ISSN 0859-600X

Volume XII No. 1 January 2007

AQUACULTURE ASIA

Integrated aquaculture-agriculture in Bangladesh

Replacing marine fish oil in feeds with tropical palm oil

Modeling the market chain for the live reef food fish trade

Cage culture of tilapia in Lake Taal

Lotus - a versatile aquatic plant

AQUACULTURE ASIA

Aquaculture Asia
is an autonomous publication that gives people in developing countries a voice. The views and opinions expressed herein are those of the contributors and do not represent the policies or position of NACA.

Editor
Simon Wilkinson
simon.wilkinson@enaca.org

Editorial Consultant
Pedro Bueno

NACA
An intergovernmental organization that promotes rural development through sustainable aquaculture. NACA seeks to improve rural income, increase food production and foreign exchange earnings and to diversify farm production. The ultimate beneficiaries of NACA activities are farmers and rural communities.

Contact
The Editor, Aquaculture Asia
PO Box 1040
Kasetsart Post Office
Bangkok 10903, Thailand
Tel +66-2 561 1728
Fax +66-2 561 1727
Email
simon.wilkinson@enaca.org
Website http://www.enaca.org

Printed by

Scand-Media Co., Ltd.

Volume XII No. 1
January-March 2007

ISSN 0859-600X

Big issues for small players

Producers know that food safety is a big issue. When a food safety scare happens, governments can close access to export markets overnight and millions of dollars of product already in transit may be rejected at the border or destroyed. A problem caused by one producer can impact on the whole industry. Even if the market remains open, consumers will go on strike and demand will plummet.

For consumers, managing risk is easy – just eat something else for a while. However, for producers and other links in the supply chain it can be a complex and difficult issue to address. Importing nations often impose stringent requirements on imported products in terms of how they are produced and handled as well as on the critical issue of how these measures are documented. The requirements vary substantially between countries; they change frequently, sometimes with very little notice and the cost of compliance can be high. Increasingly, other factors such as the environmental and social impacts of production are becoming issues subject to the requirements of importing nations, and also to intense lobbying by NGOs. Producers have little choice but to comply, if they want to sell their product in that market.

In response to the concerns that have been raised, a range of certification schemes have been developed for aquaculture products by different stakeholder groups, including by government agencies, industry associations and NGOs. However, there is no unified approach, and such schemes suffer from a lack of cross-recognition, particularly from governments who ultimately have the final say on market access.

While all major stakeholder groups want to impose their own requirements, from a practical standpoint it is simply unrealistic to expect small-scale producers to comply with a plethora of different standards or product certification schemes – particularly if a particular scheme does not help them obtain advantage in, or at least access to, their target markets. Importing nations cannot completely ignore the plight of small-scale farmers, at least not if they want to eat fish, as collectively these people produce a large proportion of the world's farmed fisheries products.

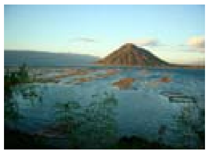
What is needed is some common ground that would-be certifying agencies can use as an agreed basis for developing certification schemes, to facilitate cross-recognition of standards, perhaps in combination with some practical approaches to facilitate implementation among small scale producers such as working through farmer groups and clusters rather than individuals.

With this in mind, NACA will co-organize an Expert Workshop on "Guidelines for Aquaculture Certification" in March, together with FAO and the Department of Fisheries of Thailand. The workshop will be the first in a series aiming to facilitate the development of international guidelines for the certification of aquaculture products. More information is available in the NACA Newsletter, but we will be sure to publish the findings and outcomes of the workshop for our readers in future issues of the magazine.

Simon Wilkinson

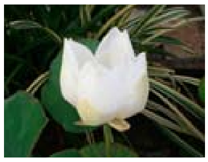
AQUACULTURE ASIA

In this issue



Page 3.

Page 8.



Page 11.

Page 14.

Page 38.

Page 45.

Peter Edwards writes on

Rural Aquaculture

Dr Edwards is a consultant and Emeritus Professor at the Asian Institute of Technology in Thailand where he founded the aquaculture program. He has over 30 years experience in aquaculture education, research and development in the Asian region. Email: pedwards@ait.ac.th or pedwards@inet.co.th

Cage culture of tilapia in Lake Taal, Philippines

Crowded cages in Lake Taal, the flooded caldera of an active volcano.

Cage culture of Nile tilapia (*Oreochromis niloticus*) began in Lake Taal, Batangas province in the 1970s, following the rapid development of cage and pen culture in nearby Laguna de Bay, the largest inland lake in the Philippines. Lake Taal has an area of 26,000 ha, and an average depth of about 60m as it is the flooded caldera of one of the country's largest and still periodically active volcanoes. In contrast to shallow and eutrophic Laguna de Bay with dense plankton and organic detritus to nurture fish, aquaculture in Lake Taal depends almost entirely on artificial feeds.

Average annual cage production of tilapia in Lake Taal from 1995-2002 was almost 20,000 t, the biggest production of tilapia from freshwater cages in the Philippines. However, a more recent and accurate estimation of tilapia production from cages in Lake Taal through an ongoing project indicates huge previous underestimation by a factor of at least five times (Patrick White, personal communication); based on aerial photography and interviews undertaken in Lake Taal in 2006, the total annual production is over 100,000 tonnes. This greatly increases the benefits from rural aquaculture in Lake Taal as well as concerns about the adverse environmental impact and sustainability of the practice.

This column is based on a case study of Lake Taal prepared as part of an Asian Development Bank (ADB) Special Evaluation Study (SES) "An Evaluation of Small-scale Freshwater Rural Aquaculture Development for Poverty Reduction". It documented the human, social, natural, physical and financial capital available to fish farmers

Pelleted feed awaiting transport to cages.

Constructing ponds to nurse tilapia adjacent to Lake Taal.

and identified channels through which the poor are affected. A survey was conducted in 2003 of 100 tilapia cage farmers and 81 nursery pond farmers in and around Lake Taal. Other key informants and stakeholders such as input suppliers, fish traders, fisheries groups and government agencies at various levels were also interviewed.

Technology and management

While almost half of the cage farmers of Lake Taal rely on seed produced in hatcheries and nurseries of neighbouring provinces, tilapia nursery ponds adjacent to the Lake have become a major source of fingerlings with many tenant farmers converting their rice farms, wholely or partially, to tilapia nursery ponds. Nursery pond farmers buy small fry from hatcheries in nearby provinces and later sell fingerlings nursed in ponds to cage growout farmers in the lake. The majority of lakeside tilapia nurseries have two to three fingerling production cycles per year.

Tilapia cage culture in Lake Taal is intensive with almost total reliance on commercial feeds and improved tilapia breeds. There were almost 7,000 cages in 2002 although over 10,000 cages were recorded in 1999. Most cages consist of bamboo for a frame and flotation with a synthetic net enclosure. The most common dimensions are 10x10 m with a depth of 6-7 m. Tilapia is grown throughout the year, usually in two 5-6 month cycles. Average production is 3 tonnes/cycle/cage or 6 tonnes/year.

Farmers consider tilapia farming to be very profitable compared with other limited livelihood opportunities around the lake but half of respondents reported absence of capital as a major impediment to starting cage farming. High costs of inputs were identified as major problems by cage farmers: feed costs by 80% and seed costs by 61% of respondents; other major production problems cited in a multiple response question were high fish mortality (93%), fish diseases (89%), bird predation (82%), parasite infestation (74%) and poor water quality (74%). The majority of respondents also reported declining profits (95%) as well as natural calamities such as typhoons (92%) and floods (50%), indicating major concerns about the sustainability of cage farming in the lake.

Tilapia nursery ponds by the side of Lake Taal.

Livelihood assets of cage and nursery farmers

Human capital

Tilapia farming in Lake Taal (cage growout) and around it (nursery ponds) is dominated by caretakers who enter into a profit sharing arrangement with the lessor of the cage site or the owner of the farm land, respectively. Most of the fish farmers who are local people are comfortable working as caretakers because of financial capital requirements to carry out aquaculture.

Natural capital

As the waters of Lake Taal are owned by the State, cage farming sites are leased. Favorable cage sites with shelter from storms and high water exchange, road and boat access, and security are limited so cages are crowded, which reduces water quality.

Farmland used for nursery ponds and support facilities for cages is under private ownership.

Cage ownership is limited to local residents but they make arrangements with non-resident or absentee-investors as most are unable to finance cage aquaculture. Local residents therefore serve either as caretakers or as permanent cage workers on farms that are usually registered in their names.

Social capital

Social networks are common and strong among cage farmers in Lake Taal with most respondents learning aquaculture from sources within Batangas province. Major sources of information reported by farmers were fellow farmers, friends, relatives and their own experience. Most cage farmers are members of a livelihood-related association, with almost half belonging to multi-purpose cooperatives catering mostly to cage farmers.

Physical capital

Most cage and nursery farmers have lived in the area for more than three decades, with over 90% owning their own dwelling units. Most owned modest household appliances and facilities.

Feeding tilapia fry in a lakeside nursery pond.

Financial capital

Tilapia farming in and around Lake Taal is mainly sustained by external funding as high operating costs and risks of fish farming deter local people from using their own limited financial assets for fish farming. Sharing of the net proceeds, and in some partnerships any financial losses, is agreed between a financier (usually a non-resident) and a farmer/caretaker (usually a local resident). Most fish farmers have multiple employment to reduce risks and provide additional financial security. The main sources of income for cage farmers in 2002 were cage farming (74%), trading (6%) and fishing (6%); for nursery farmers it was nursing fish (62%), rice farming (13%) and livestock raising (10%).

Transforming processes

Markets

Increasing consumption of tilapia among Filipinos has increased the demand for the fish, leading to the development of new technologies, market expansion and the opening of opportunities and services, including those for the poor. Markets for farmed tilapia from cages have expanded into Metro Manila and nearby towns, thus increasing access and opportunities for small-scale farmers and urban consumers.

Public and private institutions

Tilapia farming in Lake Taal has benefited greatly from public and private research and technical support, providing choices of tilapia strains and improved management practices. Two governments stations in Batangas have provided research, training and information dissemination on tilapia farming and have also increased farmer understanding of the natural hazards in the lake. Fish farming cooperatives in Lake Taal also facilitate information exchange and aquaculture-related training and discussion as well as

Harvesting tilapia from a cage.

helping in the mobilization of farmers' resources, monitoring the condition of the lake, and farmers' compliance with local regulations.

Labour and employment

Tilapia cage and nursery farming both generate direct employment opportunities for caretakers, labourers and their households. Additionally, they have created 'spin-off' jobs such as cage making and nursery pond construction, input supply, and fish harvest, transport and trading which boost local economies.

Support services, facilities and infrastructure

Social networks have been more effective than government extension services in disseminating information on tilapia farming among small-scale farmers but the national government has a credit program for small-scale farmers with loans are available to local fish farmers. Private service facilities such as boats to transport food to cages and ice plants to keep produce fresh are of crucial importance. Government develops and maintains facilities and roads to markets. Cellular phones have improved communications tremendously.

Policy and law

Official policies for freshwater aquaculture in the Philippines are pro-poor. Unfortunately, these policies are not implemented effectively as they are hindered by vested interests and by complex and confusing legislation. Furthermore, laws and regulations have been widely ignored in and around Lake Taal.

Harvested tilapia are sorted and transported by boat.

Natural resources management

Tilapia cage farming has not been adequately managed in terms of relationships with other lake users and its environmental impacts. A fish sanctuary established for conservation purposes is heavily occupied by fish cages, a source of frustration for the lake's fishers.

Sorting harvested tilapia.

Environment

Lake Taal is not a stable environment for tilapia farming. Fish are always potentially at risk from the unpredictability of the Taal volcano and associated seismic activity with ammonia and sulphide releases from the lake floor. The daily addition of large quantities of artificial feeds to the cages places large nutrient (nitrogen and phosphorus) and loadings on the lake. Fish kills are frequent, especially during overturns when deep turbid waters with low dissolved oxygen rise to the surface layers where cages are located.

Crisis and coping strategies

In the last 12 months prior to the survey, the five major types of crisis reported by households of all respondents were typhoons (75%), floods (45%), financial loss from livelihood occupation (41%), illness in the family (39%) and drought (20%) when lake water level falls. Major coping strategies were relying on family savings (51%) and obtaining loans from friends and relatives (40%). Vulnerability of farmers to external risk, especially natural calamities, might explain why most farmer respondents are caretakers.

Constructing a cage frame with bamboo.

Outcomes

All respondents were asked to assess various situations five years ago, at present and five years from now to determine perceived outcomes. Cage and nursery farmers both reported benefits in terms of employment and incomes over the last five years, enabling them to acquire household assets. However, cage farmers reported declining cash incomes. Both types of farmers share the same perception that the lakes resource base, upon which their livelihoods depend, is deteriorating.

Conclusions

Tilapia cage farming in Lake Taal contributes to reducing poverty through direct employment in farming in cages and lakeside nurseries. It also provides employment in fish hatcheries and fish feed supply and in fish harvesting and marketing.

For both cage growout of tilapia in Lake Taal and associated nurseries, access to livelihood assets, together with robust markets (input, output and labour markets), available services and infrastructure, and supportive policies and institutions, are all vital channels of effects.

The main factor that led to the rapid expansion of tilapia farming in the lake was its provision of water space, owned by the government and therefore available for occupation by would-be tilapia farmers who had no land. Moreover, the lake water was of good quality for tilapia cage farming, apart from ever-present risks of occasional volcanic activity, overturns of noxious deep waters to the surface, and typhoons: risks that farmers appear willing to carry for want of other opportunities to farm fish.

The expansion of cage farming in the lake has been largely unconstrained by attempts to limit entry and to manage cage farming in concert with the other diverse uses of the lake: principally fishing and tourism. The very success of cage farming in Lake Taal threatens its future because of overcrowded cages, leading to poor water quality and fish kills, and poor feed conversion efficiency that all contribute to worsening harvests and eroded profit margins.

Cage farming of tilapia in Lake Taal is an important contributor to keeping tilapia prices stable, thus helping to make tilapia more affordable to poor consumers as a source of animal protein, healthy lipids and micronutrients. Lake Taal tilapias also have a high reputation for quality. It is important that their contributions to rural and urban food security be maintained.

There is little to no scope for growth of cage farming in the lake because of the lake's finite carrying capacity and the need to accommodate other lake users. However, well-sited and well-managed tilapia cage farms and nurseries merit strong efforts to safeguard their future against further mismanagement, environmental deterioration, alien species, fish diseases, fish kills, and rising feed costs. Only if these manageable risks can be addressed effectively can cage farming operations in Lake Taal be sustainable and sufficiently profitable to withstand the inevitable occasional losses from adverse natural events such as overturns from seismic and climatic conditions and typhoons.

Further Information

The study on which this column is based is available on the ADB web site and as a printed book with the title "An Evaluation of Small-scale Freshwater Rural Aquaculture Development for Poverty Reduction.

To recap, the purposes of the study were to:

1. assess the channels of effects for aquaculture to generate livelihoods and reduce poverty
2. analyse enabling conditions for aquaculture to benefit the poor, and
3. distill lessons for making aquaculture more relevant for povert reduction for future development programs.

This is the fifth and final column based on the ADB study. Previous columns introduced the ADB study on aquaculture and poverty (Vol. X, No.3, 6-8, 2005), and summarized case studies on 'Small-scale pond culture in Bangladesh' (Vol. X, No.4, 5-7, 2005), 'Farming carps in leased ponds in Bangladesh' (Vol. XI, No.1, 26-29) and 'Poor Farmers culture tilapia intensively in Philippines' (Vol. XI, No.3, 15-18).

The study is available from:
http://www.adb.org/Documents/Reports/Evaluation/sst-reg-2004-07/default.asp?p=opereval

Also listed at One Fish:
http://www.onefish.org/servlet/CDSServlet?status=ND04MTU1MS4yNDk2MDomNj1IbiYzMzlkb2N1bWVudHMmMzc9aW5mbw~~#koinfo

For a hard copy contact:

Njoman George Bestari
Senior Evaluation Specialist
Operations Evaluation Department
Asian Development Bank
Email: nbestari@adb.org
Tel: (632) 632-5690
Fax: (632) 636-2161
Website: http://www.adb.org.

Development and adoption of BMPs by self-help farmer groups

Umesh N.R.

Farmers of the Astarang (Orissa) Aquaclub.

For farmers, and users and gatherers of aquatic resources, being organized into a formal association or a self-help group is to collectively achieve a strong capacity to enter and stay in aquaculture, effectively demand and absorb institutional services and technical assistance, cope with natural hazards and economic risks, address barriers to property and financial access, and acquire and effectively use capital and operating assets.

These are some hard evidence of the advantages of small farmers being organized, sharing resources, helping each other and adopting better management practices or BMPs, from NACA-assisted projects, in India and Vietnam:

Case No. 1: India

The small shrimp farmers in India are, like other shrimp farmers in the region, repeatedly hard hit by viral diseases. They are the most vulnerable to shocks and least able to rebound from adversities. Yet, other then providing 70% of the total volume of exported shrimp, they also comprise more than 80% of the shrimp farmers in India. This led to MPEDA requesting NACA assistance in developing and providing technical assistance to a shrimp health management project.

The shrimp health management project developed best health management practices for small farmers that are organized into “aquaclubs” (a group of 15-20 farmers). The BMPs were developed after studies were made of the problems of farmers. Pilot ponds were volunteered by some farmers and these served to test and then demonstrate the management practices. The project eventually evolved into a community development pilot with health management as the core technology. A project evaluation in 2004 found that the shrimp farmers that formed aquaclubs and adopted BMPs have increased yield by 33%, harvested shrimp that were 1.5 times larger, and were visited 20% less frequently by diseases than surrounding non-adopting farmers. Moreover, their products became more attractive to buyers because the shrimp had no antibiotic residues as the management practices they adopted exclude the use of banned drugs and chemicals. The project was subsequently expanded from Andhra Pradesh to four more states, namely, Gujarat, Orissa, Karnataka and Tamil Nadu. Another evaluation, of the 2005 crop results from 930 demonstration ponds spread over 484 hectares of area in 15 villages, showed an increase in production by two-fold, 34% increase in size of shrimp, and 65% reduction in disease prevalence compared to surrounding non-adopting ponds. There was a remarkable improvement in the quality of the shrimps due to non-use of drugs and banned chemicals and the adoption of better harvest and post-harvest handling practices. Economic analysis clearly demonstrate that farmers adopting BMPs have higher profitability,

Preparing the pond. Pre-stocking BMPs include sludge removal and disposal away from the pond site, ploughing on wet soil if the sludge has not been removed completely, water filtration using twin bag filters of 60 micron mesh size, maintaining a water depth of at least 80 cm at shallowest part of pond, and conditioning water for 10-15 days prior to stocking.

lower cost of production and are able to produce quality and traceable shrimp without using any banned chemicals.

Another project outcome is the contract seed production system, by which the organized small farmers could procure high quality seed at a reasonable price by entering into a contract with hatcheries for batch supplies of certified healthy seed, even offering premium price to hatchery owners for quality and reliable seed supply produced according to their specifications.

Encouraged by the success of the technical assistance project to promote BMPs among small shrimp farmers, India's Ministry of Commerce & Industry approved in May 2006 a scheme for "Registration of Aquaculture Societies for Adoption of Code of Practices for Sustainable Shrimp Farming." The aquaculture societies are the farmers' associations. The MPEDA will grant five lakhs rupees (500,000 Indian Rupees or almost US$ 12,000) as financial assistance to a society that registers with MPEDA and agrees to abide by the prescribed code of practices The assistance will be for various items including infrastructure development, hiring of technicians, capacity building of members and for activities that promote environmentally friendly farming methods. The MPEDA plans to provide such grant to 25 aquaculture societies in various parts of the country this year.

A team of young Indian technicians was trained by NACA-MPEDA-ICAR to serve as technical assistants to the farmers. They work alongside MPEDA and State technical personnel. Minimal backstopping is provided by NACA headquarters and one of the technical resource persons occasionally visiting the projects to provide field advice is a Thai farmer. Recently plans for a National Centre for Sustainable Aquaculture were unveiled by MPEDA to institutionalize the BMP scheme.

Case No. 2: Vietnam

Viet Nam witnessed an outstanding 3-fold increase in aquaculture production, from 374,000 mt in 1993 to 1,150,000 mt in 2003, and a 2-fold increase only in the 5-year period 1998-2003. Shrimp farming played a major role in this rapid development, with a production that, according to FAO data, over the 5 year period between 1998 and 2003 registered a 4-fold increase reaching over 220,000 mt and that, according to national statistics, grew constantly to reach an estimated 350,000 mt in 2005. This sharp increase in production came at a cost. Escalating environmental deterioration and the associated shrimp health problems, which in 2004 led to an estimated loss of more than 11% of the total shrimp production, began to damage the sector. Farmers usually dealt with these health problems by increasing the use of chemicals, involving sometimes the application of banned substances, which led importing countries to impose restrictions on Vietnamese aquaculture products that

Positive impacts of the project include reduced disease prevalence, more successful crops, increased profit, no antibiotic use, increased environmental awareness, reduced FCR and increased cooperation between farmers, hatchery operators and other stakeholders.

in turn most likely resulted in a negative impact on the livelihoods of farming communities.

The government of Vietnam recognized the need for promoting a more sustainable development of the sector and initiated several activities in this direction. Among these is a project that supported coastal aquaculture, which demonstrates the private and social benefits of adopting BMPs. In this project, support was given to the promotion of responsible development of the shrimp farming sector at all levels and for all links in the production chain. BMPs were developed for broodstock traders, hatcheries, seed traders and farmers. Focus was given on the development of simple and practical BMPs, which addressed the needs of less resourced small-scale farmers. Ten sets of extension material were developed and disseminated in close collaboration with the Ministry of Fisheries. The tangible outcomes include the following (MOFI/NACA.2005):

- Implementation of BMP for hatcheries was supported in six hatcheries and resulted in seed production up to 1.5 times higher and a price per unit seed of about 30-40% higher than non-BMP seed.
- BMP implementation was also supported in 7 pilot farming communities (655 direct beneficiaries). Implementation led to a remarkably lower risk of mortality, higher production and higher probability of making a profit.
- Farming communes that introduced seed testing increased their chances of making a profit of over seven times.
- BMP application led to average yields that were sometimes more than four times higher than in farms where BMP had not been adopted.
- The project BMPs were also incorporated into the draft standards for the production of organic seed.

The project also strengthened the institutions involved with seed health management by conducting training courses and by supporting the development of national and provincial-level legal documents to improve the process of seed screening and certification.

While there was no formal farmers' association, the Vietanmese farmers were informally organized at the village level and, in any case, belonged to the same communes. Through periodic meetings, the farmers in the same village became involved as a group in the adoption of BMPs.

Strengthening trust

These two projects are providing indications that the development and adoption of BMPs by small-scale farmers can be facilitated by their being well organized; it enables them to attain economy of scale to be able to comply with the best practices requirements.

More fundamental than the small farmers and the environment benefiting from BMPs is the social harmony it engenders. The above projects have arguably served to enhance trust and cooperation among the players in the market chain that include hatchery owners, the farmers, and processors/exporters. The basis for this proposition is that the supplier of inputs, the farmer, and the buyer of products stand to gain more from each one behaving responsibly towards one another than by taking advantage of each other.

