in the future, obviating the use of antibiotics and chemotherapeutants.

An important key to culture of any fed species is the development of sustainable, cost-effective and nutritionally complete feeds, along with efficient feed management systems. Current research is focusing on improved understanding of nutritional requirements, nutrient availabilities and cost-effective formulations designed to maximize food conversion efficiency. Continuing cost pressures and the acute need to find additional protein and lipid sources to augment limited fishmeal and fish oil supplies is driving an increased understanding of how different nutrients are utilized and how to use increasing amounts of terrestrial ingredients. New sources of proteins and lipids from algae and microbes can offer alternatives, as cost efficiencies improve. Use of enzymes, probiotics and prebiotics, phytogenic compounds and organic acids are being shown to change gut microflora and improve health, digestibility and performance. Improved pelleting and extrusion technologies allow the production of top-quality feeds.

Advancements in production systems, including recirculation technologies, cages and integrated multi-trophic aquaculture, are also contributing to industry expansion and sustainability. All of these production system technologies are benefitting from expanding information and communication systems which are enabling advances in every stage of production. These and other examples suggest some of the benefits that future scientific-based innovation will contribute towards meeting increasing food demands, while improving social, environmental and financial sustainability of the global aquaculture industry.

KEY WORDS: *Aquaculture, Breeding, Feeds, Genetics, Novel technologies, Pathogens, Production systems, Sustainability.*

Introduction

Aquaculture continues to be the fastest-growing food production sector. The expansion of world populations and continuing problems with food deficits in many parts of the world stresses the need for additional/new sources of protein. In parallel, current trends suggest an increasing demand for high quality seafood from an expanding middle class, as countries like China continue to experience significant economic growth. It is recognized that sustainable aquaculture can contribute to solutions which can reduce pressures on wild caught fisheries while efficiently producing high quality protein. It has been suggested that aquaculture could provide new opportunities for food production from the sea and for efficient production systems on land which could expand food production within limited land and water resource constraints. Meeting these needs and achieving these goals will require innovation to refine existing aquaculture techniques and to apply new technologies to responsibly expand production. The scientific and business communities are responding to the challenges and opportunities inherent in the growing aquaculture sector with research efforts generating novel technologies that mirror the diversity of the industry. The present review provides an overview of some of the areas of current innovation in aquaculture. Sections on genetics and breeding, health, nutrition, sustainable production systems and information technology provide a review of some of the important trends in current and emerging research and development directions.

Genetics and breeding

Breeding and genetic selection

It is well known that genetic improvements have made tremendous contributions to assuring sustainable supplies of food for expanding world populations. For example, the often cited research by Havenstein, Ferket and Qureshi (2003) elegantly demonstrated that “genetic selection brought about by commercial breeding companies has brought about 85 to 90 percent of the change that has occurred in broiler growth rate over the past 45 years. Nutrition has provided 10 to 15 percent of the change”. The selected birds were estimated to have a feed conversion ratio (FCR) of 1.62 and 1.92 on the 2001 and 1957 feeds, respectively, with average body weight (BW) of 2 672 and 2 126 g. The unselected controls demonstrated FCRs of 2.14 and 2.34, with average BW of 578 and 539 g. As described below, examples are emerging in aquaculture-related literature demonstrating rates of relative genetic gain which can equal or exceed those described above for poultry. With their high fecundity and in many cases shorter life spans than terrestrial livestock and poultry, aquatic animals are excellent candidates for selective breeding programmes. However, aquaculture, with a few exceptions, remains an industry based on the culture of mostly unselected, semi-natural stocks and/or isolated populations subject to inbreeding and/or unintentional selection (Lutz, 2001). Aquaculture producers in many rural areas in developing countries have little understanding of, or interest in genetics in general, and in the rapidly advancing science of molecular biology, in particular. Meeting future demands for sustainable supplies of farmed seafood will depend upon continued progress in implementing practical methods of genetic improvement at all levels of the industry. This can be achieved through improved training and extension,continued investment in professionally managed breeding programmes and expanded access to improved stocks.

Species selection and establishment of founder stocks

Classical breeding programmes (i.e. selective breeding, crossbreeding and hybridization) are the mainstream of finfish genetic improvement (Bartley *et al.*, 2001; Gjedrem, 2005; Hulata and Ron 2009). The impact of selective breeding programmes on the aquaculture industry can be exemplified by the wide global distribution of the Donaldson strain of rainbow trout (*Oncorhynchus mykiss*) (Parsons, 1998), the success of the Norwegian Atlantic salmon (*Salmo salar*) breeding programme (Gjedrem, 2000) and the progressing dissemination of the selectively bred Nile tilapia (*Oreochromis niloticus*) known as genetically improved farmed tilapia – GIFT (Pullin, 2007). From 2000 to 2005, global production of essentially unselected strains of giant tiger prawn (*Penaeus monodon*) has levelled at about 700 000 tonnes. On the other hand, worldwide production of whiteleg shrimp (*Litopenaeus vannamei*), predominantly from domesticated and selectively bred broodstock increased from about 200 000 tonnes to over 1.6 million tonnes over the same period (Preston *et al.,* 2009). Based on the initial isolation of specific pathogen free (SPF) founder stocks, breeding programmes with *L. vannamei* have focused on maintaining biosecure SPF breeding populations, individual selection for growth and family selection for disease resistance (Browdy, 1998). Domestication and breeding of *L. vannamei* has significantly improved the economics and reliability of shrimp farming (Wyban, 2009). Whereas in the past, improving growth rate was the most common breeding goal, new traits have been incorporated more recently into breeding programmes. These include production-related traits (such as age at maturity; eliminating vertebral deformity; feed efficiency; and resistance to stress, diseases and parasites) and consumer-related traits (such as appearance, body composition and carcass quality). As fish welfare is becoming a crucial issue for the aquaculture industry (Ashley, 2007), attention has also been given to animal welfare-related traits (Olesen, Groen and Gjerde, 2000; Bentsen and Olesen, 2002; Olesen *et al.*, 2003). Attention is also given to the possible effects of selection on the social behaviour and growth pattern of the fish (Brännäs *et al.*, 2005). Improvements also have been made in breeding programmes through the introduction of new methodology for measuring complex traits, such as flesh color or feed efficiency (in rainbow trout – Helge Stien *et al.*, 2006; Kause *et al.*, 2006).

Breeding strategies

Efforts have been made recently to optimize mating designs for reducing effects of inbreeding(Gjerde, Gjøen and Villanueva, 1996; Villanueva, Woolliams and Gjerde, 1996; Sonesson and Meuwissen, 2000, 2002; Sonesson, Janss and Meuwissen, 2003; Gallardo *et al.*, 2004; Dupont-Nivet *et al.*, 2006; Holtsmark *et al.*, 2006, 2008; D'Agaro *et al.*, 2007) and in improving the experimental designs and statistical models to enhance genetic gains (Sonesson, Gjerde and Meuwissen, 2005; Hinrichs, Wetten and Meuwissen, 2006; Martinez *et al.*, 2006a,b). In addition, emerging technologies based on molecular markers and genomic approaches progressively rise in importance, and efforts are made to involve molecular approaches in breeding programmes (Fjalestad, Moen and Gomez-Raya, 2003; Silverstein *et al.*, 2006). A step further towards improving the design of a breeding programme was taken by Hayes, Moen and Bennewitz (2006) in their comparison of different strategies for using molecular marker information in order to maximize genetic diversity in the base population. Combining available phenotypic information for the traits of interest with marker data, they would "ensure that as much genetic variance as possible, for as many traits as possible, is captured in the base population".

The use and exchange of aquatic genetic resources (AqGR) have been crucial elements in facilitating aquaculture's fast growth (the fastest in the food-producing sector) over the last three to four decades. A special issue of *Reviews in Aquaculture* featured a series of reviews on genetic resources of species and species groups of important cultured aquatic organisms, for food production purposes, and issues related to the use and exchange of genetic resources thereof (Bartley *et al.*, 2009). The papers describe a variety of uses of AqGR that include breeding and genetic improvement in aquaculture, supporting culture-based fisheries (Solar, 2009); culture of marine shrimp (Benzie, 2009), common carp (*Cyprinus carpio*) (Jeney and Zhu, 2009), Nile tilapia (Eknath and Hulata, 2009), bivalve molluscs (Guo, 2009), salmon (Solar, 2009) and striped catfish (*Pangasianodon hypophthalmus*) (Nguyen, 2009); providing bait fish (Na-Nakorn and Brummett, 2009); producing ornamental species (Nguyen *et al.*, 2009); and mass cultivation of seaweeds (Yarish and Pereira, 2008).

Issues related to biosecurity, guidelines for the transfers of stocks and assuring pathogen status of genetic strains must be considered in the development and dissemination of selected stocks and improved strains. As mentioned above, for penaeid shrimp, the exclusion of listed pathogens from breeding centers and maintenance of stocks free of specific pathogens was a critical component in the development of selective breeding for *L. vannamei*. International Council for the Exploration of the Sea (ICES) guidelines were followed in the collection of founder stocks and a hierarchy of breeding centers, multiplication centers and hatcheries supported by careful attention to pathogens of concern were critical components of the breeding programme (Browdy, 1998). Thus, attention to issues related to disease control and pathogen transfer should be an important consideration in the management and regulation of sustainable aquaculture development.

