CHAPTER 4

CASE STUDIES IN THE FISHERY SECTOR

Application of probiotics using a small boat
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CHAPTER 4.1

APPLICATION OF PROBIOTICS AS AN ENVIRONMENTAL TREATMENT AND FEED ADDITIVE IN THE PRODUCTION OF FARMED MARINE SHRIMP IN CHINA

YUAN XINHUA

Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences,
Asia-Pacific Regional Research and Training Center for Integrated Fish Farming,
9 West Shanshui Road, Wuxi, Jiangsu 214081, China.
E-mail: yuanxh@ffrc.cn

INTRODUCTION

Guangdong Province is the most important province for marine shrimp (*Penaeidae*) production in China. In 2010, it produced 400 000 tonnes of shrimp, which accounted for 30 percent of the total for the country. Since 1993, there have been numerous problems with shrimp farming in China relating to the deterioration of the environment and the occurrence of disease. With the ban on using antibiotics in aquaculture, scientists and farmers have tried to find alternative methods to improve the aquaculture environment, introduce new species for culture and improve growth performance.

Commercial shrimp aquaculture production in China is characterized by high stocking density, intensive management and high economic returns. These practices also lead to many problems in shrimp ponds, such as overfeeding, high organic content, and changes in the micro-organism ecosystem. These changes can lead to eutrophication, water quality deterioration and a reduction in shrimp growth.

To control and prevent diseases, chemicals and antibiotics were used in shrimp production. These drugs have proved effective in fighting disease outbreaks, but have also caused problems, such as drug residues in shrimp, water pollution, lower productivities and destruction of the ecological food chain. In 2002, Chinese processed shrimp products were banned for export to the European Union market because of antibiotic residues in shrimp meat (this ban was lifted in 2004). To meet the demand for environmentally friendly aquaculture practices, an alternative to antibiotic use in aquaculture was adopted. The use of probiotics was one such alternative.

In China, the first probiotic strain for aquaculture was reported by the Sichuan Agricultural University in 1995. By 2001, the Ministry of Agriculture had approved 13 probiotic strains for agriculture practices. Now, there are more than 400 companies producing *Bacillaceae*, yeast, *Rhodospirillaceae* and *Lactobacillus* probiotics for agriculture, with an annual production of 10 000 tonnes. Approximately 2 000 tonnes of probiotics are used in Chinese aquaculture. None are exported from China. Numerous research academies and universities tried to select more efficient and specific probiotic strains for use in aquaculture. Many companies have already developed their own probiotic strains and are trying to produce enough to satisfy the market demand. In 2011, there was a reported demand for 30 000 tonnes of probiotic products in China for aquaculture practices. After decades of research and development, the use of probiotics is now popular in the commercial production of farmed shrimp.

PROBIOTICS IN SHRIMP FARMING

As an alternative strategy to antibiotic use in aquatic disease management, probiotics have attracted extensive attention. In 1998, a reported application of a probiotic, *Bacillus* spp., in shrimp ponds led to higher survival rates of shrimp that had been infected by *Vibrio* bacteria, and further successful cases were reported thereafter. The probiotic is used to improve the water quality, as an additive to feed, and to improve disease prevention. It is applied in the nursery pond and grow-out pond; it can be used in different forms, such as powder, liquid or as additives to pellet feed. The probiotics improved the water quality as well as the survival rate and growth of the shrimp.

Other bacteria that have been used successfully as probiotics belonging to the genus *Vibrio*, *Pseudomonas* and *Bacillus*, and the species *Thalassobacter utilis*. Most researchers have isolated these probiotic strains from water bodies used for shrimp culture, or from the intestine of different *Penaeid* shrimp species. The use of probiotic bacteria, that outcompete and displace harmful bacteria, and the use of immune-stimulants are two of the most promising preventive methods developed in the fight against shrimp diseases. Probiotic bacteria also produce some digestive enzymes that might improve the digestive system, enhance stress resistance and improve the health of the shrimp.

The use of probiotics in aquaculture has become a promising means of balancing the micro-environment in shrimp ponds in order to improve productivity and the health of the water in an ecologically friendly manner. The practice has attracted increasing attention from aquaculture extension officers, farmers and environmentalists.

Three bacterial genera, *Bacillus, Vibrio* and *Pseudomonas*, are commonly administered as probiotics in shrimp aquaculture. Candidate probiotics are species-specific and need to be tested for their effectiveness for certain species *in vitro* and *in vivo*. A solution containing the appropriate probiotic can be used for pond disinfection and water quality improvement before filling the pond with water. Water quality can also be improved by administering the probiotic to ponds a few days before stocking with shrimp. For improvements in shrimp growth, it is best to add the probiotic directly to the shrimp feed and several techniques are used to accomplish this.

Overdosage or prolonged administration of probiotics can induce immune-suppression in shrimp. Therefore, a combination of probiotics results in better outcomes for the shrimp than using individual probiotics.

TECHNOLOGY TRANSFER

The use of probiotics was promoted through several methods. Because shrimp farming is an expensive business to operate, many farmers tried to find solutions to recover shrimp farming costs. With the support of the extension systems in China and commercial production of probiotics, the farmers can easily access the products from the market. The technical assistance on how to use probiotics was provided by the local fishery technical extension officer, and retail sale support by private companies and sales outlets. The fishery technical extension station in the province selected demonstration farms and farmers to use the probiotics and train additional farmers on the use of probiotics. The sales company promoted the probiotics to shrimp farmers by providing technical advice and extension for a better understanding of how to use probiotics in aquaculture. Farmers used probiotics as an alternative to antibiotics to fight the daily problems in water treatment and shrimp mortality. The ease of accessing training and the positive results achieved in the production of shrimp led to the increased adoption of probiotics.

There are three approaches to the financing of the application of probiotics to aquaculture.

i) Demonstration project: demonstration farms were selected and funded by the government through the fishery technical extension station. The farms would then receive intensive technical guidance and initial funds to carry out the application of probiotics to their own shrimp ponds.

ii) The sales company provided free trial probiotic liquids to demonstration farmers, and set up successful examples to advertise their products.

iii) Some farmers purchased the probiotic liquids and carried out pilot projects on their farms before applying them for the whole farm. When they succeeded in the application, they continued to finance the use of probiotics themselves and applied the new technology in their farm as part of a sustainable aquaculture business.

The research academies and universities were the major organizations in technology innovation. They had advanced research facilities and developed many probiotic strains for use in aquaculture. Commercial companies used to cooperate with research academies and universities in new technology development. The research achievements and products could be transferred to company for commercial production, through contracts or agreement. There are also some imported products, such as probiotics from Japan, Sweden and the United States. There is need for authorized sales permission or agreement between the companies.

From left to right
Preparation of probiotics before application to the pond
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Application of probiotics using a foam board
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INTELLECTUAL PROPERTY RIGHTS ISSUES

There is the potential for intellectual property rights to be applied to some specific strains of probiotics, culture methods and the formula of culture medium. There are reports of patented probiotic strains and culture methods in China, but there are no reports on the use of these patents for actual products in current aquaculture practices. The development of commercial probiotic products has involved research by private industry or cooperation between private industry and research academies, and therefore represents a public-private partnership. Intellectual property rights would need to address this aspect. In future, specific and effective probiotic products with patents should be encouraged.

CHALLENGES ENCOUNTERED IN USE OF PROBIOTICS

The challenges of using live organisms such as probiotics in treating aquaculture species, feed and ponds included:

i) The success of probiotics in water strongly depends on their density and activities. Therefore there is a need to monitor and adjust the density of the probiotics and their activities in water during the application.

ii) Some of the strains used in aquaculture were isolated from fish ponds or fish intestines, while others were developed for use with poultry. Therefore, there is a need to develop specific probiotic strains for other aquatic species.

iii) There is a need to carry out more research to improve understanding of the mechanisms of how probiotics function in the organisms and in aquaculture ponds.

iv) The production, processing, transportation and storage of probiotics should be developed for higher efficiency.

v) The advantages and disadvantages of using single strains of probiotics or combinations of strains should be studied to improve efficiency.

vi) Policies and regulations need to be formulated to increase the distribution of probiotics. The regulations and policies to use probiotics in different countries are not the same. Most countries regulate probiotics as micro-organisms and do not permit importation because of environmental risk considerations. Permission to import also may experience delays due to the time required for the safety inspection.