As the above projects illustrate, engendering trust among stakeholders, particularly the different players in the market chain as well as other users of coastal resources, water in particular, can facilitate the voluntary adoption of responsible farming practices such as Codes of Conduct and BMPs. Importantly, organized farmers are also be in a stronger position to deal with other players.

References

Ministry of Fisheries/NACA. 2005. "Reducing the risk of aquatic animal disease outbreaks: pilot project in Ca Mau, Nghe An, Ha Tinh, Quang Ninh and Khanh Hoa coastal provinces", Final Report of the Project, Support to Brackishwater and Marine Aquaculture, Ministry of Fisheries/ DANIDA/NACA. Hanoi, Vietnam.

NACA. 2006. Annual Report to the Governing Council for the Year 2005

NACA 2004. "Village shrimp health management project in Andhra Pradesh." NACA Newsletter. Oct-Dec 2004. www.enaca.org/NACA Newsletter.

Lotus – an aquatic plant of versatile qualities

R.N. Mandal, G.S. Saha, and P.K. Mukhopadhay

Central Institute of Freshwater Aquaculture, P.O. Kausalyaganga, Bhubaneswar, Orissa, India, Pin 751 002

Lotus, *Nelumbo nucifera* L. under the family Nymphaeceae, is a common floating leaved aquatic plant whose beauty attracts all and invites attention to those who know its versatile qualities. It is, however, apparently treated as weed because of its prolific growth and habit to encroach any water body within a rather short period. Lotus propagates by means of runner that can penetrate vertically about 1.0 m into the bottom and spread horizontally about 1.5 m to develop new shoots and foliage. Erect and stout leaves supported with a long stalk place its canopy over the water surface and sometimes even above it. The leaf stalk is adapted so that it is able to increase its length up to three metres proportionately with increasing water level. Once the lotus leaves cover the water surface the penetration of light into the water can be hindered. Lotus can grow in all kinds of wetlands including beels, jheel, ditches, ponds, reservoirs, karanjali and lakes and is commonly found throughout tropical and subtropical Asia, Australia and Oceania.

Significantly, lotus has been accepted as a sacred plant across much of its geographical range, particularly in Asia, irrespective of communities or religion. Lotus flowers are put in the shrines of Hindu, Buddhist, Jain, and other deities, and are often offered for worship (puja) because of their sanctity. However, despite the versatile qualities, religious and other values of lotus it has not been treated as a conventional aquatic crop. This has been something of a missed opportunity and left vast wetlands fallow. Considering the importance of lotus to human lives, this article provides the glimpse of the wealth of this plant.

Lotus – an ideal substratum for periphyton

Investigations have shown that lotus is a good substrate for growth of periphyton, particularly within the zone that falls between the suface of the water down to approximately 50cm depth, where the decomposition of plant matter provides valuable nutrients for periphyton growth, in one analysis found to consist of

21.9% crude protein, 2.8% crude fat, 9.9% ash and 41.7% nitrogen free extract (Santra, 1998; Banerjee and Mathai, 1990). Lotus leaves decompose faster than the stalks, which are more fibrous. Decomposing lotus matter has been found to be densely populated by blue-green algae such as *Oscillatoria* sp., particularly on the undersurface of the leaves, which around which other algae such as *Merismopedia* sp., *Spirulina* sp., *Chlorella* sp., *Scenedesmus* sp., *Navicula* sp., and *Kirchnerialla* sp. and zooplankton *Brachyonus* sp., and many others are found. Periphyton in turn provides a useful food and / or substrate for other potential food organisms that can enhance the natural productivity of a system, and potentially fish yield in an aquaculture situation.

Lotus-cum-fish culture: Organic aquafarming

The demand for organic aquaculture products is increasing at the present time. In organic aquaculture different plant based and organic materials including manure, mahua cake and neem cake have been used as natural alternatives to the more conventional use of chemical fertilizers, with excessive use of the latter often leading to accumulation of expensive nutrients in the mud where they are not available to phytoplankton to stimulate productivity. However, the cultivation of some kinds of rooted aquatic plants can help to tap nutrients bound in the sediment and over time release them back into the water column. In this regard, the cultivation of lotus can be important both in economy as well as ecology, and may bring similar returns to other more conventional crops (Yi and Lin, 2002). The following benefits may be harvested from lotus cultivation in combination with fish culture:

- Lotus and Indian major carps can be cultivated simultaneously to allow harvest of two crops from the same cultivable area. In composite fish culture, different fish such as catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus cirrhosus*) maintain specific niche related to their food habit. Catla and Rohu, which are surface and column feeders respectively, can be cultivated in lotus vegetated pond where lotus-associated periphyton assemblages will fall within their natural feeding range in the water column. In addition mrigal, a bottom feeder, should be cultivated along with them.
- The same water body may be used in the later stage for lotus cum catfish culture when Indian major carp culture is not possible. Over the course of time lotus vegetation will gradually spread towards the centre of the water body or as far as the depth permits. In this case, catfish cultivation is recommended in place of Indian major carps, and may give substantial yields with a similar level of profit.
- If prolific growth of lotus vegetation may make a water body unsuitable for carp or catfish culture, grass carp can be released, which will consume a large quantity of the tender lotus leave and achieve remarkable growth. In such a condition a pond can be left for one year to harvest grass carp along with lotus leaves, flowers and rhizomes.

Considering the above phenomenon, and with due regard for the need to achieve an ecological balance within a water body we have proposed a conceptual model for optimum harvest of organic yields in integrated lotus cum fish culture pond.

Lotus – a potential support for aquaculture

Cultivation of lotus vegetation will bring two advantages at a time in any fish culture pond. The roots as well as rhizome (the underground stem) will accumulate excessive nutrients deposited in the pond sediments, even to a depth of one metre. The study of Shreshtha and Lin (1996) showed clearly that 50% of the trapped nutrients could be liberated by lotus cultivation. Apart from that, decomposition of lotus plant will further add to the natural fertilisation of the water body, thereby enhancing natural productivity. As mentioned, the leaves and the upper portion of the stalks act as substrata for periphyton growth, which boosts the availability of natural foods in the pond for access by fish.

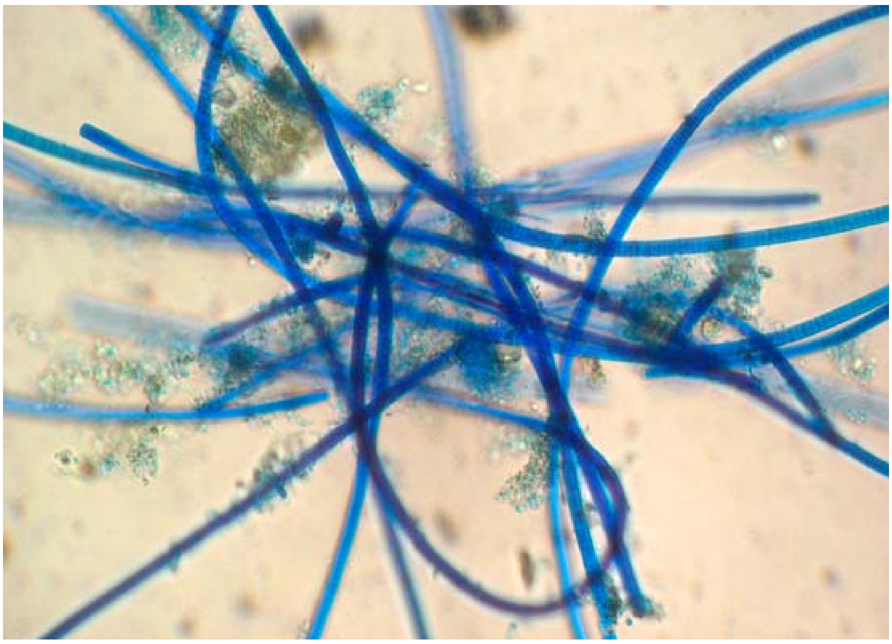
Lotus – a variety of uses

Myth

In Sanskrit language, lotus has been introduced by the names of 'Padma', and 'Kamal', for its integral relationship with nature: 'Padma' signifies that lotus manifests itself by unfolding petals along with emitting fragrance as soon as the sun rise; whereas 'Kamal' signifies that lotus presence as such adorns the water body and thus raises its state of elegance.

Therapeutic use

Medicinally, lotus flowers are cooling agent, used as astringent in diarrhea, cholera, fever and recommended as cardiac tonic. Seeds are used to check vomiting, given to children as diuretic



Oscillatoria sp. and other periphyton.

and refrigerant, as a cooling medicine for skin diseases and leprosy and are considered as antidote to poison. Lotus stalks are considered as astringent and cooling, useful for relieving burning sensations of the body and bleeding piles. The root in powder form is prescribed for curing piles, dysentery, and dyspepsia and used as a paste to treat skin infections and ringworm. Lotus honey, which has the major proportion of nectar collected from lotus flower is treated as good as 'Amrita' in Ayurveda. It is believed to be so precious that its consumption can recover people's suffering from many common ailments.

Food

People use almost all parts of lotus: rhizome and roots can be eaten after boiling, the thalamus is used as a vegetable directly or blended with other vegetables. Seeds can be consumed as soon as they are collected. Leaf stalks can be cut into pieces and mixed as one of the ingredients in preparation of pickle. Tender lotus leaves are used as fish feed, particularly for grass carp.

Household uses

Lotus leaves are used as mealtime plates (thali), with one leaf covering an area about 50 cm^2. In rural Bengal thali are served to people when mass gatherings occur to take food in any auspicious ceremony. Lotus flowers are used for various purposes including preparation of garlands offered to deities during worship and exchange between couples during marriage ceremony as an auspicious gift of love. People maintain lotus in water bodies for their aesthetic beauty.

Folklore

Hindus have a strong belief that if they go out for any work after seeing lotus flower, success will follow. Lotus has two varieties i.e. white flower and pink flower, which are equally auspicious across religion and accepted to all, commonly used in a wide range of ceremonies from funeral rites to worship of deities and temple offerings. Ponds with lotus vegetation are believed to be sacred places, and therefore most of the temple ponds preserve lotus vegetation.

Cultivation

There is no established package of practice of lotus cultivation in India, but it is reported that China and Japan cultivate lotus during fish culture (Yi and Lin, 2002). In India it generally grows in perennial wastewater bodies and in fallow seasonal wetlands. Malik (1961) mentioned a cultivation practice in Punjab, India where a low-lying plot about 154 acre was used for lotus cultivation. In this case, the rhizome along with root, known as 'bhen', was planted in silt soil during summer. Young plants were grown from bhen and became mature after three months. Mature plants showed luxuriant growth afterwards. He also reported that seeds could germinate and produce young seedlings. In embankment of lotus pond, an apiary can be set up to collect honey which will have the major constituent gathered from nectar of lotus flowers.

Economy

It has been estimated the economy of lotus cultivation is on the order of around Rs. 1,235 (US$ 25.95) earned against an expenditure of Rs. 346 per hectare, with a net income was Rs. 890.00 per hectare. Similarly, we have experience with the sale of different lotus products in Kolkata market in different seasons as per table 1.

Conclusion

Most of India's vast wetlands are either only partly utilized or abandoned. Frequently these water bodies are choked with weeds and it is somewhat surprising that little interest has been shown in the opportunity to cultivate lotus in such areas in combination with fish culture. The authors have experienced that this approach, and the harvest of different lotus products, can contribute to the livelihoods of large numbers of local people. A large area dominated by lotus vegetation remains fallow on both sides of the southeastern railway track near Kolkata, where a community is engaged to collect lotus crops that are marketed to Kolkata. Unfortunately, apathy prevails in maintaining these seasonal wetlands, which have a high natural productivity, but are not utilized properly for fish culture. Not only would this be profitable, but also source of income for poor farmers in the lag season, when virtually no work remain available for them after the kharif season. The time has come to look forward and step ahead to cultivate lotus commercially along with fish culture.

Table 1. Price of lotus products.

Lotus product	Festival season	Off season
Flower	Rs. 10/- for one flower	Rs. 2/- for one flower
Leaves	Rs. 2/- for-20 leaves of standard size	Rs.1/- for 20 leaves of standard size
Stalk	Rs.1/- for 10 pieces	Rs.1/- for 10 pieces
Fruit	Rs.1/- for 6-8 numbers	Rs.1/- for 6-8 pieces
Rhizome	Rs. 60/- for one kg. while used as medicine	Rs. 20/- for one kg. while used as food

References

Santra, S. C., 1998. Management of aquatic vegetation, In: Training Programme on Wise use of Wetlands, by British Council Division, Calcutta during 2-6 February.

Banerjee, A., and Mathi, S., 1990. Composition of Indian Aquatic plants in relation to Utilization as animal forage, Journal of Aquatic plant management, 28, 69-73.

Denny, P., 1972. Sites of nutrient absorption in aquatic macrophytes, Journal of Ecology, 60; 819-829.

Boyd, C. E., 1982. Water Quality Management for pond fish culture, Development in Aquaculture and Fisheries Science, 9. Elsevier, 318 p.

Smart, R. M., and Barko, J. W., 1985. Laboratory culture of submerged freshwater macrophyte on natural sediment. Aquatic Botany 21: 251-263.

Yi,Yang, and Lin, C. K., 2002. Lotus-Fish Culture in Ponds: Recycling of Pond Mud Nutrients, Nineteenth Annual Technical Report, PD/A CRSP, Oregon State University, Corvallis, Oregon.

Shrestha, M. K., and Lin, C. K., 1996. Determination of phosphorus saturation level in relation to clay content in formulated pond muds, Aquaculture Engoneering, 15: 441-459.

Malik, H. C., 1961. It pays to grow Sighara and Bhen, Indian Farming, 11(8): 23-24

Integrated aquaculture-agriculture systems in Bangladesh: Potential for sustainable livelihoods and nutritional security of the rural poor

Nesar Ahmed[1], Md Abdul Wahab[1] and Shakuntala Haraksingh Thilsted[2]

1. Department of Fisheries Management, Bangladesh Agricultural University, Mymensing 2202, Bangladesh; 2. Department of Human Nutrition, Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark.

Bangladesh is considered one of the most suitable countries in the world for small-scale freshwater rural aquaculture, because of its favorable resources and agro-climatic conditions. In Bangladesh, total fish production was estimated at 2.1 million tons in 2005 of which 44% from freshwater aquaculture, 34% from inland capture fisheries, and 22% from marine fisheries (DOF, 2006). Over the last two decades, there has been a steady increase in inland freshwater aquaculture. Around 400,000 ha of freshwater ponds and ditches and more than 900,000 households are involved in aquaculture (ADB, 2005). Conditions are highly favorable for the rapid expansion of aquaculture as the quantity of seed produced has risen rapidly in recent years (Muir, 2003). The main production systems for freshwater aquaculture in Bangladesh are extensive and semi-intensive pond polyculture of carps. However, a current focus is on promoting culture of giant freshwater prawn (*Macrobrachium rosenbergii*) farming due to its export potential. In addition, farmers are practicing a wide range of integrated aquaculture agriculture (IAA) systems for increased income and employment opportunities, with the added benefits of increasing food production and providing more sustainable livelihoods. This study examined IAA systems in rural Bangladesh in order to broadly understand the production systems, productivity, economic returns and social benefits thereof.

An integrated agriculture-aquaculture farm.

Harvesting of prawn and fish.

Definitions of IAA systems

IAA can be defined within the general definition of integrated farming on the basis of diversification of aquaculture-agriculture towards linkages between subsystems (Edwards et al., 1988). According to Prein (2002), IAA is defined as concurrent or sequential linkages between two or more human activity systems. Linkages between aquaculture and human activities involve not only agriculture but also include roles in sanitation, nutrient recovery and energy recovery (Edwards, 1998). Taking a wider perspective, integration has been viewed as part of 'Integrated Resources Management' (Lightfoot et al., 1993; Pullin, 1998).

Methodology

Study area

The primary area for the study was Gouripur upazila (sub-district) under Mymensingh district in north-central Bangladesh. Mymensingh district is divided into 12 upazilas. Among them only Gouripur upazila was selected for this study since it has been become an important area for freshwater prawn farming (*M. rosenbergii*) because of the availability of hatchery produced post-larvae, favorable conditions such as the availability of low lying agricultural land, warm climate, fertile soil, and cheap and abundant labor. A typical village within this upazila, Dahukigram, was selected as a study site due to its dynamic pattern of IAA systems which have been undergoing change with the adoption of new technologies developed by the Bangladesh Agricultural University (BAU).

Data collection methods

A combination of participatory, qualitative and quantitative methods was used for data collection. Data were collected for six months from June to November 2006. The participatory rural appraisal (PRA) tool focus group discussion was used with farmers and associated groups (i.e. women, children, day laborers, fish traders, fry traders, etc). Focus group discussions were used to get an overview of particular issues such as existing practices of IAA systems, socio-economic condition of farmers and livelihood situation. A total of six focus group discussion sessions were conducted where each group had from 6 to 12 persons (total 52) and duration was approximately two hours. For questionnaire interviews, a total of 25 farmers were surveyed. Farmers were interviewed at their houses and/or farm sites. The interviews focused on farming systems, production technology, productivity, constraints, production costs and returns, socio-economic benefits, etc. Data from questionnaire interviews were coded and entered into a database system using Microsoft Excel software to analyze, producing descriptive statistics.

Farmers with their harvest.

Marketing.

Mola - a highly nutritional fish mainly for household consumption.

Production systems

Marketing of fish.

Prawn-fish farming

The livelihoods of a considerable number of small and marginal farmers are associated with integrated prawn farming in the study area. Integrated prawn farming first started in this area in 2004 through the RUF project (a DANIDA-funded project) after which it spread throughout other villages. A few local farmers first converted their lands and/or ponds into prawn farms. The primary reason for prawn farming was higher economic returns. Although prawn farming started in recent years in the study area, respondents had an average of 7.5 years of experience in carp polyculture.

The size of farm may play an important role as it may reflect the availability of capital, managerial ability, and the potential to operate and use resources efficiently. The average farm size including dikes in the study area was found to be 0.08 ha (20 decimals, or 40 m^2 make one decimal), ranging from 0.06 to 0.11 ha. Most farms were owned by the farmers themselves. According to the survey, 92% of farmers owned their own farm, the remainder (8%) being leased. According to lease farmers, annual leasing rates were on average US$ 176 per ha per year (US$ 1 = Tk 70 in November 2006) usually contracted for 1 to 3 years. Lease arrangements are usually made without any documentation, but sometimes require either a written description of terms or the signing or thumb-printing on a blank sheet of revenue stamp paper.

The concept of integrated prawn farming makes it possible to incorporate a wide variety of crops together with fish, rice and dike cropping. The peak season of integrated prawn farming is from April to November, a culture period of around 6-8 months. Prawn fry are stocked when they become available in April/May and are harvested primarily from October to November. Although farmers can produce two crops annually, they are not doing so due to lack of money and inadequate technical knowledge. Farmers were observed to prefer juveniles to post-larvae for prawn stocking, due to their availability in local markets and high survival rate. The average annual stocking density was found to be 19,760 per ha (80 per m^2).

Cucumber farming on pond dikes.

Almost all farmers produced carps with the prawns. Farmers stocked Indian major carps such as catla (*Catla catla*) and rohu (*Labeo rohita*). However, they generally grow very small quantities. The average stocking density was found at 2,470 per ha (10 per m^2), at a ratio of 7:3 of catla and rohu. According to Mires (1991), integrated prawn and fish culture is suitable where prawn and fish do not compete for feed resources. Durairaj and Umamaheswari (1991) noted that the growth and yields of prawn in integrated farming systems did not appear to be influenced by fish in India, and therefore prawn-cum-fish culture has been considered both technically and economically viable. Hulata et al. (1990) obtained good results when prawn and carp were cultured in an integrated system. According to Shang and Tisdell (1997), polyculture of prawn and fish are usually ecologically sound. However, catla and rohu with prawn instead of 6-7 species polyculture is a new approach to this integration that farmers found both economically and ecologically best practice.

It is a further new approach that all respondents have started stocking of a nutrient dense small indigenous species (SIS) of mola (*Amblypharyngodon mola*), which has a good reputation for its higher vitamin A content as an additional component with prawn and carp culture. The success of this new culture approach rests upon the partial harvesting of mola throughout the year.

A farmer tends his dike crops.

This supports the nutritional requirement of the farmer's households as well as reduces competition with cultured carps for food and space. In the past, mola and all other SIS was regarded as a weed fish and eradicated from the fish ponds using pesticides such as rotenone and phostoxin. However, recently mola is considered to be an important source of essential micro and macro nutrients for the majority disadvantaged population living in both rural and urban areas who can not afford to take nutritional foods in their diet. Mola has thus been considered to play an important role in the elimination of malnutrition, especially keep the children out from night blindness and women from anemia. For integrated prawn farming, mola were stocked from natural sources (e.g. old perennial ponds, floodplains, etc) and the average annual stocking density was found to be 19,760 per ha (80 per m^2).

A variety of feeds were used for IAA systems in the study area. Currently, commercial fish feeds are widely used to increase the productivity of prawn culture. According to the farmers, the production of prawn is higher when commercial feeds are used rather than home made or other feed. The preferred feed for prawns is Saudi-Bangla fish feed, a commercial pelleted feed manufactured in Bangladesh. The feeding schedules of prawns were observed for three feeding periods: i) starter - first 4 weeks at the rate 6% of body weight, ii) grower - second 12 weeks at the rate 4% of body weight and iii) finisher - last 8 weeks at the rate 3% of body weight. The average annual feeding rate for prawns was estimated at 1,093 kg/ha. However, it was found to use a mixture of rice bran and oil cake (2:1) for carps and mola at the rate 3% of body weight. On average annual feeding rate was estimated at 3,948 kg/ha for both carp and mola. In general, feeds are given twice a day in the morning and evening.

In general, farmers used two types of fertilizer such as organic (mainly cow-dung) and inorganic (urea and TSP). The purpose of using fertilizers is to create conditions which would help increase the production of natural feeds (e.g. phytoplankton, zooplankton, and benthos) thereby increasing fish production. Most of the respondents use fertilizers in their farm, mainly cow-dung, urea and TSP at varying frequencies. On average, annual fertilization rates were 750 kg/ha of cow-dung, 105 kg/ha of urea and 64 kg/ha of TSP (Table 1).

Although farmers harvest fish throughout the year, the peak season of fish harvesting is from September to November. Farmers harvest their fish by using cast nets and seine nets. According to the survey, the average annual yield of prawn (head-on), carp and mola were estimated at 552, 1,274 and 157 kg/ha (Table 1). A number of interdependent factors affected growth rate and productivity of fish, including stocking rate, the quality of feed supply, water quality, and other aspects of farm management. The size of fish at stocking, the duration of culture and the size at which the fish are harvested also influence the total yield. The average productivity of prawn has increased in the study area over recent years due to the technical assistance and training offered to the farmers through the RUF project. Ahmed (2001) reported that the average annual yield of prawn in southwest Bangladesh was 432 kg/ha.