Risks associated with selective breeding programmes should not be ignored. Species or strains of many fish species have been translocated from their place of origin or from places to which they have been introduced, and deliberately released for stocking or escaped from culture facilities, thereby affecting wild stocks (Cross, 2000). For example, the farming of Atlantic salmon, which has greatly expanded in the last 50–60 years, resulted in large numbers of escaped farm salmon invading native salmon populations throughout the North Atlantic (Fleming *et al.*, 2000; Carr and Whoriskey, 2006; Gilbey *et al.*, 2005; Hindar *et al.*, 2006; O'Reilly *et al.*, 2006). The nature of this interaction has been investigated by McGinnity *et al.* (2003, 2004), Weir *et al.* (2004, 2005) and others. Escaped salmon from net-pen aquaculture may have various potential biological consequences, e.g. risk of feral stock establishment; risks of competition with wild fish for mates, space and prey; risk of pathogen transmission; and risks associated with genetic interactions with wild stocks (Naylor *et al.*, 2005; Verspoor *et al.*, 2006). Culture of Atlantic salmon has also been shown to genetically affect wild populations of other salmonids, e.g. sea trout (*Salmo trutta*) (Naylor *et al.,* 2005; Coughlan *et al.*, 2006). Additional concerns are the potential risks associated with Atlantic salmon selective breeding programmes and translocations of stocks in and between Europe, North America and Chile.

The effects of cultured species on their respective wild populations are visible in the last two or three decades also with the Mediterranean gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*). These effects include interaction and competition for resources by accidentally escaping fish (whose numbers are increasing according to the records) and contribution of escaped fish to reproduction in the wild (Dimitriou *et al.*, 2007).

Tilapias are a group of fish that have been widely spread around the world during the last 50 to 60 years (Pullin *et al.,* 1997). More recently, stocks of Nile tilapia were introduced from various regions in Africa into the Philippines and mixed with cultured (earlier-introduced) strains to form the base population for the GIFT breeding programme carried out by the WorldFish Center (formerly ICLARM) and collaborators (Eknath *et al.,* 1993, 2007; Eknath, 1995). Improved descendants from this programme were disseminated to several countries in Southeast Asia for evaluation against local stocks, eventually leading to commercial culture of this introduced strain, which showed superior growth rate and survival relative to that of other strains used by farmers (De Silva, 2003). Since no native wild populations of tilapia existed in those countries, escapement did not result in any damage to wild tilapia populations. Upon termination of the GIFT research programme, subsamples were transferred to several countries in the region and served as founders for separate, parallel, further breeding programmes (Gupta and Acosta, 2004). The arguments for and against using improved GIFT strain in aquaculture in Africa are summarized in Brummett and Ponzoni (2009).

Future trends and prospects

Conventional breeding programmes will continue to be the main engine driving the global aquaculture industry forward. Efforts will persist to increase efficiency and optimize the design of breeding programmes by maximizing the use of pedigree information while using both established and cutting-edge technologies mentioned above. However, since these methods are less suitable for economically important traits that are difficult to measure on candidates for selection (such as carcass and disease traits), alternative approaches will have to be further developed and optimized. Here is where incorporation of recent biotechnological tools may come into play. The potential for accelerating breeding programmes expected from applying these tools has yet to be realized in the aquaculture industry. Nevertheless, marker-assisted selection (MAS) and gene-assisted selection (GAS) methodologies, when mature, may eventually become practical in efforts towards identifying genes that underlie economically important traits and towards combining quantitative and molecular data in breeding programmes. A potentially alternative breakthrough may arise from solving containment problems, currently limiting the use of genetically modified (GM) aquacultured organisms; with education and accumulation of data, antagonism of the public to the use of genetic modification may fade.

Genome-based technologies

DNA marker technologies

DNA marker technologies have been developed to reveal and differentiate genomic variations within a population, among populations or among various other higher levels of taxa. For fisheries and aquaculture purposes, such genomic variations are studied in relation to phenotypic performance of the fisheries population or aquaculture broodstocks.

The entire task of DNA marker technologies is to provide the means to reveal genome variations, in particular the indels (involving insertion or deletion of one or more bases) and the single nucleotide polymorphisms (SNPs – substitutions in bases at any given site of DNA) represent the vast majority of genomic variations. In the last 30 years, several DNA marker technologies have been developed, including restriction fragment length polymorphism (RFLP, for recent reviews, see Liu, 2007, 2009), microsatellites, rapid amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and SNP.

RFLP is an old technology. Due to its relatively low polymorphic rate and low ability to differentiate genomic variations, RFLP is no longer frequently used in most genomic settings, although it is still used in some fisheries and aquaculture settings.

Microsatellites are simple sequence repeats (SSRs) of 1–6 base pairs. The variation of the number of repeat units causes microsatellite polymorphisms. The advantages of microsatellites include their abundance in genomes, even distribution, small locus size facilitating polymerase chain reaction (PCR)-based genotyping, co-dominant Mendelian inheritance and high levels of polymorphism (for recent reviews, see Liu, 2007, 2009). The disadvantages of microsatellites include the requirement for existing molecular genetic information, a large amount of up-front work for microsatellite development, and the tedious and labour-intensive nature of microsatellite primer design, testing and optimization of PCR conditions. Over the past decade, microsatellite markers have been used extensively in fisheries and aquaculture research, including studies of genome mapping, parentage, kinships and stock structure.

At the beginning of the 1990s, efforts were devoted to develop multi-loci, PCR-based fingerprinting techniques. Such efforts resulted in the development of two marker types that were highly popular at that time: RAPD (Welsh and McClelland, 1990; Williams *et al.,* 1990) and AFLP (Vos *et al.*, 1995). RAPD has been widely used in genetic analysis of fisheries and aquaculture species, but its further application in genome studies is limited by its lack of high reproducibility and reliability. In addition, RAPD is inherited as dominant markers and transfer of information with dominant markers among laboratories and across species is difficult. AFLP is based on the selective amplification of a subset of genomic restriction fragments using PCR (for recent reviews, see Liu, 2007, 2009). AFLP combines the strengths of RFLP and RAPD. It is a PCR-based approach requiring only a small amount of starting DNA, it does not require any prior genetic information or probes, and it overcomes the problem of low reproducibility inherent to RAPD. It is particularly well adapted for stock identification because of the robust nature of its analysis. The other advantage of AFLP is its ability to reveal genetic conservation as well as genetic variation. The major weaknesses of AFLP markers are the dominant nature of inheritance, the technically demanding procedures and the requirements for special equipment such as automated DNA sequencers for optimal operations.

SNP describes polymorphisms caused by point mutations that give rise to different alleles containing alternative bases at a given nucleotide position within a locus (for recent reviews, see Liu, 2007, 2009). Recent technology breakthroughs have brought SNPs to the center of genetic and genomic applications, becoming the markers of choice in the future. They are very abundant in genomes. They allow comparative mapping analysis and are amenable to automated large-scale genome analysis. The real challenge now is SNP discovery. As reflected in its definition, SNP discovery depends on sequencing. Sequencing a huge number of genome segments representing the same sequences from independent chromosomes was a daunting task. However, recent development of the next generation sequencing has made it readily possible for many fisheries and aquaculture species using state-of-the-art equipment.

In spite of the current lack of draft whole genome sequences for aquaculture species other than nori (Gantt *et al.,* 2010), it is anticipated that they will soon become available for other major aquaculture species. Once genetic linkage maps are well constructed, genome scans for quantitative trait loci (QTL) are expected to follow to study traits which will be important targets for marker-assisted selection. As SNP markers are great markers for the analysis of trait-genotype associations, their application to aquaculture will become essential. SNPs will likely become the major markers of choice for genome research and genetic improvement programmes in aquaculture. Marker-assisted selection or whole genome-based selection in aquaculture should provide unprecedented genetic gains and benefits.

Genetic modification

The successful transfer of foreign gene constructs into a new host has been demonstrated for several fish species over the past 20 years. Short gene constructs have been inserted into breeding populations of fish, resulting in significant gains in traits of interest such as growth, disease resistance and cold tolerance (Lutz, 2001; Rasmussen and Morrissey, 2007). A number of techniques have been developed for introducing the genetic constructs achieving incorporation, expression and passing of the genes to subsequent generations of fish. The technology for creating transgenic animals is constantly improving, overcoming current limitations and providing potential alternatives for breed improvement. While overcoming potential technical problems with transgenic fish, the major constraints to adoption of transgenic stocks in aquaculture are the development of regulatory policies, the assessment of environmental and food safety risks and the acceptance of these technologies by consumers.