IMPACT AND LESSONS LEARNED

Shrimp farmers in China are business-oriented; farmers pay rent to the government for the use of ponds. The application of probiotics became an important and common approach in ensuring adequate production and profit from the ponds both for small-scale (ponds of < 1 ha) and for large scale (ponds > 1 ha) commercial shrimp farmers. There are many reports on the successful application of probiotics in shrimp farming. The growth of the shrimp postlarvae (PL) was 17 percent higher than the control group and the survival rate improved 111 percent. The water quality improvement results were also very good, the ammonia nitrogen was reduced by 60 percent, and nitrate by 87 percent. Toxic matters were removed and the survival rate of PL and shrimp was greatly improved. After 85 days of farming, the yield of the probiotic-treated group was 227kg/667m^2 (or about 3.4 t/ha), almost double the yield of the control group. Additionally, the individual size of the treated group of shrimp was larger than the control group.

Probiotics are useful micro-organisms for improving the efficiency of aquaculture production in an environmentally friendly manner. Although there were many reports on the successful application of probiotics in aquaculture, future research and development projects are still needed to understand exactly how probiotics function.

i) The research should be based on the local farming situation, with the aim of solving the problems in sustainable production.

ii) Research academies and universities, along with private industry, have played important roles in research and development. However, government support is still needed.

iii) Commercial production, private industry and extension can be very effective at promoting technology exchange and adoption.

Adoption of the new technology was strongly assisted by the demand for high-quality shrimp and sustainable aquaculture practices. As an alternative to antibiotics use in aquaculture, probiotics helped in the recovery in the production of farmed shrimp, and allowed farmed shrimp to continue to be an important export species. In light of ongoing requests by the government, trade companies and producers, more probiotic strains will certainly contribute to future good harvests, sustainable production and strong economic returns.

Distribution of shrimp postlarvae among society farmers
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CHAPTER 4.2

PCR-BASED PATHOGEN DETECTION IN SHRIMP AQUACULTURE IN INDIA

PRAKASH C. THAKUR[1],
ARUN P. PADIYAR[2],
AMIYA K. SAHOO[3],
G. SUBBARAO[4] and
D. RAMRAJ[5]

[1] Research Scientist, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15260, United States. E-mail: pcthakur@pitt.edu
[2] Aquaculture Specialist & Entrepreneur, Padiyar Nivas, Penemangalore, Karnataka-574231, India.
[3] Scientist, Central Inland Fisheries Research Institute, Indian Council of Agricultural Research, Barrackpore, Kolkata, West Bengal- 700120, India
[4] Sree Vasudha Labs, 4-70-3, AQJ Degree College, Vishakhapatnam, Andhra Pradesh-530017, India
[5] Padmanabha Labs Pvt. Ltd., Plot No.18B, Kohinoor Complex, Vettuvankeni, Chennai, Tamil Nadu-600041, India

SHRIMP AQUACULTURE AND DISEASE SCENARIO IN INDIA

Shrimp farming continues to be the largest export-oriented aquaculture production sector in India, providing a significant contribution to the world's farmed shrimp production. The majority of shrimp farming in India is by low-income small-scale farmers (Umesh *et al*., 2010). Therefore, properly managed farming offers significant employment opportunities and helps alleviate poverty among the coastal population. Although there are many species of shrimps, only a few of the larger ones are cultivated in India. The two species currently dominating the Indian shrimp farming are: Giant Tiger Shrimp, *Penaeus monodon* (*P. monodon*), and the Pacific White Shrimp, *Penaeus vannamei* (*P. vannamei*). Intensification of shrimp farming has caused many shrimp diseases of epidemic proportion over the last two decades, particularly viral diseases in the densely populated monoculture farms. Such virus infections spread rapidly and cause massive crop losses in clustered farming, directly impacting the income of farmers (Walker & Mohan, 2009). Thus, 'better management practices' (BMPs) are immensely important to the success and sustainability of the shrimp industry (Mohan *et al*., 2008; Padiyar, 2005a; Subasinghe, 2005).

Viral pathogens reported in Indian shrimp farms and hatcheries are: white spot syndrome virus (WSSV), yellow head virus, Monodon baculovirus (MBV), Hepatopancreatic parvovirus (HPV), Laem-Singh virus and *Macrobrachium rosenbergii* nodavirus (MrNV) (Prakasha *et al*., 2007; Ravi *et al*, 2009; Walker & Mohan, 2009). Bacterial diseases, such as luminescent Vibriosis, caused by *Vibrio spp*, are also sporadically reported as affecting shrimp farms.

In India, by far the major economic losses are caused by white spot disease (WSD) epidemics, caused by WSSV, which is the most devastating pathogen due to its wide host range and ability to cause 100 percent mortalities within days. Epidemiological studies suggest high prevalence of WSSV among the shrimp broodstock and postlarvae (PL) populations (Corsin *et al*., 2003; Otta *et al*., 1999; Thakur *et al*., 2002). Stocking infected PL remains a major source of WSSV infection in the culture systems, although the virus can be effectively transmitted horizontally by water-borne contact or ingestion (Lo *et al*., 1998). BMPs developed from epidemiological studies recommend stocking pathogen-free PLs to minimize the risk of outbreak and economic loss (MPEDA/NACA, 2001). Instead of using larvae from wild catches, specific pathogen-free (SPF) brood stocks raised in captivity are being used in an effort to reduce disease risks. In India, *P. monodon* used to be the major farming species until recently. However, because of the susceptibility to WSD due to non-availability of SPF stocks, it is being gradually replaced by *P. vannamei* since 2008, as one of the measures of reducing losses in shrimp farming. Since 2010, the majority of the shrimp farmers in India have switched to *P. vannamei* culture.

ADVANCES IN PCR AS A BIOTECHNOLOGICAL TOOL FOR SHRIMP DISEASE MANAGEMENT IN INDIA

Biotechnological tools of relevance to shrimp disease management have been applied for better prevention strategies, routine pathogen screening and to develop specific disease resistant strains. Currently, these technologies are used in the Indian shrimp industry at various levels, depending on the intensity and commerciality of the farming system.

With the high prevalence of viral infections in broodstock and PL populations (Corsin *et al*., 2003; Thakur *et al*, 2002), and the fact that other crustaceans can be carriers of the virus, the most sought after biotechnological tool in shrimp farming is an effective means of pathogen screening. During the early 1990s, no techniques were available to screen effectively for pathogens. However, with the advancement of biotechnological research, there are now many DNA-based detection technologies, including polymerase chain reaction (PCR)-based diagnostics available for all the major shrimp viruses. A number of PCR tests have been developed for the detection of the prevalent viruses in shrimp farms (OIE, 2012). Similarly, using nested PCR and dot-blot hybridization techniques, viral prevalence could be detected in wild shrimp species in Indian coastal waters (Manjanaik *et al*., 2005). Multiple viral infections in apparently healthy PL samples could be effectively detected using PCR methods specific to MBV, HPV and WSSV (Manivannan *et al*., 2002). These PCR tests use a set of different primers targeted to amplify specific regions of each of the virus genomes. Several reverse transcriptase PCR (RT-PCR) tests are also available to detect RNA viruses. Similarly, PCR tests have also been developed to detect bacterial pathogens such as *Vibrio* species (Karunasagar, 2000). Recently, rapid and cost-effective immunodiagnostic strips have begun to appear for screening shrimp diseases (Patil *et al*., 2008). Advanced methods such as multiplex PCR and pond side diagnostic tools such as loop-mediated isothermal amplification (LAMP) and insulated isothermal PCR (iiPCR) are also now available for single or multiple viral detections. Using these biotechnological tools, unskilled farm personnel could be trained to diagnose shrimp disease outbreaks at the farm.