Although farmers produce a variety of fish, they mostly consume 70% of mola and 20% of carps, and none of the farmers reported to consume prawn. The market chain from farmers to consumers encompasses mainly primary, secondary and retail markets, involving sales agents, suppliers, wholesalers and retailers. Sometimes local agents buy fish from the farmers at the farm side, carrying them to the local markets. Farmers commonly use vans, rickshaws and buses to transport fish from remote villages to the

A farmer ready to market dike-grown cucumber.

Mr. Abdul Hannan - pioneer of integrated cucumber farming.

markets. Plastic containers, aluminium containers and bamboo baskets are commonly used for keeping fish during transport. Prawn and fish are traded whole, un-gutted, and fresh without processing apart from sorting and icing. The price of fish depends on quality, size and weight, seasonality, supply and demand, market structure, taste, etc. The average farm-gate price of prawn, carp and mola were found at US$ 5.0, 1.14 and 1.71 per kg, respectively.

Rice farming

Most farmers were involved in rice production in their farm and only a few farmers did not cultivate rice, mainly due to its low production or due to unsuitable land for rice cultivation. It was found that farmers cultivated boro rice in the central plateau of the farm during the dry season from December/January to March, and avoided cultivation of a monsoon season rice crop (aman rice: June to September) when the fish are in the farm. Farmers suggested that the aman rice competed with the fish for living space, and placed demands on their limited capital during the fish growing season. In addition, farmers believe that the use of pesticides for rice has a negative effect on prawn growth. However, Roy et al. (1991) noted that in integrated prawn culture in deep water rice fields, rice production was increased by the presence of fish and prawn. Nguyen (1993) stated that integrated prawn culture with rice farming is ecologically sound, and a good method of diversification in Vietnam, where prawns predate on insects and improve soil fertility. These reports suggest that the culture of monsoon season rice crop with prawn is technically possible.

The average rice productivity was estimated at 4,943 kg/ha/crop in the study area. Productivity of rice was estimated including the area of canal and water for fish farming and so actual yields would be higher typically by 20 to 25%. According to the farmers, IAA systems have increased rice productivity by 10 to 15% due to enhanced fertility and environment of rice fields as a result of alternate culture of rice in the dry season and culture of prawn and fish in the monsoon season.

Dike cropping

A remarkable scenario that is uncommon in most areas of Bangladesh is the introduction of dike cropping in the study area. Farmers themselves were trying to introduce this technology with not much success. Thanks are due to Dr Dave Little of the University of Stirling, UK and Dr Marc Verdegem of the Wageningen Agricultural University, the Netherlands and the support from EU funded 'INCO DEV PondLive project' which was implemented by the BAU research team, which is acknowledged for their untiring efforts. Farmers have now standardized the technology of pond dike systems through regular addition of pond mud and fertile pond water for culturing cucumber and other crops (e.g. gourds, beans, leafy vegetables, etc).

Almost all farmers cultivate cucumber in dikes which has been steadily increasing in the study area. Historically cucumber farming first started in 1991 in Dahukigram introduced by Mr. Abdul Hannan 'pioneer of cucumber farming'. He learnt cucumber farming from people on the char land of the Old Brahmaputra River. Abdul Hannan studied their techniques, bought seed from his uncle of that area and began cucumber farming on his pond dikes.

Around 1995, a few well-off local farmers in the study area began to experiment with cucumber farming on their pond dikes. The early innovators experimented with construction design and other technical aspects of cucumber farming, and profited

Expansion of IAA systems - employment opportunity for day laborers.

Fry trading - opportunity for rural poor.

well from their success. Finally a few farmers, some time between the late 1990s and the mid 2000s, developed cucumber cultivation on pond dikes on the long bamboo made and/or doincha tree structures. Since then cucumber farming has become one of the financially most attractive investment opportunities in this area. Cucumber farming has been expanding rapidly in recent years, at an average rate of 20% per annum.

The peak season of cucumber farming is from July to September, a culture period of around three months. Cucumber seed are planted in July and are harvested during August to September. Production costs are mainly associated with seed, fertilizer, labor, bamboo/doincha structure, etc. The average annual yield of cucumber was estimated at 1,470 kg per ha farm. Productivity was estimated, including the area of fish farming and the average area of dikes, of total farm. Farmers noted that the productivity of cucumber on pond dikes is 10-15% higher than on land due to using pond water and pond mud to raise the dikes each year.

There are a number of intermediaries involved in the marketing of cucumber. Farmers never directly communicate with consumers, market communication normally being made through local agents, suppliers, wholesalers and retailers. Local agents buy cucumber from the farmers at the farm side, carrying them to the suppliers and typically earn 1 to 5% of commission for their services. In general, a local agent carries typically 10 to 25 kg/day of cucumber. They commonly use vans and rickshaws to transport cucumbers from remote villages to the assembling centers near the main road side, which take 30 minutes to an hour depending on distance. Then suppliers commonly use trucks to transport cucumbers to the Kawran bazaar of the Bangladesh capital Dhaka which take 4 to 5 hours. Every day two trucks are used to transport cucumber from a single village, carrying an average 22,500 kg/day. The average farm-gate price of cucumber was estimated at US$ 0.17 per kg, whereas consumer paid at US$ 0.29 per kg.

Net making provides income for older people.

Constraints of IAA

A number of constraints were reported for IAA systems, including water pollution, poor water quality, flood, excessive rainfall, diseases, theft, poisoning, inadequate supply of prawn fry, marketing problems and high production costs. Farmers were requested to state their single most important constraint. According to the questionnaire interviews, 62% of respondents identified high production costs as well as lack of capital was their single most important constraint for IAA systems. It seems that investment in IAA systems is quite large. Production costs were reported to have increased significantly in recent years as a result of increased costs of prawn seed and feed, and increased labor wage rates. The main problem for farmers is the shortage of operating capital. The months from June to September are when fish feeding demands what seems to be an unlimited amount of daily expenditure to buy feeds. Inadequate and costly finance can therefore be considered a significant constraint. The proportion of respondents identifying inadequate supply of prawn fry was 22%. Only 12% and 4% of farmers reported lack of proper technical knowledge and diseases of prawns to be the most important constraint, respectively.

Production costs and returns

Table 2 shows that total costs of IAA averaged US$ 3,226 per ha per year, with variable costs and fixed costs estimated at US$ 2,785 and US$ 441, respectively. Variable costs and fixed costs averaged 86.33% and 13.67% of total costs, respectively. Variable costs include feed, seed, fertilizer, labor, harvesting and marketing, rice cultivation, dike cropping and miscellaneous. Fixed costs include depreciation (e.g. net, gear, equipment, etc), land use cost and interest on operating capital. Land use cost was anticipated by using the valuation of land at its rental or lease price. Interest on operating capital was calculated by taking into account the amount spent in cash for IAA farming. The amount of money needed to meet the expense of inputs such as seed, feed, fertilizer, labor, etc are treated as operating capital in this study. Interest on operating capital was charged at the rate of 15% per annum and was estimated for the time during the culture period.

Gross revenue is the pecuniary value of total production. Gross revenue was calculated by multiplying the total amount of production (sold and consumed) by their respective market prices (US$/kg). The average gross revenue from IAA systems was calculated at US$ 5,572 per ha per year. The gross revenue of prawn, carp, mola, rice and cucumber averaged 49.53%, 26.06%, 4.81%, 15.08% and 4.52% of total, respectively (Table 2). The average annual net return per ha of farm was estimated at US$ 2,346. Results showed that IAA systems gave particularly good returns to the farmers.

Table 1. Inputs and outputs of integrated prawn farming.

Items		Mean	SD
Farm size (ha)		0.08	0.04
Stocking rate (No./ha/year)	Prawn	19,760	4,725
	Carp	2,470	872
	Mola	19,760	5,194
Feeding rate (kg/ha/year)	Prawn	1,093	268
	Carp and mola	3,948	953
Fertilization (kg/ha/year)	Cow dung	750	134
	Urea	105	47
	TSP	64	22
Productivity (kg/ha/year)	Prawn	552	118
	Carp	1,274	356
	Mola	157	49

Source: Survey data (2006)

Table 2. Production costs and returns (US$/ha/year) of IAA systems.

Items		Amount (US$/ha/year)	
		Mean	SD
Variable costs (VC)	Seed	1,428 (44.27%)	376
	Feed	954 (29.57%)	189
	Fertilizer	41 (1.27%)	11
	Labor (family and hired)	105 (3.25%)	29
	Harvesting and marketing	57 (1.77%)	17
	Rice cultivation	109 (3.38%)	31
	Dike cropping	59 (1.83%)	19
	Miscellaneous	32 (0.99%)	10
	Sub-total	2,785 (86.33%)	411
Fixed cost (FC)	Depreciation	61 (1.89%)	18
	Land use cost	176 (5.46%)	54
	Interest on operating capital	204 (6.32%)	62
	Sub-total	441 (13.67%)	98
Total costs (TC = VC + FC)		3,226 (100%)	431
Gross revenue (GR)	Prawn	2,760 (49.53%)	526
	Carp	1,452 (26.06%)	321
	Mola	268 (4.81%)	41
	Rice	840 (15.08%)	76
	Cucumber	252 (4.52%)	38
	Total	5,572 (100%)	1,042
Net return (NR = GR – TC)		2,346	482

Parentheses indicate as percent of total. Source: Survey data (2006).

Socio-economic benefits

According to the survey, almost all farmers have improved their social and economic conditions through IAA. The introduction of IAA has increased farmer's income by 65% and infused unprecedented amounts of cash into the local economy. Clearly there have been visible qualitative and quantitative changes in standard of living, food consumption, and level of economic activities. The main benefits from IAA were an increase in economic solvency and improved social status in the community. Food security has increased, more people can afford better medical care, more children go to school, they have better clothes and their housing conditions have improved. Farmers have improved their sanitary and drinking water facilities. There have also been an increased number of bicycles, rickshaws and vans for transport, and mobile phones for communications (i.e. marketing of their products). In addition, income from IAA provides the opportunity for more investment in productive resources (e.g. poultry farming, livestock rearing, petty business, etc) as well as increased security for coping with uncertain situation such as illness and natural disasters (i.e. flood, heavy rain).

Some small farmers have experienced dramatic improvement in their living standards because the land that they have converted into IAA farms was previously not utilized due to its low-lying area. For these farmers IAA systems has obviously brought significant impact. Many farmers noted that flood risk has reduced because of the construction of higher dikes for cucumber farming. Farmers also stated that their families had benefited from selling and eating dike crops. Most small and marginal farmers have expectations of achieving a significantly better life after a few more years of IAA.

Development of IAA has provided employment opportunities for some women, and they are now able to contribute to household income. Women noted that IAA has increased their

Farmers are able to send their children to school.

workload and they would like to do more work due to its high economic return; however, it was not normally possible due to their household work obligations. Women are involved in various facets of IAA, including dike cropping, feeding of fish, fertilization, fish harvesting, farm supervision and management. Women involved in IAA activities were found to be generally more independent and they enjoy a higher social status.

Sustainable livelihoods of the poor

IAA has an important and significant role in providing food and offering income earning opportunities to large number poor people in the study area. The basis of sustainable livelihoods is income generation and food supply. If farmers and associated groups have access to a secure source of income they will escape poverty. Income helps poor farmers to buy food and gain access to social benefits, and allows farmers to withstand shocks and prevent them falling into poverty. A household is food secure when it has the capacity to procure a stable and sustainable basket of adequate food. Scoones (1998) noted that five key indicators are important for assessing the achievement of sustainable livelihoods: 1) poverty reduction, 2) well-being and capabilities, 3) livelihood adaptation, 4) vulnerability and resilience, and 5) natural resource base sustainability.

IAA farming systems have substantially improved the livelihoods of the rural poor. A range of associated groups have benefited from this IAA systems. Improvements in the standard of living which have occurred for farmers, fry traders, fish traders, prawn traders, intermediaries, women, and even day laborers. A network for prawn and fish trading – local agents, suppliers, intermediaries, wholesalers, retailers have all gained. A similar network for cucumber trading derives benefits for marketing people. The opportunities for day laborers to find work have increased significantly. Additional employment opportunities are also generated in the fish hatcheries, fish feed industries, and transport sector from rickshaw pullers to large trucks which carry farm products to local markets.

Conclusions

It is evident that IAA plays an important role in the economy of particular areas of rural communities. There is great potential for increasing family incomes and creating security through engaging in IAA systems. However, there are several primary factors concern for its sustainability which includes high production costs, low supplies of prawn fry, poor institutional support, inadequate extension services and lack of technical assistance. It is therefore necessary to provide technical assistance with institutional and policy support for sustainable IAA systems as well as for sustainable livelihoods to the farmers and associated groups.

Acknowledgement

The study was supported by the DANIDA-funded RUF project. The views expressed in this article are those of the authors and do not necessarily reflect the views of the DANIDA.

A farmer with mobile phone – IAA is improving incomes and social status.

Women's workload has increased due to IAA.

References

ADB. 2005. An Evaluation of Small-scale Freshwater Rural Aquaculture Development for Poverty Reduction. Asian Development Bank (ADB), Manila, Philippines.

Ahmed, N. 2001. Socio-economic Aspects of Freshwater Prawn Farming in Southwest Bangladesh. PhD Thesis, Institute of Aquiaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK.

DOF. 2006. Brief on Department of Fisheries Bangladesh. Department of Fisheries (DOF), Ministry of Fisheries and Livestock, Dhaka.

Durairaj, S. & Umamaheswari, R. 1991. Polyculture of freshwater prawn. In: Proceedings of the National Symposium on New Horizons in Freshwater Aquaculture, Bhubaneswar, Orissa, India, pp. 244-245.

Edwards, P. 1998. A systems approach for the promotion of integrated aquaculture. Aquaculture Economics and Management 2(1):1–12.

Edwards, P., Pullin, R.S.V. & Gartner, J.A. 1988. Research and Education for the Development of Integrated Crop–Livestock–Fish Farming Systems in the Tropics. ICLARM Studies and Reviews 16, ICLARM, Manila, Philippines.

Hulata, G., Karplus, I., Wohlfarth, G.W. & Haleve, A. 1990. Effects of size and age of juvenile freshwater prawns Macrobrachium rosenbergii at stocking on population structure and production in polyculture ponds. Journal of the World Aquaculture Society 21:295-299.

Lightfoot, C., Bimbao, M.P., Dalsgaard, J.P.T. & Pullin, R.S.V. 1993. Aquaculture and sustainability through integrated resources management. Outlook on Agriculture 22(3): 143–150.

Mires, D. 1991. Culture management for freshwater prawns, Macrobrachium rosenbergii in temperate climates, as practised in Israel. In: Proceedings of the Third Brazilian Symposium on Shrimp Culture, Joao Pessoa, Paraiba, Brazil, pp. 11-31.

Muir, J.F. 2003. The Future for Fisheries: Economic Performance. Fisheries Sector Review and Future Development Study, Commissioned with the association of the World Bank, DANIDA, USAID, FAO, DFID with the cooperation of the Bangladesh Ministry of Fisheries and Livestock and the Department of Fisheries, Dhaka.

Nguyen, Q.T. 1993. Rice-freshwater prawn (Macrobrachium rosenbergii) farms in the Mekong Delta, Vietnam. NAGA, ICLARM Quarterly 16(4):18-20.

Prein, M. 2002. Integration of aquaculture into crop-animal systems in Asia. Agricultural Systems 71:127-146.

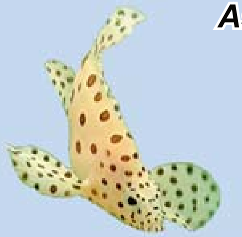
Pullin, R.S.V. 1998. Aquaculture, integrated resources management and the environment. In: Integrated Fish Farming (eds. J.A. Mathias, A.T. Charles & H. Baotong), Proceedings of a Workshop on Integrated Fish Farming, 11–15 October 1994, Wuxi, Jiangsu Province, China. CRC Press, Boca Raton, Florida, USA, pp.19–43.

Roy, B., Das, D.N. & Mukhopadhyay, P.K. 1991. Rice-fish/prawn vegetable integrated farming – viable proposition in deepwater rice ecosystem. In: Proceedings of the National Symposium on New Horizons in Freshwater Aquaculture, Central Institute of Freshwater Aquaculture, Bhubaneswar, Orissa, India, pp. 24-25.

Scoones, I. 1998. Sustainable rural livelihoods: a framework for analysis. IDS Working Paper 72, Institute of Development Studies (IDS), Sussex, UK.

Shang, Y.C & Tisdell C.A. 1997. Economic decision making in sustainable aquaculture development. In: Sustainable Aquaculture (ed. J.E. Bardach), John Wiley and Sons, Inc, pp. 127-148.

Professor Wahab of BAU discussing with farmers about their problems.



Asia-Pacific Marine Finfish Aquaculture Network

Magazine

SKRETTING

Development of a spreadsheet model of the market chain for the live reef food fish trade

Geoffrey Muldoon[1] and Bill Johnston[2]

1. CRC Reef Research Centre. Ph: +61 7 4781 5253. Fax: +61 7 4781 4099. Email: geoffrey.muldoon@jcu.edu.au;
2. Department of Primary Industries and Fisheries. Ph: +61 7 5430 4928. Fax: +61 7 5430 4994.
Email: bill.johnston@dpi.qld.gov.au

The market for live reef fish for food (LRFF) is longstanding in Southeast Asia, with demand for live fish concentrated in Hong Kong and southern China. The total regional trade in LRFF is considered to be around 30,000 tonnes per year with an estimated 15–20,000 tonnes of this going into Hong Kong (Sadovy et al., 2004). More than 20 countries in Southeast Asia and the Pacific supply fish to this market using a variety of capture techniques and transport technologies.

A key component of this project (ADP/2002/105: Economic and market analysis of the Live Reef Food Fish Trade in Asia-Pacific) is aimed at identifying and measuring major cost and revenue components along the marketing chain using a spreadsheet model. This model will be further developed to incorporate key risk factors for the various agents along the chain from the point of capture to the point of sale; and for the case of both sea and air transport technologies.

The live reef food fish trade market chain

Market chain analysis can help to identify constraints (e.g. information flows), inequities (e.g. distribution of value) and practices (e.g. handling, quality control) along the chain that can serve to enhance benefits of trade to agents, especially those upstream agents in source countries. Within the LRFF trade however, addressing these market chain constraints and inequities can be obstructed by relationships between agents along the market chain.

The characteristics of the LRFF trade have resulted in the market chain becoming quite extended and complex. These characteristics include rudimentary storage and transport infrastructure, low technology fishing gear, the remoteness of fishing grounds from supply hubs and the considerable distances of source countries from markets. After being caught by the fisher, LRFF pass through many levels of trade before reaching restaurants in Hong Kong and China. The market chain can be shorter in some countries than in others. In Southeast Asia, the supply side of the market chain includes one or two middlemen whose role is to consolidate catches from independent fishers into sufficient quantities for movement along the chain. In Australia, the middleman role is assumed by fishing firms who employ fishers to catch fish and who sell these fish directly to wholesale exporters. In Australia, LRFF fishers receive a percentage of the 'beach price' paid to the fishing firm by the export wholesaler. The chain is shorter still in the Pacific with fishers being employed directly by exporters who tranship this catch, almost entirely by sea.

As a high value commodity, there is a perceived potential for high economic gains along the chain. The high price of LRFF in Hong Kong has created an impression among suppliers from importing countries that the prices they receive from buyers one step further along the chain is too low (Chan, 2001). In the extended market chain for LRFF however, each agent requires an acceptable margin to continue trading. In practice these gains tend to be unevenly distributed among agents for a variety of reasons including: fishers' lack of knowledge of final values; transport costs incurred by traders when shipping fish across large distances either by sea or air; the high risks of mortality endured by traders during transport; health

Australian Government
Australian Centre for International Agricultural Research

APEC

scares (e.g. ciguatera); and shocks in economic conditions (e.g. Severe Acute Respiratory Syndrome (SARS)). A hypothetical market chain, showing distribution of the final value of LRFF amongst the various agents along the chain for wild-caught LRFF, is shown in Figure 1.

While horizontal cooperation at various stages along the chain does occur, vertical cooperation, or integration, is more likely in fishery chains as a result of:

- the perishability of the product.
- variations in product quantity and quality.
- consumer awareness of product quality.
- economies of scale.

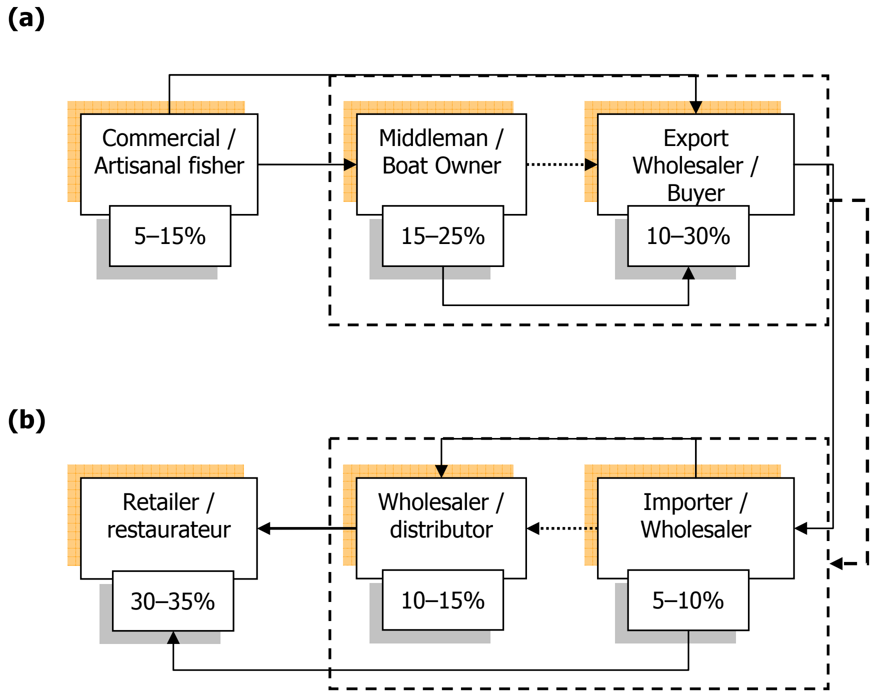
Within the LRFF trade, opportunities for vertical cooperation exist in both the wild-caught and aquaculture sectors. Vertical integration along the market chain for wild-caught LRFF usually occurs at the collection/export stage of the source country supply chain and the import/distribution stage of the import country supply chain (Figure 1). These vertical relationships will tend to obfuscate efforts to identify the distribution of final product value along the market chain. The individual agents will tend not to set prices or margins in line with their respective business operations; margins will be centrally determined for each of these 'profit centres'.

In terms of LRFF aquaculture, there has tended to be an increased dis-aggregation at the upstream end of the market chain with increased specialisation in the production process. Hatchery, nursery and grow-out phases of LRFF aquaculture tend to be distinct components in the production stage. Vertical coordination between successive stages of the chain will occur where benefits can be demonstrated in terms of flexibility to meet variant demand conditions, access to product quality information, implementation of quality control activities and access to credit (van Anrooy, 2003).

