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AQUACULTURE ASIA

Farming carps in leased ponds, Bangladesh

Seed production of mud crab in India

Mesocosm technology for grouper aquaculture

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Nursery rearing of silver barbs

Utilisation of fish wastes

Lymphocystis disease

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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.

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Lessons learned the hard way

After a recent meeting of CONSRN a number of the participants gathered for an informal discussion of 'lessons learned' in recovery from the tsunami disaster. Many issues were discussed, but there was one in particular that stood out: The need for better coordination in the delivery of assistance to tsunami-affected communities. Despite the enormous resources that donors have put into providing emergency assistance and now longer-term rehabilitation support, it is very clear that most donors do not want to fund coordination activities. They want to fund provision of material aid.

I think I understand the psychology of this: There is a natural tendency for donors to want to make sure that every last dollar is spent on the needy rather than administration or red tape. However, it is entirely obvious to everyone involved in the recovery that this isn't happening: Duplication of effort among donors is rife. Misallocation of assistance is rampant. Needy people are being given things that they don't need at all. Worst of all, some people have received no assistance to this day.

The overwhelming consensus was that coordination of donor assistance should not be regarded as some sort of administrative overhead. Coordination is essential to ensure the efficient use of resources, or if you want to look at it in a different light, to prevent wastage of resources still desperately needed by many people.

I will leave you with a few other thoughts from the discussions that caught my attention:

- Organizations (and individuals) that aren't equipped to engage in disaster recovery should seriously consider their own capacities before deciding to get involved. A lot of resources have been spent in managing people that wanted to help, but couldn't.
- Streamlined rules and procedures are needed for organizations to be able to react quickly to emergencies (see the point above!). There have been many examples where an organization's internal bureaucracy prevented it from delivering assistance in a timely way, or where donors demanded excessive reporting on the expenditure of small grants.
- Offers of cash or entitlement systems are often more useful than offers of expertise or goods, which frequently do not match actual needs. It is better to empower people to buy/select their own assistance than to procure it for them.
- Work towards helping people to help themselves. It is better to strengthen existing local organizations than to try and set up new ones, and to employ local or national staff where possible.
- Working through community groups can help avoid many of the problems encountered when working with individuals.

Probably the greatest lesson is that these lessons are nothing new. They are recurring themes in natural disasters around the world that should have been learned by now, at least by international donors. Unfortunately it seems that human nature is that we always have to learn things for ourselves. The hard way.

Simon Wilkinson

AQUACULTURE ASIA

In this issue


Page 7


Page 11


Page 15


Page 17


Page 22


Page 26


Page 36

Notes from the Publisher

The investment that is NACA

NACA did not go the way many projects had gone; it flourished after project funding ended. This largely reflects the correctness of the strategy of FAO and UNDP in establishing a network organization based on Technical Cooperation among Developing Countries, and the wisdom of the NACA Governments' decision to continue to invest in NACA. This is the third of a series on "The Lessons from NACA." The earlier ones are in the previous issues of Aquaculture Asia. It casts NACA as a public investment and, with some figures, tries to answer the question as to whether it is paying off.

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The annual percent rate of growth or APR of the value of aquaculture production in Asia between 1988 and 1997 (in the wake of the operation of the UNDP/FAO Regional Project to establish NACA) was 11 percent by volume from 13.4 to 34 million metric tonnes and 9 percent by value from US$ 19.3 billion to US$ 42 billion. In perspective, the total cash input over 11 years between 1981 and 1991, from donor and government funds to the NACA Project and the UNDP/FAO Seafarming Development Project (this 4-year project was also managed by NACA), was US$ 9 million.

Carrying on the investment

The NACA Project evolved into an autonomous organization and expanded its scope and operations after project funding ceased. For example, the Regional Seafarming Project was absorbed into the NACA program and the seafarming centres became part of the regional network. As an independent body owned and operated by its member-governments, NACA adopted a change in operational strategy. It had to (i) become self-sustaining in order to finance core activities such as technical advice, information exchange, and network coordination and administration, (ii) generate revenues by provision of services against payments, (iii) develop programs and projects for collaborative assistance, and (iv) forge partnerships with other institutions. These measures made it possible for NACA to continue as a focal point for the implementation of multilaterally and bilaterally funded regional and national projects.

NACA also operates, on request, government-funded or bilaterally-funded national projects. Two cases can provide an example of this aspect of its program, in India (government-funded) and Vietnam (donor-funded). An important point to be made of these two cases is that the results of national projects are shared among countries through NACA's networking and TCDC activities. The experiences in India had in fact informed the work in Vietnam and Iran on shrimp health management. In turn, these have been benefited by the results of ACIAR-assisted projects (in which NACA is also involved) on shrimp disease in Thailand and Indonesia. ACIAR has also embedded a research and capacity building project into the project in India. A second point is that external expert assistance is minimal; a cadre of young local professionals and techni-

Start up funding

UNDP/FAO project funding to the NACA Project totaled US$ 7.2 million, with an additional US$ 800,000 for the Seafarming Development and Demonstration Project that NACA also managed from 1987 to 1991. Participating governments contributed to the NACA Project US$ 804,500 from 1985 to 1989 including US$ US 400,000 by China PR (TCDC/IPF) and the Thai Aid Programme in support of special activities, and voluntary contributions from Bangladesh, Indonesia, India, DPR Korea, Malaysia, Nepal, the Philippines, Singapore, Sri Lanka, and Viet Nam, which were then participants of the Project. In-kind contributions could not be estimated but the Chinese, Indian and Thai governments upgraded from their own national funds, with assistance from UNDP, the Regional Lead Centres (in Wuxi, Bhubaneshwar and Bangkok) to very high standards in line with their participation in the network as lead centres. The Philippines' Aquaculture Department of SEAFDEC hosted the Regional Lead Centre in the Philippines as well as the Senior Aquaculturist Training Programme, which ran for 9 years and graduated 137 senior personnel from eight classes (the degree was awarded by the University of the Philippines in the Visayas (UPV), which collaborated in teaching the program). Investments to the 10-year project and an additional year for the Seafarming Project (1987-1991) was therefore around US$ 9 million. Various other sources of assistance, mostly from donor organizations like IDRC, ADB, CIDA, Commonwealth Fund, JICA, USAID, ODA (now DFID), the AusAid and ACIAR, were generated for the numerous specific training, research, and information activities and for exchange of experts.

cians is trained to provide the technical assistance to the farmers. Capacity building activities includes the farmers associations and the institutions providing farm services.

Since 2000, NACA has been providing technical and management assistance to India's Marine Products Export Development Authority (MPEDA) in a shrimp health management program for small farmers. Starting with a few farmers in Andhra Pradesh, it has expanded to 900 farmers in five states. The project is fully funded by the Government's Ministry of Commerce, which has disbursed, in 2000-2005, the sum of US$ 81,400 (paid directly to NACA for expert assistance on the various studies that led to the formulation, start-up and initial assistance to the project) and Indian Rupees 3.05 million (or around US$ 70,000) for in-country activities including the salaries of a team (at present 15) young Indian technicians, recruited and trained by NACA, MPEDA with expert assistance from ICAR through its Central Institute of Brackishwater Aquaculture. This team provides technical assistance to farmers. The technical team works with MPEDA's field officers and is provided backstopping from ICAR's CIBA and the NACA Headquarters (HQ). One of the technical backstopping personnel is a shrimp farmer from Thailand. Funds for HQ and external assistance personnel came from an ACIAR grant of AU$ 56,000 AUD. The project has recently expanded to five states and now includes a marketing component. After the study and planning phase, NACA has not taken a single Rupee out of India in helping operate this project. A second phase has been approved by MPEDA.

The other case is NACA's support to a donor-funded (DANIDA) Fisheries Sector Support Program in Viet Nam. NACA was also requested by the Government to provide technical support to the Brackishwater and Mariculture components of the project. A NACA-recruited aquatic health management officer was fielded and took the lead role in the health management component of the project, which essentially was focused on shrimp. A NACA field office was established in Hanoi and from implementing DANIDA-supported project, has diversified into providing assistance to other donor-funded projects, also with backstopping from HQ. The value of the NACA-implemented component of the project was US$ 326,500 from 2003 to 2005. The Government has made the same request to NACA for continuing its support for the second phase of the project.

Ongoing contributions

The total government core contribution to NACA from 1991 to 2005 has been US$ 4.42 million. The total external and other non-core sources of funding it generated over the same period was US$ 10.53 million or a ratio of 2.38, which is in effect the amount generated for every dollar invested by governments. It has been increasing: The average ratio for 2000-2005 was 2.63, and those in 2004 and 2005 were 2.97 and 3.24, respectively.

The in-kind contribution of members has not been quantified, but can be illustrated: China P.R., starting in 1992, took over and funded under its Technical Cooperation among Developing Countries (TCDC) program the 3-month training course on integrated fish farming (IFF) at the NACA Regional Lead Centre in Wuxi. The course intake is usually 40 from Asia-Pacific, Latin America, Africa, the Near East and Eastern Europe. Over 25 years of uninterrupted yearly offering, the IFF has trained nearly 1,000 personnel. Centres in Thailand, Indonesia, and India also offer or host regular and periodic courses for personnel from government, industry, farmer associations and NGOs. Their courses are partially supported by the governments. Secondly, regional projects require national coordinators and the governments and sometimes universities provide the institutional (and a person) focal point for these regional projects, on an honorary basis.

Investment focus in Asian Aquaculture

The viability and relevance of NACA as an investment can be seen in a better perspective in the context of the progress of the sector. Between 1976 - when the idea of a network organization was hatched during the 1976 Kyoto Global Conference on Aquaculture organized by FAO/UNDP - and today there have been four discernible areas of emphasis in Asian Aquaculture: (1) Higher productivity and better returns; (2) better environmental performance; (3) enhanced livelihood opportunities and socially responsible farming; and (4) market access and trade. These four areas are not mutually exclusive; while emphasis shifted over the years, the focus of investments broadened to eventually embrace all four, described as follows:

Higher productivity and returns

As expected, in a newly emerging industry, the years after the Kyoto Conference of 1976 saw the establishment of pilot-scale models to test technical and economic viability of commercial-scale operations. These were initially based on existing basic information on new aquaculture species and farming systems. To improve productivity and attract public investments in more research and private sector investments in commercial farming, R & D gave priority to better production technologies as well as species development. Traditional production systems largely developed as an art by farmers through the ages, e.g. integrated fish farming in China and composite culture of several carp and other species in the Indian subcontinent which produced much more biomass and used farm energy and wastes, began to be studied by scientists. This enabled technological improvement on the systems and made them more susceptible to dissemination and adaptation to other countries.