RECOMMENDED AND COMMONLY USED PCR DETECTION METHODS IN INDIA

PCR-based pathogen detection methods in shrimp farming were commercially introduced in India in 1996 by the Marine Products Export Development Authority (MPEDA), fisheries research institutes and various private agencies. During the initial years, farmers used to screen for MBV, HPV and infectious hypodermic and haematopoeitic necrosis virus aside from WSSV. However, since it became apparent that the crop losses were primarily attributed to WSD, farmers resorted to routine PCR screening of WSSV in broodstock and PL in hatcheries and farms as one of the most common health management strategies.

The best life stages of crustaceans for PCR detection of WSSV are late PL stages, juveniles and adults. Persistent infection occurs commonly and lifelong infection has been shown. In such cases, probability of detection can be increased by exposure to physiological or environmental stress (Joseph & Philip, 2007; Vidal *et al*., 2001). Since the major WSSV target tissues are of ectodermal and mesodermal origin (Lo *et al*., 1997), the pleopods (swimming legs) are most commonly used for PCR screening as a non-invasive method for adult shrimps. The dead and moribund samples from the culture ponds can also be effectively used for PCR tests, as they often provide indication of a disease outbreak (Mohan *et al*., 2002). Similarly, epidemiological investigations reveal that PCR screening of shrimp samples during middle of the culture period may serve as an indicator of crop status and help in predicting disease outbreaks and optimizing harvest strategies (Sahoo *et al*., 2010; Turnbull *et al*., 2005). The PCR protocol developed by Lo *et al*. (1996, 1997) is recommended for WSSV diagnosis in most situations (OIE, 2012). A 1st-step PCR-positive result using this method indicates a serious WSSV infection, whereas, a 2nd-step nested PCR-positive result may indicate a latent or carrier-state infection. For PL or broodstock screening, a 2nd-step nested PCR is necessary for the detection of carrier status. Two-step PCR and sequencing are the recommended methods for declaring WSSV-free status, although extremely low viral loads during latent infection are sometimes undetectable even by sensitive methods such as real-time and nested PCR.

Several commercial PCR kits are now available for WSSV diagnosis and are acceptable provided they have been validated. OIE guidelines are used to bring uniformity in PCR screening practice and the OIE Register may be consulted for certified kits. Currently, there are four PCR detection kits available for shrimp pathogen screening in the Indian market: IQ2000 WSSV Kit (GeneReach, Taiwan), Bangalore Genei kit, Mangalore Biotech kit and Poseidon Biotech kit. However, it is important to adhere to uniform standards, recommended quality control measures with appropriate positive and negative controls during PCR detection to reduce chances of false positives and false negatives leading to confusion among the farmers about which method to use. With the knowledge of various geographical isolates of WSSV with genotypic variability (Marks *et al*., 2004; Walker *et al*., 2011a), DNA-based diagnostic methods such as PCR requires rigorous standardization to detect all the virulent strains.

from left to right
Farm site collection and preservation of shrimp samples for PCR test
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P. monodon broodstock in hatchery
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POPULARIZATION OF PCR TO SCREEN SHRIMP PATHOGENS IN INDIA AND ITS EFFICACY IN CONTROLLING DISEASE OUTBREAKS AND CROP LOSSES

Over the past decade, PCR-based rapid detection of shrimp pathogens has given a new dimension to the Indian shrimp industry. Beginning in 2002, MPEDA promoted the establishment of PCR labs by offering subsidies. About 45 private entrepreneurs and corporate entities started PCR diagnostic labs in Andhra Pradesh and Tamil Nadu, where most of the shrimp hatcheries were located. Subsequently, with the involvement of the Indian Council of Agricultural Research (ICAR), Australian Centre for International Agricultural Research (ACIAR), Network of Aquaculture Centres in Asia-Pacific (NACA) and FAO collaborations, MPEDA made PCR labs mandatory for *P. monodon* hatchery registration and offered required training and resources. This subsequently increased the popularity and accessibility of PCR among the small-scale farmers. As a result, there are now more than 100 hatcheries with on-site PCR labs to screen broodstock and PL. This initiative also facilitated increasing awareness among farmers of PCR screening as a method of choice. As the usage of PCR diagnostics increased, the retail cost of PCR tests significantly decreased, making it more affordable to the small-scale farmers.

In the pilot projects on the Indian east coast conducted by MPEDA/NACA and FAO, the use of PCR screening was the crucial BMP for the small-scale shrimp farming in Andhra Pradesh. The MPEDA/NACA project in collaboration with FAO and ACIAR, initiated during 2000 to support small-scale shrimp farmers in disease management, resulted in the increased usage of PCR screening. Contract hatchery systems promoted by the MPEDA/NACA project helped farmer societies/aquaclubs to procure large quantities of PCR-screened PL with improved field-level management efficiencies, providing significant improvements in profits and reduced risk of disease outbreaks (Padiyar *et al*., 2003, 2005b). An economic analysis of the farmer groups in Andhra Pradesh indicated that farmers adopting BMPs, including the use of PCR screening of PL, had better profits and were able to produce quality crops (Umesh *et al*., 2010). Such participatory projects have helped disseminate the PCR techniques among farmers and resulted in increased participation, with more than 10 000 farmers now active in about 150 aquaculture societies in five coastal states of India. Since 2007, the MPEDA/NACA collaborative project became the National Center for Sustainable Aquaculture (NaCSA), which serves as an outreach organization of MPEDA for the small-scale aquaculture sector, providing technical support to farmer groups to enhance shrimp production in a sustainable and profitable manner. The success is built on reduction of losses from disease outbreaks in production systems, and to a large extent this has been made possible by PCR technology for screening and detecting major viral pathogens in broodstock and PL.

One of the recent trends of Indian shrimp farming sector is the introduction of *P. vannamei* and its increasing popularity among shrimp farmers. This has culminated in conversion of the majority of *P. monodon* farms into *P. vannamei* farming. Although this may be attributed to the relatively faster growth rate and lower cost of production of *P. vannamei*, the availability and sourcing of SPF stocks of *P. vannamei* has also contributed to this preferential change among farmers. Since large-scale commercial introduction of *P. vannamei* in 2009, many private PCR labs reported decline of their business, because farmers were given to understand that PCR tests are not needed for *P. vannamei* SPF stocks. However, MPEDA and the Coastal Aquaculture Authority now make it mandatory for all the *P. vannamei* licensed hatcheries to have an in-house PCR lab and test for all the OIE listed shrimp pathogens on the SPF broodstock and PL batches. Currently, the imported *P. vannamei* broodstock are quarantined at the Aquatic Quarantine Center of the Rajiv Gandhi Center for Aquaculture and screened for OIE listed pathogens by PCR. Then the stocks are released to importing hatcheries after confirmation of freedom from the listed pathogen.

Though PCR proved to be a helpful tool to contain the diseases at farm and cluster levels where it was used as one of the BMPs, there was stagnancy in shrimp production during 2005-2009. The authors feel that such stagnation may be attributed to: (1) PCR was not used in the right place. *P. monodon* brooders from the wild were rarely tested by hatcheries, and the PCR test was restricted to only PL by farmers, (2) large numbers of open farming system with very loose biosecurity, increasing the risk of horizontal infections in the culture areas, (3) poor farmer cooperation and farming discipline at farm cluster levels, especially among the small-scale farmers.

Crop loss due to disease outbreaks in some states, e.g. Andhra Pradesh, Tamil Nadu and Gujarat, were significantly reduced due to high level of awareness about recommended PCR screening methods and BMPs among farmers. The prevalence of disease outbreak among farmer societies promoted by NaCSA has come down to 20 percent from a baseline of 80 percent prior to BMP implementation (Umesh *et al*., 2010). There was a significant increase in national shrimp production from 2008 to 2012 (MPEDA, 2012). This may largely be attributed to the use of *P. vannamei* SPF stocks and a strong quarantine system which uses PCR to screen the imported SPF animals. From analysis of the current trends, and through the first-hand information from our farm-level questionnaires, we reason that PCR is an important health management tool for preventing crop losses due to viral disease outbreaks. However, proper use of the technology and the compliance of farmer/hatchery owners to adopt PCR screening and BMPs is equally important to gain benefits from these biotechnological applications in the shrimp farming industry.