Schematic of the market chain model

The current trade in LRFF is largely unregulated resulting in over-exploitation of fish stocks. It has been argued that this lack of regulation has meant

Figure 1: Economic value chain model for wild-caught live reef food fish for: a) the supply or export; and b) the demand or import sectors of the market chain. Percentage is an estimate of the final consumption value extracted at that link of the chain. The dashed boxes at export and import stages along the chain and the dashed line between these stages indicate vertical linkages between market chain agents.



small scale fishers are not receiving fair economic returns. The rationale for this has been that downstream agents bear the trade risks (i.e. fish mortality, exchange rate fluctuations and high transport costs) and these costs are passed back along the chain, leading to the relatively low prices paid to fishers.

The key objective of this project component is to measure cost and risk components of the market chain to enable options for risk reduction, improved price transparency and improved returns for small scale fishers to be examined. There are two approaches to deriving what constitutes a fair economic return:

i) A bottom-up approach of determining the costs of catching fish to derive a 'fair' beach price that captures this cost plus a suitable margin.
ii) A top-down approach based on the equitable distribution of the final product value (i.e. retail price) between agents based on risks and costs (e.g. transport, holding etc.).

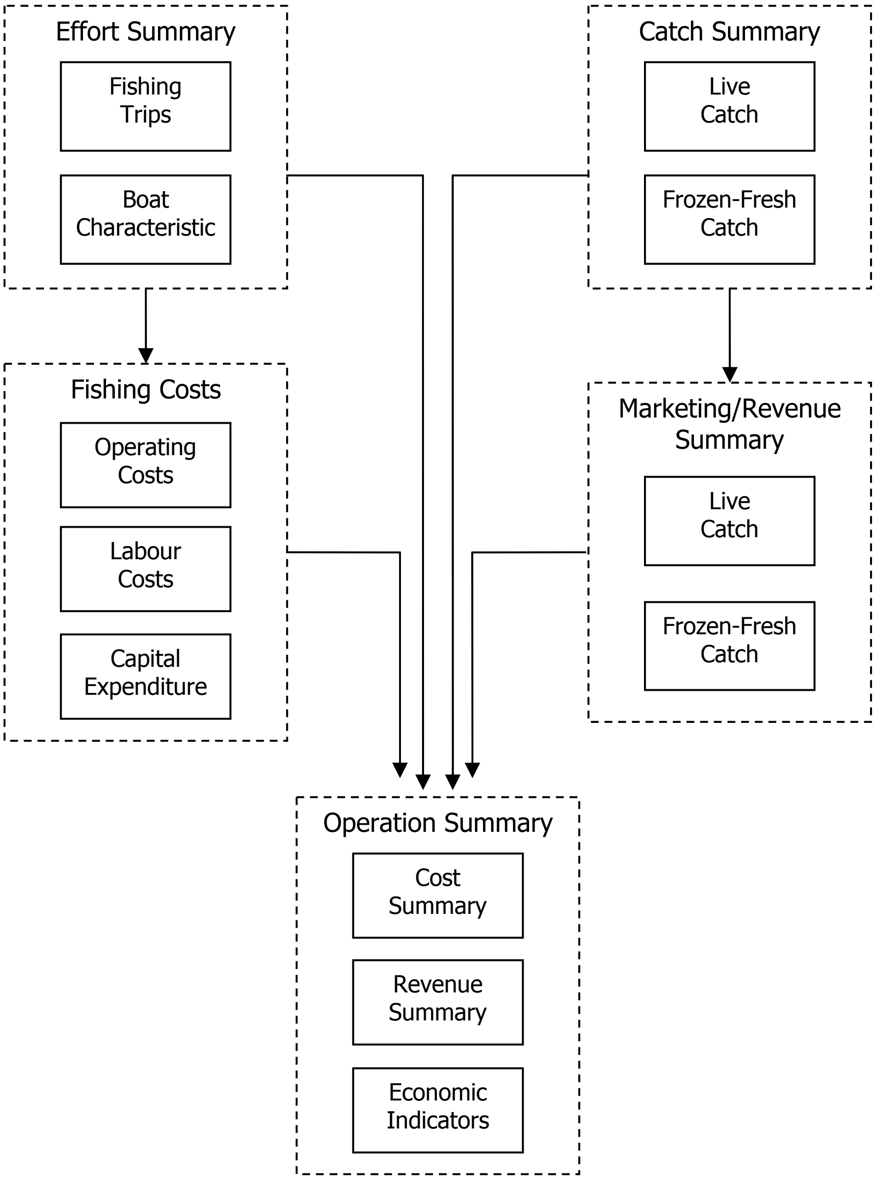
Developing a bottom-up market chain model for the LRFF fishery is problematic for two related reasons. Firstly, there is a paucity of usable data, with the exception of fishing and export operations in Australia, that would enable a market chain model to be fully populated. Secondly, vertical integration between agents hampers the development of discrete sub-models for specific agents. The LRFF market chain is slightly more complex than traditional food industry value chains through the inclusion of middlemen (Sadovy et al., 2004). Traditional food industry chains consist of: producer, wholesaler, exporter, importer and retailer.

The initial spreadsheet models developed have used a hybrid top down approach. The model suite consists of two sub-models: one for fishers and fishing operations or middlemen (Figure 2); and the other for remaining market chain agents consisting of exporters, importers, distributors and retailers (Figure 3).

The fisher/middleman or fisher/fishing vessel sub-model

The fisher sub-model allows for costs to be derived using effort parameters and total revenues to be derived using catch parameters. Revenues can be based on either empirical beach price data or by using a margin-based approach, again

Figure 2: Schematic of fisher sub-model showing relationship between fishing costs and fishing effort and between fishing revenues and fish catch. Costs and revenues generate economic performance indicators.



using empirical evidence. Beach prices can also be used to derive margins based on costs. Total cost and revenue information are subsequently used to develop indicators of economic returns including: net present value; annualised returns; internal rates of return and rates of return on capital (Figure 2).

The supply chain sub-model

In recognition of the lack of data available, a simplified model has been developed to schematically represent the supply chain (Figure 3). The current model incorporates wholesaler/exporter, importer/distributor and retailer margins based on empirical evidence. The model allows for these margins to be adjusted to explore the impacts of different margins on returns to agents and also to aid in examining the issue of 'fair price'. The option to validate these margins based on key cost parameter will also be made available. Exchange rate movement risks have been accounted for using an expected value probability model (Figure 4).

Distribution of value and risk

The distribution of value of marine products has been recognised as an issue of concern in many developing country export fisheries, in terms of the percentage of final value accruing to agents along the chain and the under-pricing of resources (Jacinto, 2004). Even so, margins and value need to be considered in the context of risk borne by the respective agents along the market chain.

In the case of wild-caught fisheries, it has been suggested that fishers are usually poorly paid based on the final value of seafood products (Wood, 2001). Several factors give explanation for those receiving a relatively smaller percentage of final value. The remoteness of fishing grounds and small individual catches requires a middleman who can consolidate catches into sufficient quantities for transfer to exporters. Often these middlemen provide credit to fishers in the form of gear etc. to facilitate their fishing activities, although credit arrangements are usually not 'mutually beneficial'.

For export fisheries, financial risks increase as the product moves along the market chain. The middlemen and exporters bear mortality risks and the costs of holding fish post-harvest. The costs of transportation to markets are borne by middlemen, wholesalers and exporters and/or importers. Shipping and freight costs can make up between 50–65% of landed price paid by importer. At the consumption end of the chain retailers incur considerable rent and wage costs (MacFadyen et al., 2003). The greater downstream risks of financial losses from mortality, prior to the product reaching consumer markets, partly explains the inequitable distribution of value.

Within the spreadsheet model, risk has been incorporated both for the fisher/fishing vessel and the supply chain sub-models. Within the fisher/fishing vessel sub-model, two types of risk have been accounted for: fish catches and fish prices. The first can account for increases or decreases in catches as a result of policy (management regulations) or environmental (overfishing) factors. The second recognises changes in demand that influence prices. Within the supply chain model, risk is associated with mortality, exchange rate fluctuations and downstream price expectations.

For each of the various risk components, an expected probability approach is used to calculate an expected value under a range of anticipated outcomes.

These expected values are used in turn to generate a cumulative probability distribution (Figure 4b). In the case of the fishing operation, risk analysis models have been incorporated for price and catch. These are reflected in the annual returns to the vessel (Figure 3). For exporters and importers, it is intended that risk analysis will be incorporated in the form of estimating expected survival rates for a consignment of live fish. The cumulative probability distribution will likely be expressed in terms of both volume (quantity) and value of a consignment and also as an annual return based on the number of monthly or annual consignments.

Conclusions

Despite the widespread use of market chain analysis as a means of identifying cost and revenue flows and value distribution for agents along the chain, idiosyncrasies of the LRFF trade constrain development of market chain models for use in identifying inefficiencies and distortions along the chain, in particular, inequities in value distribution.

Taking these limitations into account, this paper has outlined the initial development of an Excel spreadsheet that will be used to examine costs and revenues, and risks in the chain from the point of capture to the point of sale of LRFF.

Data limitations have dictated the form of the spreadsheet modelling approach, but in this first iteration, there are two models: for fishers and fishing vessel/middlemen; and for other agents in the chain. Adopting this approach is intended to overcome, to a degree, the lack of existing data on the operations of these downstream agents and also the need to collect the data required to populate the spreadsheet; data which will prove difficult to procure. There are a number of next steps planned for this research. These will be to modify and expand on these initial spreadsheet models to:

- include and validate where possible, the economics and risk for both sea and air transport.
- investigate trends in air transport that could lower mortality risks and assist more remote supplying countries (e.g. Pacific) to participate in the market for LRFF.
- explore the impacts of policies that can improve market performance and distribution of product values.. (Market structures in developing fisheries with complex market chains tend to be fixed so that reducing links in the market chain to the benefit of small-scale fishers will be difficult. Governance and distributional outcomes are often skewed toward wholesalers and exporters leading to marginalisation of small-scale fishers. Opportunities for horizontal cooperation are greater in aquaculture, where supply is more able to meet variant demand and farming cooperatives, have more control over their production and supply activities).
- incorporate results from demand and supply modelling on the impacts of increased aquaculture production; most likely on beach prices for specific species or species groups.

Lastly, it is anticipated that, if there is sufficient data, spreadsheet models will be constructed not only for Australia but for at least two Southeast Asian countries and two Pacific countries. These models will aid capture fishery managers and the aquaculture sector in assessing future viability of the live reef capture and aquaculture fisheries within their countries.

References

Chan P.S.W. 2001. Wholesale and retail marketing aspects of the Hong Kong live seafood business. In B. Paust and A. Rice (eds). Proceedings of the second international confer-

Figure 3: Schematic of the supply chain sub-model showing relationships and information flows between market chain agents and value distribution.

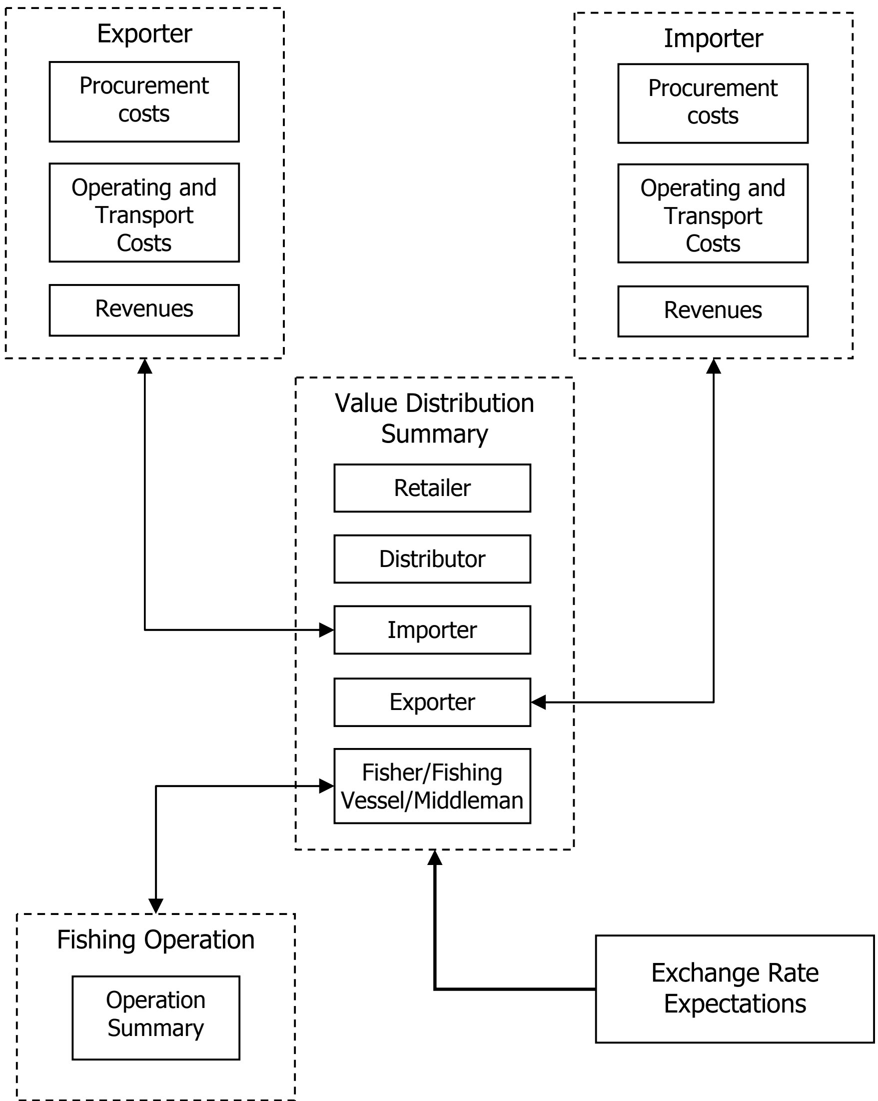
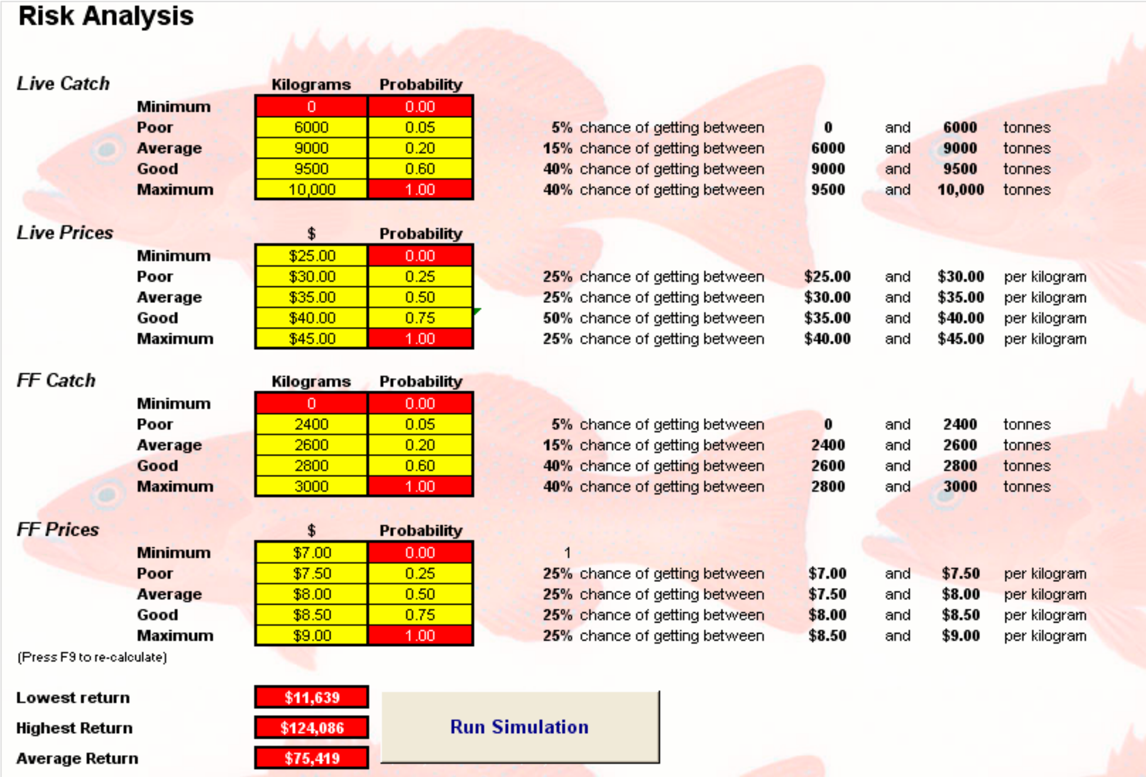


Figure 4: Risk analysis for fishing vessels which: (a) uses expected probabilities of a range of catch and fish price scenarios to estimate lowest, highest and average annual returns; and (b) generates a cumulative probability distribution of expected annual returns.

(a)



Risk Analysis

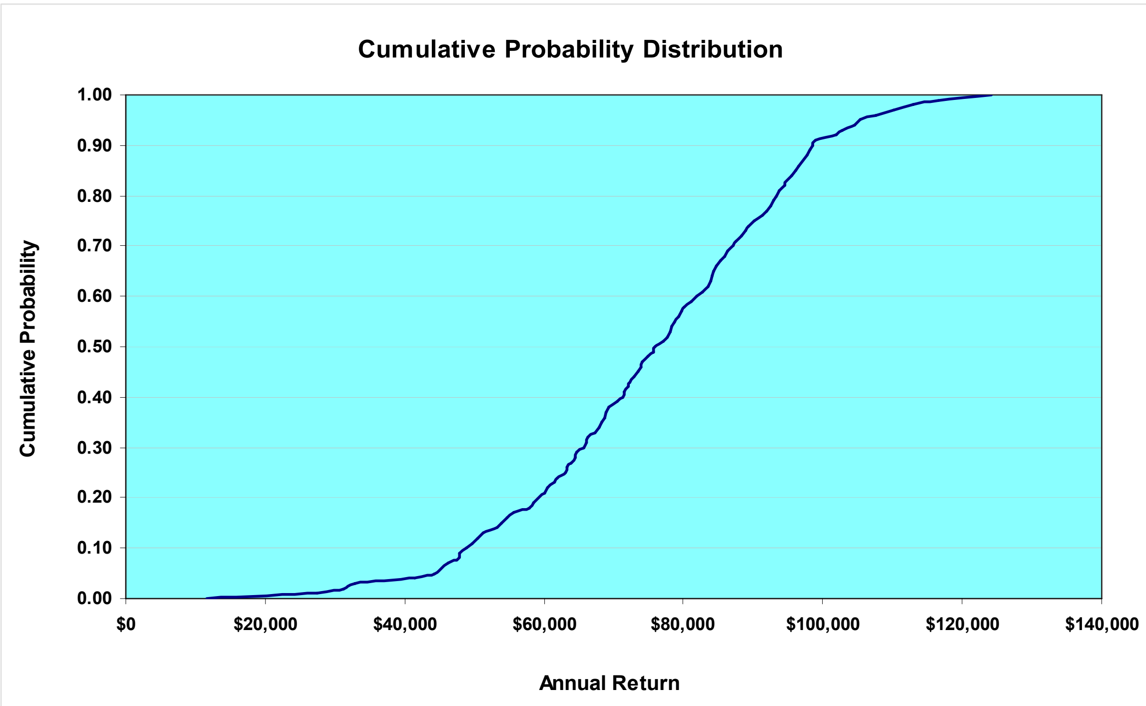
Live Catch		Kilograms	Probability						
	Minimum	0	0.00						
	Poor	6000	0.05	5%	chance of getting between	0	and	6000	tonnes
	Average	9000	0.20	15%	chance of getting between	6000	and	9000	tonnes
	Good	9500	0.60	40%	chance of getting between	9000	and	9500	tonnes
	Maximum	10,000	1.00	40%	chance of getting between	9500	and	10,000	tonnes
Live Prices		$	Probability						
	Minimum	$25.00	0.00						
	Poor	$30.00	0.25	25%	chance of getting between	$25.00	and	$30.00	per kilogram
	Average	$35.00	0.50	25%	chance of getting between	$30.00	and	$35.00	per kilogram
	Good	$40.00	0.75	50%	chance of getting between	$35.00	and	$40.00	per kilogram
	Maximum	$45.00	1.00	25%	chance of getting between	$40.00	and	$45.00	per kilogram
FF Catch		Kilograms	Probability						
	Minimum	0	0.00						
	Poor	2400	0.05	5%	chance of getting between	0	and	2400	tonnes
	Average	2600	0.20	15%	chance of getting between	2400	and	2600	tonnes
	Good	2800	0.60	40%	chance of getting between	2600	and	2800	tonnes
	Maximum	3000	1.00	40%	chance of getting between	2800	and	3000	tonnes
FF Prices		$	Probability						
	Minimum	$7.00	0.00	1					
	Poor	$7.50	0.25	25%	chance of getting between	$7.00	and	$7.50	per kilogram
	Average	$8.00	0.50	25%	chance of getting between	$7.50	and	$8.00	per kilogram
	Good	$8.50	0.75	25%	chance of getting between	$8.00	and	$8.50	per kilogram
	Maximum	$9.00	1.00	25%	chance of getting between	$8.50	and	$9.00	per kilogram

(Press F9 to re-calculate)

Lowest return	$11,639
Highest Return	$124,086
Average Return	$75,419

Run Simulation

(b)



ence: Marketing and shipping of live aquatic products. November 1999, Seattle. University of Alaska Sea Grant (AK-SG-01-03) p 201–205).

Jacinto E.R. 2004. A research framework on value chain analysis in small scale fisheries. Paper presented to the 10th Biennial Conference of the International Association for Study of Common Property, Oaxaca, México, 9-13 August 2004. 27p

MacFadyen G., Banks R, Banks R., Phillipps M., Haylor G., Mazaudier L. and Salz P. 2003. Background paper on the international seafood trade and poverty. Prepared under the EC-PREP project (EP/R03/014), 'International Seafood Trade: Supporting Sustainable Livelihoods among Poor Aquatic Resource Users in Asia'. Poseidon Aquatic Resource Management Ltd (UK), Network of Aquaculture Centres in Asia-Pacific and STREAM Initiative.

Sadovy Y.J. and Vincent A.C.J. 2002. Ecological issues and the trades in live reef fishes. In: P.F. Sale (ed.) Coral reef fishes. Dynamics and diversity in a complex ecosystem. Academic Press, San Diego, p391–420.

Sadovy Y.J., Donaldson T.J., Graham T.R., McGilvray F., Muldoon G.J., Phillipps M.J., Rimmer M.A., Smith A. and Yeeting B. 2004. While stocks last: the live reef food fish trade. ABD Pacific Studies Series. Asian Development Bank, Manila.

van Anrooy R. 2003. Vertical cooperation and marketing efficiency in the aquaculture products marketing chain: A national perspective from Vietnam. Paper presented to the Aquamarkets 2003 Conference. Manila, Philippines 2–4 June 2003.

Wood E.M., 2001. Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society, UK. 80pp.