To increase the impact of innovations, a mechanism to coordinate and integrate the various and increasing number of R & D initiatives that began to get underway was devised. This would also avoid duplication of efforts and therefore waste of investments. It enabled researchers, otherwise isolated and working alone, to exchange results and collectively improve on them. It used scarce national resources, pooled through cooperation, more cost-effectively. The model, designed and implemented through a UNDP/FAO global programme on aquaculture development coordination, was regional networking among aquaculture centres run by governments and operating under the principle of TCDC - technical cooperation among developing countries - in short, NACA. To bring aquaculture on a par with livestock husbandry became the long-term objective of NACA. This required intensified disciplinary and interdisciplinary research. The mechanism encouraged and promoted basic and applied research, as well as the application to aquatic organisms of knowledge from research and farming systems development of terrestrial animals. The orientation of technology development and transfer and capacity building during this stage was the expansion of regional aquaculture development in general and commercial–scale aquaculture enterprises in particular. The provision of credit was linked to a feasible business plan.

Better environmental performance

If the global growth of aquaculture between 1980 and 1990, which was sustained at 10 percent, can be an indicator, the NACA strategy, as advocated by the Kyoto Declaration, had succeeded and the investment on the NACA project had paid off. More than that, this growth owed much to aquaculture becoming an increasingly science- and technology-based activity. The rapid growth from the mid-80s was carried into the mid-90s and spurred further expansion and intensification. This rapid and generally uncontrolled growth however raised concerns of its effects on the environment, natural resources, other sectors, and its own sustainability.

Increasing fish disease problems drove home the message that aquaculture as practiced is threatening its own continued viability. An ADB-NACA regional study in Asia-Pacific[1] in 1989-90 for the first time came up with an estimate of losses to aquaculture from diseases of US$1.4 billion a year and made farmers aware of the links between disease occurrence and environmental deterioration. This spurred investments into improving policies, regulations, management systems, and regional and national capacities for aquatic animal health management. The harsh spotlight trained on shrimp aquaculture drew investments away from production technology to more environmentally benign systems and technologies, and more efforts on the development of regulations and policies to lessen or manage the impacts of aquaculture on the environment and on itself.

UNDP/FAO/NACA spearheaded a regional development project (1987-1991) that promoted exchange of seafarming technologies among countries, training of technicians and farmers, and the planning and orderly management of coastal aquaculture[2]. Guidelines were developed for planning. Coastal aquaculture was then beginning to expand with increasing emphasis on shrimp aquaculture and cage culture of finfish. This regional seafarming development and demonstration project, apart from promoting the exchange of technology among countries and training seafarming practitioners, provided or developed regulatory and management guidelines that improved the environmental performance of aquaculture farms.

FAO and NACA, through a regional TCP[3] in 1992-1993 conducted a regional study on Environmental Management and Assessment of Aquaculture Development that focused on the different impacts of aquaculture on the environment and on itself and the other sectors impacts on aquaculture. The study highlighted technologies, practices, and capacities that needed to be developed.

The earlier ADB/NACA study on health management and the FAO/NACA TCP on environmental assessment and aquaculture development prompted governments to look for more guidelines for sustainable farming systems and the policies that would ensure them. This was met by an ADB/NACA Regional study[4] on "Aquaculture Sustainability and the Environment". A product of this study was the Aquaculture Sustainability Action Plan adopted by the Developing Member Countries of ADB. The farmer representatives to the final workshop of this project (October 1995, Beijing) also requested NACA and ADB to initiate activities to organize a regional aquafarmers' network. NACA has since taken this recommendation up with a number of activities.

With greater awareness of the importance of health management governments and private sector began to invest more in capacity building for disease prevention and control. At this stage NACA, OIE or World Animal Health Organization and the FAO formed a stronger alliance for health management and implemented in Asia-Pacific various collaborative regional projects. Notable among these were (i) an FAO TCP[5] project with NACA on Responsible Movement of Live Aquatic Species that led to the formulation of the Technical Guidelines and National Strategies for Responsible Movement of Aquatic Animals, Disease Diagnostic Guide, and Surveillance, Reporting and Information System for Aquatic Animal Pathogens; and (ii) a capacity building project for import risk assessment with APEC[6] as well as the SPC, ASEAN and SEAFDEC and various other organizations, that involved APEC economies, other non-APEC countries in Latin America and the Caribbean as well as in Asia-Pacific. Others included harmonization of procedures on introductions; development of action plans to implement the various policies on introductions, early warning and preparedness; specific studies on introduction of certain species like *P. vannamei*; a workshop on aquatic invasive alien species; molluscan health, and policy development on introductions, etc. Expertise from countries with advanced knowledge and experiences in these areas such as Australia, Canada, France, Japan, New Zealand, Japan, U.K and the U.S.A were brought into the region through these various linter-linked and collaborative projects.

It was during this period that the environmental and socio-economic issues took primacy over the productivity, economic viability, and profitability concerns. Economic concerns broadened from cost- and-returns (private benefit) to internalizing environmental and social costs of polluting and resource-degrading practices (environmental benefits). The influence of this period on attitudes of farmers is that it made good business sense to be environmentally responsible.

Better livelihoods and social responsibility

At the close of the decade of the 90s, a regional aquaculture planning workshop (in August 1999) attended by 19 Asia-Pacific governments came up with the assessment that aquaculture in most Asian countries generally had become a better-organized economic sector, characterized by stronger private sector participation and increasing state support. It noted a number of fundamental shifts:

- that farmers' aspirations for higher yields and better returns from innovations in production technology have been tempered with concerns for sustainability;
- that the aim of gaining higher returns has been joined by schemes to share benefits equitably; and
- that the primary purposes of producing more food, earning higher incomes and improving economies have expanded to ensuring that enough food is produced and made accessible to the masses and that the poorer participants in the aquaculture sector gain a better livelihood.

The observation on equity and livelihoods reflected the increasing attention by development assistance institutions, donor agencies, civil society, and governments on the impact of aquaculture and aquatic resources exploitation on the societal objectives of poverty alleviation and assuring food security. The review on financing and institutional support for aquaculture development made at the 2000 Conference on Aquaculture in the Third Millennium noted that international development assistance was increasingly directed towards poverty alleviation, and urged that the assistance needs to adhere to basic principles of social equity, including gender, environmental sustainability, technical feasibility, economic viability and good governance.

The FAO Code of Conduct for Responsible Fisheries was adopted in 1995. During this stage, research, technology, policy and institutional services were increasingly oriented to the needs of small producers including subsistence farmers, and paid more attention to the circumstances of the disadvantaged groups (the landless, the women especially those that are heads of families, aquatic product gatherers, farm workers, etc.). Social responsibility, in addition to environmental friendliness of aquaculture, began to permeate project planning during this period. Ways to focus aquaculture on poverty alleviation (or not having it exacerbate poverty) were studied.

It was at this period when the trend began in the down-sizing of most public-funded initiatives. Among other responses, assessments were carried out on the impacts of the R & D efforts of the past 20 years. Generally, because of the regional coordination mechanism, it was found to be cost-effective: resources were pooled, results were shared, efforts were not duplicated, and governments did not have to go through the costly exercise of reinventing the wheel. Mixed results however were found in local applications. The findings highlighted that the effectiveness of research application depended on institutional capacities. The outcome was to include institutional strengthening as a researchable issue. Thus began the research studies and pilot programs on co-management, voluntary management mechanisms, and more broadly participatory planning and implementation mechanisms. Aquaculture planning became integrated into overall rural development planning.

Better access to markets and fair trade

This is the current area of priority concern and in which governments have been investing resources, spurred largely by food safety issues that ramified into broader market access and trade issues. They now include eco-labeling to enable consumers to express their environmental and social concerns. Farmers now have to contend not only with the quality and price of products but how they are produced and what impacts the farming practice has on the environment, biodiversity and welfare of farm workers (now also of the fish).

The global trade liberalization agenda has had a marked impact on seafood trade. Resolutions and agreements on market access issues, regulatory measures on health and food safety requirements, and a host of other forms of technical barriers to trade are expected to affect seafood exports, especially from developing countries. A driving force has also been the need to comply with an ever-increasing number and stringency of market requirements. The flashpoint likely had been the rejection of shrimp exports by EU but a combination of technical barriers of trade, Sanitary and Phyto-Sanitary measures, and non-tariff barriers to trade prompted the broadening and hastening of initiatives that were already in place such as ASEAN's focus on competitiveness in trade, and the Consortium (of FAO, NACA,WB, WWF and UNEP) on Shrimp Farming and the Environment's work on international principles for responsible shrimp farming that are aimed at developing uniform certification standards and best management practices.

Government and private sector institutions are developing policies on and embarking in food safety programmes (i.e. Thailand's "Farm to Plate") like HACCP, investing in research, extension, hardware (to detect banned antibiotics and drugs at the level required by importers and train personnel to run the HACCP schemes and operate the equipment) investing in IT program development (for traceability, as in Thailand), and developing regulations as well as promoting voluntary management mechanisms such as Codes of Conduct and Best Management Practices to support producers, especially the small-scale, address the complex issues surrounding food safety and ecolabelling.

The response of Governments and of the production and processing sectors are seen as beneficial to aquaculture in the long run, largely by making the sector more competitive and environmentally responsible. On the other hand, apprehensions have been expressed as to their impacts on the small and poor farmers, which do not enjoy the economy of scale to be able to comply cost-effectively with the requirements. On this point, experiences from NACA projects and those under the STREAM Initiative[7] are providing examples that organizing small farmers and poor aquatic gatherers and adoption of voluntary mechanisms like BMPs and Codes of Practices can improve their productivity and quality of their product, provide environmental benefits, enable them to attain economy of scale and be able to transact with suppliers and buyers on a stronger footing and at less cost, and comply with increasingly stringent market access requirements.

NACA's development coincided with these four areas of emphasis and its regional work programme addressed the associated issues. To operate the work programme and address the issues, NACA generated support for major regional and national activities from bilateral, multilateral and investment agencies; since 1990 there have been more than 65 collaborative projects, workshops, assessments, and information development activities of regional, subregional and national as well as inter-regional scope. In the aftermath of the tsunami, NACA became involved in various planning, learning, and management activities to restore livelihoods and develop the stricken communities. It joined a consortium8 comprising BOBP-IGO, FAO, SEAFDEC and World Fish Centre that has since jointly organized regional activities to develop, with affected countries, NGOs and other organizations, strategies and guidelines for rehabilitating and developing livelihoods based on aquaculture and capture fisheries.

No attempt has been made to measure the internal rate of return to FAO and UNDP's, other donors, and the subsequent Governments' investments in the NACA Project and in the NACA Organization, but the multiplier effects of this small investment, which is little more than US$ 13 million spread over 25 years or some US$ 500,000 a year, could not be small.