FUTURE CHALLENGES AND EMERGING PATHOGENS

Shrimp aquaculture in India is continuously evolving and new species have been introduced, which increases the chances of new pathogens and disease outbreaks. For instance, recent crop losses due to distinctive pattern of mortalities in *P. monodon* and *P. vannamei* farms of China and Southeast Asia, attributed to early mortality syndrome or acute hepatopancreatic necrosis syndrome, indicates plausible presence of new pathogens, although the causative agent is not yet known and the disease is still described as idiopathic (NACA, 2013). There have been reports of emerging viral infections caused by MrNV and extra small viruses (XSV) in *M. rosenbergii* and *P. indicus* PL batches in Indian hatcheries and PCR techniques are being developed and popularized for detection of such emerging pathogens (Ravi *et al*., 2009). Realizing the loss in freshwater prawn at early stage due to MrNV, an OIE reference laboratory has been set up in Tamil Nadu for routine screening by RT-PCR. In addition, LAMP and multiplex RT-PCR methods have been developed (Haridas *et al*., 2010; Pillai *et al*, 2006). However, PCR screening for such emerging pathogens is rarely practised and farmers should be made aware of potential new pathogens and proper biosecurity measures to control future outbreaks. Finally, despite using PCR-screened broodstock and PL, WSSV infection and disease frequently occur through various horizontal and poorly understood dynamics of infection modes in the culture system (Walker *et al*., 2011a, b). Therefore, juveniles and adults in the culture ponds, as well as other potential horizontal sources such as crustaceans in the pond area and feed, should be periodically monitored for WSSV contamination during the culture period.

ACKNOWLEDGEMENTS

The sponsorship received from FAO and the participation of expert consultants from ICAR, Indian shrimp aquaculturists and entrepreneurs are greatly appreciated.

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from top left, clockwise

Farmers harvesting the hybrid catfish
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A farmer sacrificing a male African catfish to collect testes
©Sathid Chatchaiphan

A farmer preparing sperm solution of African catfish
©Sathid Chatchaiphan

A farmer squeezing eggs from the hormone-injected female
©Sathid Chatchaiphan

A farmer grading the hybrid after harvesting
©Sathid Chatchaiphan

CHAPTER 4.3

INTERSPECIFIC HYBRID CATFISH IN THAILAND

UTHAIRAT NA-NAKORN

Kasetsart University Research and Development Institute
Kasetsart University, 50 Ngamvongwan Road, Chatujak, Bangkok 10900, Thailand
E-mail: uthairatn@yahoo.com

Interspecific hybridization is an approach to producing living organisms that rarely exist in nature. The general objective is to combine desirable traits from maternal and paternal species, to make use of hybrid vigour or heterosis (a phenomenon where the hybrid performs better than the average of the parental species).

Interspecific hybridization occurs more easily in fish than other vertebrates, probably because of the flexible sex-determining system of fish, which enables the better survival of the hybrids. Although interspecific hybridization has been done in laboratories, very few hybrids have been used at a commercial scale. However the hybrid between the broad-head catfish, *Clarias macrocephalus* and male African sharp-tooth catfish, *Clarias gariepinus* is a good example of a commercially successful hybrid.

REASONS FOR THE HYBRIDIZATION BETWEEN THE BROAD-HEAD CATFISH AND AFRICAN SHARP-TOOTH CATFISH

The broad-head catfish is native to Southeast Asia. It is found in shallow freshwater throughout the region. It is a favourite food fish in Thailand and the neighbouring countries because of its desirable yellowish colour and the texture of its flesh, and it thus commands a relatively high market price. However, the culture of this species was not successful on a commercial scale due to its slow growth that required at least seven months reaching a marketable size of 200 grams. Moreover, it is very susceptible to bacterial diseases that reduced the survival rate to about 30 percent. These were the main reasons for hybridizing the broad-head catfish with the African sharp-tooth catfish, *C. gariepinus* which was introduced from Vietnam via Laos in 1987 (FishBase, 2007).

The African sharp-tooth catfish is native to Africa. It was introduced to Southeast Asia because of its high growth rate (reaching 200 grams body weight within 3 months) and low mortality due to diseases (survival rate about 80-95 percent). However, its meat quality (white and soft meat) was not well accepted by local people. Therefore, it was hybridized with some local catfish species in the hope of producing fast-growing, disease-resistant hybrids with acceptable meat quality.

ESTABLISHMENT OF THE TECHNOLOGY

There has been no record of the first hybridization attempt between these two species. Anecdotal reports state it was first done by a local farmer in northeastern Thailand by trial and error. The technology has been rapidly transferred among catfish hatcheries because the protocols were similar to the well-known breeding practice of the broad-head catfish. In brief, the hybridization is done by artificial insemination where a mature female broad-head catfish is injected once with a hormone (LH-RH analogue, Trade name: Suprefact) at 25 µg/kg plus Domperidone at 5 mg/kg while the male African sharp-tooth catfish is injected with the same hormone at 10 µg/kg and the same dosage of Domperidone. About 15 hours after injection the females are made to release their eggs by the breeder gently pressing on the belly. The eggs come out easily through the urogenital opening. Then the eggs are mixed with sperm solution squeezed from the minced testes of the sacrificed male. The sticky fertilized eggs are simply spread on the fine mesh net immersed under water. Hatching occurs 24-36 hours after fertilization. The fry are then fed with live food (water fleas) and will reach fingerling size of 1 inch within 10 days. The hybrid is very similar to the broad-head catfish and is always misidentified by local people as this species.

Nukwan *et al*. (1990) were the first group to perform scientific investigations on this hybridization. They found that only the hybrid having the broad-head catfish as the mother hatched and survived well; the hybrid did not show heterosis for growth (i.e. the growth rate of the hybrid was not higher than average growth rate of the parents). The advantages of the hybrid over the maternal species (the broad-head catfish) was the improved growth rate (reaching 200 grams body weight within 4 months) and survival rate, while the meat quality was better accepted than that of the paternal species (the African catfish).

CONTRIBUTIONS OF THE HYBRID CATFISH

The hybrid has been widely accepted by the farmers and as such, it enhanced the country's annual production of walking catfish (mixed species of the genus *Clarias* are often generally referred to as walking catfish) from 17 900 metric tonnes in 1990 to the peak of 159 314 metric tonnes in 2004 despite a slight decline during the recent years (Figure 1).

The beneficiaries of this technology are the catfish hatcheries and the grow-out farmers. The catfish hatcheries that practise the hybridization technology are located mainly in central

Thailand where the water flea *Moina macrocopa,* an essential food for the fry, is commercially available. Although the exact number is not known, the number of hatcheries is estimated to be at least 1 000 throughout the country. The number of grow-out farms is much higher. In 2010, there were 79 288 catfish farms in Thailand covering an area of 14 432 hectares (Research and Statistical Analysis Division, 2010). At present, among freshwater commodities in Thailand, hybrid catfish production is second only to Nile tilapia, and Thailand has been ranked first among the walking catfish producing countries (FAO, 2010). This has made this technology a successful case study of the application of biotechnology to aquaculture.

The culture of the hybrid has been practised at either commercial or backyard scale. The commercial farms stocked the hybrid in earthen ponds of varying sizes (large ponds of more than one hectare in the central provinces and small ponds in northeastern provinces). The stocking density of the 2-cm long fingerlings is very high (about 1 000 000 fingerlings per hectare). The culture period is about 4-5 months and the yield is about 70 000 kg/hectare in the central provinces, slightly lower in the northeastern provinces. The survival rate ranges from 40 to 60 percent. Feeding differs according to parts of the country. For example, the farms in central Thailand have access to cheap, high nutritional value wastes (e.g. chicken viscera, waste from fish processing plants), thus they rely on these wastes. The additional reason is the low market price of the hybrid catfish in central Thailand (US$0.8/kg) that forces the farmers to reduce the cost. In northeastern provinces where the hybrid demands higher market price (e.g. US$1/kg), farmers use commercially available pellet feed only.