The Report of the 4th Regional Grouper Hatchery Production Training Course 2006

The 4th Regional Grouper Hatchery Training Course was another success. A total of 20 participants from 13 countries attended the training course which was hosted by the Brackishwater Aquaculture Development Centre (BADC)-Situbondo, Indonesia. The participants came from Australia, Hong Kong, Indonesia, India, Malaysia, Maldives, Myanmar, Philippines, Qatar, Saudi Arabia, Singapore, Thailand and Vietnam. The full report of the training course, which contains a lot of photographs of the activities, can be downloaded from the NACA web site.

Overall the training course met participants' expectations. Five considered the training course was excellent and 12 said it was well organized. Sixteen participants considered the lectures organized cover all aspects of grouper hatchery production. All participants believed they have increased their knowledge and practical experience on grouper hatchery production after the course. They also agreed that they have received sufficient level of technical support throughout the training course, in spite of some language differences.

All participants were able to gather contacts for their future aquaculture activities after they returned home. The training course and the field trips provided an opportunity for the participants to obtain future contacts for supplies and marketing. Field trips were well organized based on the feedback provided by participants.

The 5th Regional Grouper Hatchery Training Course: June 11-July 1, 2007

Building on the success of the 2006 course, NACA is pleased to announce the 5th Regional Grouper Hatchery Production Training Course for the Asia-Pacific Marine Finfish Aquaculture Network is currently planned for 2007 and tentative schedule for the training course is June 11-July 1, 2007.

The course structure will be similar to the previous year. For further information please download the report of the 2006 course from the NACA website. If you wish to register for the training course please contact Mr Sih Yang Sim at grouper@enaca.org. More information will be posted on the web site in mid-February 2007.

Immunological and nucleic acid based methods for detection of food borne pathogens

P. R. Sahoo[1] and S. Tanuja[2]

1. Senior Research Fellow, Central Institute of Freshwater Aquaculture, Bhubaneswar, India; 2. PhD Scholar, Central Institute of Fisheries Technology, Cochin, India.

The analysis of foods for the presence of both pathogenic and spoilage bacteria is a standard practice for ensuring food safety and quality. Conventional methods require several days to give results because they rely on the ability of microorganisms to multiply to visible colonies. Moreover, culture medium preparation, inoculation of plates, colony counting and biochemical characterization make these methods labour intensive. Another disadvantage of traditional methods is that cells which are viable but otherwise nonculturable cannot be detected. In the food industry there is a need for more rapid methods to provide adequate information on the possible presence of pathogens in raw materials and finished food products, for manufacturing process control and for the monitoring of cleaning and hygiene practices (Enn and Reiikelt, 1999). Immunological and nucleic acid based detection methods offer alternative approaches that can meet these needs.

Immunological methods

Immunological methods rely on the specific binding of an antibody to an antigen. Both polyclonal and monoclonal antibodies are used in this method. However, one of the disadvantages of using polyclonal antisera in immunoassays is the variability found in the animal's immune response.

Homogenous and heterogenous Immunoassays: Immunoassays can be classified as homogeneous (eg: reverse passive latex agglutination (RPLA) or heterogeneous (eg: enzyme linked immunosorbent assay (ELISA). In a homogeneous assay the antigen–antibody complex formed is directly visible or measurable and there is no need to separate the bound from the unbound antibody. Incubation times are usually very short. In latex agglutination tests, latex beads are coated with antibodies that agglutinate specific antigens and form a more easily visible precipitate. These tests are available for most common pathogens. In a heterogeneous assay, the unbound antibody must be separated from the bound antibody using labeled reagents. ELISAs for pathogens have detection limits ranging from 103 to 105 colony forming units (cfu)/ml. Therefore direct detection of pathogens in foods is not possible and enrichments are required for at least 16–24 hours. Kits for the detection of bacterial toxins are based mainly on immunoassay systems, but the use of these kits does not give any information on the biological activity of the toxins (Brett, 1998).

Immunocapture based methods: These techniques include immunological binding (capture), followed by physical separation of the target organisms from a mixed enrichment culture. One application is immunomagnetic separation (IMS) in which a sample is mixed with beads coated with antibodies for the target organism. The target organisms in the sample bind to the immunomagnetic beads, which are then isolated from other sample material and microorganisms in a magnetic field. The beads are then plated on medium and incubated overnight.

Automated immunoassays: In one type of this assay, an aliquot of boiled enrichment medium is placed into a reagent strip, which is coated with antibodies. The strip contains all the ready-to-use reagents (wash solution, conjugate and substrate) required. The instrument performs all assay steps automatically. Finally, the fluorescence is measured by the optical scanner in the apparatus and analyzed automatically by the computer.

Biosensors: Biosensors consist of a microchip-based system for analyzing the formation of antigen–antibody complexes (Malmqvist, 1993). The sensor chip consists of a glass support, an overlaid gold film and a dextran matrix to which antibodies can be immobilized. The antigen is injected over the chip surface. The antibody–antigen complex changes the refraction index at the chip surface, which can be measured optically (Robison, 1997).

Nucleic acid based assays

Generally, DNA based methods have the advantage over phenotypic identification methods of not being influenced by the environmental conditions of the cells because the nucleotide sequence of the DNA is kept constant during growth. Genetic detection methods are based on the hybridization of target DNA with a specific DNA probe. Common targets for the identification of various pathogenic microorganisms are genes determining the production of toxins (Willshaw et al. 1985, Koch et al. 1993). Genes for specific enzymes, for example; thermonuclease (Liebl et al. 1987), b-galactosidase (Bej et al. 1990a), b-glucuronidase (Bej et al. 1991a) etc have also been described. Ribosomal RNA (rRNA) genes are also suitable as targets. These genes are ubiquitously distributed but show differences due to their phylogenetic divergence. A significant advantage of using rRNA as the target nucleic acid is the high copy number (>104/cell). Thus rRNA-based detection can be used for in situ and colony hybridization (Betzl et al. 1990, Salama et al. 1993) alternatively randomly chosen DNA can be used as a target region (Schmidhuber et al. 1988, Fitts et al. 1983, Scholl et al. 1990). Such random DNA-fragments may be a part of a gene of vital importance or a DNA sequence without any essential function.

Nucleic acid hybridization

Nucleic acid hybridization is typically between a DNA or RNA molecule present in the target organism and a probe DNA that has a sequence complementary to the target sequence. Probe DNAs usually contain 15 to 30 nucleotides. The first step in these genetic methods usually is lysis of the cells and often also purification to free the nucleic acid, so that it can hybridize with the DNA probe. Direct hybridization uses a labeled DNA probe to hybridize to nucleic acid within the sample. Radioactive and fluorescent probes allow direct detection of hybrids. Examples of direct hybridization techniques are colony hybridization and the colorimetric DNA hybridization (cDNAH) assay. The most common solid-phase formats are the southern blot and the various dot blot formats in which the target nucleic acids are immobilized on a membrane. After hybridization, positive colonies can be identified with a labeled DNA probe. The colorimetric DNA hybridization assay is based upon a sandwich hybridization assay that employs a three-component system consisting of a solid-phase bound capture probe, the target RNA and a signal generating probe (Curiale and Klatt 1990). Hybridisation kits are available for the detection of a number of microorganisms (e.g. *Salmonella* spp., *L. monocytogenes*, E. coli, S. aureus, Campylobacter spp. and *Y. enterocolitica*). Generally these direct hybridization assays, although specific, lack the necessary sensitivity for many applications. Even when probes are directed at the highly expressed 16S and 23S rRNA, at least 105 to 106 microorganisms / ml are required (Mabilat et al. 1996).

Amplification methods

Sensitivity can be greatly improved through the use of the different in vitro amplification methods. The most popular method of amplification is the polymerase chain reaction (PCR) technique. In this method, first double stranded DNA is denatured into single strands and specific short DNA fragments (primers) are annealed to these DNA strands, followed by extension of the primers complementary to the single stranded DNA with a thermostable DNA polymerase. Starting from a single target DNA or RNA sequence, more than one billion product sequences can routinely be synthesized by PCR. This quantity of DNA can be visualized as a band on an ethidium bromide-stained electrophoresis gel. To increase the sensitivity and more importantly to confirm the identity of the amplification product, Southern blotting and hybridization with a specific probe should follow. Drawbacks of PCR are the inability to distinguish between live and dead cells, the presence of polymerase inhibitors in food samples leading to false negative results, and the accessibility of the target organisms. Pre-enrichment prior to PCR analysis overcomes most of these problems. For the sensitivity of PCR, the matrix of the food sample is decisive (Way et al. 1993), PCR inhibition can be prevented by separating bacteria from the food matrix prior to DNA extraction by differential centrifugation, IMS, dilution and addition of bovine serum albumin or by immunomagnetic separation of the target organism (Grant et al., 1993). PCR detection systems based on multicopy genes (e.g. rDNA sequences, IS elements) are generally more sensitive than those based on single copy genes. The simultaneous amplification of multiple regions of a DNA template templates by adding more than one primer pair to the amplification reaction mixture is a process termed multiplex PCR. Multiplex assays, however, usually do not exceed a total of six primer sets because of limitations in the ability to resolve many fragments in agarose and because of the potential for generating nonspecific products that make interpretation difficult. In the field of infectious diseases, the technique has been shown to be a valuable method for identification of viruses, bacteria, fungi, and/or parasites. But the presence of more than one primer pair in the multiplex PCR increases the chance of obtaining spurious amplification products, primarily because of the formation of primer dimmers. Thus, the optimization of multiplex PCR should be done to minimize or reduce such nonspecific interactions (Elnifro etal, 2000)

Real Time PCR: While conventional PCR-based methods are highly sensitive and specific, they require post-PCR detection procedures, such as gel electrophoresis. Also the results based on size discrimination may not be very accurate. Real time PCR eliminates the need for post-PCR processing by measuring the accumulation of PCR amplicons during each cycle of PCR in real time, thus decreasing analytical time and labor. In addition, because fluorescence increases in direct proportion to the amount specific amplicons, real time PCR can be used for quantitation (Campbell and Anita C Wright, 2003). Since fluorogenic probes target gene-specific sequences internal to the primer sites, real time PCR imparts an added degree of specificity compared to conventional PCR-based methods. Real time PCR has shown to be useful in detection of pathogenic food borne bacteria.

Reverse transcriptase polymerase chain reaction (RT-PCR): is a laboratory technique for amplifying a defined piece of a ribonucleic acid (RNA) molecule. The RNA strand is first reverse transcribed into its DNA complement or complementary DNA, followed by amplification of the resulting DNA using polymerase chain reaction. In the first step of RT-PCR complementary DNA is made from a messenger RNA template using dNTPs and an RNA-dependent DNA polymerase, reverse transcriptase, through the process of reverse transcription. The above components are combined with a DNA primer in a reverse transcriptase buffer for an hour at 37°C. After the reverse transcriptase reaction is complete, and complementary DNA has been generated from the original single-stranded mRNA, standard polymerase chain reaction is initiated. A thermostable DNA polymerase and the upstream and downstream DNA primers are added. The reaction is heated to temperatures above 37°C to facilitate sequence specific binding of DNA primers to the cDNA. Further heating allows the thermostable DNA polymerase ('transcriptase') to make double-stranded DNA from the primer bound cDNA. The reaction is heated to approximately 95°C to separate the two DNA strands. The reaction is cooled enabling the primers to bind again and the cycle repeats. After approximately 30 cycles, millions of copies of the sequence of interest are generated. The original RNA template is degraded by RNase H, leaving pure cDNA (plus spare primers).

Use of reverse transcriptase PCR (RT-PCR) in detection of viable but nonculturable bacterial cells (VBNC): The viable but nonculturable (VBNC) state is a recently described survival mechanism of bacteria facing environmental stress conditions (Oliver, 2005). The VBNC state has been described for numerous gram negative bacteria, including bacteria of medical interest such as vibrios, Shigella dysenteriae, *Campylobacter jejuni*, *Helicobacter pylori*, and *Escherichia coli* O157:H7. When in this state, bacteria are no longer able to grow and form colonies on conventional culture media but demonstrate metabolic activity maintain their pathogenicity and, in some cases, may return to active growth when optimal conditions are restored. In this state, genetic expression may be modified, and it has been reported that DNA, RNA, and protein synthesis, as well as the concentration of ribosomal and nucleic acids, decreased drastically in VBNC cells. Even though DNA based techniques can detect VBNC cells, a major disadvantage of these DNA-based detection methods is that they also may amplify DNA from dead microorganisms. mRNA is a short-lived molecule (half life of few minutes) due to the presence of nucleases that digest it very rapidly . The presence of mRNA can be regarded as a valid and convincing criterion for assessing cell viability. mRNA detection by RT-PCR is one of the most appropriate methods for evaluating presence of VBNC bacteria. This has been successfully tried in the pathogens *Legionella pneumophila*, *Vibrio cholerae*, *V. vulnificus*, *Mycobacterium leprae*, *Listeria monocytogenes* and others. Yaron and Mathews (2002) have targeted the mRNA that codes for tdh1 and tdh2 genes of *Vibrio paraheamolyticus* to not only detect VBNC cells but also to allow estimation of the potential virulence of the bacteria. The retention of virulence by VBNC cells has been demonstrated in several studies (Patel et al. 1991). However, due to the short half-life of prokaryotic mRNA, it is difficult to obtain intact RNA (Belasco and Higgins 1988), and rapid lysis of the microorganisms needed for its extraction (Patel et al. 1991). Because of the much higher stability of ribosomal RNA (rRNA), the difficulties involved with handling mRNA may be circumvented by using rRNA as a target. The supposition that rRNA can be used to determine viability is supported by the observation that cell degradation is accompanied by ribosome disappearance (Silva et al. 1987).

Nucleic Acid Sequence Based Amplification (NASBA): An alternative to PCR is the isothermal amplification system NASBA. NASBA is specifically designed to detect RNA and employs three enzymes: a reverse transcriptase, RNaseH and T7 RNA polymerase, which act in concert to amplify sequences from an original single-stranded RNA template. Oligonucleotide primers, complementary to sequences in the target RNA, are incorporated in the reaction. One primer also contains a recognition sequence for T7 RNA polymerase. The reaction contains both dNTPs and NTPs. The first primer binds to the RNA, allowing the reverse transcriptase to form a complementary DNA strand. Then the RNase digests away the RNA and the second primer binds to the cDNA, allowing the reverse transcriptase to form a double-stranded cDNA copy of the original sequence. This double-stranded DNA then acts as a kind of mini gene, which is transcribed by the T7 RNA polymerase to produce thousands of RNA transcripts, which then cycle through the reaction. The reaction is performed at a single temperature, normally 41°C. At this temperature, the genomic DNA from the target microorganism remains double-stranded and does not become a substrate for amplification. This eliminates the necessity for DNase treatment, which is required when using RT-PCR for RNA detection (Uyttendaele, 1997) and it also offers specific detection of viable cells. The product of NASBA reaction is mainly single-stranded RNA. This may be detected on by gel electrophoresis followed ethidium bromide staining. A confirmatory step of probe hybridization can also be involved. The detection of messenger RNA has been proposed as an indicator of cell viability (Coutard etal, 2005) as defined by capability of cell division, metabolism or gene transcription. Messenger RNA can have a short half-life within viable cells, and is rapidly degraded by specific enzymes (RNases), which are themselves very stable even in environments outside the cell itself showed that NASBA can selectively amplify mRNA sequences in a background of genomic DNA, which indicated that NASBA amplification of mRNA could be used to specifically detect viable cells.

Molecular subtyping methods

A limitation in the use of nucleic acid based assays is that they indicate only the genetic potential to produce toxin or to express virulence and do not give any information on toxins already present in foods or expressed virulence. They also do not lead to the isolation of the organism and so no further characterization can be done. On the other hand, molecular based techniques may be more reliable for the detection of viable but nonculturable bacterial cells. DNA fingerprinting methods like restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) analysis are suitable for grouping of isolates in transmission studies or outbreak investigations. In the RFLP technique, DNA is cleaved by restriction enzymes and the resulting fragments are separated by gel electrophoresis. Different binding patterns (polymorphism) may be observed after transferring the DNA from the gel to hybridization filters by blotting and hybridization with labeled probes. After visualization of the label, a typical banding pattern can be observed. DNA probes used in RFLP analysis are often based on highly conserved genes coding for rRNA (in ribotyping) (Rodrigues ct al., 1991: Rodtong and Tannock. 1993). RAPD is a technique based on PCR. In the RAPD assay patterns are generated by the amplification of random DNA segments with single small e.g. 10-base primers of arbitrary nucleotide sequence, and a subsequent gel electrophoresis of the amplified DNA (Williams et al., 1990). Repeated cycles of heating and cooling, generally 45 cycles per RAPD assay, lead to an exponential synthesis, and thus many copies of the amplified segments. An amplification of IV-fold copies can be expected (Kocher and Wilson, 1992). A related approach is the amplification of fragments by PCR with oligonucleotides specific for simple repetitive DNA sequences (PCR fingerprinting). Repetitive DNA sequences have been described recently in eubacteria. Based on these sequences oligonucleotides have been designed, matching repetitive extragenic palindromic (REP) elements and enterobacterial repetitive intergenic consensus (ERIC) sequences, which enabled the generation of fingerprints with PCR. Since these repetitive sequences are mainly present in enteric bacteria and some related Gram-negative bacteria, as observed by hybridization experiments with REP and ERIC probes, the applicability of this fingerprinting method is restricted to these organisms (Versalovic et al., 1991). The PCR-fingerprinting method is more robust than the RAPD method since the annealing temperature is higher, 55°C instead of 37°C, which is closer to the optimal temperature (72°C) of the Taq polymerase so the primer is extended before increase in temperature may denature the primer from the template DNA. Pulsed-field gel electrophoresis (PFGE) is considered to have both good reproducibility and sufficient resolving power for the epidemiological typing of bacterial isolates. In this technique, restriction enzymes digest the complete genome and large DNA molecules are resolved by continuous reorientation of the electric field during gel electrophoresis (Tenover et al., 1995) Basically, the continuous reorientation of the electric field causes the DNA molecules to stretch in the direction of the field and hook when the field has changed.

This results in a migration velocity in the net field direction depending primarily on the size of the DNA molecule. Long molecules need more time to reorientate than smaller molecules. Amplified fragment length polymorphism (AFLP) is a recently developed method for genotyping based on selective amplification of restriction fragments of digested total genomic DNA. This technique combines the reliability of RFLP with the advantages of PCR (Vos et al., 1995). DNA is extracted from cells and fragmented by a restriction enzyme. The DNA fragments are separated by electrophoresis and transferred to a membrane. Pattern detection occurs after hybridization with a DNA probe. First, a chemiluminescent label is introduced and the emission of light from the hybridized fragments is captured by a camera. This image pattern can be compared to others in a database and the identification of the organism is based on a pattern match.

DNA microarrays

Microarrays are composed of many discretely located probes on a solid substrate such as glass. Each probe is composed of a sequence that is complimentary to a pathogen-specific gene sequence. Probes are typically deposited on glass surfaces using a contact printing system such as quill pins, solid pins, or ring-and-pins. Targets may be PCR products, genomic DNA, total RNA, rRNA, cDNA, plasmid DNA, or oligonucleotides. In most cases, the targets incorporate either a fluorescent label or some other moiety such as biotin that permits subsequent detection with a secondary label. Direct or chemical labeling with Cy-3 and Cy-5 fluorescent dyes is the most common means for detecting targets on microarrays. Targets are then hybridized to the array to identify species-specific polymorphism within one or more genes. Once post-hybridization steps are completed, then arrays are imaged using a high-resolution scanner. Microarrays can also be used to “fingerprint” bacterial isolates and they can be used to identify diagnostic markers suitable for developing new PCR-based detection assays.

Conclusion

The progress in recombinant DNA techniques offers opportunities for their application as analytical tools in food microbiology and food control. Improvements in the field of immunology, molecular biology, automation and computer technology continue to have a positive effect on the development of faster, more sensitive and more convenient methods in food microbiology.
The possibilities of combining different rapid methods, including immunological and DNA methods, should be further exploited. More research is needed on techniques for separating microorganisms from the food matrix and for concentrating them before detection by immunological or nucleic acid-based assays. As positive results of PCR tests do not indicate if the virulence or toxin gene was actually expressed, future studies should focus on the development of assays that measure biological activity. Development of the DNA chip approach is continuing at a rapid pace and for the microbiologist, the DNA chip technology will be one of the major tools for the future.

References

Bej, A. K., DiCesare, J. L., Haff, L. and Atlas, R.M. (1991). Detection of Escherichia coli and Shigella spp. in water by using the polymerase chain reaction and gene probes. Appl. Environ. Microbiol. 57, 1013–1017.

Bej, A. K., Steffan, R. J., DiCesare, J., Haff, L. and Atlas, R. M. (1990). Detection of coliform bacteria in water by polymerase chain reaction and gene probes. Appl. Environ. Microbiol. 56,307–314.

Belasco, J. G. and Higgins, C. F. (1988). Mechanisms of mRNA decay in bacteria: A perspective. Gene 72, 15–23.

Betzl, D., Ludwig, W. and Schleifer, K. H. (1990). Identification of lactococci and enterococci by colony hybridisation with 23S rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 52, 1190–1194.

Brett. M. M., (1998). Kits for the detection of some food poisoning toxins: problems, pitfalls and benefits. J. Appl. Microbiol. Symp. Suppl. 84, 110S–118S.

Coutard, F., M. Pommepuy, S., Loaec, D., Hervio, H., (2005). mRNA detection by reverse transcription-PCR for monitoring viability and potential virulence in a pathogenic strain of Vibrio parahaemolyticus in viable but nonculturable state. J. Appl. Microbiol., 98, 951-960.

Crimont, F. and Grimont, P .A. D. (1986). Ribosomal ribonucleic acid gene restriction as potential taxonomic tools. Ann. L’inst. Pdsteur / Microbiol. 137B, 165-175.

Curiale, M. S. and Klatt, M. J. (1990). Colorimetric deoxyribonucleic acid hybridization assay for rapid screening of Salmonella in foods: Collaborative Study. J. Assoc. Off. Anal. Chem. 73, 248–256.

Dalsgaard, A. and Olsen, J. E. (1995). Identification of food borne pathogens by nucleic acid hybridization. Int. J. Food Microbiol., 12,67–76.

Elfath M. Elnifro, Ahmed M. Ashshi, Robert J. Cooper, and Paul E. Klapper (2000). Multiplex PCR: Optimization and Application in Diagnostic Virology., Clinical Microbiology Reviews.13, 559-570.

Enne de Boer and Rijkelt R. Beumer, (1999). Methodology for detection and typing of foodborne microorganisms. Int. J.food. Microbiol., 50, 119-130

Fitts, R., Daimond, M., Hamilton, C. and Neri, M. (1983) DNA-DNA hybridization assay for detection of Salmonella in foods. Appl. Environ. Microbiol. 46, 1146–1151.

Gianluca Bleve, Lucia Rizzotti, Franco Dellaglio, and Sandra Torriani, (2003). Development of Reverse Transcription (RT)-PCR and Real-Time RT-PCR Assays for Rapid Detection and Quantification of Viable Yeasts and Molds Contaminating Yogurts and Pasteurized Food Products. Appl. Environ. Microbiol ., 69, 4116-4122

Grant, K. A., Dickinson, J. H., Payne, M. J.,Campbell, S., Collins, M. D. and Kroll, R. G.(1993) Use of the polymerase chain reaction and 16S rRNA sequences for the rapid detection of Brochothrix spp. in foods. J. Appl. Bacteriol. 74, 260–267.