References

1. ADB/NACA.1991. Fish Health Management in Asia-Pacific. Report of a Regional Study and Workshop on Fish Disease and Fish Health Management. ADB Agriculture Department Report Series No.1. Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.
2. TCP/RAS/87 and 90. Regional Seafarming Demonstration and Development Project, with nine participating governments including China, Hong Kong , Indonesia, India, Korea DPR, Korea Rep, Malaysia, Philippines, Singapore, Thailand; it operated from 1987 to 1991.
3. TCP/RAS/2253 Environmental Assessment and Management of Aquaculture.
4. ADB-NACA. 1998. Report of the Study and Workshop on Aquaculture Sustainability and the Environment (RETA 5534). Bangkok. 492p.
5. TCP/RAS/6714 (A) and 9065 (A) Assistance for the Responsible Movement of Live Aquatic Animals launched in 1998 and participated by 21 countries (Australia, Bangladesh, Cambodia, China P.R., Hong Kong SAR, India, Indonesia, Iran, Japan, Korea (DPR), Korea RO, Lao PDR, Malaysia, Myanmar, Nepal, the Philippines, Pakistan, Singapore, Sri Lanka, Thailand, Viet Nam).
6. Arthur, J.R., M.G. Bondad-Reantaso, F.C. Baldock, C.J. Rodgers and B.F. Edgerton. 2004. Manual on risk analysis for the safe movement of aquatic animals (FWG/01/2002). APEC/DoF/NACA/FAO. 59 p. APEC Publication Number: APEC # 203-FS-03.1.
7. STREAM (short for Support to Regional Aquatic Resources Management) was established by a consortium of DFID, FAO, NACA and Voluntary Services Overseas, an international NGO. The fore-runner of STREAM in NACA was an earlier FAO-NACA programme called Aquaculture for Sustainable Rural Livelihoods Development, which was subsequently recast into an initiative based on DFID's Sustainable Livelihoods Approach. See www.streaminitiative.org.
8. CONSRN is the acronym of the Consortium to Restore Shattered Livelihoods in Tsunami-Devastated Nations. See www.apfic.org. Go to the tsunami web page for CONSRN Reports.

Nursery rearing of Puntius gonionotus: A preliminary trial

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Carps form the backbone of freshwater aquaculture in India constituting about 85% of the total freshwater aquaculture production. Ever since the standardization of carp breeding technology in India during the early seventies, the major share of freshwater fish production in the country has been through six species of carps; namely the three Indian Major Carps (rohu, *Labeo rohita*; catla, *Catla catla* and mrigal, *Cirrhinus mrigal*) and three exotic carps (grass carp, *Hypothalmichthys molitrix*; silver carp, *Ctenopharyngedon idella*, and common carp, *Cyprinus carpio*). The need of the hour in the freshwater aquaculture sector in India is species diversification. The country is endowed with 10-12 varieties of economically important medium and minor carps suitable for exploitation in freshwater aquaculture and that have tremendous scope to complement or substitute for the six prevailing carps varieties in Indian freshwater aquaculture system. Among them, *Puntius gonionotus* is an important medium carp with high consumer preference. This fish was introduced to India during 1972 from Indonesia for controlling aquatic weeds. Later on during the 1990s the culture potential of this species was realized. The advantage of this species over other carp is that it is a self-recruiting species that can breed naturally in lentic water after one year and can grow to a marketable size 0f 200-300g within 3-4 months.

Nursery rearing is an important aspect of carp culture that can greatly affect the total fish yield at the final harvest. Most Indian farmers use carp fry as a stocking material in grow out ponds, which are easily susceptible to predation by carnivorous and weed fishes that are found in the ponds. Even when farmers attempt control measures to eliminate these unwanted fishes during pond preparation (before stocking the seeds) they often manage to enter again. However, it is often observed that when advanced nursery-reared carp fingerlings are stocked in ponds the survival rate of fish at the final harvest is greater than 90% as the larger size sized seed are less vulnerable to predation. Therefore, the nursery rearing of carp for a period of 2-3 months is prerequisite for successful carp culture. Although there are many reports of nursery rearing of three Indian major carps and three exotic carps, there is little information available on this aspect of culture for the silver barb *Puntius gonionotus*. Therefore, in the present study an attempt was made to document strategies for the nursery rearing of advanced *P. gonionotus* fry for a period of two months in an earthen nursery pond using a balanced formulated feed. The growth, survival, and other nutritional indices of the fish were studied in detail.

Approach

Pond preparation

A 0.04 ha nursery pond was selected for the experimental trials. The water was pumped out completely during the month of July then bleaching powder (30% active chlorine) was applied at 30 kg/ha in order to eradicate unwanted predatory and weed fish. Almost all fishes were killed within 2-3 hours of bleaching application and removed by hand picking. After seven days of bleaching power application the pond was refilled to a depth of 1m depth by pumping. The pH level of water was maintained at 7.6 by application of lime at 100 kg/ha. After two days of lime application, the first dose of manure in the form of row cow dung was applied at 10,000 kg/ha. Subsequently, urea and single super phosphate was applied at 30kg and 100kg/ha, respectively. Plankton was collected using a plankton net (100 m mesh size) from other fish culture ponds and then released to the experimental nursery pond as an innoculum. After seven days sufficient plankton had developed, assessed by filtering 50 litres of water from different part of the experimental nursery pond. The plankton density was found to be 1.5 ml-2.0 ml/ 50 litres of water. Coir ropes were tied in 1 m intervals above the pond using bamboo poles across the length and breadth of the dykes so as to scare the predatory birds and check their entry in to the pond.

Procurement of seed

Fry of *P. gonionotus* (average weight 0.4±0.02 g) were procured from the hatchery of the Central Institute of Freshwater Aquaculture, Bhubaneswar during the morning hours. The stocked fish were acclimatized to the ambient temperature of nursery pond for three hours and then released at a stocking rate of 100,000/ha.

Feeding and other management practices

Twenty-four hours after stocking, the fish were fed with a powdered basal diet. The percent and chemical compositions of experimental diet are shown in Table 2 and Table 3, respectively. The feed was supplied to the fish at 10% of body weight for a period of 30 days and then reduced to 5% for the next 30 days of the experiment. The fish were fed twice daily at 10.00 and 15.00 hours. The growth of the fish was observed in every 15 days and the daily ration was adjusted accordingly.

Male (above) and female (below) Puntius goniotus after 120 days of culture.

Intermediary application of fertilizer was conducted at 50kg and 100 kg/ha of urea and single super-phosphate, respectively. The pond was manured with a second dose of raw cow dung at 5,000 kg/ha after one month of stocking.

The growth data indicated that the fish grow from an initial average weight of 0.4 ± 0.05 g to 15.6 ± 0.43 g within 60 days. The observed survival rate was 89.64%. The total weight gain (g/fish), average daily weight gain (mg/day/fish), percent weight gain, nitrogen intake (mg/day/fish), food conversion ratio, specific growth rate, net protein retention, protein efficiency ratio, energy retention efficiency (%), lipid retention efficiency (%), normalised biomass index, average hepatosomatic index, average viserosomatic index, thermal growth coefficient and instantaneous growth rate were studied and are presented in Table 4. The initial and final carcass compositions of the fish are shown in Table 5. The range of water quality parameters are depicted in Table 1.

Discussion

In India, the practice of raising the fry to fingerling size and to sell them at a better market price (Rs.10,000-15,000/thousand fingerlings of size 15.0 to 20.0g) is now fast becoming a profitable seasonal business for small and marginal farmers particularly during the monsoon season. These days many farmers prefer to stock advanced fingerling over the conventional method of stocking fry in cultured fish ponds. When stocking advanced fingerlings in grow-out ponds the survival rate found to be better as they are big enough to protect themselves from predation by birds and carnivorous fish generally found inside the culture pond. Another benefit is that fish can also attain the marketable size in 7-8 months instead of 10-12 months by stocking advanced fingerlings. Usually farmers rear the fish in nursery pond at a higher stocking density. In the present experiment, the fish were stocked at a stocking density of 1,00,00/ha and supplemented with good quality balanced feed having 30.27% crude protein and 3.85 kcal/g energy levels. Optimum water quality was maintained throughout the experi-

Table 1. Range of water quality parameters during the study.

Parameters	Range
Temperature (°C)	26.5 – 28.3
PH	7.5 –7.8
Dissolved oxygen (DO) (mg/l)	5.2-5.6
Alkalinity (mg $CaCO_3$/l)	112.23-117.29
Hardness (mg $CaCO_3$/l)	105.13-108.93
NH_3N (mg/l)	0.06-0.15 mg/l
NO_3-N (mg/l)	12-18 mg/l
NO_2-N (mg/l)	0.08-0.12 mg/l
P_2O_5-P (mg/l)	0.05 mg/l
Transparency (cm)	35-42

Table 2. Percent composition of feed used in the experiment.

Feed Ingredients	Percent composition
Fish meal	10
Ground nut cake	25
Soybean	30
Rice bran	32
Mineral and vitamin mixture	3

Table 3. Proximate composition of feed used.

Parameter	Percent composition
Dry matter	89.25
Crude protein	30.27
Crude fibre	9.55
Ether extract	8.95
NFE	40.48
Energy (kcal/g)	3.85

Table 4. Growth performance of *Puntius gonionotus* fry in nursery pond.

Parameters	Value
Average weight gain (g) of fish	15.20
Average daily weight gain (mg/day/fish)	253.33
Percent weight gain	3800
Nitrogen intake (mg/day/fish)	11.07
Food conversion ratio	0.98
Specific growth rate	6.105
Condition factor	1.11
Protein efficiency ratio	3.365
Protein retention efficiency (%)	45.41
Lipid retention efficiency (%)	61.25
Energy retention efficiency (%)	30.11
Normalised biomass index	13,484
Average hepatosomatic index	2.95
Average viscerosomatic index	9.42
Thermal growth coefficient	1.005×10^{-3}
Instantaneous growth rate (g/day/fish)	0.114

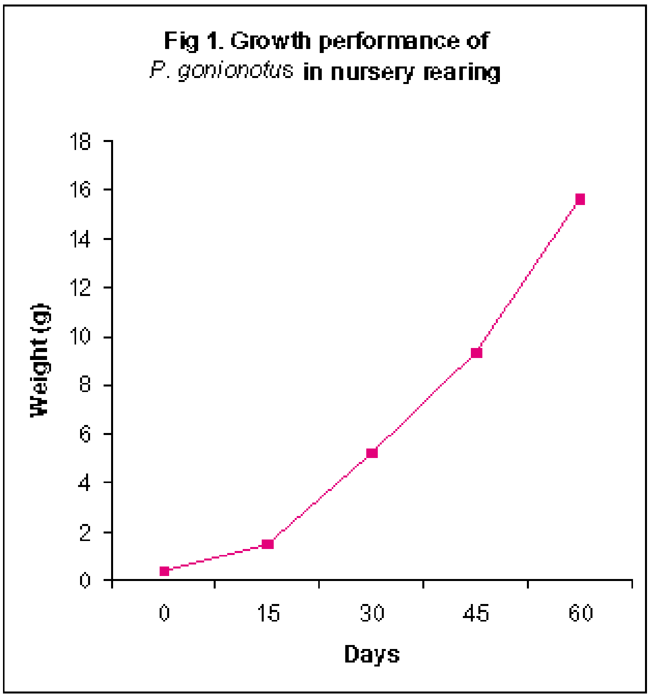
mental period (Table 1). The incidence of disease and abnormality were not found in the cultured fish. The observed survival rate 89.64% of *P. gonionotus* from fry to fingerling in the present experiment was found to be better than the survival rate obtained in the nursery rearing of other carps as reported elsewhere by earlier workers. For calculation of food conversion ratio, the nutritional contribution of planktons in pond was not considered. Therefore, the food conversion ratio found in the present experiment might have less than 1.0. The other nutritional indices of growth performances are also found to be satisfactory (Table 4). Aside from ether extract, which increased from 14.25 to 22.92 %, there was not much difference in the initial and final body carcass compositions of the fish, which indicated that as the fish grows the lipid content in the muscle increases.