Recently, Kapuscinski *et al.* (2007) have published a book detailing options for comprehensive science-based risk assessment and risk management for genetically modified fish. The authors conclude that, realizing the potential of transgenic aquaculture to be of best use for society, its risks must be honestly and accurately analyzed and understood. The book details transparent, flexible, participatory and scientifically sound processes of risk assessment and management. They suggest practical guidelines to begin the process proactively using a safety-first approach and proceeding on a case-by-case basis. As the technologies for gene transfer continue to advance, there will be a growing need for these types of approaches to focus on reducing probability of unanticipated and unacceptable environmental risks while facilitating responsible utilization.

Genome mapping

The genome of a species of interest can be mapped genetically using recombination points as references, or physically using DNA segments as references. Both genetic linkage maps and physical maps are very important. Genetic linkage maps are required to study performance and production traits, while physical maps are required to study the genes involved in the determination of performance and production traits. Genetic linkage mapping involves analysis of performance trait(s) in relation to the markers on the chromosomes. Genetic linkage maps have now been made with many of the aquaculture species, such as Atlantic salmon, tilapia, catfish, rainbow trout, Atlantic cod (*Gadus morhua*), seabream and European seabass. Mapping performance traits by genetic linkage analysis is referred to as QTL mapping, as most, if not all, performance traits are controlled by multiple loci. QTL mapping provides information as to where the genes controlling the performance trait(s) are located in relation to the segregating markers. However, without a physical map, one can just get some information as to which markers are close to the QTL, but cannot easily conduct detailed analysis of candidate genes controlling the traits. Once the physical map is available, sequence-tagged markers on the genetic linkage map can be located on the physical map, and this process is referred to as map integration. Upon integration of genetic linkage and physical maps, genomic segments involved in QTL can be identified. If the genomic segments involving the QTL are relatively small, one can determine what genes are included in the segment(s), thereby identifying candidate genes for the involved performance traits. Practical application of QTL mapping is marker-assisted selection. Fuji *et al.* (2007) reported an example of practical application of marker-assisted selection to develop a population of lymphocystis disease-resistant Japanese flounder (*Paralichthy olivaceus*). It is anticipated that in the future whole genome-based selection programmes will be developed for aquatic species, as is already occurring in terrestrial livestock species (Liu, 2009).

Genome sequencing

The purpose of whole genome sequencing is to decode the entire genetic composition of an organism through DNA sequencing. Whole genome sequencing used to be very expensive, so it was not financially possible for fisheries and aquaculture species. However, the availability of the next generation sequencing technologies has made it much cheaper to sequence the genomes of aquatic organisms, most often within a million dollars. Most recently, several whole genome sequencing projects involving aquaculture species are underway, including Atlantic cod, Pacific oyster (*Crassostrea gigas*), Atlantic salmon, channel catfish (*Ictalurus punctatus*), tilapia, nori (*Porphyra*) and several other species. Whole genome sequences will serve as the most detailed linkage and physical map of the genome, with every base pair of the vast majority of the genome known. Whole genome sequencing also generates large numbers of SNPs for the analysis of trait(s). Once the association of SNPs with traits is known through genetic studies, candidate genes can be identified and tested.

Functional genomics

Environmental or physiological stimuli including physical, chemical biological, metabolic hormonal or disease stresses induce changes in the expression of an organism's genome, the results of which determine the type, level and effectiveness of the response. The application of new genomic analysis technologies to aquaculture species can be applied to generate a wealth of data on molecular response mechanisms. The study of the function of genes and genome segments has been facilitated by the increasing data on genome sequences. Sequencing of expressed sequence tags (ESTs) has been the primary approach to gene discovery in aquaculture species. New approaches based on next generation sequencers should quickly increase our understanding of genes of important aquaculture species through *de novo* sequencing of whole transcriptomes. ESTs are single pass sequences of random complementary DNA (cDNA) clones. Random clones are sequenced from cDNA libraries extracted from target tissues of organisms of interest. The rate of gene discovery is rapid at first, but it drops precipitously once commonly expressed genes have been collected. Normalization techniques can then be used to collect more rarely expressed genes. The most immediate information gained from analysis of EST collections is the existence of genes structurally related to those present in other organisms, which are likely to play roles in important physiological processes. A second level of information arising from EST analyses relates to levels of expression, as well as tissue distribution of specific transcripts. Abundance of an mRNA is often (but not always) directly related to the frequency at which ESTs representing it are present in a particular library. From this information, relative levels of expression for different genes can be inferred, which provides a first level of functional insight, even for genes for which activities cannot be predicted from sequence alone. This becomes particularly important in the study of invertebrates, where less fundamental information may be available (Robalino *et al.*, 2009).

Even with every single base pair sequenced, the function of genes and genome segments is largely unknown. However the development of new tools for functional genome analysis is proving new ways to gain insights into gene function. One of the most important of these tools is the use of genome scale expression analysis using microarrays or next generation sequencing. Liu (2009) provides a tabular summary of the current status of microarray development in aquaculture and aquatic species. A microarray is an arrayed series of thousands of tiny spots of DNA which can then hybridize with messages in an unknown sample, providing information on the abundance of nucleic acid sequences in the target sample. This then corresponds to up or down regulation of genes, providing data on tens of thousands of genes simultaneously. This information can be used to classify the physiological state of the organism from which the sample was collected or to generate data on the up and down regulation of specific genes. For example, in shrimp, our understanding of antiviral responses is quite limited, this despite the tremendous economic significance of viral epizootics in shrimp culture. Using advanced genomic tools including a first and second generation microarray, much has been learned about specific genes and genetic pathways, about the importance of antimicrobial peptides and about the function of double-stranded RNA as an inducer of antiviral immunity (see Robalino *et al.*, 2009 for review).

In well-studied model species such as the mouse or rat, gene functions are most often studied by gene knockout, i.e. upon knockout of a gene, one can determine what functions are lost. These types of studies are being carried out in shrimp using gene silencing to better understand the function of genes and proteins (e.g. de la Vega *et al.,* 2008). However, in most fisheries and aquaculture species, gene knockout has not been possible, although some studies on model species are ongoing.

Future trends and prospects

In the future, genetics approaches will allow identification of the genomic locations that are involved in certain functions through QTL or whole genome association studies. Coupling of location candidate genes with expression candidate genes may allow further narrowing down to the real candidate genes. Combining direct approaches and comparative genomic analysis will be very useful. For instance, if a gene is well studied to have certain functions in one organism, it is possible and perhaps likely that the ortholog of this gene would have the same or similar functions in related organisms. In this regard, functional studies using model species such as zebrafish (*Danio rerio*), pufferfish (*Fugu rubripes*), and medaka (*Oryzias latipes*) can lend much to functional studies in fisheries or aquaculture species. Upon the availability of the whole genome sequence assembly, the assignments of orthologs will become possible.

Although the pace of advances in genetic enablement has been accelerating as its potential is realized in aquaculture, significant challenges remain:

- The tremendous variety and diversity of aquaculture species often results in competition and division of limited resources among an expanding number of species. In some cases, much can be learned from closely related organisms, but much effort must be invested in each target species to achieve maximum results. Achieving consensus on highest priority species could improve the pace of discovery.
- Despite continuing improvements in lowering the cost of high throughput genetic technologies, the expense of a well-designed selection programme and the investments necessary for application of advanced genomic tools will limit private-sector adoption to large-scale integrated companies or well-funded specialty firms. National and multinational scientific consortia could accelerate advancement and transfer of technologies to the private sector.
- Biosecurity and problems with controlling pathogens in the aquatic environment will continue to constrain genetic improvement efforts unless carefully controlled.

- Breeding programmes and genomic tools quickly generate very large volumes of complex data. Attracting skilled individuals and applying necessary computing resources to aquaculture bioinformatics applications will be a key to future success.
- A final critical prerequisite to the safe and sustainable application of genetic technologies for aquaculture continues to be the development of and investment in educational resources and policy and regulatory tools. Implementation of the great potential of genetically improved aquaculture species will depend upon its practitioners, consumers and regulatory authorities having a clear understanding of the risks and benefits. This, in turn will allow the reasoned application of practical and precautionary approaches which will enable safe and sustainable implementation.

Health

Managing the health of aquatic organisms has proven to be one of the greatest challenges and opportunities for expansion of sustainable production of cultured seafood. Epizootic outbreaks of disease continue to represent one of the most important limiting factors for the success of aquaculture production systems in different countries in the world. The worldwide movement of live (i.e. eggs, gametes, larvae, juveniles and broodstock) and frozen aquatic animals is necessary for the development of aquaculture. However, it has also provided opportunities for rapid transmission and trans-boundary spread of diseases, causing adverse socio-economic losses in the aquaculture food-producing industry (Bondad-Reantaso *et al*., 2001; OIE, 2009a, b; Lightner *et al*., 2009; Walker and Mohan, 2009). In response, aquaculture researchers and industry have developed new technologies and improved management techniques. The efforts have focused on diagnostic technologies, epidemiology and disease exclusion. This section elaborates on some recent developments and their potential application for improving aquaculture sustainability.