James D. Oliver, (2005). The Viable but Nonculturable State in Bacteria. J. Microbiol., 43, 93-100

Jennifer M. Warner and James D. Oliver, (1998). Randomly Amplified Polymorphic DNA Analysis of Starved and Viable but Nonculturable Vibrio vulnificus cells. .Appl. Environ. Microbiol., 64, 3025-3028.

Koch, W. H., Payne, W. L., Wentz, B. A. and Cebula, T. A. (1993). Rapid polymerase chain reaction method for detection of Vibrio cholerae in foods. Appl. Environ. Microbiol. 59, 556–560.

Kocher. T.D. and Wilson. A.C. (1992). DNA amplification by the polymeruse chain reaction. In: A. R. Hoelzel (editor), Molecular Genetic Analysis of Populalions. A Practical Approach, IRL I’rcsh.Oxford University Press. Oxford. pp 185 --207.

Liebl, W., Rosenstein, R., Gotz, F. and Schleifer,K. H. (1987). Use of staphylococcal nuclease gene as DNA probe for Staphylococcus aureus, FEMS Microbiol. Lett. 44, 179–184.

Mabilat, C., Bukwald, S., Machabert, N., Desvarennes, S., Kurfurst, R. and Cros, P. (1996). Automated RNA probe assay for the identification of Listeria monocytogenes. Int. J. Food Microbiol. 28, 333–340.

Malmqvist., M., (1993). Biospecific interaction analysis using biosensor technology. Nature 361, 186–187.

Mark S. Campbell and Anita C. Wright, (2003). Real-Time PCR Analysis of Vibrio vulnificus from Oysters Appl. Environ. Microbiol,., 69, 7137-7144,

Masahito Oda, Masatomo Morita, Hajime Unno, and Yasunori Tanji, (2004). Rapid Detection of Escherichia coli O157:H7 by Using Green Fluorescent Protein-Labeled PP01 Bacteriophage .Appl. Environ. Microbiol., 70, 527-534

Oliver, J. D., and R. Bockian. 1995. In vivo resuscitation, and virulence toward mice of viable but nonculturable cells of Vibrio vulnificus. Appl. Environ. Microbiol., 61:2620-2623

Patel, B. K. R., Banerjee, D. K. and Butcher, P. (1991). Extraction and characterization of mRNA from mycobacteria: implication virulence and gene identification. J. Microbiol. Methods, 13, 99–111.264–273.

Robinson.B.J, (1997). Immunodiagnostics in the detection of food borne pathogens. In: Tortorello, M.L. and Gendel, S.M. Editors, 1997. Food Microbiological Analysis — New Technologies Marcel Dekker, New York, pp. 77–89.

Rodrigues, U.M., Aguirre. M., Facklam. R.R. and Collins, M.D. (1991). Specific and intraspecific molecular typing of lactococci based on polymorphism of DNA encoding rRNA. J. Appl. Bactcriol.71, 509- 516.

Rodtong. S. and Tdnnock,G. W. (1993) Diffcrcntiation of Lrrc,/ohtrc,i//rr strains by ribotyping. Appl. Environ. Microbial. 59, 3480-3484.

Salama, M. S., Sandine, W. E. and Giovannoni, S.J. (1993) Isolation of Lactococcus lactis subsp. cremoris from nature by colony hybridization with rRNA probes. Appl. Environ. Microbiol. 59, 3941–3945.

Schmidhuber, S., Ludwig, W. and Schleifer, K. H. (1988). Construction of a DNA probe for the specific identification of Streptococcus oralis. J. Clin. Microbiol. 26, 1042–1044.

Scholl, D. R., Kaufmann, C., Jollick, J. D., York, C. K., Goodrum, G. R. and Charache, P. (1990) Clinical application of novel sample processing technology for the identification of salmonellae by using DNA probes. J. Clin. Microbiol. 28, 237–241.

Silva, M. T., Appelberg, R., Silva, M. N. T. and Macedo, P. M. (1987). In vivo killing and degradation of Mycobacterium aurum within mouse peritoneal macrophages. Infect. Immun. 55, 2006–2016.

Tenover., F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H. and Swaminathan, B., (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33, pp. 2233–2239.

M. Uyttendaele, A. Bastiaansen and J. Debevere.,(1997). Evaluation of the NASBA® nucleic acid amplification system for assessment of the viability of Campylobacter jejuni. Int. J. Food. Microbiol., 37, 13-20.

Versalovic. J., Koeuth, T. and Lupski. J.R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res., 19, 6823-6831.

Vos., P.,Hogers, R., Bleeker, M., Rijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res., 23, pp. 4407–4414.

Way, J. S., Josephson, K. L., Pillai, S. D., Abbaszadegan, M., Gerba, C. P. and Pepper, I. (1993) Specific detection of Salmonella spp. By multiplex polymerase chain reaction. Appl. Environ. Microbiol. 59, 1473–1479.

Williams. J.G.K., Kubelik, A.E., Levak, K. J., Rafalski, J.A. and Tingey, S.C. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 6531-6335.

Willshaw, G. A., Smith, H. R., Scotland, S. M. and Rowe, B. (1985). Cloning of genes determining the production of erocytotoxin by Escherichia coli. J. Gen. Microbiol., 131, 3047–3053

Yaron, S and. Matthews, K.R., (2002). Reverse transcriptase polymerase chain reaction assay for detection of viable Escherichia coli O157:H7: investigation of specific target genes. J. Appl. Microbiol., 92, 633-638.

Quantitative evaluation of APP for the standardization of stress in naturally and artificially EUS infected *Channa gachua*

Alok Tripathi* and T.A. Qureshi**

**Department of Biotechnology, Saroj Institute of Technology & Management, PO: Aurjunganj, Lucknow 225 008, India; **Department of Applied Aquaculture, Barkatullah University, Bhopal 26, India, E.mail: aquaimmuno@yahoo.co.in*

In many pathological states, circulating white blood cells may show alterations in their morphology, function or concentration. Although changes in the absolute count of various types of white cell are commonly found in disease conditions and are usually non-specific, they may still be helpful in indicating the cause of a particular syndromatic condition.

The hematological and biochemical parameters of normal and UDN infected salmon were examined by Mulcahy (1969), Roberts (1972) and Wilkins et al, (l970) and all agreed that UDN infected fish had significantly lowered total serum protein levels. The degree of reduction varied, but since each group used a different technique for measurement, and it is probable that the degree of lesion development in the fish they examined varied also, this does not invalidate the general finding. Mulcahy (1969) has also found a “UDN specific protein pattern", the marked increase of a minor fraction in polyacrylamide gel electrophoreses serum from fish with early lesions. Other workers have unfortunately not described this specific feature, which could be of great value in diagnosis. Conroy (1972) studied the hematology of a small sample of UDN infected fish and found it to be very similar to that of the normal fish entering freshwater. There was a small but statistically significant increase in cells of the granulocyte series and Conroy (1972) suggested that this might be due to requirement of enzymes for autolysis of necrotized epidermal tissue. This does not seem likely, however, in view of the complete lack of invasion of lesions by granulocytes in most cases. Flemming (1958) has reported hypoptoteinaemia as a feature of other fish diseases and Carbery (1970) and Mulcahy (1971) recorded it in wild trout (*Salmo trutta*) affected with a condition presumptively diagnosed as UDN. The marked oedema found in adult stages is also explicable simply on the basis of haemodilution effect. Hodkinson and Hunter (1970) tested serum from adult salmon and parr by the Ouchterlony double diffusion method. Against antigens prepared from diseased salmon tissues and from *Saprolegnia* cultures. Roberts et al. (1972a) followed up the resemblance observed in the grade I lesions to the acantholytic bullae seen in early pemphigus lesions in man, by seeking anti epithelial auto-antibodies in the serum of diseased fish and in the fish artificially sensitized in salmon epithelium, by means of a double indirect immuno-fluoresecence technique.

Thus under the present work, hematological and serological studies are conducted in naturally and artificially EUS infected fishes to find out the similarities between the impacts caused by the pathogens, involved naturally as well artificially, on the blood cells count and serum proteins.

Material and methods

For the purpose of present work, naturally infected fish was collected from local fish ponds while for experimental infection trial, snakehead, *Channa gachua*, was procured from a local fish market. Experimental infection trials were conducted on a total population of 170 fish ranging between 8-10 cm in total length and 25-35 g in weight. They were acclimatized in plastic pools in the laboratory for 15 days before subjecting them to experiments. During experiments, they were kept in glass aquaria of the size of 60 x 30 x 30 cm filled with underground freshwater. They were fed on pelleted feed and minced fresh goat liver @ 2-3% of their body weight. A record of main physico-chemical parameters of water such as pH temperature and dissolved oxygen (DO) was maintained.

The experimental infection trials were designed to find out the actual pathological picture of the syndrome. For this purpose, the suspected pathogens were divided into three different groups viz viruses, bacteria and fungi and four concentrations of each pathogen are used to assess the impact of concentration on the secretion of APP (Table 1). They were injected to experimental specimens intramuscularly either singly and in various possible combinations.

For the isolation of virus (SHRV), tissue cells infected with it were harvested together with the liquid medium. Viruses with harvested cell were rapidly frozen and thawed three times and centrifuged at about 400 x g for 10 minutes to remove cellular debris. This was followed by ultra centrifugation at 40,000 x g for1 hours at 4°C. The viruses were identified by the help of neutralization test of polyclonal antisera and isolated viron particle as per procedure followed by the authors (Tripathi and Qureshi 2006). Bacterial suspension was prepared by culturing the isolates on TCA plates at 300°C for 24 hours and harvesting them with 500 ml of 0.85 % physiological saline solution. The colony forming unit per nil (cfu/ml) of this solution was determined by plating 10-fold dilution series. For this purpose, the solution was diluted with distilled water to give the cell number 10^3/ml which was also used for inoculation to fish. The fungus, *Aphanomyces invadans* was cultured up to the asexual stage and allowed to ooze out sporangia before its suspension was prepared in physiological saline. The counting and staining were done by the malachite green method.

A total of 170 fish was inoculated with pathogens in pure as well as in mixed form. The fishes were kept in aquaria with temperature ranging from 14 to 20°C. The fish were divided into 17 groups, each containing one aquaria of ten fish. Feeding was stopped one day before starting the experiment. 0.01ml of suspension was inoculated intramuscularly per gram of fish body weight. The fish were first anesthetized in solution of Tricono-methonosulphomatic (8 ml of 0.5 % solution of TMS /500 ml sterile water). The counting and inoculation of pathogens was done following the methods given by Miller (1994), Qureshi and Mastan (1998) and Robert (1994).

The hematological parameters of fish affected with EUS and artificially infected were compared with apparently healthy specimens with no visual disease symptoms. For this purpose, blood samples were taken fourth day after the inoculation, from the caudal peduncle and heart with the help of 2 ml disposable syringe. Red blood cells and white blood cells were counted using a haemocytometer. Blood smears were prepared and stained by method followed by Anderson (1974) for the differential count of white blood cells. Hemoglobin concentration was measured using a hemoglobin meter. For the purpose of electrophoresis the serum was separated from the blood of healthy, naturally infected and experimentally infected fish as per the procedure given by Gupta and Arunen (1997). The SDS-PAG-electrophoresis was conducted with the help of standard molecular weight markers (4363602T: BDH) at 200 V for 90 minutes. Barbitol buffer was used at pH 8.0 and the stripe stained with comassic brilliant blue. The quantification of serum protein was conducted through densitometry. For the estimation of transferrin and ceuloplasmine, polyclonal antisera were generated as per procedure given by the Gupta and Arunan (1992).Transferrin and ceruloplasmine are quantified by the immunodiffusion method as per procedure given by Ouchterlony (1962).

Observations

In healthy *Channa gachua*, the total erythrocyte count (TEC) was 2.44-0.452 x 106 /µl^3 and total leucocytes count (TLC) was 16.20x103 /µl^3. The total hemoglobin value was 11.0 gm/100 ml blood and haematocrit value was 39.4 %. In naturally infected *Channa gachua*, the values of TEC, TLC, hemoglobin and haematocrit value were 1.86±0.172 x 10 x 106 /µl^3, 27.20 x103/µl^3, 8.0 gm/100 ml and 36.8%, respectively. Among the artificially infected fish, the minimum value of TEC in set FE (1.78±0.220 x 106 /µl^3) and the maximum was in set FF (2.14±0.840 x 106 /µl^3). The minimum value of TLC was in set FA4 (2.410 x 103 /µl[3]), and the maximum value was in set Sig. (2.88 x 103 /µl3). The minimum value of hemoglobin was found in set FB4. (8.2 gm/100ml blood) and the maximum was in set FF (10.0 gm/100 ml) blood. Similarly, the haemocrit value was the minimum in set S I a (36.01%) while the maximum in set FB4 (39.0%) (Table 2).

In healthy *Channa gachua*, the differential leucocyte count was more or less normal. The lymphocytes, monocytes, eosinophils, basophils and neutrophils were 52.94, 32.43, 6.18, 0.29 and 7.45% respectively. In naturally infected fish these values were found to be deviating significantly from the normal values. An increase was observed in the number of lymphocytes and eosinophils while a decrease was noticed in the number monocytes and neutrophils. In the fish subjected to experimental infection trails, the maximum and minimum number of leucocytes and lymphocytes was 75.65% and 38.66% in set Sle and Sib, respectively. Similarly the number of monocytes was the minimum in set FD (18.78%) and maximum in set FA1 (24-16%). Eosinophils were the minimum in set FE (2.150%) and the maximum in FC1 (18.18%). Neutrophils were the minimum in FD (2.13%) while the maximum in FA1 (9.87%). Variations in the number of basophils were insignificant (Table 3).

The serum protein profile of healthy, naturally infected and artificially infected fish was assessed by acetate cellulose paper electrophoresis. Total protein value was highest in healthy fish i.e. 5.2. In naturally infected fish, it was only 4.2, while lower values were recorded in the fish subjected to experimental infection trials. The lowest value was in set FB2 (4.20) and the highest in set FH (5.1). The albumin and globulin

Table 1. Experimental inoculum.

Set	Inoculum	LD50	Amount of pathogen inoculated / 25g B.W.
FA1	Pure viral culture	1.52×10^2	1.12 x 102 pfu
FA2	Pure viral culture	do	1.22 x 102 pfu
FA3	Pure viral culture	do	1.32 x 102 pfu
FA4	Pure viral culture	do	1.42 x 102 pfu
FB1	Pure bacterial culture	4.78×10^2 cfu	4.12 x 102 cfu
FB2	Pure bacterial culture	do	4.22 x 102 cfu
FB3	Pure bacterial culture	do	4.32 x 102 cfu
FB4	Pure bacterial culture	do	4.42 x 102 cfu
FC1	Pure fungal culture	1.62×10^2 spores	1.22 x 102 spores
FC2	Pure fungal culture	do	1.32 x 102 spores
FC3	Pure fungal culture	do	1.42 x 102 spores
FC4	Pure fungal culture	do	1.52 x 102 spores
FD	Mixed culture of virus and bacterium	As above	@ 0.71 x102 pfu + 2.25 x 102 cfu
FE	Mixed culture of virus and fungus	As above	0.71 x 102 pfu + 0.81 x 102 spores
FF	Mixed culture of bacterium and fungus	As above	2.25 x 102 cfu and 0.81 x 102 spores
FG	Mixed culture of virus, bacterium and fungus	As above	0.71 x 102 pfu,1.5 x 102 cfu and 0. 54 x 102 spores
FH	Only PBS	As above	As standards

Table 2. Hematological parameters of naturally and artificially EUS infected *Channa gachua*.

No.	Fishes	TEC x 106/µ³	TLCx103/µ³	Hb (gm /100ml)	Haematocrit %
1	Normal	2.44 ± 0.452	16.2	11.0	39.4
2	Nat.dis. Fish*	1.86 ± 0.172	27.2	8.0	36.8
3	FA1	1.97 ± 0.171	24.4	8.4	36.0
7	FB1	1.99 ± 0.192	23.6	8.2	39.0
11	FC1	1.76 ± 0.421	24.1	9.8	38.1
15	FD	1.89 ± 0.840	26.1	9.4	39.4
16	FE	1.78 ± 0.220	28.0	8.4	37.4
17	FF	2.14 ± 0.840	24.8	10.0	34.6
18	FG	1.82 ± 0.260	28.2	8.4	38.0
Nat .dis. Fish*=Naturally Infected fish					

Table 3. Differential leukocyte count of healthy, diseased and experimentally infected *C. gachua*.

No.	Sets	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %	Neutrophils %
1	Normal	52.94	33.63	6.18	0.29	7.45
2	Nat. dis. fish*	54.80	30.88	8.79	0.75	5.77
3	FA1	56.28	24.16	9.48	0.00	9.87
4	FB1	38.66	20.83	8.30	0.27	2.32
5	FC1	55.76	25.98	18.18	0.00	3.68
6	FD	61.70	18.78	16.65	0.69	2.13
7	FE	75.65	13.44	2.15	0.26	4.76
8	FF	59.22	21.33	4.26	0.21	5.19
9	FG	63.45	20.48	10.50	0.00	5.48
Nat. dis. fish* = Naturally Infected fish						

ratio was found to be the lowest in naturally infected fish (FG). The albumin fraction was the highest in set FF and the lowest in naturally infected fish. It was very low in set FE. Protein alpha 1 was the maximum in set FE (46.09%) while the minimum in set FF (3.24%). Alpha 2 was also the as alpha 1. The beta protein was the maximum in set FF (38.02%) while the minimum in set FE (5-179%). The gamma (γ-globulin) was the maximum in set FG (32.55%) while the minimum inset FE (4.58%) (Figure 1).

No sharp rise or fall was observed in case of total protein (TP) in particular pathogenic inoculation with the value ranging from 4.2-5.2 g/dl. However, a dose dependent change was noticed in different protein fractions, in case of all pathogenic inoculation. In case of viral inoculation, there was a gradual decrease in serum albumin while rest all four fractions showed proportional relationship with the doses. In case of bacterial inoculation too, same trend was notice. And in case of fungal inoculation an inverse proportional relation was observed with serum albumin and alpha 1, while proportional relation was observed with alpha 2, beta and gamma proteins.

To establish the quantitative relation between dose of a particular pathogen and acute phage protein, the graphs were obtained for three selected proteins viz. albumin, transferrin and ceuloplasmine. Under the present work, nearly straight-lines are recorded when graphs are plotted against the different doses of three selected pathogens ie.SHRV-19E, *A. hydrophila* and *A. invadens*. The constant of proportion-

Table 4. Dose dependent change in quantities of serum albumin against the three selected pathogens.

No.	Set	Amount of pathogen inoculated /25g B.W.	Protein (albumin) Fraction	Content mg/100ml blood	K .Tan α = S.A / dose & unit	S.D.
1	FA1	1.12 x 10^2 pfu	29.38	1.4	0.111	0.1344098
2	FA2	1.22 x 10^2 pfu	14.55	0.698	0.526	
3	FA3	1.32 x 10^2 pfu	7.03	0.352	0.225	
4	FA4	1.42 x 10^2 pfu	3.87	0.19	0.115	
5	FB1	4.12 x 10^2 cfu	25.89	1.29	0.262	0.133577134
6	FB2	4.22 x 10^2 cfu	21.72	1.01	0.278	
7	FB3	4.32 x 10^2 cfu	19.36	0.92	0.278	
8	FB4	4.42 x 10^2 cfu	16.22	0.77	0.190	
9	FC1	1.22 x 10^2 spores	22.33	1.07	0.077	0.382547
10	FC2	1.32 x 10^2 spores	19.00	0.895	0.061	
11	FC3	1.42 x 10^2 spores	16.33	0.38	0.048	
12	FC4	1.52 x 10^2 spores	13.23	0.64	0.037	

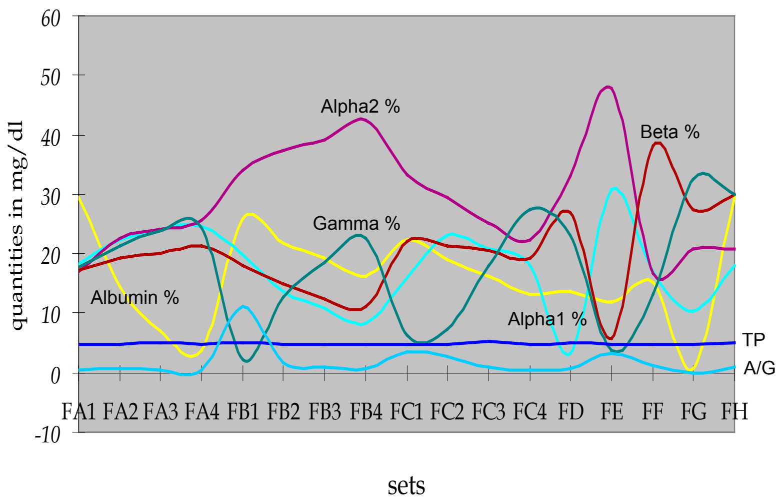
Table 5. Dose dependent change in quantities of serum transferrin against the three selected pathogens.

No.	Set	Amount of pathogen inoculated / 25g B.W.	Protein (transferrine) Fraction	Content mg/100ml blood	K x Tan α = S.A / dose & unit	S.D.
1	FA1	1.12 x 10^2 pfu	17.33	0.83	0.085	0.0037
2	FA2	1.22 x 10^2 pfu	19.36	0.93	0.085	
3	FA3	1.32 x 10^2 pfu	20.11	1.00	0.085	
4	FA4	1.42 x 10^2 pfu	21.32	1.17	0.085	
5	FB1	4.12 x 10^2 cfu	17.88	0.89	0.021	0.008
6	FB2	4.22 x 10^2 cfu	14.82	0.69	0.018	
7	FB3	4.32 x 10^2 cfu	12.36	0.59	0.015	
8	FB4	4.42 x 10^2 cfu	11.01	0.52	0.013	
9	FC1	1.22 x 10^2 spores	22.11	1.06	0.096	0.023
10	FC2	1.32 x 10^2 spores	21.39	1.00	0.086	
11	FC3	1.42 x 10^2 spores	20.5	0.998	0.077	
12	FC4	1.52 x 10^2 spores	19.36	0.94	0.014	

Table 6. Dose dependent change in quantities of serum ceuloplasmine against three selected pathogens.

No.	Set	Amount of pathogen inoculated / 25g B.W.	Protein (ceruloplasmin) Fraction	Content mg/100ml blood	Kx Tan α = S.A / Dose & Unit	S.D.
1	FA1	1.12 x 10^2 pfu	17.10	0.82	0.055	0.01
2	FA2	1.22 x 10^2 pfu	22.46	1.07	0.066	
3	FA3	1.32 x 10^2 pfu	24.01	1.20	0.066	
4	FA4	1.42 x 10^2 pfu	25.65	1.23	0.066	
5	FB1	4.12 x 10^2 cfu	34.15	1.70	0.030	0.38
6	FB2	4.22 x 10^2 cfu	37.29	1.75	0.030	
7	FB3	4.32 x 10^2 cfu	39.25	1.89	0.032	
8	FB4	4.42 x 10^2 cfu	42.36	2.01	0.034	
9	FC1	1.22 x 10^2 spores	33.25	1.59	0.099	0.05
10	FC2	1.32 x 10^2 spores	29.36	1.38	0.080	
11	FC3	1.42 x 10^2 spores	25.23	1.22	0.064	
12	FC4	1.52 x 10^2 spores	22.30	1.08	0.053	

Figure 1. Dose dependent changes in serum protein profile.



ality found in this experiment for a particular protein fraction, was within in limit of standard deviation (SD<1), Tables 4-6.