Conclusion

The outcomes of our study indicate that in nursery rearing of *Puntius gonionotus* fry at a moderate stocking density of 100,000/ha with a provision of balanced feed and optimum water quality maintenance, a survival rate of as high as 89.64 % with 3800 % weight gain over initial can be achieved within 60 days.

Table 5. Initial and final carcass compositions (%) of the experimental fish.

Parameters	Initial values	Final values
Moisture	78.0	76.0
Crude protein	56.82	57.73
Crude fibre	6.53	5.86
Ether extract	14.25	22.82
Total ash	15.25	11.10
Acid insoluble ash	6.28	5.92
Gross energy (kcal/g)	4.18	4.85



A haul of Puntius goniotus after 120 days of harvest.

Artemia enrichment and biomass production for larval finfish and shellfish culture

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In aquaculture operations, feed is an important input and typically accounts for 50—60% of the recurring investment. Feed organisms play an important role in the dietary regimen of cultivable fish and shellfish, particularly in the larval stages, with zooplankton, such as copepods, rotifers, larval stages of molluscs and small crustaceans routinely utilized as feeds during larval development. The most important live feed organisms are *Artemia salina*, rotifer *Brachionus plicalitis* and various freshwater cladocerans such as *Moina* spp. Although many artificial feeds have been formulated and compounded none have been found to perform better than the brine shrimp *Artemia*, which is given as a live feed to 85% of species cultivated in aquaculture around the world. Because of its unique nature and role, *Artemia* has revolutionized the aquaculture industry; especially prawn culture and salt production in many advanced countries. The recent developments in aquaculture production have further resulted in increased demands for *Artemia*.

Among the live feed organisms, the brine shrimp *Artemia* comes in for prime consideration because of its nutritional and operational advantages. *Artemia* nauplii are considered to be the best food for mysis larvae and pelagic post-larvae of shrimp and contain about 40—55% protein, 4-20% fat and sufficient amounts of essential amino acids and fatty acids. Although expensive, *Artemia* provides good survival and is more consistent in hatchery production than any other larval food. The ease of feeding *Artemia* and its superior nutritional quality ensure that it will be used in hatcheries for many years to come if supplies can keep up with the demand.

Biotype distribution and zoogeography:

Brine shrimp *Artemia* have a discontinuous distribution in more than 300 natural biotopes in temperate and tropical regions of the world. In nature they are found in hyper-saline lakes, brine ponds and lagoons and man made salterns. Due to geographical isolation, populations of *Artemia* have diversified into more than 150 reported strains. Of the 250 reported areas reported to have *Artemia* populations only a few are exploited for aquaculture purposes. Most of the others have not been surveyed for potential exploitation. Recent find spots of *Artemia* are scattered throughout the tropical, sub tropical and temperate climatic zones along coastlines as well as inland, sometimes at 100 miles from sea. There are numerous scattered reports about brine shrimp occurrence, both in inland salt lakes and in coastal salt works from the Indian sub—continent with over 14 find spots reported from the states of Tamil Nadu, Maharashtra, Gujarat and Rajasthan.

Exploration of *Artemia* populations have been carried out in Europe, USA and Canada thorough inventory campaigns in recent decades. Information about Africa, especially the sub-Saharan part of continent remains limited. Intensive exploration work has conducted in several countries of South America, China, Iran and in the countries of the former Soviet Union with a focus on the Central Asian Republics and Southern Siberia. Although valuable characterization work has been done for many of the main commercially exploited populations and strains, the status of many of the non-exploited populations is less well known, with only fragmentary information available.

Collection from natural habitat

Natural populations of *Artemia* are found in large salt lakes and coastal saline areas. Different geographical strains have adapted to widely fluctuating conditions with regard to temperature and ionic composition of medium. In salt works, *Artemia* is normally only found in evaporation ponds at intermediate salinity levels from about 100 ppt onwards up to 200-250 ppt. At high salinity levels depending on the local strains as well as hydrological conditions in the ponds, cysts are eventually produced and are driven by the wind and accumulate on the shore of ponds. The quality of *Artemia* differs from strain to strain and location to location. *Artemia* are exploited in the form of cysts of adult biomass, mainly for use in aquaculture and shrimp hatcheries. Adult *Artemia* are mainly collected from shallow salt ponds, either directly in water or after being thrown on shore where they accumulate in reddish brown layers, several cm thick and many meters in length.

In the 1970s commercial supplies were mainly from natural sources in the United States and Canada to the extent of 30—50 metric tones/year, but production later improved to 100 metric tones as a result of commercial aquaculture. Many commercial harvesters and distributors sold brands of various qualities. Approximately 90% of the world's commercial harvest of brine shrimp cysts comes from the Great Salt Lake in Utah. It is reported that normally 200,000 to 300,000 nauplii might hatch from each gram of high quality cysts. Good quality cysts can fetch up to US$ 80 per kilo.

On-grown cultured artemia. Resting cysts are beginning to form.

Artemia culture in India

Artemia are widely distributed and found in saltpans and salt lakes throughout the world. The success of rearing technology greatly depends upon the availability of *Artemia* nauplii. Apart from being easy to handle, eggs of brine shrimp are capable of being dormant over a long period and hatching out readily when suitable conditions are given. The eggs in the brood pouch develop into either free-swimming nauplii that are set free by the parent, or when reaching gastrula stage they are surrounded by a thick shell and are deposited as cysts measuring 0.2—0.3 mm in diameter, which are in a state of diapause. The eggs are generally collected from natural sources, dried and stored in containers in a cool and dry place. When introduced into normal seawater, they hatch out within 48 hours at room temperature into tiny nauplii. Instead of seawater, a solution prepared from common salt dissolved in freshwater can also be used as a medium for hatching eggs. After hatching, the free-swimming nauplii are separated from the unhatched cysts and empty shells, since these can interfere with the digestion of larvae and may even cause blockage of the gut.

Although the main source of *Artemia* eggs continues to be the natural grounds in salt pans, attempts are being made to culture them in artificial conditions so as to ensure a steady supply of eggs and nauplii as and when required. The dried *Artemia* cysts are introduced into filtered seawater at the rate of 1 gm/litre and provided with aeration and light, which stimulate the embryonic development. The free-swimming nauplii hatch out within 18-36 hours after hydration in seawater. The nauplii are collected and introduced into a plastic pool or tank at the rate of 1000 nauplii/litre. The salinity of the culture tank is raised gradually up to 80% until that time the larvae mature at around 12—14 days post-hatching. The fertilized eggs develop into nauplii in the brood sac and are released into the culture tank every 4—5 days. The adults may survive up to three months and release around 100—180 young, depending upon the culture environment. Continuous mass culture can be maintained with partial water changes and harvesting. The single cell algae *Chlorella*, *Tetraselmis*, yeast or bacteria cultures are used to feed *Artemia*. Under unfavourable conditions, such as very high salinity and low oxygen levels, *Artemia* switch from production of live young to production of dormant cysts, through secretion of chlorine by a gland in the brood pouch. The cysts released into the water and adhere into sides of the culture tank, where they can be collected, dried and stored in a closed container or in saturated high saline water for up to 6—8 months for future use.

Considerable progress has been achieved in the Marine Prawn Culture Laboratory of the Central Marine Fisheries Research Institute (CMFRI) at Narakkal, where it has been possible to maintain generations of *Artemia* under laboratory conditions. The brine shrimp can easily be maintained in fiberglass tanks or plastic pools containing seawater and fed on yeast and unicellular algae. *Artemia* nauplii and larger stages are fed to penaeid prawn larvae from mysis stage onwards. It has been found that on average an early shrimp postlarvae eats around 80 brine shrimp nauplii per day. Assuming the hatch rate of *Artemia* eggs to be around 50%, it is estimated that around 5 kg of *Artemia* eggs along with 70 kg of minced bivalve meat are required to raise 1 million advanced post larvae to 20 days in age.

Enrichment of *Artemia*

Artemia strains differ in size and nutritional quality, particularly in content of highly unsaturated fatty acid (HUFA). *Artemia* composition is generally in the range of 51—55% protein, 14—15% carbohydrate, 13—19% fat and 3—15% n-3 HUFA. When analyzed on a dry weight basis, cysts of a well-known *Artemia* strain contained 28% crude protein, 10% crude fiber and 10% crude fat. Enrichment techniques for improving the nutritional value of live foods are widely used in marine fish hatcheries. Small *Artemia* cysts with high HUFA content originate from USA and are produced in several parts of the world under controlled conditions in salt operations. Freshly hatched *Artemia* is used as feed as they are most nutritious. However, the lipid level and fatty acid composition of newly hatched *Artemia* nauplii can be highly variable, depending upon the strain. The performance of larval fish is directly related to the level of HUFA in *Artemia* being fed to them and essential fatty acids are the principal food value of *Artemia*. When level of HUFA is low, survival of larval fish also declines.

The premium quality *Artemia* with high hatching rates, small size and greater HUFA levels are relatively expensive and sometimes difficult to find in quantity. To compensate for a poor HUFA level in *Artemia*, they can be enriched with omega yeast, vitamins, marine oils, vitamin B-12 producing bacteria and commercial enrichment media. As an alternative, a hatchery can purchase a commercial enrichment product to treat lower quality *Artemia*. In 1980s, researchers found that fish larvae fed strains containing more than 4% eicosapentaenoic acid (EPA) 20:5 n-3, had significantly better growth than those fed with *Artemia* containing less than 3% EPA. Recent research has suggested that docosahexaenoic acid (DHA) 22:6 n-3 is the most important HUFA for marine fish larvae. These high EPA *Artemia* are equal to HUFA enriched *Artemia* in terms of nutritional value but are helpful in bridging the gap between rotifers and enriched day old *Artemia*.