Diagnostic technologies

Most currently available aquaculture diagnostic technologies are based on traditional methods used in bacteriology, virology, mycology and parasitology. Over the last two decades, significant efforts have been invested in development of more advanced methods (OIE, 2009a, b). As a result, routine histopathology and classical microbiology have now been widely supported by a significant number of immunodiagnostics (immunohistochemistry (IHC), direct or indirect fluorescence antibody (FAT/IFAT), enzyme-linked immunosorbent assay (ELISA), immunochromatography (ICT)) and conventional nucleic acid-based approaches such as *in situ* hybridization using pathogen-specific gene probes, polymerase chain reaction (PCR), reverse transcription-PCR and quantitative real-time PCR (qPCR) (OIE, 2009a, b). The last is the latest improvement over the standard PCR techniques. Perhaps the most refined diagnostic technology currently available is the development of qPCR, especially using TaqMan® probe, because it provides quantitative detection of a specific target with higher specificity and sensitivity. A limited but growing number of protocols, reagents and kits are currently available for aquaculture pathogen detection based on some of the technologies listed above. Monoclonal antibodies (mAbs) are being produced as standard reagents for diagnostic tests and are available commercially (Adams and Thompson, 2008). Aside from more secure diagnosis, their commercial production will make a significant contribution to sustainability of aquaculture when used for disease surveillance, as large numbers of animals can be screened non-destructively for previous exposure to selected pathogens. Furthermore, they can be used for post-vaccination efficacy testing, as well as for testing wild stocks.

Today, laser-based capture micro-dissection is an emerging technology enhancing histopathology to allow researchers to precisely isolate specific pathogens from tissue sections, even with mixed infections. These then can be isolated for nucleic acid extraction and molecular diagnostic, genetic and proteomic analysis (Small *et al.,* 2008). The implementation of histology-based virtual microscopy (VM) is also an emerging technique. VM allows storage of a complete clinical and pathology workup consisting of several images which are stored in a dedicated server database. This facilitates rapid effective case management and communication for teaching or for off-site diagnostic review. The use of digital slides also represents a powerful tool for the assessment of diagnostic accuracy and quality control programmes for diagnostic laboratories in different parts of the world (see the European Union (EU) funded research programme BEQUALM available at www.bequalm.org/fishdisease.htm, Rocha *et al.,* 2009). Time consuming conventional methods for bacterial identification are being replaced by a strip-concept of dehydrated biochemical tests (enzymatic and assimilation) in miniaturized microtubes (e.g. API 20 E). Moreover, a fully automated microbial identification and susceptibility system (VITEK) has been introduced for busier clinical laboratories and aquaculture certification programmes (Kuen, 2007).

An emerging platform combines end-point nucleic acid amplification such as PCR or loop-mediated isothermal amplification (LAMP) with dot-blot hybridization (DBH) or ICT. These emerging methods are allowing the development of highly specific, sensitive, rapid and cost effective methodologies for detection of pathogenic microorganisms which are less prone to contamination. In addition, these methods can be applied in resource-poor and "point-of-care" diagnostic settings (Teng *et al.,* 2007; Srisala *et al.,* 2008; Andrade and Lightner, 2009; Soliman and El-Matbouli, 2010). New dimensions are being opened for diagnostics with powerful multiplexing platforms for simultaneously testing for multiple different pathogens using emerging Luminex xMAP® and microarray technology. Although these technologies are just beginning to be applied for aquaculture, they are likely to become more widely used in aquatic animal diagnostic laboratories in the future (Adams and Thompson, 2008).

Many of these new diagnostic technologies can be tools in our efforts to improve the health of aquacultured animals. It is important to understand the advantages and disadvantages of each of these technologies, what kind of test is the most appropriate to apply in a specific disease situation and the type of conclusion that can or cannot be drawn from their results. Certification programmes for diagnosticians, for laboratories and for the methods themselves are currently limited, and governmental accreditation programmes would improve the outlook for more accurate and appropriate use of these powerful tools (Lightner *et al.,* 2009).

Epidemiology

The contribution of new diagnostic technologies to better understanding disease transmission and to epidemiological modelling can inform regulators and therefore contribute to determining constraints on movements of stock to better control spread of diseases across borders. Aquaculture epidemiological information has been routinely supported by a combination of molecular biology, bioinformatics and taxonomy to identify specific names and biological properties of the new and emerging infectious agents or strains. For example, retrospective molecular sequence analysis of the evolutionary story of etiological agents corroborated suspected transboundery routes of disease transmission and the characterization of emerging circulating strains in aquaculture operations around the world (McBeath Alastair, Bain and Snow, 2009; Wertheim *et al.,* 2009; Muller *et al.,* 2010).

Surveillance has become more important since the formation of the World Trade Organization (WTO) and subsequent implementation of various multilateral agreements on trade aimed at reducing the risk of international spread of important aquatic animal diseases, early warning of disease outbreaks, planning and monitoring of disease control programmes, provision of sound aquatic animal health advice to farms, certification of exports, as well as international reporting and verification of freedom from particular diseases. Geographic information systems based on remote sensing and mapping have also emerged as a powerful analytic and decision-making technology to assist epidemiologists in government, industry and reference laboratories to minimize the likelihood of rapid spread of disease in aquaculture operations (Kapetsky and Aguilar-Manjarrez 2007; Bayot *et al.,* 2008).

Vaccines

Vaccine development is benefiting from new technologies in three main ways, i.e. by specific antigen determination, more efficacious adjuvants and vaccine delivery. Most commercial vaccines are against bacteria, a few against viruses and none against parasites. Most are inactivated bacterial pathogens, and there are a few commercial vaccines which are live attenuated pathogens. Using molecular technology, pathological organisms can be genetically modified to remove the virulence genes to avoid reversion and, therefore, are more sustainable. The advances in DNA recombinant vaccines are most promising and more sustainable because they reduce concerns for the environment and for the consumer (Sommerset *et al.,* 2005; Kurath, 2008). DNA vaccines, based on administration of a plasmid encoding the gene for the selected antigen, have been under development for a number of years. Progress has been restrained by environmental and safety concerns by regulators and by confusion with genetically modified organisms (GMOs) by consumers (Lorenzen and LaPatra, 2005). Once these problems have been overcome, DNA vaccines may make a considerable contribution to fish welfare. These new technologies coupled with proteomics may well open up the way for parasite vaccines. Until recently, these vaccines have been constrained by difficulties in finding protective antigens, but breakthroughs for parasites like sea lice may be on the horizon (Ross *et al.,* 2008). Proteomics and epitope mapping can be used for precise identification of specific antigens and to monitor efficacy and duration of response.

Adjuvant research has accelerated in recent years, benefitting from advances in mammalian vaccinology. This challenging research aims to improve vaccine response by increasing immunogenicity, focusing on co-stimulatory signals received from dendritic cells. Activity has concentrated on finding agents that activate dendritic cells to enhance effectiveness of vaccines as molecular adjuvants. Application of molecular tools is enabling cytokine discovery and elucidation of their role in the expression of co-stimulatory molecules (Secombes, 2008). Alongside the study of co-stimulatory molecules, there is the possibility of adjuvants which act to inhibit negative regulators.

Currently, the most common procedure for vaccine delivery is by immersion or injection, both of which have their drawbacks. However, oral delivery systems are improving. Whereas the environment of the intestine has, to date, been seen as hostile to antigen integrity, it is now possible to protect it and release it in the most suitable environment, the hindgut. Poly (I:C) coated micro particles (PLGA) are revolutionizing delivery of antigens to immune cells for the induction of a long-lasting immune response for vaccination by promoting innate and adaptive immune responses in fish (Behera *et al.,* 2010).

Dietary supplements

The use of dietary supplements and nutritional strategies which may modulate overall fitness, gut health and immune responses is discussed below in the Nutrition section. Use of immunostimulants and stress diets to improve the defense of animals during critical stressful periods, have been promoted in the commercial feed sector. Compounds have been suggested such as β-glucans, bacterial products and plant derivatives which have the potential to activate the innate defense mechanisms by acting as receptors which trigger gene activation (Galindo-Villegas and Hosokawa, 2004). Probiotics and prebiotics are at a similar stage in research, attracting much attention (Balcázar *et al.*, 2006). Organic acids and essential oils have been suggested to modulate gut microbial communities, improving resistance to some opportunistic enteric pathogens (Luckstadt, 2008). More information is necessary on the mode of action and the host/microbe interactions. It may be envisaged that useful products will be available in the future, contributing to greater sustainability by avoiding the use of drugs.