Discussion

Hematological data of naturally and experimentally infected fishes showed a resemblance that indicated the presence of virus, bacteria and fungi at the stage at which the sample had been taken ie. ulceration stage, in both cases. However, the number of total erythrocyte count, which may be taken as an indicator of extent of severity, was found to be equal in case of the experimental fishes of set FG inoculated with virus, bacteria and fungi and set FE, inoculated with virus and fungi. This observation gives an indication that the bacterium has no role to play in this situation. The differential leukocyte count in naturally infected fish was found to be considerably higher then that of healthy fish. Lymphocytes, the indictors of viral and bacterial infection were found to be quite high in number in naturally infected fishes as compared to healthy fishes, which might be due to stimulation of viral pathogen. The monocyte count was found to be significantly reduced indicating that the bacterium has no role to play in EUS. The observed increased in eosinophils count was due the inflammation which occurred before the viral inflammation. The leucocytes count in experimentally infected fishes was also found to be quite high and DLC in set FE was nearly the same as that of naturally infected fishes. The above observations get support from the facts quoted by Hughes-Jones and Wickrinasinghe (1991).

The protein profile of naturally EUS affected fish was found to be in accordance with the interpretation given by Laurell (1985) for the stress conditions. The total protein of naturally infected fishes was found quite low as against the healthy fish. Albumin, a transport protein was found to be quite reduced in naturally infected fish as compared to the healthy fishes, which might be due to the conversion of albumin to protective protein (γ globulin). The acute phase protein, CRP having α2 mobility, was found to be high in naturally infected fish, thereby indicating an extreme stress condition. The increase in γ globulin was a clear cut sign of the infectious nature of disease. The protein profile of naturally infected fishes was found to be comparable to the protein profile of fishes experimentally inoculated with virus and fungus of set FE except γ globulin. However, the globulin produced in response to viral and fungal pathogens was nearly equal to that produced in naturally infected fish. This observation supports the findings of Laurell (1985).

No sharp rise or fall was observed in the case of total protein (TP) in particular pathogenic inoculation and it ranged between 4.2-5.2 g/dl throughout the whole set of experiments. However a dose dependent change was noticed in different protein fractions in almost all pathogenic inoculations. In the case of viral inoculation, there was a gradual decrease in serum albumin while in the rest all four fractions increased in proportional with the doses. In case of bacterial and fungal inoculation too the same trend was noticed. Such a dose dependent change was also reported by the Gerwick (2002). Hence by quantifying the serum protein fraction, the extent of pathogenicity or level of infection of a particular pathogen, with relation to a particular host can be evaluated, providing the condition that all the hosts should be of the same physiological stage.

References

Anderson. D.P. (1974). Fish Immunology; T.F.H, Publication Hong Kong - 66-73.

Conroy, D.A. (1972). Studies on hematology of Atlantic Salmon (Salmo salar) Symp. Zool. Soci. Lond., 30 101-127.

Flemming,H.(1958). Untersuchungen uber die Blutieweisskorper gesunder und bauch wassersuchtskrank Karpen Z. Fisch 7: 91-152.

Gupta, S.K. and Arunan, K. (1997). A Handbook of practical and clinical Immunology.

Gerwick, L., R. Steinhauer, S. Lapatra, T. Sandell, J. Jourtuno, N. Hajiseyedjavadi and C.J. Bayne (2002). Fish and Shellfish Immunology 12 229-242.

Hodkinson, M. and Hunter A, (1970). Immune response of UDN infected Salmon to Saprolegnia, J of Fish Biol.5 305-311.

Hughes-Jones, N.C. and Wickramasinghe (1991). Lecture notes on Hematology, (5th edn.) Blackwell Scientific Publication 103-13 1.

Laural C.B., (1985). In recent advances in clinical biochemistry. Vol. 3 (Price C.P. Alberty K.G.M.M. edn.) 1-103 Edinburgh Churchill Livingstone.

Madeley, C.R. (1995). Traditional Techniques of Viral diagnosis (Medical Virology: Edited by Desselberger U.) Oirle Press Oxford. 1-32.

Mastan, S. A. and Qureshi, T.A, (1999). Bacterial flora of different EUS prone water bodies of Bhopal. Indian J Applied & Pure Biol.14(2) : 122-128.

Millar, S.D.(1994). Joint Viral/Fungal induction of EUS in snakeheads. ODA Regional seminar on Epizootic Ulcerative Syndrome. 272-278.

Mulcahy M.F. (1971). Serum protein change in Ulcerative Dermal Necrosis (UDN) of trout (S Trutta) J. Fish Biology 3 199-203.

Continued on page 41.

Replacing marine fish oil in aquafeeds with tropical palm oil products

Wing-Keong Ng, Ph.D.

Fish Nutrition Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia. Email: wkng@usm.my.

Marine fish oils are a major ingredient used in the formulation of commercial aquaculture feeds. These conventional sources of dietary oils for aquafeeds are produced from small pelagic fish that are a finite fishery resource. According to industry estimates, aquafeeds currently use about 70% of the global supply of fish oil and by the year 2010, fish oil used in aquaculture is estimated to reach about 97% of the world supply. It has been estimated that over 75% of the world's major capture fisheries are now fully exploited, over-exploited or depleted. Fisheries biologists have recently raised concerns about the sustainability of these capture fisheries, especially as fishmeal and fish oil resources, and the harm that over exploitation could do to the delicate ecological balance of the world's oceans. The stagnation in global fish oil production, coupled with increased demand for its use in aquaculture feeds, has therefore greatly inflated fish oil prices. Fish oil production is heavily localized in specific regions of the temperate world resulting in it becoming increasingly expensive and difficult to obtain in many tropical countries practicing aquaculture. The rapidly growing aquaculture industry in Asia and elsewhere cannot continue to rely on finite stocks of marine pelagic fish for fish oil supply. Therefore, there is currently great urgency within the aquafeed manufacturing industry in finding suitable sustainable alternatives to marine fish oils.

One potential substitute for marine fish oils in aquafeeds are tropical palm oils. Palm oil is the generic name given to a range of products derived from the processing and refining of crude palm oil. Global production of palm oil has now exceeded 33.3 million tons and is currently the most abundant vegetable oil produced in the world. Malaysia is currently the largest producer and exporter of palm oil, contributing about 46% of the global supply. Since 1998, the Fish Nutrition Laboratory at Universiti Sains Malaysia has embarked on a comprehensive research program to evaluate the potential use of palm oil products in aquafeeds. The aim of this article is to give an overview of some of the major issues involved in the use of palm oil in the diets of various fish species.

Various varieties of high oil yielding palm oil fruit on display at a recent international palm oil congress in Malaysia.

Palm oil products for aquafeeds

Crude palm oil (CPO) is extracted from the mesocarp of the fruit of the oil palm tree. CPO has a deep orange-red color due to the high content of carotenoids and is a rich source of vitamin E. Both beta-carotenes and vitamin E are potent natural antioxidants. During refining of CPO, the carotenoids present are thermally destroyed and the oil bleached to produce the desired color for a refined, bleached, deodorized (RBD) palm oil. Two by-products produced during the refining of CPO are spent bleaching clay (SBC) and palm fatty acid distillates (PFAD). Both SBC and PFAD still contain significant amounts of valuable nutrients such as lipids and vitamin E. To widen the range of its use, RBD palm oil can be fractionated by thermomechanical processes to produce RBD palm olein (RBDPO) and RBD palm stearin. Other than these major products of CPO, more than 30 different palm oil products are produced from various degrees of refining, bleaching, deodorizing and hydrogenation. The physical, chemical and nutritional properties of the various palm oil products are different and have varied commercial uses.

The ever expanding oil palm cultivation in Malaysia and other tropical countries offers the possibilities of an increased and constant availability of palm oil-based feedstuffs for aquafeed formulations. Novel sources of energy, lipid, vitamin E and carotenoids from palm oil products and by-products will contribute to the development of cost-effective feeds for the aquaculture industry.

Advantages of palm oil use in aquafeeds

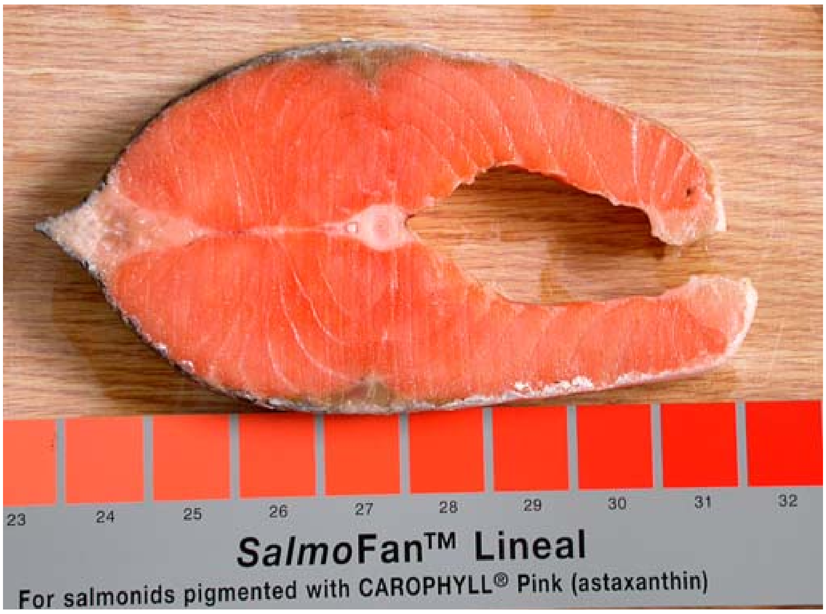
Sustainable production and lower cost

The oil palm industry had been expanding at a rapid pace and there are currently more than four million hectares of oil palm plantations throughout Malaysia alone. Currently, palm oil accounts for more than 23% of the total world production of oils and fats and is the most important commercial vegetable oil. The rapid rise in the importance of palm oil in the global oil market is due to several factors, including the fact that the oil palm is the most prolific oil producing plant. In Malaysia, about 3,800 kg palm oil and 424 kg palm kernel oil/ ha/ year are produced. Through modern tissue culture techniques, palm oil productivity of up to 8 tons per hectare per year is now possible. The average oil productivity of soybeans at 378 kg soybean oil/ ha/ year pales in comparison. Being a perennial, once planted, the crop will be in production for about 25 years, which guarantees reliability of oil supply and lowers production costs as compared to other major oilseed plants which are mostly annuals. Palm oil is one of the cheapest vegetable oils in the global market. In 2005, CPO costs about US$422 per ton compared to US$545 and US$669 per ton for soybean and rapeseed oils, respectively. In the same year, marine fish oils cost US$750/ton. Fish oil production is predicted to decline further in 2006 and exporters are expecting further price increases. The cost savings for using palm oil in aquafeeds instead of fish oil is obvious.

Red hybrid tilapia fed palm oil-based diets from stocking to marketable size grew equally well compared to fish fed fish oil-based diets.

Reduces feed rancidity and oil leakage

The traditional use of fish oils that contain a high proportion of polyunsaturated fatty acids (PUFA) in commercial aquafeeds makes these feeds highly susceptible to oxidation and subsequent rancidity. This is especially problematic for salmonid feeds that can contain up to 40% dietary fish oils. Feeding fish rancid oils will trigger deleterious effects of lipid peroxidation in cellular biomembranes. CPO contains about 48% saturated fatty acids and the low concentrations of PUFA give it exceptional resistance to oxidation. Together with the protective effects of potent natural antioxidants (carotenoids and vitamin E) present in palm oil, the incidence of feed rancidity should be substantially reduced when palm oil is used in aquafeed formulations. The use of CPO in high-fat diets is one practical and cost-effective way to produce high-energy diets without the high risk of feed rancidity. These feeds could therefore be stored longer while maintaining freshness and palatability. We also observed that increasing the dietary level of CPO in extruded salmon feeds reduces the incidence of oil leakage from these high lipid diets. This is because CPO is semi-solid at room temperature and has a higher melting point compared to fish oils. CPO can therefore be used to reduce the migration of lipids to the pellet surface that causes loss of valuable nutrients, and staining of packaging materials and feed equipment.



Salmon fillets from fish fed crude palm oil-based diets maintained good texture and coloration.

Comparable fish growth performance

To date, we have demonstrated that various palm oil products such as CPO, RBDPO, PFAD and residual oil present in SBC could effectively replace fish oil in the diets of various catfishes, carps and tilapia without compromising growth performance. It would seem that for freshwater fish species which may have lower requirements of n-3 fatty acids (from fish oil), the use of palm oil in the diets of these fish have great potential. Even for marine fish species, research carried out in collaboration with the Institute of Aquaculture at the University of Stirling (Scotland) on Atlantic salmon and rainbow trout had shown similar encouraging results as long as the fish are fed fish meal-based diets. It is possible to use diets containing CPO up to 100% of added oil without significant effects on the growth rate or feed conversion ratio of Atlantic salmon. Recent feeding trials with groupers (in collaboration with Universiti Sabah Malaysia) had also showed similar results for this high value marine food fish.

Our research seems to indicate that CPO can replace up to 100% of added fish oil without any marked negative effects on fish growth, feed utilization efficiency, survival or important fillet quality parameters such as lipid content, texture and color. In this respect, palm oil is similar to other vegetable oils that had been reported to be able to replace a significant part of fish oil in fish diets.

Palm oil is a superior energy source

Palm oil is a superior source of dietary energy. In vitro studies done on mitochondrial β-oxidation in fish suggest that there exist a substrate preference for saturated and monounsaturated fatty acids over PUFA. Unlike vegetable oils such as soybean or rapeseed oil which contains high PUFA content, palm oil contains an abundant supply of saturates (48%) and monoenes (42%). Studies conducted in our laboratory had shown the protein sparing effect of palm oil in catfish diets. We found that growth and feed efficiency of African catfish responded significantly in a positive manner to palm oil additions of up to 8% (in isoenergetic diets) with no further improvement beyond this dietary level. Protein sparing effects resulting in higher protein retention in fish fed RBDPO-supplemented diets were also observed.

Deposition of beneficial vitamin E in fish tissues

Vitamin E is a generic descriptor given to a group of lipid-soluble, structure-related compounds, which occur naturally as tocopherols and tocotrienols. While most vegetable oils contain almost exclusively tocopherols, palm oil is unique because tocotrienols represent about 70-80% of the vitamin E content. We were the first group of researchers who reported the deposition of tocotrienols in fish tissues. During CPO refining, vitamin E is preferentially accumulated in the PFAD fraction which makes it a suitable energy and vitamin E source for aquafeeds. PFAD is about 15% cheaper compared to CPO. We observed a linear increase in total vitamin E concentrations in the fillet of African catfish fed practical diets with increasing levels of PFAD at the expense of fish oil. We were then able to show that the deposition of palm vitamin E in the fillets of tilapia fed a tocotrienol-rich fraction extracted from palm oil imparted higher oxidative stability compared to the fillets of fish fed diets supplemented with equivalent levels of dietary synthetic α-tocopherol acetate, the current standard in vitamin E supplementation in commercial aquafeeds. The perivisceral adipose tissue of tilapia was found to be the major depot for vitamin E among the various tissues examined with tocotrienols making up almost 50% of the total vitamin E deposited. Other than increased concentrations of vitamin E, fillets of fish fed increasing levels of dietary palm oil products also showed increasing reduction in lipid peroxidation products. This would translate to longer shelf life for seafood products. The level of vitamin E in fish tissues is known to influence the freshness and long-term storage properties of fish fillets. The use of palm oil, and especially PFAD, in fish diets has great potential as a practical and cost effective means of adding value to the flesh quality of aquaculture products.

The deposition of tocotrienols in fish fillets also adds value to the product, especially if eaten raw as sashimi, since the potential health benefits of tocotrienols in the human diet may include beneficial effects on the prevention of cardiovascular diseases, cancer and stroke. It may also increase the market value and consumer acceptance of salmon steaks in which pigmentation is a quality parameter. The potential accumulation of palm vitamin E in salmon flesh would slow down the oxidation of these pigments thereby maintaining coloration for longer periods.

Beneficial to fish and consumers' health

Feeding high levels of fish oils and vegetable oils that contain a high proportion of PUFA that are highly susceptible to oxidation can also lead to increased oxidative stress for the fish that may result in pathological conditions. One practical and cost-effective way to produce high-lipid diets without the damaging side effects of increased lipid radicals is to use a more saturated lipid source that contains high levels of natural antioxidants such as vitamin E and carotenoids. This is found in palm oil.

Modern human diets have caused our intake of n-6 fatty acids to increase, drastically altering dietary n-3 to n-6 ratios. These ratios vary from 1:2.5 for Eskimos on a fish-based diet to 1:20 for modern diets rich in vegetable oils. Eskimos who rely on their traditional diets are relatively free of degenerative diseases common to urban dwellers. It is generally known that the fatty acid composition of fish fillets reflects the fatty acid composition of the dietary oil used. Similar influences of dietary CPO on the fatty acid composition of fish fillets were observed in our research studies. Since our human diets already have too much n-6 PUFA, a good fish oil substitute should limit the deposition of these undesirable fatty acids in fish fillets. This makes palm oil superior to most conventional vegetable oils that contain high proportions of n-6 PUFA that would be deposited in fillets when fed aquafeeds containing such oils.

Disadvantages of palm oil use in aquafeeds

Lack of beneficial n-3 fatty acids

Palm oil does not contain n-3 highly unsaturated fatty acids (HUFA), which are required by some fish, especially marine species. Consequently, palm oil in the diets of these fish should be formulated in conjunction with HUFA sources such as fish oil and fishmeal to assure that minimal HUFA requirements are met. Apart from not prejudicing the health and welfare of fish, the use of palm oil products in aquafeeds should also not affect the health promoting benefits to the consumer. Farmed fish such as Atlantic salmon is currently of high nutritional quality with an abundance of n-3 HUFA. The protective effects of fish lipids, especially n-3 fatty acids, eicosapentaenoic acid [20:5(n-3); EPA] and docosahexaenoic acid [22:6(n-3); DHA], against cardiovascular and other degenerative diseases are well-documented. Clearly, producers and consumers of salmon will want to minimize any reduction in quality, in terms of its health benefits in the human diet, arising from the substitution of fish oil with palm oils. As mentioned earlier, fish fed palm oil diets generally showed a fatty acid profile similar to the fatty acid profile present in their diet. The use of high levels of palm oil in fish diets will decrease the concentrations of beneficial n-3 HUFA in fish fillets destined for the human consumer. Consequently, palm oil in fish diets should be formulated in conjunction with HUFA sources such as fish oil and fishmeal. Another strategy that can be used to normalize the flesh levels of beneficial n-3 HUFA is to revert back to a fish oil-based finishing diet at an appropriate time before harvest. This feeding strategy will allow the use of higher levels of palm oil in fish diets for the major part of the grow-out phase thus providing feed costs savings without significantly altering the health benefits of the resultant fish fillet in the human diet. We were able to show that the n-3 concentrations in tilapia fillets were substantially restored when fed a fish oil-based finishing diet after feeding palm oil-based grow-out diets.

Lowered nutrient digestibility in cold waters

The high melting point of CPO due to its high saturated fatty acid content may reduce fatty acid digestibility and subsequent energy availability, especially when such CPO-based diets are fed to coldwater fish species during the winter season when water temperatures are low. In a pilot scale study conducted in Norway, we observed that decreasing water temperatures (11 to 6ºC) did not markedly affect protein, lipid or energy apparent digestibilities of the diets with 10 or 25% CPO (as % of added oil). However, dry matter digestibility decreased significantly in fish fed the diet with CPO at 25% of added oil. With the exception of protein digestibility, significantly reduced nutrient digestibility in Atlantic salmon fed the 50% CPO diet was observed. Increasing dietary CPO levels and decreasing water temperature significantly reduced the apparent digestibility (AD) of saturated fatty acids. The AD of monoenes and PUFA were only affected in salmon fed the 50% CPO diet. The results of this study showed that the inclusion of CPO up to about 10% (wt/wt) (25% of added oil) in Atlantic salmon feeds resulted in negligible differences in nutrient and fatty acid digestibility that did not affect growth performance of fish at the range of water temperatures generally encountered in the grow-out phase. In another study conducted with rainbow trout, we found that increasing dietary CPO level and decreasing water temperatures significantly increased the triglycerides content in trout fecal lipids, with saturates constituting more than 60% of the fatty acid composition. The reduction in apparent digestibility of saturated fatty acids was therefore due in part to the increasing resistance of dietary triglycerides rich in saturated fatty acids to digestion. Further research on nutrient digestibility in palm oil-based diets is needed to enable feed formulators to better calculate for dietary energy availability and to optimize the use of CPO in coldwater fish diets according to environmental temperatures.

Conclusion

Information on the use of palm oil products in fish diets is currently limited to a few species. Some fish species seems to be able to effectively utilize high levels of palm oil in their diets both as dietary energy and fatty acid sources without adverse effects on growth and feed utilization efficiency. Since different fish have different abilities to utilize various dietary lipids, more research work is necessary to fully exploit the use of palm oil in fish diets. Considering the economic advantages, reliability of supply and added benefits of palm oil over many other vegetable oils, its potential as an alternative dietary lipid source in aquafeeds warrants further investigation. Enhancing the use of palm oil in aquafeeds will decrease feed costs by reducing the demand for fish oil which will subsequently alleviate fishing pressure on many capture fisheries. This will have a positive impact on the global aquaculture and aquafeed industries as well as the palm oil industry.

Further readings

Ng, W.K., Lim, P.K. and Boey, P.L. (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, *Clarias gariepinus*. Aquaculture 215: 229-243.

Ng, W.K., Campbell, P.J., Dick, J.R. and Bell, J.G. (2003). Interactive effects of dietary palm oil concentration and water temperature on lipid digestibility in rainbow trout, *Oncorhynchus mykiss*. Lipids 38: 1031-1038.

Ng, W.K., Sigholt, T. and Bell, J.G. (2004). The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different blends of fish oil, rapeseed oil and palm oil. Aquaculture Research 35: 1228-1237.

Ng, W.K., Wang, Y. and Yuen, K.H. (2004). Replacement of dietary fish oil with palm fatty acid distillate elevates tocopherol and tocotrienol concentrations and increases oxidative stability in the muscles of African catfish, *Clarias gariepinus*. Aquaculture 233: 423-437.

Ng W.K., Koh C.B. & Zubir B.D. (2006). Palm oil-laden spent bleaching clay as a substitute for marine fish oil in the diets of Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 12: 459-468.

Quantitative evaluation of APP

Continued from page 37.