The essential fatty acids DHA and EPA, and also DHA/EPA ratio, are important for normal pigmentation and affect metamorphosis and stress tolerance in marine fishes. Feeding *Artemia* to cold-water species may have certain negative effects as reported in species like halibut and cod. Halibut fed exclusively on brine shrimp for the first few weeks often appear malpigmented with unnatural morphological appearance. However, natural zooplankton produces high quality juveniles. Consequently, worldwide research is undergoing to develop a feed that can replace *Artemia*. *Artemia* enriched with DHA-rich phospholipid extract are currently being tested in the first feeding of halibut larvae. Larvae of cold water species, e.g. Atlantic halibut, can contain high levels of DHA (25—30% of total lipids) and also have a high DHA/EPA ratio at onset of exogenous feeding, indicating that these larvae need high amounts of DHA and DHA/EPA ratios in their feed. However, such high levels have not been achieved when using traditional feed enrichment products based on emulsified fish oils. Work carried out to test enrichment products that facilitate high content of DHA and DHA/EPA ratios in *Artemia* shows that DHA spray dried phospholipid diets have resulted in the highest DHA content and highest DHA/EPA ratio reported so far. The DHA content of enriched *Artemia* was 17.2% of total fatty acids, which is almost similar to that present in halibut larvae at onset of exogenous feeding. Maximal DHA/EPA ratio in *Artemia* enriched with DHA phospholipid diets, in both sets of experiments, ranged from 2.78—3.8, which is even higher than that found in halibut larvae. These findings suggest that cold-water species and flatfishes need high levels of DHA and high DHA/EPA ratios in the feed.

Through the years *Artemia* have proved to be one of the easiest to prepare and most nutritious foods available to the hatchery managers for rearing larval finfish and crustaceans. There are a number of enrichment products in the market. *Artemia* systems Inve. (Gent, Belgium), Aquafauna Bio-marine Inc. and Sanders Brine Shrimp Company are among the companies that carry these products. A large number of companies are also producing algal pastes or concentrates that can be used as food supplements for *Artemia*.

Evaluation of *Artemia* cysts

The quality of *Artemia* cysts varies according to strain or commercial brand. Lack of information on hatching performance of cysts could also lead to uneconomical utilization. Determination of cyst quality is therefore essential to the efficient use of *Artemia*. Cyst quality can be assessed on the basis of its moisture content, hatching efficiency, hatching percentage and output. Adults as well as cysts can be contaminated with high levels of heavy metals and chlorinated hydrocarbon. Thus quality assessment of adult *Artemia* is essential before its commercial exploitation. *Artemia* cysts and biomass harvested from the lake often have low hatchability and survival percentage. Quality assurance approaches such as HACCP covering all aspects of *Artemia* production including cleaning and washing, processing and drying, storage and decapsulation procedures are important to ensure a consistent quality end product.

Development of farming practices

In India, no systematic attempts have been made in exploitation and scientific culture of *Artemia* so far. Cultivation technology has been at an experimental stage in many of the Indian laboratories for several years. Several research organizations including the Central Marine Fisheries Research Institute (CMFRI), Central Institute of Brackishwater Aquaculture (CIBA) and National Institute of Oceanography (NIO) have done some basic research on laboratory and small-scale field production. The fatty acid (HUFA) contents of the cysts from such trials were about 3.2 mg/g and yields too low at 0.5-2 kg/ha/month compared to international standards of more than 4 kg/ha/month. Tata Chemicals Ltd. and M/s Ballarpur Industries Ltd have also made some attempts at cultivation and produced about 0.5 kg of cyst/ha/month. The low rate of production of *Artemia* cyst is due to the lack of input of scientific techniques in the culture and processing of *Artemia*.

India is reported to have 176,848 ha of saltpan with the current salt production of around 987,000 lakh/tones/year. Due to the low return from salt farming, about 46% of commercially exploited saltpans have been abandoned in India. About 88,420 ha condenser area is available capable of producing more than 1,000 tonnes of cysts, which can be used to produce shrimp in the reservoir through use of *Artemia* biomass as feed. The present requirement for *Artemia* in India is estimated to be around 150 tonnes/year and this is expected to increase to more than 300 tonnes by 2010. This requirement for *Artemia* cysts can be met with an investment of Rs. 1.2 billion on its import. Shortages in the supply of *Artemia* are an impending development in aquaculture due to high global demand and high price of cysts. In India, commercial exploitation has not been carried out in earnest and only a small quantity is harvested in Gujarat for use in the pet fish industry. Thus, there is no serious commercial exploitation of *Artemia* biomass production in India.

Global projection and requirement

Presently the world's yearly consumption of *Artemia* cysts is estimated over 2,000 tonnes. The principle source of the *Artemia* is the Great Salt Lake, Utah, USA. About 10 companies that control the harvesting produce more than 1,500 tonnes of cysts per year, which accounts for more than 90% of the world's commercial production. Although other large salt lakes featuring populations of *Artemia* exist, these lakes have not been commercially exploited, mainly because little is known about their productive potential. On the other hand there is a new market for *Artemia* cysts of high nutritional value, for first feeding in aquaculture operations. Besides cysts, *Artemia* biomass is in high demand due to its high nutritional value for fish and shrimp larvae. This biomass is also utilized by the ornamental fish industry. Live biomass is sold at US$ 2/kg in USA and Europe and frozen biomass at the rate of US$ 5/kg. Cultivation of *Artemia* biomass was developed in China around the Bohay Bay area. More than 20,000 tonnes were collected in the salt works and sold at the rate of US$ 0.2/kg for use as food in larval culture and grow out shrimp culture operations.

Economics and economy in *Artemia* production

The demand for *Artemia* is very high and one kg of cyst produced in India costs Rs. 1,000 to 1,500. Culture and trade in cysts and biomass could generate substantial earnings from domestic and export markets. *Artemia* farming in India is expanding very slowly due to a lack of information on culture techniques. By the turn of century, India is aiming to produce 100,000 tonnes of cultured shrimps, for which seeds have to be produced. Higher production and sales are expected as a result of fast development in shrimp and prawn culture industry and further diversification of *Artemia* products. In view of the rapid expansion of the aquaculture industry it is projected that demand for biomass will increase very significantly in coming years.

The annual production of *Artemia* cysts in India is currently less than one tonne and the quality is low compared to that of imported cysts (6.2 mg/g HUFA and <50% hatch rate) due to poor processing, packing and lack of quality control measures. However, the growth of aquaculture, the unstable world cyst market and the potential for *Artemia* culture within the vast area of India's saltfields suggests that the country can emerge a major exporter of *Artemia* cysts enabling significant earning of foreign exchange. Use of the saltpan land with integrated production will improve the economy (6-8 times). However, appropriate technology packages need to be developed to support the development of this industry.

Establishment of *Artemia* processing plants

Artemia cysts are used to feed shrimp larvae from 3-21 days after hatching in the form of decapsulated and nauplii stages of *Artemia* (live feed). It is estimated that 1 million shrimp seed requires 5-10kg of *Artemia* for each cycle of 21 days. This *Artemia* is being imported from USA (Salt Lake strain) under high import duties. Each kg costs about Rs. 6,000/-. Due to this high cost and difficulty in obtaining sufficient quantities of cysts on time hatchery operators are led to compromise in looking to alternative cheaper feeds, which adversely affects the health of shrimp seed, increasing susceptibility to disease. There is therefore an urgent need to establish a processing plant in India for *Artemia salina* that is grown in the Salt Lake of USA. The Andhra Pradesh Government is planning to establish one such plant on the East Coast with technical expertise from Thailand in the processing of *Artemia* cysts.

Research needs on *Artemia*

Over the past two decades brine shrimp have become a key resource in the industrial expansion of fish and crustacean larviculture. Annual consumption of *Artemia* cysts has increased from a few tons in the mid seventies to over 2,000 tons in recent years. Exploitation of new natural sources, introduction of selected species/strains in suitable biotopes and improved techniques for harvesting and processing have all contributed to the increase the availability of high quality cyst products. The techniques developed to improve hatchery use and maximize the nutrition of *Artemia* nauplii e.g. cyst decapsulation, nauplius cold storage, and nauplius enrichment with selected fatty acids and vitamins have contributed to the fast expansion of the industrial farming of several aquaculture species. Although brine shrimp are mostly used in the form of freshly hatched nauplii, more and more use is being made of *Artemia* biomass collected from salt lakes, salinas, managed pond productions and intensive culture systems for use in shrimp nursery and maturation facilities.

Baseline information on the culture aspects collected at both laboratory and field studies have revealed that Indian strain of *Artemia* is parthenogenetic and size of cyst and nauplii are bigger than their US counterparts. Apart from this, *Artemia* is considered as an extremophilic organism and its larvae and adults are among the best of all animal osmo-regulators, surviving in severely hyper-saline waters and resisting extreme environmental stress factors that no other animal can tolerate. These aspects need to be critically looked into for ways to improve the survival, hatchability rate and development of farming practices suitable for different agro-climatic conditions.

Further reading

1. Anon. (1978) Summer Institute in Breeding and rearing of marine prawns, CMFRI special publication No. 3: 128 pp.
2. Anon. (1983) Mass culture of Brine shrimp in salterns, CIFRI Newsletter Vol. 6 (5), Sept.—Oct.: 10 pp.
3. Anon. (1995) Live feed: Handbook on Aquafarming, A MPEDA publication: 61 pp.
4. Anon. (1999) New Feed for farmed Marine Fish—may improve efficiency and profitability, Publication from Norweign Institute of Fisheries and Aquaculture Ltd. (INFA). Fiskeriforskning info, No. 9: July 1999 issue
5. Anon. (2002) AFI News Letter—A publication of Aquaculture Foundation of India, Chennai, Vol. 5 (3):1—18.

6. Granvil, D., Treece (2000) *Artemia* production for Marine larval fish culture, SRAC publication no. 702: October 2000.
7. Hudinaga, M. and Kittaka, J. (1966) studies on food and growth of larval stage of a prawn, Penaeus japonicus with reference to the application to practical mass culture, Inf. Bull. Planktol. Japan, 13: 83—94.
8. Hudinaga, M. and Miyamoura (1962) Breeding of he Kuruma prawn (Penaeus japonicus Bate), J. Oceanogr. Soc., Japan, 20: 696—706.
9. Moti, Harel, Sureyya, Ozkizilcik, Eric, Lund, Paul Behrens and Allen R., Place, (1999) Enhanced Absorption of Docosahexanoic acid (DHA, 22:6n-3) in *Artemia* nauplii using a dietary combination of DHA-rich phosphplipids and DHA-Sodium salts, Comparative Biochemistry and Physiology, Part B, Vol. 124 (2): 169-76.
10. Palanichamy, S. (1996) Continuous mass culture of live feed-to-feed different stages of prawn and fishes, Bull Cent. Mar. Fish Res. Inst., 48: 117—119.
11. Sahul Hameed, A. S., Murthi, B. L. M., Rasheed, M., Sathish, S., Yoganandhan, K., Murugan, V. and Kunthala Jayaraman (2002) An investigation of *Artemia* as a possible vector for white spot syndrome virus (WSSV) transmission to Penaeus indicus, Aquacuture, 204:1—10.
12. Sorgeloss, P., Lavens, P., Legar, P., Tackaert, W. and Versichela, D. (1986) Manual for culture and use of brine shrimp *Artemia* in aquaculture, *Artemia* Reference Centre, State University of Ghent, Belgium.