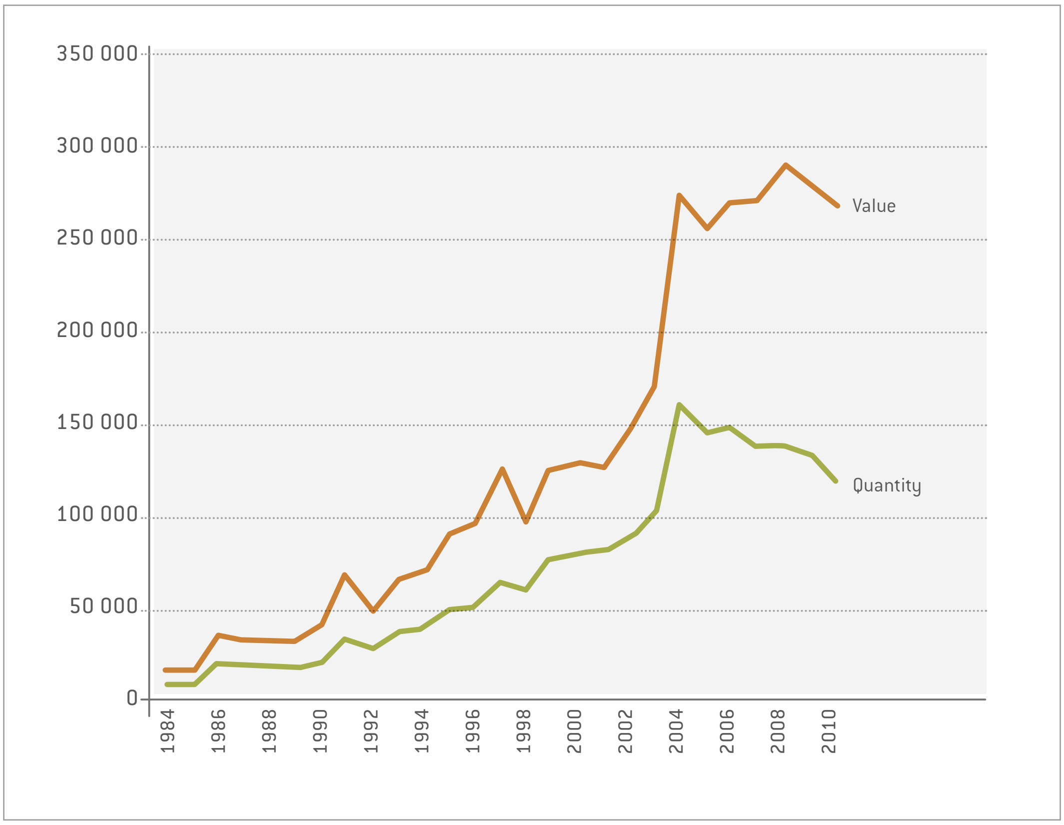
The backyard culture of the hybrid catfish is practised in rural areas and the production does not significantly contribute to the country's production. However, it does provide poor people with a cheap protein source and small income from limited resources available. The fingerlings (mostly of large size about two inches) are stocked in small ponds (100-200 square metres), tanks or cages, at relatively low stocking density. They are fed mainly with commercially available pellets while locally available protein sources (e.g. silk worm pupae, termites) are occasionally used as feed whenever available. The culture period is about three months when the 100-gram fish are harvested. At present, backyard culture is being promoted throughout the country as a part of a drive for self-sufficiency in agriculture.

CURRENT PRODUCTION TREND

As previously mentioned, the country's annual production of walking catfish has declined slightly. The major obstacle is the reduction of its market price. Since the export market of catfish is very limited, a majority of the hybrid catfish production is consumed domestically.

As such, the market is easily oversupplied, which affects the market price. In addition, deteriorating pond environments are triggering disease outbreaks with increasing frequency. These are the major causes of the recent reduction in annual production.

Figure 1. Quantity (metric tonne) and value (US$) of hybrid catfish annual production during 1984-2010



Source: FAO (2010)

ADVERSE IMPACTS OF THE HYBRID CATFISH

A dark side of the practice of hybridization also exists. Despite the fact that a majority of the interspecific hybrid animals are sterile, the female hybrid catfish produces mature eggs although in numbers far lower than the parental species; the males show a higher degree of sterility. As such, there was a concern that if the hybrid escaped into natural water bodies, it might backcross with the native catfish species including the broad-head catfish. The repeated back-crossing could eventually result in genetic introgression which may compromise the fitness of the local species.

During the past decades, Thailand has frequently faced severe floods, which resulted in the escape of millions of the hybrids into natural water bodies. The adverse impact of the escapees was revealed by the detection of gene-products in the wild broad-head catfish collected from habitats in every part of the country (Senanan *et al*., 2004; Na-Nakorn *et al*., 2004) that were found previously only in the African sharp-tooth catfish. This supports the hypothesis that the escaped hybrid may be contributing to the decline of native walking catfish by reducing their fitness. However, other factors such as habitat loss and overharvesting would also contribute to the decline of the native catfish. So far, there are no measures to mitigate the problem of genetic introgression, while the escape of the hybrid from grow-out farms still occasionally occurs.

Moreover, the expansion of the hybrid culture has adversely impacted the environment through the release of aquaculture waste. The grow-out ponds are always stocked with the hybrids at very high density and fed with high-protein feed. Therefore, wastewater from catfish farms is heavily loaded with organic matter and rich in nutrients. As such, when it is released to natural waters it causes rapid depletion of dissolved oxygen and thus causes mass mortality of aquatic animals. The wastewater also interrupts the flowering of rice due to excess nitrogen.

CONCLUSION

Overall, the hybridization technology has made a highly significant contribution to the aquaculture production of walking catfish in Thailand. The technology has triggered enormous expansion of the aquaculture business of walking catfish, feed industries and other related businesses. Moreover, it enhances the access of poor rural people to cheap and high-quality protein from the hybrid. Nevertheless, the farming of hybrids also has an adverse impact on native species and the environment. The adoption of this technology in the future should therefore be accompanied by an awareness of the adverse impacts. Good guidelines for the breeding of the hybrid should be established and endorsed for the sake of the ecological sustainability of aquaculture.

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Farmer catching the market-sized Jian carp from the cage
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next page
Farmers harvesting Jian carp from the pond for sale
©Zaijie Dong

CHAPTER 4.4

USE OF WITHIN-FAMILY SELECTION AND GYNOGENESIS TO DEVELOP THE JIAN CARP (*CYPRINUS CARPIO* VAR. *JIAN*) IN CHINA

ZAIJIE DONG

Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences
Asia-Pacific Regional Research and Training Center for Integrated Fish Farming
9 East Shanshui Road, Wuxi, Jiangsu 214081, China
E-mail: dongzj@ffrc.cn

INTRODUCTION

Heterosis, also known as hybrid vigour or outbreeding enhancement, is the improved or increased function of any biological quality in hybrid offspring. Heterosis has been used as an effective way to improve fish quality and increase fish production. Since the 1970s, Chinese fishery scientists have made broad studies of the utilization of heterosis in the production of common carp (*Cyprinus carpio*) and have made great achievements. Some hybrids of common carp have been successfully used in aquaculture. Heyuan carp is one hybrid which is obtained from the cross between purse red carp (*C. carpio* var. *wuyuanensis*)♀ × Yuanjiang carp (*C. carpio* var. *yuankangensis*)♂. It has several advantages, including high growth rate, good body shape, good feed conversion rate and high seinability (easily collected by nets in ponds). To produce Heyuan carp, pure populations of purse red carp and Yuanjiang carp must be maintained and the sexes in both populations chosen carefully during mating. The resulting Heyuan hybrid cannot be used as brood fish because the traits will segregate when the hybrids are mated. How to fix the hybrid vigour in the brood fish, i.e. pass on the same combination of desired traits in the offspring, and make the mating of brood fish easier for farmers is very important for smallholder farmers to produce quality seeds for aquaculture.

APPROACH USED: THE MATING PROGRAMME

Funded by projects from the Chinese Government, scientists at the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences made efforts to develop a population of carp that would allow farmers to utilize the heterosis of Heyuan carp by means of an easy way of crossing males and females in the population. The methodology of combining within-family selection and chromosome engineering (gynogenesis) was adopted to increase the homozygosity of the loci controlling the desired traits. The detailed mating programme (Figure 1) is summarized below.