Chemotherapeutants

The greater efficacy and widespread use of vaccines will have the greatest impact on sustainability, by obviating the use of antibiotics and chemotherapeutants. There is little enthusiasm for the licensing of new antibiotics, and antiviral drugs have attracted little research interest in animal production industries. Chemotherapeutants have been, to date, essential for the control of parasitic diseases. However, issues relating to environment and consumer safety have been a powerful influence on the newer products under development. Avoidance of topical treatments using bath immersion applications have given way, where possible, to oral in-feed products for greater control of the active ingredients, less pollution and cost saving. Despite the need for new effective chemotherapeutants, costs and complexities of licensing constrain development. Owing to the concern for the natural environment, history of reduced sensitivity and product misuse in aquatic environments, the reaction from environmentalists and consumers has resulted in substantial regulation. The regulation of timing and rate of application of chemicals is likely to intensify. This, coupled with better monitoring, will encourage aquaculture to utilize more non-chemical control methods as part of an integrated pest management strategy (Sommerville, 2009). The use of multiple tactics against infection and greater regulation of drugs and chemicals will be major steps towards sustainability.

Disease exclusion

In the early years, aquaculture was plagued by misdiagnosed diseases in wild broodstock and seed. Presently, a variety of improvements have been made in applying biosecurity principles, best management practices (BMPs) and disinfection for control of pathogens. This has been facilitated by the development of more reliable and accurate diagnostic methods, application of educational approaches for training, use of better low water exchange management systems which reduce opportunities for pathogen introduction, improvement of feed formulations and advances in overall routine biosecurity and sanitation. Thus, over the past two decades strategies have been refined and adopted by many aquaculture operations based on use of a combination of i) early detection of specific pathogens over the time, ii) development of infrastructure for commercial supplies of healthy or SPF stocks, iii) improvement of stocks for desirable performance traits (i.e. disease tolerance, growth rate, feed conversion efficiency) and iv) development of consistent documented history for a particular stock assuring freedom from specific listed pathogens over time. As described above, major breakthroughs have been made in molecular techniques in recent years which make the genetic selection for disease-resistant fish stocks a realistic possibility for the future, and this is accelerating as pedigree families become more available (Jones *et al.,* 2002). The rapid expansion of culture of whiteleg shrimp in Asia over the past decade exemplifies the potential for improvement of productivity through the use of healthy improved seed stocks coupled with biosecurity and disease management strategies. More detailed reviews on this topic can be found in Lightner *et al.* (2009) and Benzie (2009).

Future trends and prospects

The rapid expansion of aquaculture has provided opportunities for increased pathogenicity of existing infections and additional exposure to emerging disease etiologies. Although future success in realizing effective diagnostic or exclusion technologies for emerging diseases cannot be predicted, experience over the past 20 years suggests that many of the current strategies and advances reviewed here will facilitate future success in assuring aquatic animal health. This will depend upon continued advancement in several areas including:

– Developing accredited biosecure breeding programmes and expanding systems for health certification of stocks.
– Establishing and accrediting international reference laboratories and virtual international, national and regional surveillance systems.
– Accreditation and certification of diagnosticians, diagnostic laboratories and diagnostic methods.
– Developing improved reliable, rapid, accurate and ready-to-use multiplex kits for pond-side diagnostics.
– Identifying markers and exploring mechanisms of disease resistance.
– Expanding registration and availability of effective vaccines and of new methods for disease control and treatment.

Application of improved diagnostic technologies coupled with more thorough expanded epidemiology and disease exclusion efforts should continue to contribute to a more advanced and sustainable aquaculture industry for wholesome food production in the years to come.

Nutrition

The future of aquaculture nutrition will be based on a better understanding of the basic nutritional requirements and the role of gut microflora in the fish digestion process of a growing list of important cultured species, coupled with innovative solutions for delivering these nutrients in ways which minimize environmental impacts. The increasing demand for sustainable aquaculture products has focused attention on the need to improve feeds and feeding to allow increased production and productivity. Traditionally, aquaculture feeds, particularly for carnivorous and omnivorous species, were based on fishmeal and fish oil. These excellent ingredients are still the basis for many feeds today, but supply of fishmeal and fish oil is static. While there is strong evidence that current production is sustainable, there is little prospect that additional production is likely. Inclusion rates are declining for major farmed species, but demand for protein and lipid (including essential fatty acids found in fish oil) is increasing rapidly as production of aquaculture species grows. Total replacement of fishmeal for some species (e.g. catfishes, carps and tilapia) is possible, and replacement of a significant proportion of the fishmeal and a lesser proportion of the fish oil for most species is relatively easily achieved. However, as availability declines and the need for more replacement increases, the task will become more difficult, particularly for fish oil. Hence, further research on suitable alternatives remains a very high priority (Tacon and Metian, 2008). A key driver for aquaculture production is the increasing need to minimize negative environmental impacts. As production intensifies, the impacts from uneaten feed and faeces on the receiving environment become more critical. Unfortunately, most ingredients available to partially or totally replace fishmeal and fish oil are less well utilized, increasing production of wastes. To address both these challenges, an improved understanding of the digestive physiology and nutritional requirements of key species is needed, a greater range of potential feed ingredients and new technology to improve their value needs to be evaluated and developed and continuing improvements made to processing technology used for producing feeds.

Nutrient requirements

Aquafeed development mirrors the history of development of prepared feeds for terrestrial agriculture. Over the past 50 years, terrestrial rations have reduced or eliminated the use of fishmeal as the price of this limited commodity has risen. Formulations have been consistently improved based on a fundamentally increasing understanding of the digestive physiology and nutritional requirements of poultry, ruminants and swine. One of the key accomplishments has been the ability to continue to meet the nutritional demands imposed by performance enhancements and physiological challenges resulting from aggressive selective breeding programmes. With recent advancements in the development of molecular genetic tools, the physiological demands of better-growing stocks will continue to increase along with more powerful scientific methods for the fine tuning of animal feed development. The ability to use a wide range of protein sources for terrestrial animal feeds, many of them inferior in terms of amino acid profile, was made possible by the development of cheap, effective crystalline amino acids that could be added in small amounts to meet deficiencies in lower cost ingredients. All of these trends have direct relevance to aquafeed advancement. In fact, many of these processes are occurring concurrently and, in some cases, at a faster pace. On the other hand, there are some fundamental differences which must be understood in the unique context of aquaculture. Perhaps the major difference is that aquaculture species are cold blooded and their aquatic habitat means they require less energy for thermoregulation, locomotion and protein catabolism. With some obvious exceptions, most species are not adapted to utilizing carbohydrates for energy. This means that the total protein contents for nutritionally complete feeds are much higher than for terrestrial animals, limiting the choice of ingredients. Environmental variables directly influence nutritional demands, and species often face unique osmoregulatory challenges. For aquaculture species, feeds must be water stable, and poor-quality feeds can have the double negative of reducing growth performance and reducing water quality in the culture environment. Solubility in water can limit successful incorporation of key nutritional additives used in terrestrial animal feeds. Clearly, the number and variety of target species adds significant challenges in that research and development efforts must split between very different animal models. Thus, some of the most basic requirements remain undefined for many highly significant species. Meeting the needs of growers facing shrinking profit margins will depend upon the successful paradigm shift from formulation on the basis of ingredients to feeds based on a sound fundamental understanding of nutrition and physiology of the animal. This transition is well on its way with species like Atlantic salmon, tilapia, white leg shrimp and trout, while much more work is needed for emerging species like striped catfish and some marine carnivores.

Evaluation of ingredients

Evaluation of ingredients was not particularly important when feeds were composed primarily of fishmeal as a protein source and fish oil as a lipid source. Those ingredients are well digested and utilized by most species. However, alternative sources of protein and lipid are usually inferior in terms of matching amino acid and fatty acid composition to requirements. In addition, many alternative ingredients contain high levels of carbohydrate or ash that are not well utilized by most species. Antinutritional factors add an additional level of complexity. Key advances in this field have occurred with more structured methodology for ingredient evaluation and the identification of some additional ingredients that have high potential for increased use in aquaculture. Glencross *et al.* (2007) outline the steps involved in evaluation of ingredients. This starts with measurement of the energy and nutritional composition and examination for any contaminants. Secondly, the utilization of an ingredient and potential negative impacts on feed intake needs to be assessed to allow feed formulators to estimate maximum inclusion levels for different ingredients or combinations of ingredients. Different ingredients can affect energy or nutrient utilization and/ or they can affect diet attractiveness and palatability. Both have an important impact on their value in practical diets. To discriminate these different effects, the inclusion of different ingredients at different concentrations needs to be assessed based on performance, feed intake and feed conversion efficiency. Finally, ingredient functionality should also be evaluated. This refers to the effects on physical properties of processed feeds. Ultimately, functionality also restricts the potential use of an ingredient. Regardless of how well an ingredient is utilized, if it cannot be used beyond a certain concentration because it negatively affects pellet stability, buoyancy or structure, the ingredient value is reduced.

New areas of ingredient evaluation include the application of molecular science, genomics and proteomics, where gene and protein expression are measured in response to different ingredient or dietary treatments. This study is often called nutrigenomics and is described by Pansert, Kirchener and Kaushik (2007). New advances in ingredient evaluation also include application of different techniques of analysis. Rapid analysis of ingredient composition, such as near-infrared spectroscopy (NIRS) is allowing real time analysis of ingredients from different batches and allows feed managers to fine tune formulations on the basis of small changes in ingredient composition for different batches (Glencross, 2009).