Vembanad Lake: A potential spawner bank of the giant freshwater prawn *Macrobrachium rosenbergii* on the southwest coast of India

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In India, seed of the giant freshwater prawn *Machrobrachium rosenbergii* (often known locally as scampi) is produced with variable levels of success as no specific criteria are followed to evaluate broodstock quality for hatchery seed production. Moreover, commercial hatcheries obtain brooders either from wild stock[1] or from grow-out ponds[2]. The soaring demand for seed cannot be fully met from the wild. Moreover, wild stocks of *M. rosenbergii* have declined rapidly in recent years due to over exploitation[3], habitat loss and increased pollution particularly in southeast Asia[4]. This has been documented from countries including Bangladesh, India, Indonesia, Malaysia, the Philippines and Thailand[5]. These days, commercial farms are the main supply source of broodstock for hatcheries. Inevitably, the stocks used by commercial farms are experiencing a decline in productivity due to inbreeding depression as hatchery-produced siblings are 'recycled' as broodstock over multiple generations[5]. Farm reared females also attain sexual maturity much earlier than their wild counterparts[6]; the size at first maturity is also smaller and characterized by relatively poor fecundity and larval viability. These issues generally result in a reduction in the mean size of cultured scampi in subsequent generations. The recent occurrence of 'white tail disease' in farm reared *M. rosenbergii* is also a major threat to its culture in India. These issues need to be addressed, and over fishing of the wild stock reduced, in order to improve the sustainability of the industry and to lay a foundation for genetic improvement programs of farmed stocks.

Vembanad Lake, situated in Kerala (Lat 9º 28' & 10º 10' N and long 76º 13' & 31' E) is the largest brackish water body on the southwest coast of the Indian peninsula. The lake is approximately 60km in length and covers an area of around 21,050ha[1]. It acts as a giant reservoir, protecting against floods and is in itself a major ecological resource. The lake has a unique feature of possessing two different ecosystems, retaining an estuarine condition in the downstream region (the Cochin backwaters) and a freshwater habitat in the upstream regions (Thanneermukkom to Alleppey). In the 1950s and early 1960s giant freshwater prawn were a lucrative component of the fishery in Vembanad Lake and its confluent rivers. As an important component of shrimp exports and earner of foreign exchange for Kerala, it emerged as the most valuable species in the inland waters of the state. However, since the 1980s stocks of the giant freshwater prawn have declined substantially due to various interventions including over fishing[7]. From literature it is known that Vembanad Lake is endowed with commercial-scale capture fisheries for both *Palaemonid* and *Penaeid* shrimp. The *Macrobrachium* fishery itself is one of the major fishery activities with several species present, accounting for about 1.63% of the exploited fishery resources of the lake. The annual yield of different freshwater prawns include: *M. rosenbergii* 39.27 tons, *M. idella* 68.3

Macrobrachium rosenbergii broodstock of more than 100g, collected from Vembanad lake.

tons, *M. scabriculum* 6.78 tons and *M. equidens* 3.34 tons[8].

Berried females start appearing in the catches in July-August and increase until October-November. The slow moving ovigerous females that become abundant in the lake after august are compelled to undertake a lengthy breeding migration. It has been reported that over 23 tons of berried females are exploited annually from the lake, the highest being in October. Males are predominantly found between January to June and the females are predominant during the period August to December. Females of around 100-150g dominate the catch, representing approximately 44%[7]. Only about 30% of the total spawner stock is thought to successfully reach the breeding grounds.

It is noteworthy that the brooders of Vembanad Lake are characterized by a greater size and higher fecundity than wild brooders from other areas. Females between 157-258mm and weight of 34.2g-202g have an absolute fecundity ranging from 30,000 to 227,000 eggs[9]. Unfortunately, removal of wild brooders for hatchery operations reduces the supply of wild seed to support the natural population.

Recently it has been realized that wild brooders give much better quality seed. The practice of carelessly selecting broodstock from hatchery populations, often from the same batch, has created vicious cycle of inbreeding, leading to poor quality seed. Vembanad Lake has the potential to provide high-quality broodstock for the industry, until such time as appropriate broodstock management strategies are in place to maintain the genetic integrity of hatchery-reared populations. Restrictions on the fishing of *M. rosenbergii*, at least during breeding season, would enhance and conserve the wild stock. It would also be useful to encourage the construction of hatcheries adjacent to the breeding grounds, where berried females are found, and the establishment of sanctuaries and hatcheries would be a useful step towards the establishment of a broodstock enhancement program[10]. Broodstock purchased from the fisherman for seed production should be returned to the waters so as to minimize the impact on the wild stock. Harikrishnan and Kurup[1] have emphasized that the temporal and spatial availability of berried prawns in the lake for establishing berry procurement centers. In this connection, it would be useful for the state government and freshwater prawn fishery authority to take necessary steps to regulate and monitor stock levels and the fishery activity in the lake, particularly during the breeding season, in order to avoid over exploitation of this valuable resource.

References

1. Harikrishnan, M and M.B. Kurup, 1996. Temporal and spatial availability of ovigerous females of *Macrobrachium rosenbergii* (de Man) in Vembanad lake. Proceedings of the Eighth Kerala Science Congress. pp 441-443.

2. Balamurugan, P., Mariappan, P. and C. Balasundaram.2004. Impacts of mono-sex *Macrobrachium* culture on the future of seed availability in India. Aquaculture Asia., April-June, Vol. IX(2): 15-16.

3. Hien, T.T.T., Minh, T.H., Phuong, N.T., Wilder, M.N., 1998. Current status of freshwater prawn culture in the Mekong River Delta of Vietnam. JIRCAS Journal, 6, 89-100.

4. Mather, P.B., de Bruyn, M., 2003. Genetic diversity in wild stocks of the giant freshwater prawn *Macrobrachium rosenbergii*: Implications for aquaculture and conservation., 26 (4), 4 -7.

5. New, M. B., 2000. History and global status of freshwater prawn farming. In M.B.New and W.C.Valenti (Eds). Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford. pp. 1-11.

6. Wilder, M.N., Wei- Jun, Y., Do Thi Thanh, H., Masachika, M., 1999. Reproductive mechanisms in the giant freshwater prawn, *Macrobrachium rosenbergii* and co-operative research to improve seed production technology in the Mekong Delta Region of Vietnam. UJNR Technical reports, No.28, pp. 149-156.

7. Kurup, B.M. and M. Harikrishnan. 2000. Reviving the Macrobrachium rosenbergii (de Man) fishery in Vembanad lake, India. Naga, The ICLARM Quarterly (Vol. 23, No 2). April-June.

8. Kurup, B.M., Sebastian, M.J., Sankaran, T.M. and P. Rabindranath. 1992. Fishery and Biology of *Macrobrachium spp* of the Vembanad Lake. In: E. G. Silas (Ed) freshwater prawns. Kerala Agricultural University. Pp 78-89.

9. Sureshkumar, S. and B.M. Kurup. 1998. Fecundity indices of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). J. Aqua. Trop. 13(3): 181-188.

10. John, M. C. 1957. Bionomics and life history of *Macrobrachium rosenbergii* (de Man). Bull. Cent. Res. Inst. Univ. Travencore, Tiruvandrum Ser. C5(1): 93-102.

Seed production of mud crab Scylla serrata at the Rajiv Gandhi Center for Aquaculture, Tamil Nadu, India

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Mud crabs or mangrove crabs belong to the Family Portunidae under the genus *Scylla* with four known species, *S. serrata*, *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. In India, the common species are *S. serrata* and *S. olivacea*, based on the taxonomic identification of Keenan et al (1998).

Mud crabs have gained a niche in the international market. In the late 1990's, 4-5 tons of marketable size crabs were airlifted from India daily (Marichamy and Rajapackiam, 2001). At present, one of the several exporters in Chennai has been air lifting 750-1000 kg of live marketable size crabs daily. Crabs are exported to Singapore, Malaysia and sometimes Taiwan. Mud crabs are sourced mainly from the shallow coastal waters, estuaries, intertidal swamps, and mangrove areas. The demand for crabs has resulted in overfishing in many parts of southern India. Assessment of catches has revealed that only a low percentage is of larger sized crabs and that there has been a decrease in production rate. This situation has created interest among aquaculture ventures to produce seed of mud crab to reduce the fishing pressure on wild stocks. In previous years, several trials on seed production had been conducted mostly in Kerala and Tamil Nadu but crablet production was not sufficient to make farming viable.

The Rajiv Gandhi Center for Aquaculture (RGCA), a society functioning under the Marine Products Export Development Authority (MPEDA), based in Tamil Nadu has always been in full support to the development of culture technologies of important aquaculture species to support resource management and sustainable aquaculture. In the last quarter of 2004, RGCA started to conduct trials on the mass seed production of the mud crab *Scylla serrata* in an attempt to establish a pilot scale hatchery. The establishment of hatcheries and nursery production of juvenile mud crab as source for farming up to marketable size will open the door to sustainable development of crab aquaculture.

Sourcing of crab broodstock

Adult crabs (500-1000 g body weight; 14.4-18.0 cm carapace width) were obtained from Cochin, Chennai, Thirumullaivasal, Karaikal and Chidambaram. The crabs varied in the state of ovarian maturity, which was examined by gently pushing down the first abdominal segment adjacent to the carapace where the color of the ovary can be seen. Mature ovary is a dark orange colour while immature ovary is yellow. Crabs with immature ovary were ablated on one eyestalk to induce maturation.

Spawning/hatching

The facilities from a former shrimp hatchery were modified for use in the maintenance of mud crab broodstock and culture of larvae.

Crabs were disinfected in a formalin bath of 150 ppm in tanks or basins for 30 min and stocked in two units of 2 m x 5 m concrete tanks located in an enclosed room. The crabs were provided with 4-5 cm sand substrate and bricks as a refuge. Water depth was maintained at 40-50 cm with a water exchange rate of 30-80% daily. The water temperature and salinity in the maturation tanks ranged from 26-29.8 °C, 23-34 ppt, respectively. Dissolved oxygen fluctuated from 3.0 to 5.8 ppm while pH was 7.2-8.0. Sampling for egg carrying (berried) crabs in the tanks was done twice daily, in the early morning and late afternoon with an aid of a flashlight. Berried crabs could easily be recognized during sampling as their abdominal flap extended outwards. Berried crabs were transferred individually to 300-liter fiberglass incubation tank with treated aerated seawater.

Of the 32 crabs that spawned five hatched their eggs, in which most had partial hatching. The egg mass of the remaining 27 crabs had unfertilized eggs with no further egg embryonic development. Fouling by filamentous bacteria and sessile protozoans were observed in eggs usually at around one week after spawning and fungal infection was often observed in unfertilized eggs. Fertilized eggs are less susceptible to infection than the unfertilized eggs probably due the hardened fertilized membrane restricting fungal penetration. Bath treatment with formalin and fungicide were applied to minimize infestation of fouling microorganisms and fungal infections.

Limb movement and beating of the heart at more than 160 beats/min were found to be indicative of imminent hatching. Incubation time was typically 10-11 days at a temperature of 25.4-31°C. Hatching usually occurred in the morning. The female was removed from the incubation tank after hatching and zoeae were collected thereafter.

Common mud crab species in India: Scylla serrata (above) and S. olivacea (below).