Step 1: establish four families. Choose four female purse red carp and four male Yuanjiang carp, cross purse red carp♀ × Yuanjiang carp♂ to produce the first filial generation (F1) of four hybrid families.

Step 2: conduct within-family selection. Select males and females with good traits for fast growth, grey colour and long body shape from the F1 generation in each of the four families. Cross the males with females from the same family to produce the second filial generation (F2) of four families.

Step 3: conduct within-family selection. Select males and females with good traits from the F2 generation in each of the four families. Cross the males with females from the same family to produce the third filial generation (F3) of four families. Meanwhile, conduct gynogenesis. (Gynogenesis is a special form of sexual reproduction in which insemination is necessary but the head of the sperm penetrating into the ovum does not transform into male pronucleus; and the gynogenetic embryo develops at the expense of the ovum nucleus only, i.e. the male gamete contributes no genetic material to the embryo. Consequently the gynogenetic offspring are all females, identical to the mother). Select two good females based on the growth, colour and body shape. Strip matured eggs from the two fish and inseminate them with inactivated sperm. Induce two lines of gynogenetic fish by cold shock treatment at 0-2°C (G1 and G2).

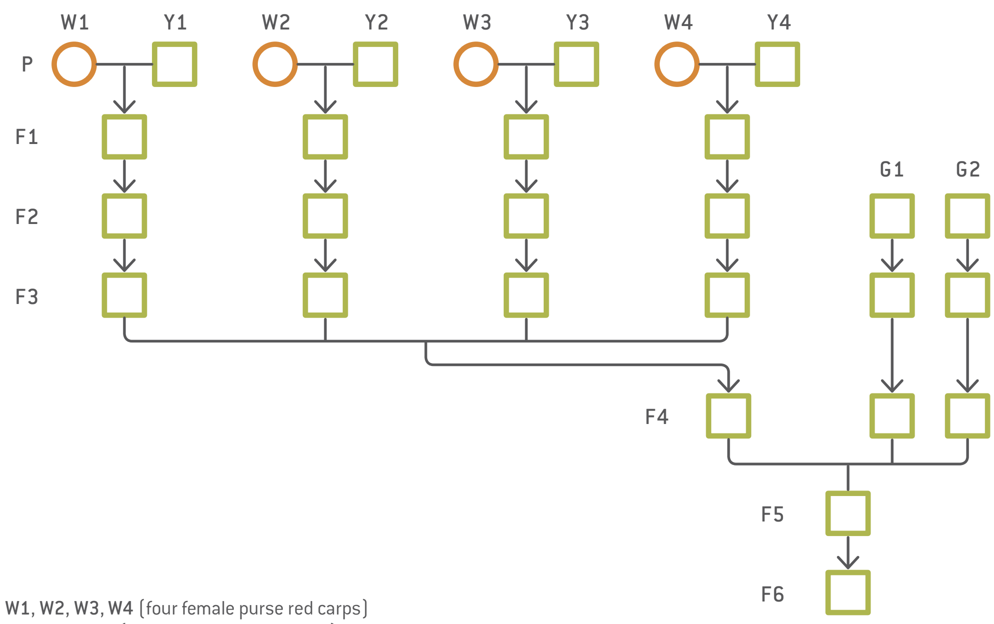
Step 4: conduct within-family selection and gynogenesis. Select and merge males and females with good traits from all the F3 families, they randomly mate with each other to produce the fourth filial generation (F4). Meanwhile, select one good female fish in each of the two gynogenetic lines, induce the second generation of two gynogenetic lines (boxes below G1 and G2).

Step 5: conduct random mating. Select good fish from the F4, as well as select good females from the second generation of two gynogenetic lines. Merge them together and mate randomly to produce the fifth filial generation (F5).

Step 6: conduct random mating. Select good fish from F5, and randomly mate them to produce the sixth filial generation (F6).

In F6, the traits are stable, more than 95 percent of fish were grey in colour and had a long body shape (ratio of standard length to body depth was 2.68±0.38). From this generation onward, the fish were officially designated as Jian carp (*Cyprinus carpio* var. *jian*).

Figure 1. Schematic diagram of Jian carp breeding method



W1, W2, W3, W4 (four female purse red carps)
Y1, Y2, Y3, Y4 (four male Yuanjiang carps)
G1, G2 (two gynogenetic lines)
P (parent generation)
F1, F2, F3, F4, F5, F6 (the first, second, third, fourth, fifth and sixth filial generation, respectively)

DIFFERENCE MADE

After six generations of selection for growth, colour and shape, Jian carp is a good stock of cultivated fish species with stable genetic traits for grey colour and long body shape. The growth of Jian carp outperformed its original parents and their hybridized filial generation, i.e. purse red carp, Yuanjiang carp and Heyuan carp, by a factor of 141-250 percent, 80-96 percent and 40-42 percent respectively. More importantly, the male and female individuals in the population could be easily mated by farmers to perpetuate the strain. Jian carp was first distributed to farmers in 1988. At present, Jian carp has been distributed to 27 provinces, municipalities or autonomous regions in China with great social and economic benefits. Approximately 160 000 farms use the Jian carp, many of which are smallholder farms. The specific yield of this carp is more than 30 percent greater than other varieties of common carp and the production of Jian carp is 1.35 million MT and accounts for more than 50 percent of total common carp production with an approximate value of 12.8 billion RMB.

Although within-family selection and gynogenesis are helpful to fix good traits in selection programmes, they could result in inbreeding depression if farmers do not pay close attention to the selection of brood fish. At present, inbreeding depression, i.e. retarded growth, short body and orange colour of Jian carp, has been observed in some places. The inbreeding depression was due to a lack of careful selection of brood fish which accelerated the homozygosity of loci controlling the desired traits. It is highly recommended that smallholder farmers should select brood fish each generation when they produce seeds for aquaculture. Alternatively, they may purchase seeds from authorized hatcheries that have well-managed breeding programmes in order to get high-quality seeds.

Training on proper selection of brood fish by smallholder farmers and larger producers has been undertaken by the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences. The training consists of lectures and fieldwork. On average, four to five batches of such training were conducted every year. Each course lasted three days and some 50 farmers and technicians participated. Until now, more than 5 000 farmers have received training on artificial propagation of Jian carp.

Fermented fish (the bonga, *Ethmalosa fimbriata*) drying on racks in Tanji, The Gambia
©FAO/ Alhaji M. Jallow

CHAPTER 4.5

SMALL-SCALE FISH FERMENTATION AND PROCESSING IN WEST AFRICA

GBOLA R. AKANDE

Post-harvest fisheries specialist, Nigerian Institute for Oceanography and Marine Research,
3 Wilmot Point Road, Victoria Island, Lagos, Nigeria.
E-mail: akandegra@yahoo.com

SUMMARY

Small-scale fish[1] processing, including fermentation, in West Africa contributes to solving problems of food insecurity that impede national development and threaten peace in the region. Due to limitations in infrastructure and the prevailing poor technologies, rural areas in West African countries have been slow in keeping abreast of global developments towards industrialization. The lack of standardization of the processing methods and poor hygiene during processing, with their detrimental effects on the quality and the safety of the end products, are the major problems to overcome to ensure the promotion of fermented fish products supply chains in West Africa. Quality, safety and acceptability of traditional fermented fish may be significantly improved through the choice of raw material, good handling practices and the use of starter cultures selected on the basis of multifunctional considerations. Enriched by inputs from genetics and/or genomics research, biotechnology is a major force for development in fish fermentation in West African countries. There is a need for new areas of research focused on the standardization of traditional fermentation techniques and packaging of end products to meet the expectations of modern consumers, enhance value-added products and increase the market share of traditional fermented fish products.