Ingredients

One of the greatest challenges for aquafeed development is reducing reliance on marine fish protein and lipid sources. Aquaculture feeds represent about 4 percent of total animal feed production while consuming over 68 percent of global reported fishmeal production and over 82 percent of reported fish oil production (Tacon and Metian, 2008). Moreover, continued growth of the sector has generated increasing price pressure on these limited commodities, particularly in El Niño years when supplies are limited. Higher prices coupled with increasing awareness of sustainability issues are resulting in decreasing inclusion rates and growing research into use of alternative protein and lipid sources (Tacon and Metian, 2008). In general, aquatic species have high protein requirements and low tolerance to carbohydrates in feeds (a large proportion of plant ingredients). For many warm-water species, there is also intolerance for high lipid contents, particularly those with high concentrations of saturated fatty acids. Depending upon the species, increasing use of many sources of vegetable proteins can limit availability of essential amino acids, cause problems with digestibility, increase concentrations of antinutritional factors, reduce palatability and affect physical properties of the feed. Many species, particularly marine carnivores, have high requirements for highly unsaturated fatty acids. Essential fatty acids such as docosahexaenoic acid (DHA) must be supplied from marine fish unless new alternatives are developed. Thus, there is an acute need for new nutritional technologies in this sector.

Despite limitations, a large and increasing number of ingredients have been evaluated for aquatic species, and use of these is increasing (see Gatlin *et al.,* 2007; Lim, Webster and Lee, 2008; Hardy, 2009, for reviews). The most common plant protein ingredient is soybean, soybean meal, and increasingly, soybean protein concentrate. This is a particularly valuable ingredient because of the huge volume of the grain produced in many countries and the global trade and availability. However, use in some species is restricted because of intestinal inflammation and the high content of non-starch polysaccharides and other carbohydrates that are poorly utilized by aquatic animals. Other plant ingredients that are being increasingly used include corn products (such as corn gluten meal), lupins and peas, canola, cottonseed meal and cereal products (wheat, rice and barley). Blending of ingredients can help to balance nutrient availability while minimizing potential negative effects of individual plant-based ingredients. Protein concentration, through removal of the husk and other carbohydrate fractions, tends to improve the potential for use of plant-based ingredients, and future improvements may involve enzyme hydrolysis to improve digestibility. Some ingredients contain antinutrients that reduce their potential. Many are inactivated through heat (e.g. trypsin inhibitors); some (e.g. glucosinolates and erucic acid) have been reduced through breeding programmes. Other antinutrients include phytic acid, a mineral antagonist which may be overcome for some species using enzyme supplements and organic sources of minerals (Gatlin *et al.,* 2007).

Rendered animal products can be an excellent source of protein and lipid. Ingredients such as blood meal, meat and bone meal, poultry by-product meal and poultry oil have all been very effective in feeds for a number of aquatic species (see Li, Robinson and Lim, 2008; Shiau, 2008; Yu, 2008 for reviews). High protein meat meals (produced using processing by-products with less bone), have effectively replaced all the fishmeal in diets for some species (see Hernandez *et al.,* 2010 for a recent example). Constraints to use of rendered products include variability of composition, high content of total lipid and saturated fatty acids or ash in some products and potential contamination. In addition, use of rendered products can be constrained by labeling and regulatory issues and consumer acceptance. Other types of ingredients being used in aquaculture feeds include by-products from distilleries (including for biofuel production), microbial proteins, seafood processing waste and plankton and krill. New technologies for cost-effective production of microbial proteins from waste streams of food production may offer future opportunities to convert waste nutrients into valuable ingredients.

Alternative lipid sources to fish oil are being used in greater amounts (see Corraze and Kaushik, 2009 for review). Key alternatives include vegetable oils, preferably those with high omega-3 contents (e.g. canola) and poultry oil. Neither vegetable nor animal oils have comparable fatty acid profiles, and it is likely that fish oil will still be required for high-value species, larval stages with very high requirements for highly unsaturated fatty acids and for finishing diets. The production of marine microalgae, fungi or bacteria with very high contents of highly unsaturated fatty acids is currently prohibitively expensive for use in most aquaculture feeds but as production methods become more cost-efficient and competition increases, the situation is likely to change.

Prices for food and feed ingredients have been increasing and are likely to continue to increase due to rising demands from growing population, diversion of some grains for use in biofuels, increasing costs of production and transport, and changes in global trade. This will present challenges and opportunities in the aquaculture feed sector. The focus on carbohydrate-rich fractions for some products (e.g. biofuels) may provide an opportunity to use protein fractions for feed ingredients. As mentioned above, new technologies are being developed to potentially improve the digestibility and nutritional quality of alternative feed ingredients. Protein concentrates, use of rendered ingredients and pretreatment with enzymes can offer higher quality alternative ingredients which improve performance, offering effective options when return on investment is factored in with feed ingredient costs. New sources of proteins and oils from algae and microbes may offer novel alternatives for meeting amino acid and highly unsaturated fatty acid (HUFA) requirements (Patnaik *et al.,* 2006; Kuhn *et al.,* 2009).

Other ingredients include enzymes which can act in the gut of the animal to improve digestibility, to minimize antinutritional factors or to release otherwise indigestible nutrients. For example, an increasing body of literature demonstrates efficacy of phytases in releasing phosphorus and improving mineral availability (Cao *et al.*, 2007). Low-cost enzymes are needed which can function in the gut of cold-blooded animals and are heat stable enough to withstand the rigors of the feed manufacturing process. Emerging technologies for improving the gut environment are being rigorously studied and are beginning to be applied in aquaculture feeds. Use of probiotics in feeds, although successful in human and animal nutrition, is not well accepted in aquaculture. Improved delivery methods and better understanding of gut microflora of aquatic animals could change this in the future (Balcázar *et al.* 2006). Similarly, prebiotics, essential oils and organic acids are being shown to change gut microflora, improving conditions for healthy gut flora while reducing concentrations of potentially pathogenic strains of bacteria (Luckstadt, 2008; Ringo *et al.,* 2010). With increasing use of alternative ingredients, addition of palatability enhancers and attractants may improve feed consumption (see Gatlin and Li, 2008 for a review on use of diet additives).

Feed production technologies

There are a number of different processing technologies to prepare ingredients and feeds. Washing, drying, grinding and classification are used to prepare some ingredients and to improve the nutritional value of others. Washing can remove water-soluble starch fractions in cereals, increase the protein content and remove some contaminants. Heating or cooking can remove trypsin inhibitors and other heat-labile antinutritional factors. Similarly, as protein molecules are heavier than non-protein fractions, fine grinding followed by air-classification has been used to produce protein concentrates for a number of plant protein sources. Removal of bones from source material for rendering plants will improve the protein content, and classification of dried, rendered product can be used to separate ash, also increasing the protein content. Clearly, altering processing conditions and source material can affect the composition of processing waste products.

There have been rapid improvements in processing technology for aquaculture feeds. For many years, feeds were produced using pellet presses, sometimes with steam conditioning to improve binding. The adoption of extrusion and expansion technology has greatly improved the pellet quality of aquaculture feeds, the digestibility of some nutrients, particularly starch, reduced the amount of fines, and allowed some control of pellet buoyancy. Application of post-pelleting technologies such as vacuum coating, has allowed production of feeds with much higher lipid contents (e.g. for salmonid feeds) and opened the way for addition of enzymes, attractants, carotenoids and other heat-labile supplements.

Feeding systems

Improved feed management offers the potential to reduce feeding costs and improve environmental performance. Recent research has focused on determining optimal feeding frequencies and ration sizes for different species under different water temperature regimes. Improved feeding technologies based on automatic or demand feeding can reduce labour costs, decrease variability in application and offer new alternatives to reduce the soak time for bottom-feeding species such as shrimp. New feeding systems use technology to electronically monitor the number of uneaten pellets falling through sea cages and use those data to control additions of pellets. This technology has greatly improved apparent feed conversion ratios for some species. Even newer systems are being developed to use hydrophones to detect uneaten pellets in turbid ponds. This technology is likely to reduce feed wastage and improve the cost-effectiveness of aquaculture. Development of functional feeds designed for periods of stress or for different stages of the fish life cycle will provide new opportunities and new challenges for management of feeds and feeding in production facilities.

Future trends and prospects

The increasing volume of research publications and the application of new research tools is providing more information for researchers and industry. The development of alternative protein and lipid sources, development of new water-stable supplements and use of enzymes are providing more options than ever for least-cost high-performance formulations. An improving understanding of interactions between gut environment, nutrition and disease is providing alternatives to antibiotic therapies and holds promise for helping to control other diseases by improving host immunity, fitness and digestive health. Exigencies of the marketplace will drive the industry along the same lines as livestock, improving production efficiencies and allowing for greater output of high-quality sustainable products. Aquaculture will need to provide an additional 29 million tonnes per year of food fish just to maintain current consumption levels by 2030. New and innovative nutritional technologies will be an increasingly critical link in supporting future sustainable expansion of the sector.