Larval rearing

Crab larvae were reared in tanks from 1- to 3-ton capacity. Larvae were selected for stocking by lighting the hatching tank and collecting those near the surface and swimming vigorously. Larvae were stocked at a density of 60/liter. All tanks were provided with moderate aeration. Zoeae were fed with rotifers from day 1 to 12, overlapping with Artemia nauplii from day 6 to early megalopa stage. On-grown *Artemia* (3-5-day old) provided a larger sized prey and were also fed to zoea 5 and megalopa. The megalopae stage was reached at 15 –16 days and crab instar (C) at 21- 22 days after hatching. The survival rate of C3-C4 (32-35 days after hatching) ranged from 0.23 to 8.25% (Table 1). *Nannochloropsis salina*, *N. oculata* or *Chlorella marina* at 50,000-100,000 cells/ml were added to condition the water and provide food for the rotifers and *Artemia*.

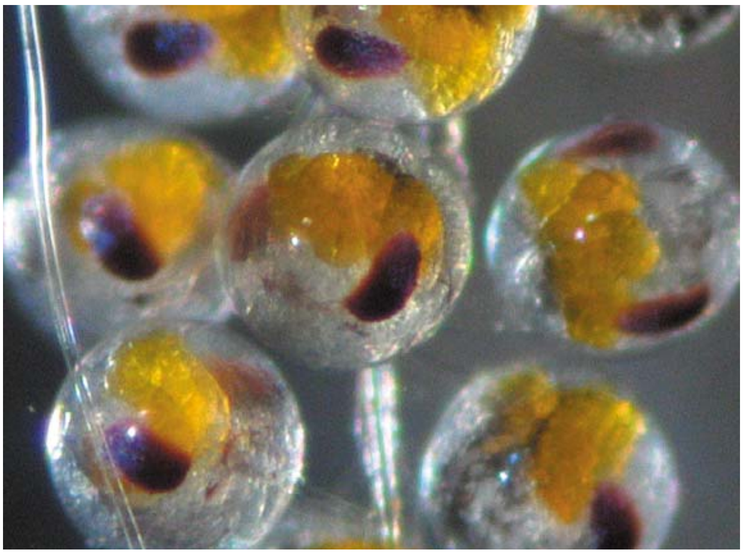
Water exchange varied between 30-50% depending on the water quality. Prophylaxis was applied only from zoea to crab instar 1 (C1). All seawater used in culture was sand filtered, chlorinated overnight and neutralized with sodium thiosulfate. Seawater was also treated with 10 ppm ethylene diamine tetraacetic acid to chelate heavy metals. Water temperature in rearing tanks (1- and 3-ton tanks) fluctuated from 26.0 to 30.7 °C and salinity from 21 to 33 ppt. Natural photoperiod was provided. The continuous rain from 28 October to 13 November 2004 caused the drop in salinity to 21 ppt in the water source and temperature to fall to 25.3°C. S. serrata larvae can survive well as low as 21 ppt salinity (Parado-Estepa and Quinitio, 2005). However, the rate of metamorphosis slowed down when the temperature remained below 26°C for several days.

Cannibalism of the zoeae by the more aggressive megalopae was often observed and accounted for a spike in mortality at metamorphosis. Thinning out the population and feeding the megalopae with bigger *Artemia* reduced such mortality.

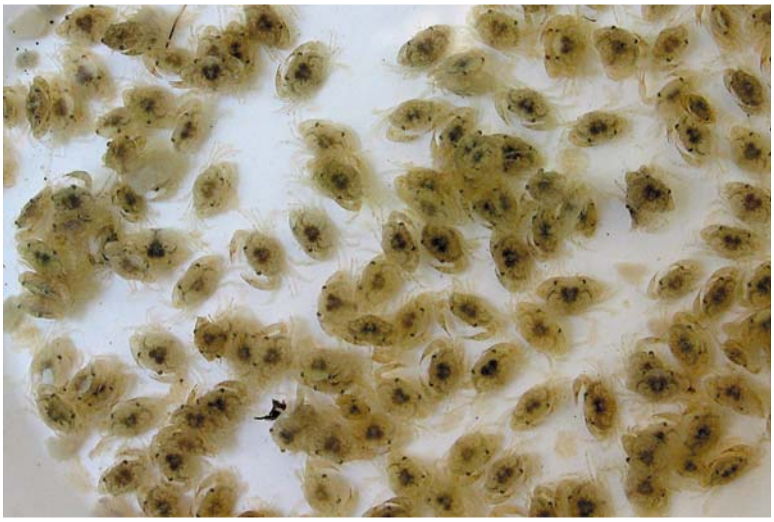
A failure and delay in molting from zoea 5 to megalopa were observed in some of the tanks. Old shells/exoskeletons that remained attached to the megalopa for several hours caused

Maturation tank with sand substrate and bricks as shelters.

Culture tanks (1 and 3-ton capacity) for mud crab larvae.



Scylla serrata embryo with developed limbs and heartbeat.



Scylla serrata crablets produced at RGCA.

Table 1. Production of *Scylla serrata* crab instar .

Crab tag	Spawning to hatching (days)	Number of zoeae/spawn	Number of zoeae stocked for larval rearing	Number of crabs (C3-C4)
3	10 days	580,000	580,000	1,352
9	11 days	355,000	355,000	8,766
17	10 days	400,000	2,000	165
29	11 days	100,000	100,000	1,270
30	11 days	4,000,000	1,040,000	9,170
32	Unknown (wild crab)	1,000,000	115,000	7,530

Inability of zoea 5 to completely shed the old exoskeleton.

abnormal swimming behaviour and failure to feed. Many of the dead megalopae siphoned out from the tank were unable to molt. Low temperature, insufficient nutrients, disease, and high dose of chemicals are implicated in the delay/failure of molting (Quinitio et al., 2001; Quinitio and Parado-Estepa, 2003).

Nursery

Crab instar were transferred in 10-ton concrete tanks for further culture. Food items comprised of minced fish and molluscs. N. salina or N. oculata was introduced in the tank and allowed to bloom prior to transfer of crabs. Excess feeds were siphoned out daily and water changes were conducted 3-4 times per week. Settlement substrates and shelters such as aquamats, green shade nets, shells, and pebbles were provided and crabs were graded by size to reduce cannibalism. Unfortunately, the performance of the hatchery-reared crabs could not be evaluated due to the destruction of ponds by the tsunami.

Conclusion

The recent trials in seed production of mud crab at RGCA can be regarded as very encouraging because 28,253 crabs were produced in a single run in 1-3 ton tanks from September to November 2004. Broodstock quality and cannibalism in crablets were the greatest constraint hence; these are being addressed in ongoing research and production runs. Since this first attempt, refinements in the larval rearing protocol have been made and the survival rate from zoea to crab instar has improved.

Acknowledgement

Mr. G. Mohan Kumar, Chairman of MPEDA and President of RGCA for his encouragement and full support of this project and the rest of the staff of RGCA for their assistance.

References

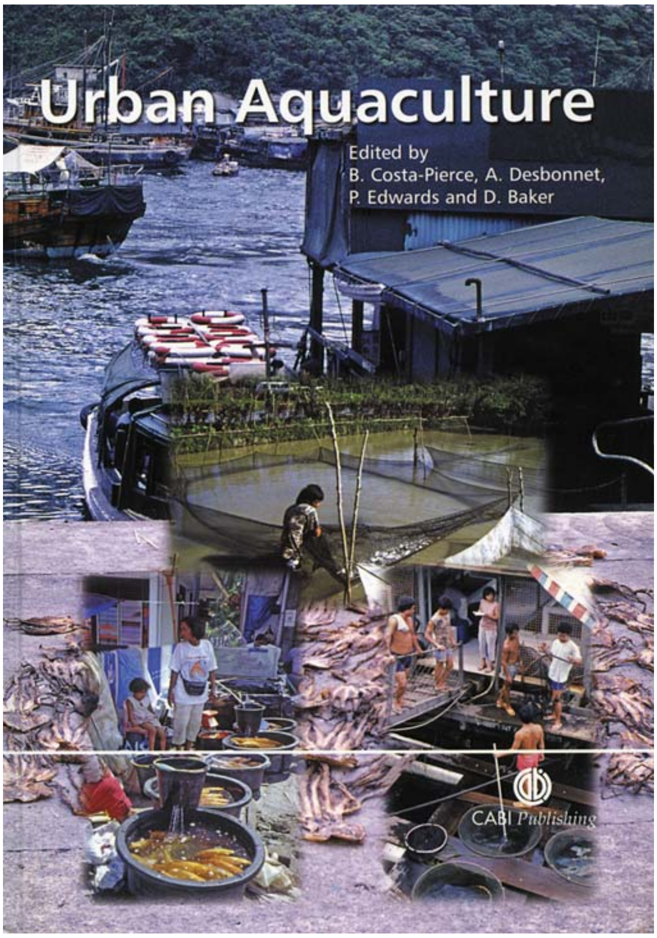
Keenan, C.P., Davie, P.J.P. and Mann, D. 1998. A revision of the genus Scylla de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). Raffles Bulletin of Zoology 46:217-245.

Marichamy, R. and Rajapackiam, S. 2001. The aquaculture of *Scylla* species in India. In: Proceedings of the International Forum on the Culture of Portunid Crabs, Aklan Philippines 1-4 December 1998, Oseni Millamena, Emilia T. Quinitio and Alan Blackshaw (eds). Asian Fisheries Science 14(2): 231-238.

Parado-Estepa, F.D. and Quinitio, E.T. 2005. Salinity requirement for larval rearing of *Scylla serrata*, *S. tranquebarica* and *S. olivacea*. In: International Workshop on Culture, Fisheries and Stock Enhancement of Portunid Crabs, 20-22 January 2005, Iloilo City Philippines. p.51.

Quinitio, E.T., Parado-Estepa, F.D., Millamena, O.M. and Rodriguez, E.M. 2001. Seed production of mud crab *Scylla serrata*. In: Proceedings of the International Forum on the Culture of Portunid Crabs, Aklan Philippines 1-4 December 1998, Oseni Millamena, Emilia T. Quinitio and Alan Blackshaw (eds). Asian Fisheries Science 14(2): 161-174.

Quinitio, E.T. and Parado-Estepa, F.D. 2003. Biology and Hatchery of Mud crabs *Scylla* spp. Southeast Asian Fisheries Development Center, Aquaculture Department, Tigbauan, Iloilo, Philippines. 42 pp.



Urban Aquaculture

Edited by B. Costa-Pierce, A. Desbonnet, P. Edwards and D. Baker.

Fishery products are the world's most important source of animal protein, especially for the poor. Meeting the basic human needs for protein foods in the future will be difficult challenge, especially as demand for fish has doubled since the 1950s. Realistically we cannot expect to catch much more food from the sea, so we must now turn to farming the waters, not just hunting them. The new challenge for planners is to accelerate aquaculture development and to plan for new production.