INTRODUCTION

Fermentation is one of the oldest known uses of biotechnology. Fermented fish can be described as any fishery product that has undergone degradative changes through enzymatic or microbiological activities either in the presence or absence of salt (Zakhia and Cuq, 1993). Fermentation is a process by which beneficial bacteria are encouraged to grow. These bacteria increase the acidity of the fish and therefore prevent the growth of spoilage and food-poisoning bacteria. Additionally, salt is used to prevent the action of spoilage bacteria and allows the fish enzymes and the beneficial acid-producing bacteria to soften (break down) the flesh. Fermentation processes are believed to have been developed over the years by women to preserve food for times of scarcity, impart desirable flavour to foods and reduce toxicity (Rolle and Satin, 2002). Today, fermentation is still widely practised as a household or village-level technology in many countries, but comparatively very few operations are carried out at an industrial level (Holzapfel, 2002).

1 "Fish" is used here in the broad sense to include fin fish and other aquatic animals e.g. molluscs.

Fish has been an important part of the diet of West Africans. It is estimated that 15 to 20 percent of all animal proteins come from aquatic sources (FAO, 2012). There is a strong preference for fresh fish. However, cured fish products such as smoked, salted, sun-dried and fermented fish are also popular.

In countries such as Chad, Côte d'Ivoire, Gambia, Ghana, Mali, Nigeria, Sierra Leone, Sudan and Uganda, fermented fish products have been relatively popular (Watts, 1965; Eyo, 1993; Zakhia and Cuq, 1993). For example, fermented fish are reported to have a ready market in the Lake Chad region of northern Nigeria (Azeza, 1986) and in the Delta State of Nigeria with preference for fermented *Heterotis niloticus* (Nwabueze and Nwabueze, 2010).

APPLICATION OF FERMENTATION IN SMALL-SCALE PROCESSING

In West Africa, hot smoking is the main method of fish processing. However, curing by salting, fermentation coupled with drying constitutes the second most important method of preserving fish after smoking. The processes of fermentation and drying preserve and add value to the fish, giving a product which has a characteristic odour and aroma. More income is derived by fish processors from fish fermentation than from smoking or drying (FAO, 1992). This, however, may depend on a wide range of factors such as the species, geographic location, seasonality and physiological state (gravid or not) of the fish, the market structure and the target consumer's purchasing power.

The quantity of fish that is processed into fermented products in any particular country is influenced by the food habits of the people and market demand. Table 1 shows the total domestic annual fish supply, the quantities consumed as fresh or frozen, and the quantities processed into smoked and fermented products by West African countries.

Côte d'Ivoire: About 35 percent of the total annual production of fish is consumed fresh while 50 percent is smoked (FAO, 1992). Only about 10 percent of the total fish supply is eaten as salted, dried and fermented products, mainly as a condiment. However, small quantities are exported to Burkina Faso. The remaining 5 percent may been eaten as fried or charcoal grilled fish, especially for outdoor facilities (restaurants, chop bars and street foods)

The Gambia: About 50 percent of the annual fish production in the Gambia is processed into salted, partially fermented and dried products mainly for export to Côte d'Ivoire, Ghana and Mali. The price of the product on the Gambian local market is high compared to other cured fishery products.

Ghana: About 15 percent of the total annual fish production is fully or partially fermented into either dried or salted dried products for the local market. The product is used as food fish and as condiment in local dishes. Salted and fermented dried fishery products are imported from the Gambia, Norway and Senegal to meet shortfalls in local supply.

Mali: Of the total annual fish production, as much as 75 percent is processed by smoking, wood grilling and dried fermentation. The rate of processing is high in Central Niger Delta where 80-90 percent of fish is processed, but lower in other areas where fresh fish is preferred. Fresh fish consumption in Mali is between 10 and 25 percent compared with 60 percent for smoked fish, which constitutes the largest share of the domestic fish supply.

Senegal: About 10 percent of the annual domestic supply is processed into various dried and fermented products. A large proportion is distributed locally, but significant quantities, representing 2-5 percent of the total volume of fish exports, go to countries such as Burkina Faso, Congo, Ghana, Mali, Togo and the Democratic Republic of Congo.

Nigeria: About 7.4 percent of the annual domestic supply is smoked (FDF, 2010) while the majority is consumed fresh. Fermented fish are mostly imported. Nigeria imports about 60 percent of Chad's annual production of partially fermented, sun-dried fish.

Table 1: Total domestic annual fish supply, the quantity consumed as fresh or frozen, and the quantities processed into smoked and fermented fish products by West African countries (in tonnes).

COUNTRY	ESTIMATED ANNUAL DOMESTIC FISH SUPPLY	QUANTITY CONSUMED AS FRESH OR FROZEN FISH	QUANTITY PROCESSED AS SMOKED FISH	QUANTITY PROCESSED AS FERMENTED FISH
Côte d'Ivoire#	75 000	26 250	37 500	7 500
The Gambia#	46 000	23 000	Not reported	23 000
Ghana#	350 000	54 000	243 500**	52 500
Mali#	100 000	20 000	60 000	15 000
Senegal#	420 000	42 000	75 000**	42 000
Nigeria*	820 000	754 400	60 680	Not reported

#FAO (1992); FAO (2010)
*FDF (2010)
** Estimated

Adapted from FAO (1992) and updated from FAO (2010).

JUSTIFICATION OF FERMENTATION TECHNOLOGY, IMPORTANCE FOR FOOD SECURITY AND THE LOCAL ECONOMY

Fermentation prolongs the shelf-life of fish in addition to improving its nutritional value. Small-scale fermentation technologies contribute substantially to food security and nutrition, particularly in regions that are vulnerable to food shortages (FAO, 1998). Fermentation also improves fish food quality through greater digestibility to increase essential amino acids, vitamins and protein. In addition, fermentation technology provides direct employment for processors and supports others who provide indirect services to the industry in the areas of packaging, distribution, marketing etc.

Fermented fish are generally appreciated for their pleasant flavour, aroma, texture and improved cooking and processing properties. It can even have beneficial health effects when the fermenting micro-organisms possess probiotic activity. Micro-organisms, by virtue of their metabolic activities, contribute to the development of characteristic properties such as taste, aroma, visual appearance, texture and shelf-life. Enzymes indigenous to the raw materials may play a role in enhancing these characteristics (Hammes, 1990).

PROCESS FLOW IN FERMENTED FISH PRODUCTS, SPECIES AND END PRODUCT USAGE

Three basic methods were identified for fish fermentation in Africa: fermentation with salting and drying, fermentation with drying without salting and fermentation with salting but without drying (FAO, 1992; Dirar, 1993 and Anihouvi *et al.*, 2005). The first method is predominantly employed for traditional fish fermentation in West Africa (Figure 1). Anihouvi *et al.* (2012) summarized the major fermented fish products in West Africa, which we shall now consider.

Lanhouin processing: The first step consists of the scaling, gutting and washing of fresh fish. This is followed by 10-15 hours of ripening, during which the fish is left in a bowl without water (Figure 1). The ripening influences the texture and aroma of the end product. After ripening, the fish is salted before fermentation and the salted fish arranged in containers (baskets, cans, baskets lined with cement, concrete vats and earthenware jars), wrapped with jute sacks or cloth, or buried in a 2-m deep hole and allowed to ferment for 3 to 8 days, depending on the local conditions and the kind of product desired (Anihouvi *et al*., 2005; Kindossi *et al*., 2012).

The species of fish include; Cassava croaker (*Pseudotolithus senegalensis*), Lesser African threadfin (*Galeoides decadactylus*), Atlantic bumper (*Chloroscombrus chrysurus*), Spanish mackerel (*Scomberomorus tritor*) and Crevalle jack (*Caranx hyppos*).

Momone processing: The processing of Momone is similar to Lanhouin (Figure 1). It is usually carried out to salvage large quantities of fish that would otherwise have been discarded due to poor quality. Momone processing involves the use of whole fish cut into smaller pieces or split dorsally. Dressed fish is washed and either left overnight before salting (15-40 percent by fish weight) or salted immediately after washing and allowed to ferment for 3 to 8 days, followed by drying on raised platforms for 1 to 3 days (FAO, 1992; Abbey *et al.*, 1994).