Sustainable production systems

Traditional Asian aquaculture

Traditional Asian aquaculture systems have been reviewed recently by Edwards (2009). These systems are based on the use of locally available wastes and by-products as nutritional inputs for the target crop. Edwards (2009) describes integrated agriculture/aquaculture systems, focusing on rice/fish integration, crop/livestock/fish integration in China and livestock/fish integration in many Asian countries. A second area of traditional practice is wastewater-fed peri-urban aquaculture, although reluctance and opposition to this type of culture system is growing as improving economic status leads to increasing demand for higher value fish. A third area of traditional culture is integrated fisheries/aquaculture fed low-value fish ("trash fish"). This practice expanded rapidly over the past two decades in Asia, but continued expansion is not sustainable due to problems with overfishing of vulnerable small wild fish, as well as issues with contamination of culture systems, introduction of pests and pathogens, generation of wastes and the availability of improved feed formulations. There is a significant research effort directed to reducing direct feeding of low-value fish to aquaculture species (Hasan and Halwart, 2009).

Research and development (R&D) has improved consistency and productivity in several areas. New methods are being developed to produce seed locally for expansion of small-scale traditional farming practices (Barman *et al.,* 2007). Opportunities exist for use of genetically improved strains and incorporation of health screening and management technologies to improve productivity. Better organization of the small farming sector locally and regionally can facilitate opportunities for application of new technologies to increase yields and reduce disease problems. Research on fertilization regimes has demonstrated financial and productivity advantages of supplementing organic inputs with small amounts of chemical fertilizers. Complexities increase as growers increase densities and begin to add formulated feeds. Traditional farming in many places is incorporating more modern methods, including the use of supplemental feeds that allow producers to increase productivity while maintaining principles of traditional aquaculture which utilize natural inputs and reduce wastes associated with more industrial monoculture (Edwards, 2009). Although traditional small-scale integrated agriculture/aquaculture systems allow for some productivity within a limited resource base, this type of aquaculture typically can support mainly household subsistence. This type of small-scale farming system will have a continuing role to play in providing contributions towards relatively poor rural household nutrition and income while allowing for a low-risk mechanism for farmers to gain aquaculture experience. However, Edwards (2009) suggests that future trends will be characterized by increasing motivation for maximizing income, leading to efforts to increase productivity, importation of nutrients from off the farm, specialization and a reduction in on-farm subsystems. Future development and research efforts should focus on medium-scale producers and application of appropriate technologies throughout the value chain to provide a basis of healthy seed, quality supplemental feeds and encouragement of cooperatives while enhancing ecologically based principles of traditional aquaculture which maximize cycling of nutrients within the system.

Integrated Multi-Trophic Aquaculture

Integrated Multi-Trophic Aquaculture (IMTA) is a technological innovation that builds upon the principles of some of the most ancient traditional agriculture and aquaculture practices which utilize waste from one sector of the farm as inputs/resources for another. Applying this ecologically based approach, modern aquaculturists envision IMTA systems as a promising means to utilize the nutrient waste from one feed receiving species to support grazers, filter-feeding organisms and primary producers. Whether land-based or around open water cages such organisms represent additional trophic levels, able to utilize what would otherwise be waste, and to allow added value for more efficient and sustainable production. Economic advantages include diversification of crops to provide additional income or a financial safety buffer in the event of problems with the primary crop. Environmental advantages include better efficiency of uptake of nutrients, reducing ecological footprint. Social and marketing advantages include improvement of perceptions of industrial aquaculture systems by local stakeholders and consumers. The aim is to increase long-term sustainability and profitability per cultivation unit (rather than per species in isolation, as in monoculture), as the wastes of one crop (fed animals) are converted into fertilizer, food and energy for the other crops (extractive plants and animals), which can in turn be sold on the market (Neori *et al.,* 2004; Robinson and Chopin, 2004; Yarish and Pereira, 2008; Abreu *et al.,* 2009).

Barrington, *et al.* (2009) have provided an excellent review of the work being done in several parts of the world on the laboratory and commercial-scale demonstration of technologies which apply this concept. A wide variety of genera of with high potential for development in IMTA systems in marine temperate waters include:

- **Seaweeds:** *Laminaria, Saccharina, Undaria, Alaria, Ecklonia, Lessonia, Macrocystis, Gigartina, Sarcothalia, Chondracanthus, Callophyllis, Gracilaria, Gracilariopsis, Porphyra, Chondrus, Palmaria, Asparagopsis and Ulva.*
- **Molluscs:** *Haliotis, Crassostrea, Pecten, Argopecten, Placopecten, Mytilus, Choromytilus and Tapes.*
- **Echinoderms:** *Strongylocentrotus, Paracentrotus, Psammechinus, Loxechinus, Cucumaria, Holothuria, Stichopus, Parastichopus, Apostichopus and Athyonidium.*
- **Polychaetes:** *Nereis, Arenicola, Glycera and Sabella.*
- **Crustaceans:** *Penaeus and Homarus.*
- **Fish:** *Salmo, Oncorhynchus, Scophthalmus, Dicentrarchus, Gadus, Anoplopoma, Hippoglossus, Melanogrammus, Paralichthys, Pseudopleuronectes and Mugil.*

Selection of species is based on established husbandry practices, habitat/site appropriateness, ecosystem functions, biomitigation ability, economic value and their acceptance by consumers.

The IMTA concept is very flexible in that it can be land-based or open-water, marine or freshwater systems, and may comprise several species combinations

(Chopin, 2006). For example, in Israel, research and development efforts towards land-based integrated aquaculture systems have focused on the combined use of algae and bivalves (with or without the addition of grazers) to treat effluent from land-based aquaculture systems (Shpigel, 2005; Shpigel and Neori, 2007). Three practical approaches of land based IMTA have been developed: 1. Fish-Bivalve-Seaweed (Shpigel *et al.*, 1993; Shpigel and Neori, 1996; Neori *et al.*, 2000). 2. Fish-Seaweed-abalone/sea urchins (Shpigel and Neori, 1996; Neori *et al.*, 1998; Neori *et al.*, 2000; Stuart and Shpigel, 2009) and 3. Fish-Constructed Wetland with Salicornia (Stuart and Shpigel, 2009). These authors have demonstrated that land-based systems can be engineered in such a way as to maintain different organisms and processes in separate culture units. Waste from the production of primary organisms becomes a readily available input, allowing for intensification. Optimization of biological processes and adjustment of parameters in the secondary units provides for the effective treatment of effluents for recirculation or before discharge. Emphasis in production may shift from one organism to another according to practical or economical considerations (Shpigel and Neori, 2007; Neori *et al.*, 2007). In Canada, a project has demonstrated the integration of culture of salmon, blue mussels (*Mytilus edulis*) and kelps in an open-water system (Chopin and Robinson, 2004). Innovative kelp culture techniques have been developed and improved both in the laboratory and at the aquaculture sites. Increased growth rates of kelps and mussels cultured in proximity to fish farms, compared to reference sites, reflected the higher food availability and energy. Nutrient, biomass and oxygen levels are being monitored to estimate the biomitigation potential. Salmonid solid and soluble nutrient loading is being modeled as the initial step towards the development of an overall flexible IMTA system. The extrapolation of a mass balance approach using bioenergetics is being juxtaposed with modern measures of ecosystem health. Long-term research is documenting food safety, animal health benefits and consumer acceptance of products from these systems (Barrington *et al.* 2009).

Several research and development strategies have been proposed with the goal of moving these concepts towards widespread commercial implementation (Troell *et al*., 2003; Barrington*et al.* 2009). These include:

- Study biological, biochemical, hydrographic, oceanographic, seasonal and climatic processes and their interactions for selected site and production system types.
- Conduct R&D at scales relevant to commercial implementation or suitable for extrapolation, while still not being irreversible.
- Develop models to estimate the appropriate biological and economic ratios between fed organisms, organic extractive organisms and inorganic extractive organisms at the aquaculture sites.
- Adapt and develop new technologies to improve operational efficiencies.
- Encourage multidisciplinary input from biologists, engineers, statisticians, economists, farmers and marketing experts in developing design and operations.

- Analyze roles and functions of IMTA systems for improved environmental, economic and social acceptability within the broader perspective of integrated coastal zone management (ICZM) and ecosystem carrying/assimilative capacity.
- Develop and harmonize appropriate animal/plant health and food safety regulatory and policy frameworks to enable more universal development of commercial-scale operations.
- Develop incentive approaches to facilitate outreach and technology transfer of these novel and somewhat complex technologies from scientists to industry, government and the public.