Ouchterlony, 0. (1968). Hand-book of Immunodiffusion and Immunoelectrophoresis. Ann. Arbor Science Publications Michigan.

Qureshi, T.A., Mastan, S.A, Prasad, Y. and Chauhan, R. (1999). Sensitivity of bacteria isolated from EUS affected fishes against various antibiotics J Ecotoxical Environ. Monit. 9(2): 149-153.

Roberts, R.J (1972). Ulcerative Dermal Necrosis (UDN) of Salmon (Salmo salar) Symp. Zool. Soci. Lond -, 30 53-81.

Roberts, R.J., Frerichs, G.N., Tonguthai K., Chinabut, S. (1994). Epizootic Ulcerative Syndrome of farmed and wild fishes. In: Recent Advances in Aquaculture 5 (Muir, J.F. and Roberts, R.J. eds.). Blackwell Scientific Publication, Oxford, M 207-239.

Wilkins, N.P., Sangster, G.R. and Conroy, D.A. (1970). some problems in the application of blood grouping techniques to the Atalontic salmon. Paper read at E.S.A.B.R. Congress: I A I.

NACA Newsletter

ISSN 0115-8503

Published by the Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

Volume XXII, No. 1
January-March 2007

Iranian officers train in advanced marine shrimp farming and meet Iraqi delegation at NACA

18 technical officers from the Iranian Fisheries Organizations (Shilat) have visited Thailand for an intensive two-week training course in advanced marine shrimp farming, from 5 to 20 November, and met with an Iraqi aquaculture delegation at NACA.

The course included ten days of classroom lectures, discussions and case study analysis taught by internationally recognised experts in shrimp health and farming systems, including Khun Pornlerd Chanratchakool, Professor Tim Flegel and Dr Charlor Limsuwan.

The course covered:

- Shrimp culture systems.
- Pond environments and preparation.
- Water and feed management.
- Shrimp product quality, food safety and inspection.
- Record keeping to aid decision making.
- Health management and disease diagnosis.
- Farm management and business risk management.
- Hatchery management.
- Assessment of postlarval quality and handling.
- Environmental management for intensive shrimp farming.
- Seawater irrigation and drainage systems.
- Overview of issues and problems in the Thai shrimp farming industry.
- International Principles for Sustainable Shrimp Farming.

Left to right: Dr Basim J. Hussain from the General Board of Fish Resources Development, Iraq Ministry of Agriculture; Prof. Sena De Silva, Director General of NACA; and Mr Omid Reza Asghari, Head of Nutrition and Live Food, Aquaculture Department, Iranian Fisheries Organization, and team leader for the Iranian delegation.

Participants also spent 10 days on the road to observe different aspects of the Thai shrimp farming practices. Participants visited farms and hatcheries to discuss culture practices; government institutions and research stations involved in health monitoring, inspection and certification of product; shrimp wholesale markets; processing facilities; and feed producing companies. Discussions were also held with local shrimp farmer clubs. As our Iranian friends prepared to depart a five-member delegation arrived from Iraq, as part of a three-week visit to study freshwater aquaculture in Thailand and China.

NACA would like to thank the staff of the Thai Department of Fisheries, the resource persons and other people that made this training course possible.

See the following page for a group photo of participants.

Green award recognises NACA work on shrimp farming

On November 8, 2006, the Consortium Program on "Shrimp Farming and the Environment" received a World Bank Green Award for their efforts towards responsible shrimp farming and the recent release of "International principles for responsible shrimp farming".

The Annual Green Award was instituted in 2001 by the World Bank's Environment Sector Board to recognize leadership and personal commitment among Country Directors and Task Teams to environmental sustainability and success in assisting World Bank client countries to integrate environment into development programs.

The Shrimp Farming and the Environment Consortium was formed in 1999 as a partnership between NACA, the World Bank, Food and Agriculture Organization of the United Nations, the World Wildlife Fund and recently the United Nations Environment Programme/GPA. The objective of this global program was to identify issues around shrimp farming and broadly advise on better management of the shrimp farming sector. The Consortium conducted a wide range of case studies and multiple stakeholder consultations involving governments, private sector, academia and NGO's totaling over 100 researchers in 20 countries over the past 6 years. One outcome from the Consortium Program is the International Principles for Responsible Shrimp Farming, recently welcomed at the FAO Committee on Fisheries Sub-Committee on Aquaculture in September 2006, as an overarching international framework for improving the sustainability of the shrimp farming industry.

The World Bank Green Award for the Consortium Program is a recognition of the work of many stakeholders who have collaborated over the past few years to develop and implement a common vision for better management of the aquaculture sector. The program has focused on shrimp farming to date but, following recommendations from FAO Sub-committee on Aquaculture, the scope of this collaborative work is expected to expand in future to include a wider range of aquaculture commodities and farming systems.

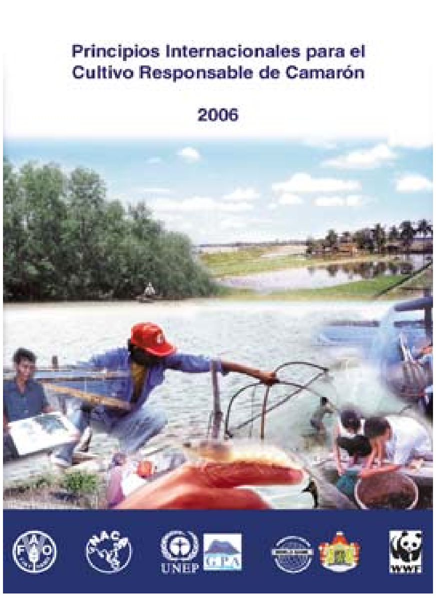
The staff of NACA would like to thank Dr Michael Phillips for his substantial efforts and contributions to the Consortium.

International Principles for Responsible Shrimp Farming now available in Spanish

The International Principles for Responsible Shrimp Farming has been translated into Spanish and is available for download at the link below. The principles document is currently being translated into several other languages and they will be available in the publication section of the NACA website in due course.

Español:

Los Principios Internacionales para el Cultivo Responsable de Camarón han sido desarrollados por el Consorcio sobre Cultivo de Camarón y el Ambiente, el cual consiste de la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO), la Red de Centros de Acuicultura en Asia y el Pacífico (NACA), el Programa Global de Acción para la Protección del Ambiente Marino frente a Actividades Realizadas en Tierra del Programa de las Naciones Unidas para el Medio Ambiente (UNEP/GPA), el Banco Mundial (WB) y el Fondo Mundial para la Vida Silvestre (WWF).



http://www.enaca.org/modules/wfdownloads/singlefile.php?cid=142&lid=762

The combined delegations from Iran and Iraq together with NACA staff (from previous page).

Building capacity on aquatic animal epidemiology in I.R. Iran

The recent outbreak of white spot disease which affected Bushehr, the largest shrimp producing province of I.R. Iran, has prompted the Iranian government to tackle capacity building on aquatic animal health management. Through its Aquatic Animal Health Programme, NACA has been supporting capacity building for development of strategies to effectively control the impact of aquatic animal diseases. As part of these efforts, a training course on Aquatic Epidemiology was conducted in Bushehr province from 16-19 September 2006. The training course was supported by the Iranian Fisheries Research Organization (IFRO) and was delivered by Dr Flavio Corsin of NACA, who is also a Regional Resource Expert in Aquatic Epidemiology.

Participants in the training course on aquatic animal epidemiology.

The course was attended by 14 participants. These were staff from seven IFRO offices located across I.R. Iran and two offices of the Iranian Veterinary Organization (IVO) in Tehran and Bushehr.

After a brief introduction on what epidemiology is and how it can help to answer important questions for the management of aquatic animal diseases, the trainees used real life examples to learn how to design, implement and analyse epidemiological studies. The participants were also given a chance to strengthen their knowledge and put it into practice by working as a group. As part of this activity the preliminary design of two epidemiological studies was conducted. These studies were aimed at:

- Identifying the prevalence of monodon baculovirus (MBV) in shrimp hatcheries in I.R. Iran.
- Identifying the effect of introducing mechanisms to lower water temperature and increase DO on the occurrence of mortality and strepto-coccosis in rainbow trout farms.

The active participation and enthusiasm of the trainees made the training course a real success, leading IFRO and IVO to request NACA for several follow up activities including a second, more advanced, training course on aquatic epidemiology and support for the development of epidemiological studies aimed at controlling diseases in shrimp and marine, coldwater and warmwater fish.

NACA Fisheries Team meets with new Governor of Aceh

NACA Fisheries Implementation Specialist, Dr Musri Musman, of the NACA Fisheries Team in Aceh, met with the new Governor-elect of Aceh, Irwandi Yusuf and Vice Governor, Mohammad Nazar. Dr Musri joined Mr Pieter Smidt, Head and other senior staff of the Asian Development Bank Project Management Office/Extended Mission in Sumatra, to brief the recently elected Governor and Vice Governor on the fisheries and agriculture components of the ADB Earthquake and Tsunami Emergency Support Project (ETESP).

Continued on page 48.

Governor-elect Irwandi Yusuf (left), with Mr Pieter Smidt (Head of ADB PMO/EMS), Dr Musri Musman (NACA team) and Richard Beresford (ADB)

Aquaculture Certification:

Programme to implement the COFI Sub-Committee on Aquaculture recommendation

Concerns over food safety and the environmental and social sustainability of aquaculture production have, over the years, lead to attempts to respond to public perceptions and market requirements. Food safety standards have been elevated and international trade regulations tightened. Policy and regulations governing environmental sustainability have been put in place in many countries, requiring aquaculture producers to comply with more stringent environmental mitigation and protection measures. In some countries these changes were initiated by the aquaculture sector itself, usually within the more organized private industry sector to ensure its sustainability and to protect operations from poorly managed activities. The private sector has made significant advances in the management of its activities and there are many examples of improved management of farming systems that have reduced environmental impacts and improved efficiency, including profitability.

A sample of the many fisheries product certification schemes that exist. Harmonization is an issue, particularly for small-scale producers.

Owing to the need for responding to these environmental and consumer concerns on aquaculture production and in order to secure better market access, there is increasing interest in certification of aquaculture production systems, practices, processes and products. Many markets increasingly recognize that some form of certification is a way of assuring buyers, retailers, and consumers that fishery products are safe to consume and originate from responsibly managed aquaculture farms or capture fisheries. Certification programmes have been underway in capture fisheries for some time and guidelines for eco-labelling of capture fishery products were developed by FAO in 2005. Efforts are also being made to develop eco-labelling guidelines for inland fisheries.

In several countries, aquaculture producers are introducing environmental certification of aquaculture products, either individually or in a coordinated manner, in order to credibly demonstrate that their production practices are non-polluting and/or non-ecologically threatening. Some countries are attempting to introduce state-mediated certification procedures to certify that aquaculture products are safe to consume and farmed in accordance with certain environmental standards. Most of the work done on improved management has been on salmon and shrimp, mainly due to their high commodity value and the importance attached as the most internationally traded products.

Within the context of the application of the Code of Conduct for Responsible Fisheries (CCRF), the FAO Committee on Fisheries Sub-Committee on Aquaculture (COFI/SCA) requested FAO to organise Expert Workshops to make recommendations regarding the development of harmonised shrimp farming standards and review certification procedures for global acceptance and transparency, which will also assist in elaborating norms and reviewing the diverse options and relative benefits of these approaches. In this regard, the Sub-Committee encouraged FAO to play a lead role in facilitating the development of guidelines which could be considered when national and regional aquaculture standards are developed. Several members of the Sub-Committee as well as a number of inter-governmental organizations offered to cooperate at national, regional and international level, and requested FAO to provide a platform for such collaboration. The Sub-Committee also requested setting up of an expert group on reviewing certification of shrimp farming systems. An Expert Workshop on "Guidelines for Aquaculture Certification", as recommended by the COFI Sub-Committee on Aquaculture will be held in Bangkok from 27-30 March 2007. The Bangkok Expert Workshop will be hosted by the Government of Thailand. It will be conducted as a joint FAO/DOF-Thailand/NACA Expert Workshop. The workshop is the first in a series of workshops/consultations as needed to prepare the international guidelines for the certification of aquaculture products. This initial workshop, being hosted in Asia, will have a strong emphasis on aquaculture products from Asian aquaculture producers. Further workshops are planned for Brazil during July 2007, and possibly elsewhere with the intention of bringing together global consensus on the guidelines and to address other issues and needs around aquaculture certification. Further background is provided in a separate Concept Note available at www.enaca.org/certification. The programme secreteriat invite you to send your comments and views on aquaculture certification to certification@enaca.org.

Current Developments in the Aquaculture Feed Industry

Proceedings of the Asian Aquafeeds Seminar, 12-13 April 2005, Kuala Lumpur, Malaysia.
Editors: Wing-Keong Ng and Chee-Kiat Ng
223 pages, ISBN 983-99563-8-8.

This book is a compilation of invited papers presented at the inaugural Asian Aquafeeds 2005 regional seminar held in Kuala Lumpur, Malaysia.

The aquafeed manufacturing industry is widely recognized as one of the fastest expanding agricultural industries in the world. With Asia accounting for more than 90% of global aquaculture production, this publication presents a comprehensive analysis of some of the current issues facing the Asian aquafeed industry and its contribution to the rapidly growing global aquaculture industry. The information contained within the pages of this book will add a small but important piece to the huge puzzle of how the global aquafeed industry works and the challenges and opportunities it provides for industry players. They consist of topics such as:

- Concepts in aquafeed formulation and feeding management.
- Alternatives to fishmeal and fish oil in aquafeeds.
- Twin screw extrusion technology.
- Current issues such as traceability, replacement of trash fish in caged marine fish culture, the establishment of an Asian Aquafeed Network, new FAO initiatives ; and
- Comprehensive reviews on the status and developments in aquafeed production in some Asian countries.

These technical proceedings provide a valuable reference for fish and shrimp feed manufacturers, nutritionists, feed mill operators, feed milling and processing equipment suppliers, feed ingredients suppliers as well as researchers, aquaculture producers, entrepreneurs and policy makers.

Purchasing a copy: RM 35.00 for local orders and US $15.00 for orders outside Malaysia; excluding postage and handling charges. Payment by crossed cheque or bank draft should be made payable to the Malaysian Fisheries Society. For Telegraphic Transfers (TT) procedure, please email or fax us for more information. All orders for Asian Aquafeeds can be made to:

Malaysian Fisheries Society
Aquatic Animal Health Unit
Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 Serdang, Selangor
Malaysia. Telephone: (603) 89468288,
Fax: (603) 89488246,
Email: myfisoc@time.net.my and wkng@usm.my
Website: http://www.vet.upm.edu.my/~mfs

Global Advances in the Ecology and Management of Golden Apple Snails

Edited by Dr. Ravindra C. Joshi and Dr. Leocadio S. Sebastian.

Here in one publication is all information so far known about golden apple snails (GAS) and the rice systems and countries they have afflicted. Around 500 pages of information are devoted to this species that continue to expand their distribution. With this complete publication, the knowledge vacuum on the ecology and management of GAS will be filled. Some 24 chapters cover various aspects of snail taxonomy (traditional as well as molecular tools), impacts of GAS on aquatic ecosystems and farmers' health, and pesticide abuse/misuse. Even GAS-invaded countries have submitted their separate country reports. Further, some chapters are dedicated to the utilization of GAS as food and as natural paddy weeder, with some information available on the biorational approach in its management and control. The publication ensures comprehensive reference on the topic, written by experts around the world for the benefit of all researchers, students, and various organizations and libraries who need information on the subject. 600 pages, hardbound.

ISBN 978-971-9081-31-9
Price: Developed countries US$102, Developing countries US$52.

NACA Mission to Myanmar

NACA's Director General and R&D Programme Manager undertook a visit to Myanmar in December 2006 to explore possible areas of support to the emerging aquaculture sector. Based on the discussion, a number of areas of support to Myanmar were identified and activities will be initiated on these within the first half of 2007. The specific initial areas of support will include:

- A mission by an expert in grouper hatchery techniques to visit the coastal hatchery at Kyun Su Township in Myeik to make an assessment of its suitability to be used as a grouper hatchery and to make relevant recommendations in this regard to the Department of Fisheries (DOF).
- A mission by a mollusc expert to the Rakhine area to assess and determine the feasibility of introducing mollusc culture of suitable species into the area.
- Arrangement of a study tour for a group of farmers practicing crab fattening / soft shell crab production to two countries in the region, with additional support for two DOF officials to accompany the group.
- Funding of a fish nutrition expert to visit Myanmar and assess the range of farm made feeds used in freshwater fish culture, assess nutritional quality of the feeds and recommend suitable improvements. This expert will work in conjunction with nutritional specialists in the DOF.
- Seeking funding in conjunction with the FAO for expert inputs into two workshops on Good Aquaculture Practices for shrimp and freshwater fish, to be held in Yangon in the second half of 2007. In this regard, NACA has already made arrangements to translate the "International Principles for Responsible Shrimp Farming" into Myanmar language, which will be used as one of the key resource materials for the workshop.
- Support two young people to study Masters Degree in the areas of fish health and application of genetics in broodstock management.

It was also apparent that Myanmar is going through some rapid changes in inland aquaculture, particularly in response to the market demand for carp species such as *Labeo rohita* and *Catla catla* in the Middle East. These new developments are driving changes in culture techniques and systems and are in turn imacting on local consumers. These effects will soon be reported in an article in Aquaculture Asia Magazine.

Workshop on insurance in aquaculture to be held in Bali, May 2007

The small scale farming sector hardly benefits from insurance coverage. Recent surveys, studies and forums all show that aquaculture insurance services do not reach the small-scale aquaculturists.

Taking cue from the findings of the Regional Conference on Insurance and Credit for Sustainable Fisheries Development in Asia, Tokyo November 1996, the Review of the Current State of World Aquaculture Insurance, the 10th Aquaculture Insurance and Risk Management Conference, Spain April 2006, and the FAO report Livestock and Aquaculture Insurance in Developing Countries", FAO, NACA and the Asia-Pacific Rural and Agricultural Credit Association or APRACA have agreed to hold a workshop on the Promotion of Aquaculture Insurance in Asia. It will be organized in cooperation with the Government of Indonesia's Directorate General of Aquaculture and is scheduled for 30 April-2 May in Bali, Indonesia.

Participants have been invited from governments, the insurance industry, credit institutions and aquaculturists and their organizations, as well as from development assistance agencies. The Bali workshop aims to:

1. Raise awareness among policy makers of the positive aspects and limitations of insurance as a risk management tool for the sustainable development of the sector as well as awareness of other risk management tools such safe and remunerative savings and deposit facilities at banks or credit cooperatives that might, in some instances, be more attractive and provide better alternatives for small-scale aquaculturists.
2. Present the aquaculture sector to the insurance industry and illustrate its potential as a profitable segment to insurers in search of new markets.
3. Discuss ways and means to support aquaculture management and development through the introduction of insurance as an additional risk management tool. Under this objective, two issues will be tackled: i) The constraints of establishing insurance programmes such as high administration costs for the insurance companies due to small and dispersed farmers and poor infrastructure and limited ability of fish farmers to pay premiums; and ii) how insurance can contribute to improving livelihoods.
4. Arrive at a consensus with regard to guidelines for insurance in support of aquaculture development in Asia and what such guidelines should include.

The prospectus and draft program of the Workshop are available for download. Inquiries may also be sent to raymon.vananrooy@fao.org or to NACA at: pedro.bueno@enaca.org.

Catfish 2007 Vietnam

An international technical and trade conference on catfish will be held from 12-14 June 2007 in Ho Chi Minh City, Vietnam, in conjunction with VIETFISH 2007, 14-16 June 2006. The conference is aimed at industry players, policy makers and planners, farmers and export-processors, imports, investors and suppliers of inputs and services.

The catfish has emerged as a bright new star in the international seafood industry in the last few years. This first ever comprehensive business-oriented international conference on the commodity will take a close look at the interesting developments that are taking place in the industry. Among topics that will be discussed are:

- Production and trade trends.
- Status in various producers countries and regions.
- Recent developments and trends in major markets
- The "catfish wars" and their effects.
- New products and marketing strategies.
- Technological developments and issues.
- Environmental aspects and sustainability.
- Prospects for the future.
- And many, many more.

The meeting is organized by FAO-GLOBEFISH, VASEP, the Ministry of Fisheries of Vietnam, INFOFISH and NACA.

Tilapia 2007 Kuala Lumpur

An international technical and trade conference and exposition on tilapia will be held from 23-25 August 2007 in Kuala Lumpur, Malaysia. The meeting will provide an important forum for industry leaders, policy makers and planners, aquaculturists and producers, export-processors and importers, investors and suppliers of inputs and services.

Significant developments have taken place in the global tilapia industry since 2001 when the first comprehensive, business-oriented international technical and trade conference on the species was held. Production of farmed tilapia increased by more than 40% from 1.27 million mt in 2000 to 1.82 million mt in 2004. Meanwhile, landings from capture fisheries rose modestly from 0.68 million mt to 0.72 million mt in the same period. The total production from both capture and culture thus rose from 1.95 million mt to 2.54 million mt, an increase of 30% within a period of just five years.

Total farmed production in 2004 alone was estimated to be worth US$ 2.2 billion, up 30% from five years earlier. Major developments have taken place in the form of growing domestic and international markets, introduction of a wide range of value added products, expansion of farming and advances in culture technology. All these, and more, will be discussed in depth at TILAPIA 2007 KUALA LUMPUR, the much awaited follow-up to the 2001 event. The conference will be accompanied by a trade exhibition featuring companies involved in growing, processing and marketing tilapia and providing various inputs and services to the industry. The conference is being co-organized by INFOFISH and NACA. For more information, please contact the conference secretariat.

Further information:

For further information on both conferences please contact the conference secretariat at: INFOFISH, 1st Floor, Wisma PKNS, Jalan Raja Laut, P.O. Box 10899, 50728 Kuala Lumpur, Malaysia, Tel: (603)- 26914466, Fax: (603)-26916804, E-mail: infish@po.jaring.my; or visit www.infofish.org.

Network of Aquaculture Centres in Asia-Pacific

Mailing address:
P.O. Box 1040,
Kasetsart University
Post Office,
Ladyao, Jatujak,
Bangkok 10903,
Thailand

Phone +66 (2) 561 1728
Fax +66 (2) 561 1727
Email: naca@enaca.org
Website: www.enaca.org

NACA is a network composed of 17 member governments in the Asia-Pacific Region.

NACA Fisheries Team meets with New Governor of Aceh

Continued from page 44.

The meeting was highly positive with strong interest in the support for livelihood recovery being provided by ETESP, with the Governor-elect showing special interest in aquaculture and fisheries activities. NACA is contracted by ADB to support implementation of the Fisheries Component of ETESP and looks forward to working with the new Governor and his team in this new phase in the development of the province of Nanggroe Aceh Darussalam.

Four issues per year

Available as a free download from

www.enaca.org

Support us with a hardcopy subscription:

US$20/year in NACA member countries

US$30/year elsewhere

To order, write to:

The Editor, Aquaculture Asia

PO Box 1040

Kasetsart University Post Office

Ladyao, Jatujak

Bangkok 10903 Thailand

Fax: +6 (2) 561 1727

Email: simon.wilkinson@enaca.org