Figure 1. Flow diagram for traditional processing of fermented fish products in West Africa

Source: adapted from Anihouvi *et. al.*, 2012

Guedj processing: Guedj is a Senegalese and Gambian traditional fermented fish product, used as a flavouring agent and very much appreciated by the local populations because of its exceptional flavour and taste (Diop, *et al*., 2010). Dressed fish are salted and allowed to ferment for about 2 to 3 days, followed by the drying on raised platforms for about 3 to 5 days (Figure 1). For marketing purposes, the women also smear the fish with an oil-based substance that gives it a reddish colour, conforming to the preferences of Senegalese consumers. Different fish species are used for the preparation of guedj, including mackerel, seabream, threadfin, croaker, mullet, catfish, meagre, herrings, skate, rays and shark, which are caught with passive nets, beach seines and lines.

Yeet: Yeet is mainly a Senegalese fermented product prepared from cymbium, a sea snail (gastropod). The flesh is removed from the shell, split into two to four parts and fermented by burying the product for three to four days in sand and covered with a canvas to prevent exposure to air. In response to better quality product, fermentation takes place in plastic containers filled with seawater and dried on raised platforms for a month to give a reddish coloured product, as demanded by Asian consumers (ICSF, 2002).

Other West African fermented fish products: There are many other fermented fish products which have received little scientific investigation. These products are: Malian Djege/Djadan and Ivorian Gyagawere/Adjonfa. Dressed and headed fish are salted and then put into water in an earthenware pot or oil drum and allowed to ferment for 12 hours (Figure 1). Fish species such as *Tilapia*, *Clarias* spp., *Alestes* spp., *Schilbe* spp. and *Hydrocynus* spp. are commonly used to process Djege and Djadan. Some major fermented fish products from West Africa and micro-organisms involved in their production are presented in Table 2.

SAFETY, QUALITY AND MARKETING CONSIDERATIONS IN SMALL-SCALE FISH FERMENTATION PROCESSING

Traditional fish fermentation processes are typically uncontrolled and dependent on micro-organisms from the environment or the fermentation substrate to initiate it, and as such can result in products of variable quality. Fermentation is generally considered to preserve or keep the hygienic quality and safety of foods, but if it fails, spoilage may result and pathogens survive, thereby creating unexpected health risks in food products which would otherwise be considered safe (Holzapfel, 2002). There is a widespread perception among fish operators that spoilt fish can occur through smoking, drying or fermentation. Besides, there is confusion in most instances between fermentation and spoilage. All these do not provide room for good quality raw material, and therefore lead to poor quality end product. At all times, the theory of starting with good quality raw materials resulting in good quality end products should always hold. Quality, safety and acceptability of traditional fermented fish may be significantly improved through the use of starter cultures selected on the basis of multifunctional considerations.

Poor processing of traditional fermented fish products constitute health hazards to consumers. Such hazards can arise through the processing technique, environment, the waste disposal system, the unhygienic nature of processing materials and improper packaging of the end products. The use of water from lagoons, rivers, lakes or the sea can introduce products to possible chemical and microbial contamination (Dirar, 1993; Anihouvi *et al.*, 2005; Anihouvi *et al*., 2012). Drying on the ground leads to contamination with sand and micro-organisms as well as blowflies and other insects, which lead to the illegal use of insecticides (FAO, 1992; Anihouvi *et al.*, 2005; Abbey *et al.*, 1994). Inappropriate technologies and lack of standards in the fermented fish products industry could be potential vehicles for transmission of food-borne diseases. There have been confirmed high levels of biogenic amines, mainly histamine, in various fermented fish products (Anihouvi *et al.*, 2005; Abbey *et al.*, 1994; Wootton *et al.*, 1989).

According to Yin *et al*. (2002) the use of starter cultures of lactic acid bacteria to ferment minced mackerel could suppress the growth of spoilage bacteria and pathogens, and substantially inhibit the development of volatile basic nitrogen. Using starter cultures for fish fermentation could also reduce the fermentation time, enhance the inhibition or elimination of food-borne pathogens, and improve the shelf-life and sensory quality of products in terms of taste, aroma, appearance and texture.

In the West African sub-region, the flow of fermented dried fish is mainly from the Gambia, Mali and Senegal to Burkina Faso, Côte d'Ivoire, Ghana, Nigeria and Togo. Senegal exported fermented fish to various West African countries. Small quantities of salted/fermented dried fishery products were exported from the Gambia and Senegal to France where they were patronized by the resident West African communities (FAO, 1992). The bulk of fermented fish produced in Côte d'Ivoire, Ghana and Mali were marketed locally. The product lends itself to easy packaging, transportation, distribution and marketing without chilling or any other expensive method of storage.

Table 2. **Some major fermented fish products from West Africa and the micro-organisms involved in their production.**

TYPES OF FISH	PRODUCTS LOCAL NAME	COUNTRY	FERMENTATION DURATION	MICRO-ORGANISMS INVOLVED
Catfish, croaker, meagre, shark, mullet, skate, rays, triggerfish, horse mackerel, octopus, tuna, sole, Spanish mackerel, seabream, herring	Gyagawere, adjuevan	Côte d'Ivoire	6 hours to 3 days with salting	Lactic acid bacteria *Leuconostoc lactis* *Lactobacillus fermentum* *Pediococcus* sp, *streptococcus* sp
Cassava croaker/Cassava fish, kingfish	Lanhouin	Benin	3-8 days with salting	*B. subtilis, B. licheniformis, B. megaterium, B. cereux, B. Mycoides, Micrococcus luteus, Staphylococcus lentus, Staphylococcus xylosus, Streptococcus; Corynebacterium spp.,*
Catfish, barracuda, seabream, threadfin, croaker, grouper, bonito, mackerel, herrings, squid, octopus, bumper, snapper, ribbon fish	Momone	Ghana	Overnight to 3 days with salting	*B. subtilis, B. licheniformis, B.megaterium, B. cereux, B. mycoides, Micrococcus luteus, Staphylococcus* spp, *Lactobacillus, Pseudomonas, Pediococcus, Klebsiella, Debaryomyces, Hansenula and Aspergillus*
Carp, threadfish	Djegue, jalan	Mali	Overnight, no salting	unknown
Alestes Nile perch, parch	Aku	Ndokwa-East in Southern Nigeria	Overnight, with little salt	unknown
Mackerel, seabream, threadfin, croaker, mullet, catfish, meagre, herrings, skate, rays, shark	Guedj, tambadiang, yet	Gambia, Senegal	Overnight to 2 days with salting	*Proteus* spp., *Shewanella, putrefaciens, Bacillus* spp.

adapted from Anihouvi *et al.*, 2012

PERSPECTIVES AND VIABILITY OF BIOTECHNOLOGY IN SMALL-SCALE FISH FERMENTATION IN WEST AFRICA

The greatest drawback in the development of fermented fish products in West Africa is that many are produced under primitive conditions, resulting in low productivity, poor quality and short shelf-life. With improved technologies, it should be possible to be innovative in adding value, such as increased shelf-life, flavour and appealing packaging and labelling, to fish products using fermentation and indigenous knowledge systems.

There are many opportunities for biotechnological innovation in the microbiology of fermented fish such as isolation, characterization and preservation as a germplasm and collection of all the micro-organisms involved; understanding the metabolic role of each of the strains involved, and their full potential in other fields of biotechnology. The powerful technique of monoclonal antibodies for the characterization of different strains of the same species can be of great help in this area.

Fermentation biotechnology has also given rise to great innovations in engineering designs of bioreactors. Most of these designs deal with liquid reaction media, but traditional fermented fish products in West Africa are produced through solid-substrate fermentation. Bioreactors to simulate such process are needed for the modernization of the traditional fermented fish production.

CONCLUSION

Small-scale fish fermentation is desirable in terms of food preservation, quality and nutrition. However, the safety, nutritional and flavour profile of the products needs to meet the expectations of consumers. The lack of standardization of the processing methods and hygiene impairs the quality and safety of the products. The packaging of the end products needs to be improved while the development of local food industries through the improvement of traditional fermentation technologies is one of the ways to enhance value-added products and the market share of traditional products. In this regard, the culture, culinary traditions and the current level of development of the processing methods in each country should be taken into account.

from left to right

Dried fermented fish arranged on elevated racks in the Gambia
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In Ghana retail trading of fermented fish is exclusively a female activity
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