Biofloc technologies

One of the intrinsic features of aquatic ecosystems is the almost complete recycling of feed materials through the biological food web. Fish excretions are metabolized by microorganisms, consumed in turn by different animals and eventually eaten by the fish. Although an essential feature in extensive ponds, cycling of wastes has declined as pond production intensified. Organic loads in more intensive ponds are high, creating extra oxygen demand and settling to the pond bottom as anaerobic sludge where they slow down the bio-recycling sequence, leading to the production of toxic compounds and the buildup of ammonium and nitrite. Trends towards further intensification of aquaculture will continue. Extensive and even semi-intensive production systems demand increasingly limited land and water resources in comparison to more efficient intensive systems (Avnimelech *et al.*, 2008). Furthermore, demands of biosecurity, effluent management, quality control management efficiencies, transparency and profitability drive producers to intensify.

Biofloc systems are based upon integration of the target crop and microbial community within a pond and can be considered as ecosystem management (see Avnimelech, 2009 for review). Water treatment is accomplished within the pond, with no need for a separate water treatment component. A dense microbial community develops when water exchange is limited and organic substrates accumulate. With appropriate aeration and mixing, an aerobic microbial community develops in the water column reaching 10^7–10^{10} microbial cells per cm^3 of pond water (Burford *et al.,* 2003; Avnimelech, 2009). Inorganic nitrogen build up is controlled through nitrogen assimilation by adding carbonaceous materials. Under such conditions, microbes take up the ammonium from the water, cycling it to less toxic forms and creating microbial protein. In addition, ammonium and nitrite accumulation are controlled through the development of an efficient nitrifying community in the biofloc system. The bioflocs are micro-environments very rich in organic matter and nutrients embedded within a relatively poor water phase. The bioflocs are made of a wide assemblage of bacteria, algae, protozoa and various zooplankters. Ongoing research is being directed towards achieving a better understanding of the components of this community and methods to manage the assemblage to minimize potential negative components while maximizing benefits (De Schryver *et al.,* 2008; Ray *et al.,* 2009). A healthy and diverse biofloc community may reduce potential for dominance of pathogenic strains and contribute probiotic effects.

An important feature of biofloc technologies (BFT) is the ability to recycle proteins. The micro-organisms in the water tend to aggregate and form bio-flocs that can be harvested by tilapia, penaeid shrimp and filter-feeders. Protein utilization rises from 15–25 percent in conventional ponds to 45 percent in BFT. Flocs can provide proteins, vitamins and minerals (Tacon *et al.,* 2002). The doubled feed efficiency and nutritional contributions are increasingly important as feed costs rise and pressures on limited resources increase. The elimination of water exchange is an important benefit with potential to enhance environmental sustainability of pond-based culture systems.

Information technology

The increasing pace of innovation and development of information technologies continues to expand the range of general and specialized applications for aquaculture. The applications of information and communication technology for the aquaculture industry are as diverse as the industry itself, ranging from highly specialized feedback and decision-making systems for high technology salmon farming operations to the increasing availability of information and learning resources for small-scale rural farmers. The topic was recently reviewed by Bostock (2009), whoprovided a detailed review summarizing the use of information technology in aquaculture; the following section provides a summary of this excellent synopsis.

New developments in the application of information technology for monitoring, control and automation are improving the ability of large industrialized production systems to manage crops and improve production efficiencies. Recent trends towards consolidation in some of the more industrialized sectors of aquaculture production have resulted from increasing cost competitiveness and associated demands for reducing production costs. Sensors and monitoring tools are being applied to better control water quality and to better protect against catastrophic loss. These may be individual units tied to a production system or networked centralized systems for monitoring multiple units and multiple sites. New sensors are being developed and marketed for monitoring of the target crops. Coupled with automated feeding systems, these technologies can be applied for counting fish, measuring fish, monitoring mortalities, sensing feeding behaviour and uneaten feed, even down to the monitoring of individual fish using electronic telemetry tags. As these sensors decrease in size and cost, their application may expand beyond highly industrialized salmon farms to wider applications with corresponding opportunities for improving efficiencies and reducing waste, thereby contributing to financial and environmental sustainability.

Computer-based systems for managing stocks and production data, optimizing production schedules, controlling feed purchases and making harvest decisions are becoming more common, even in medium-scale operations. Information and communication technologies are increasingly used to manage the array of complex business processes in a typical medium or large-scale aquaculture operation, with some moving towards integration of major business functions through enterprise resource planning software. Availability of better software tools will improve business planing, allowing future developers to better model everything from potential production dynamics to site section factors, potentially improving the outlook for sustainable project development.

One of the most important areas in which emerging information and communication technologies will contribute to future aquaculture sustainability is in assuring quality and traceability (Bostock, 2009). As the implementation and public acceptance of codes of practice and labeling expands, a corresponding demand is developing for databases, verification records and operational logs for traceability, management and reporting purposes. Technologies that support these efforts are becoming more powerful and cheaper to implement. More sophisticated systems are using real time links between traceability and stock management tools, automated data capture and networking technologies for linking database elements and customizing entry and reporting. With the wide array of traceability and labeling standards that are in effect or under development and the number of companies developing systems to provide tracking, tracing, and management information solutions increasing, future efforts to develop standards and management tools will need to focus on harmonization to reduce inefficiencies and facilitate data transfer.

The expanding role of the Internet is becoming an ever more important tool for remote management of production systems; for connecting with customers for marketing, sales and public relations; and for facilitating research, education and extension. Even the smallest-scale producers will increasingly be able to access better information and training as information technologies improve, availability expands and costs decline. Vast amounts of knowledge are available through the Internet, and the challenge continues to be managing the quality of the information and developing tools to deliver it in formats necessary for the diverse aquaculture communities in need of training. New virtual learning environments and educational tools are being developed, providing improved opportunities to train practitioners and provide extension assistance to growers, from rural cooperatives to mid-level producer groups to remote production facilities within a larger integrated company.

Finally, information technology is providing a fundamental foundation for the process of aquaculture innovation and technology development in and of itself. Better real time communications are linking universities, research laboratories and industry like never before. Research results are being disseminated faster and faster through electronic outlets, allowing the sharing of innovative advances and faster market implementation. This communication can also serve to focus research and development efforts. As discussion lists, personal networking tools, and partnering tools between cooperatives or companies expand, consensus on looming long-term issues, technology gaps and productivity bottlenecks can be reached. Benchmarks can be developed to track progress in overcoming obstacles or in improving standards. Embracing and enhancing these tools and trends can provide some of the most important opportunities for improving sustainability and productivity of the aquaculture sector.

Conclusions

The pace and scope of technological advances in aquaculture has increased over the decade since the publication of *Aquaculture in the Third Millennium* (NACA/FAO, 2001). Continued advances in genetics, health, nutrition, production systems engineering and information technology have had profound effects on aquaculture production. However, technology development and associated improvements in sustainability and productivity have, in many cases, been implemented for and by large-scale industrial aquaculture production systems. As a large proportion of aquaculture production comes from small farmers, particularly in Asia, increased efforts must be devoted to improving the development of technologies specifically for small and medium-scale systems, as well as extending the availability of existing applicable knowhow and technologies. In many cases this will require better organization of the sector and an investment of resources in expansion of medium-scale entrepreneurial aquafarming businesses where economic returns can drive industry improvement and expansion. Successful examples include the application of diagnostic technologies for regional farmers' associations, use of sex reversal and genetically improved strains of tilapia for local seed production centers, and shifting of production from trash fish and mash feeds to well-formulated pelleted or extruded feeds (FAO, 2010). These types of opportunities can and should be expanded along with classical improvements in management practices to improve productivity, socio-economic benefits and environmental sustainability of small and medium-scale aquaculture.

To focus and track progress in innovation and application of technologies, the scientific community, industry, government and NGOs should work towards consensus on common goals. An example of a consensus-building workshop which prioritized goals for technological innovation in aquaculture can be found in Browdy and Hargreaves (2009). Priority goals may address many areas of importance to future aquaculture development including: i) improving productivity and financial sustainability to encourage entrepreneurism and industry expansion; ii) increasing environmental responsibility, preparing for climate change effects and improving resource utilization efficiencies; and iii) raising socio-economic benefits to communities and improving food security.

Once goals are set, a series of criteria and quantitative metrics should be developed to focus research efforts and evaluate progress, outcomes and impacts for each objective. For example, use of pedigrees coupled with heritability metrics allows the tracking of performance improvements in traits of interest for selective breeding programmes. In developing feeds and feeding programmes, metrics focusing on efficiency can have a huge impact on financial success, as well as environmental sustainability. These could include improving feed conversion efficiencies or tracking "fish in fish out" (FIFO) ratios to quantify the amount of fish from capture fisheries necessary to produce a unit of cultured fish. A third example could be the evaluation of carbon, nutrient or energy inputs for production of a kilogram of fish to provide focus on energy usage and carbon/nutrient footprints. In many cases, improved application of technologies can contribute to environmental stewardship and efficient resource utilization while concurrently improving economic opportunities and returns. This review provides numerous examples of these types of potential win/win opportunities that can arise from focused research and development efforts. As costs of technologies drop, communication and information technologies expand and the pace of innovation increases, new and expanding opportunities will continue to emerge for the expansion of sustainable aquaculture production to meet world food needs.

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