As millions of people are moving from rural, inland areas to coastal cities we need to make urban areas not only centres of marketing and distribution but also of production, particularly using recycled urban wastewater.

This book on urban aquaculture includes papers from authors in the USA, Europe and Asia that review these emerging issues from the perspective of both developed and developing countries.

Available from www.cabi-publishing.org/bookshop. 304 pages, hardback, Price US$120. ISBN: 0 85199 829 1.

Fish wastes in urban and suburban markets of Kolkata: Problems and potentials

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Recent advances in various disciplines of aquaculture have led to remarkable increases in finfish and shellfish production in India. With the application of modern biotechnological inputs, financial investments and entrepreneurial interests it has been possible to enhance the freshwater fish productivity levels in the country from a mere 600-800 kg/ha per year to a national average of more than 2,000 kg/ha per year. Under controlled culture conditions involving use of supplementary feed, provision of aeration, use of biofertilizers and steps to check parasitic infections, production rates of more than 15 tonne /ha/year have been achieved[1]. The states that have undertaken aquaculture in a big way and have contributed significantly to the national production include Andhra Pradesh, Punjab, and West Bengal in particular. Since feed is the single largest input in aquaculture, nutrition and dietetics are often regarded as the key for future research to assist in the further expansion of aquaculture. As the industry grows, the demand for readily available and inexpensive animal protein sources will also increase. The processing of fish and stray dressings in the market place lead to generation of huge quantities of wastes much of which are grossly discarded in public places. Recycling of these wastes into fish production systems can significantly reduce pollution, raise hygiene standards and reduce feed and fish culture cost.

A retailer dressing fish before sale.

Demand for fish in Kolkata and its suburbs

With the exception of West Bengal, none of the states leading in fish production are significant consumers of fish. Most of the fish eating population of India belong to the eastern states[2] and West Bengal, with an average per capita consumption of 18.0 g fish per day as compared to the national level of 7.0 g per day, tops the consumer list. People of Kolkata (formerly known as Calcutta) and its suburbs form the bulk of the consumers of West Bengal. However, production within the state is inadequate to meet the increasing demand for fish in the markets of Kolkata and its suburbs, compelling the wholesalers to depend on supply of fish from other states. Everyday about 40 trucks carrying near around 200 tonnes of fish deliver to the wholesale markets of Kolkata from Andhra Pradesh, Punjub and places like Chennai, Nagpur, Allahabad, Jabbalpur, Belpahari, Bhopal and Delhi. The three principal wholesale fish markets in Kolkata located at Howrah, Sealdah and Patipukur together receive a supply of about 137 tonnes of carps, comprising mainly Rohu and Catla, daily from these places. Although the relative proportion of supply and wholesale price of the fish varies between the markets, total supply of Rohu is always higher than that of Catla notwithstanding the later is sold at a higher price than Rohu in all three wholesale markets of Kolkata.

Lack of enough environmental planning on disposal of fish waste

Keeping the supply and demand of fish in view a fairly efficient marketing system has developed in the wholesale as well as retail sectors of Kolkata and its suburbs. However, a sound environmental planning system is yet to be established for the disposal of the huge quantities of waste generated from these markets. A significant portion of the fish is left off as fish waste, particularly in the retail markets, and these are in general disposed carelessly with hardly any concern for the environment or for the possibilities of recycling these resources for production purposes. The average net weight of the consumable parts available from Rohu and Catla, excluding the head portion, are around 657 and 626 g/kg of fish respectively, while the amount that is unusable and is generally disposed of as waste is around 180 and 150 g/kg of Rohu and Catla respectively. Therefore, unusable wastes potentially generated from the supply of carps to the three wholesale markets of Kolkata ranges from 3,175 to 11,907 kg / day.

Need to develop recycling process for sustainable aquaculture

Disposal of these huge wastes in raw condition not only results in environmental pollution and sanitation problems but also leads to loss of enormous amount of potential nutrient and energy sources contained in the stray dressings. Considering the growing population pressure and subsequent generation of organic wastes there is urgent need to find environmentally sound and economically viable recycling proc-

Table 1. Average daily supply (in kg) of carps from outside states in the three different wholesale markets of Kolkata.

Markets	Total Supply	Rohu	Catla
Howrah	19,600	12,740	5,880
Patipukur	49,000	39,200	9,800
Sealdah	68,600	53,900	14,700

Table 2. Average wholesale price of two species of carps from outside supply in different markets of Kolkata.

Markets	Rohu		Catla	
	Weight (kg)	Price (Rs.)	Weight (kg)	Price (Rs.)
Howrah	1-2	34-36	1-2	38-40
	2-3	50-52	2-3	60-65
	5 & above	60-65	5 & above	65-70
Patipukur	1-2	35-36	1-2	40-45
	2-3	45-47	2-3	50-55
	5 & above	54-55	5 & above	60-65
Sealdah	1-2	34-35	1-2	40-42
	2-3	46-48	2-3	54-57
	5 & above	52-56	5 & above	62-66

Figure 1. Potential amount of wastes generated from the supply of carps from the three principal wholesale fish markets of Kolkata.

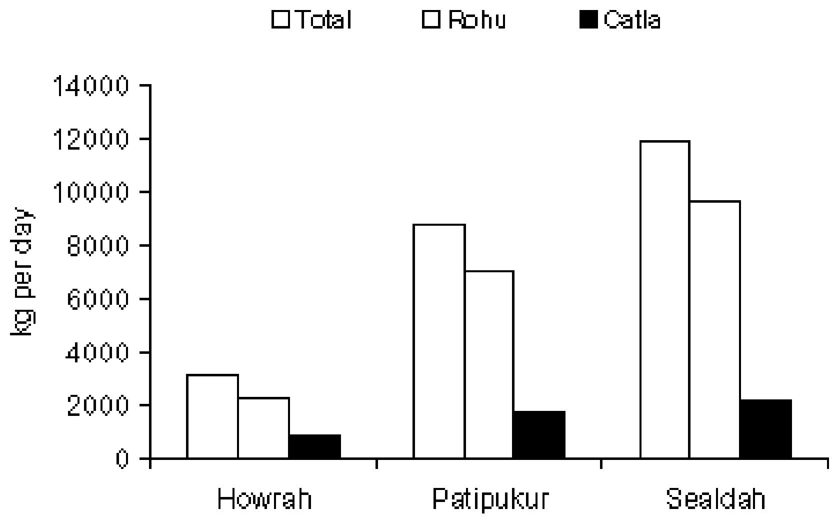
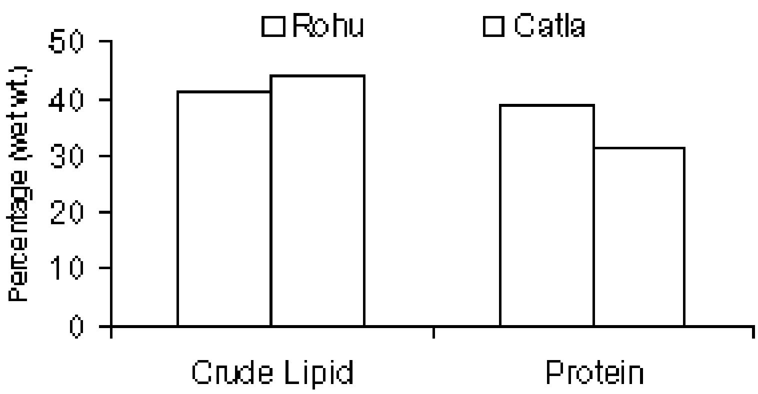


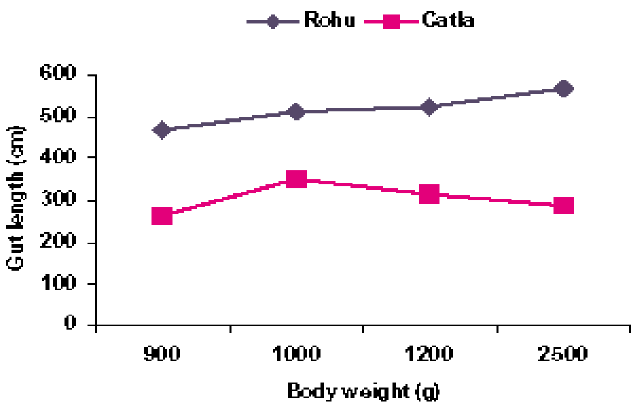
Figure 2. Lipid and protein content of the viscera of Rohu and Catla.



Viscera from a 3kg catla.

Rohu and catla dressed to sell the head and body separately while viscera are disposed of.

Figure 5. Changes in gut length with the increase of body weight in Rohu and Catla



ess that can return nutrients from these wastes thereby generating wealth out of it. Recycling of organic wastes from the fish markets through aquaculture is both potentially viable and cost effective from fish production viewpoint.

Intensive aquaculture is currently largely dependant upon the use of fishmeal and other fisheries resources as the sole or major source of dietary protein and lipid for formulated aqua feeds. Since fishmeal is also competitively purchased by the much larger poultry / livestock production sector, there is a tremendous pressure on the supply of raw materials, which is gradually declining because of over fishing of the marine resource with the result that fishmeal is becoming prohibitively expensive. Therefore, an alternative and more sustainable protein source needs to be urgently identified if intensive farming systems are to be sustainable in the long term[3].

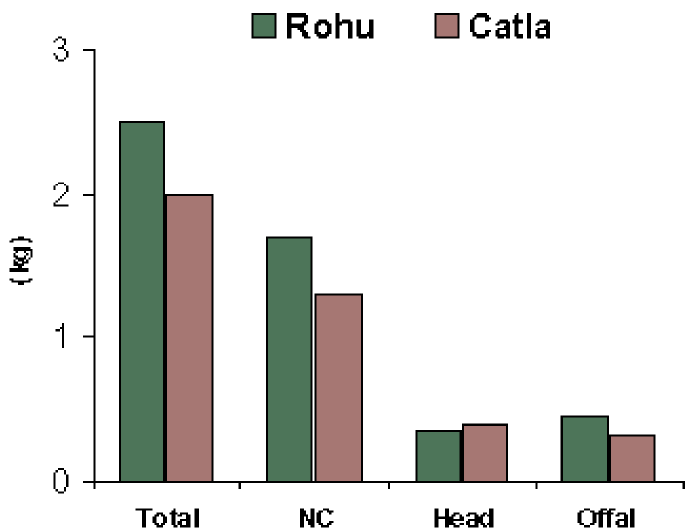
A substitute for fishmeal ?

Fish wastes including the viscera, fins, skin, portions of head, tail, and air bladder available in fish markets have immense potential to be used as a substitute for fishmeal. This waste is a rich source of protein (N X 6.25), lipid (ether extract) and energy. Viscera typically contain 31.5 to 38.9 % protein and 40.6 to 43.8 % lipid. Viscera of Catla have a slightly higher content of lipid than that of Rohu while the later contain a higher amount of protein due to its greater gut length. Huge quantities of these resources are wasted daily or lost for the opportunity for the production of a number of valuable products. Use and recycling of these wastes as a substitute for fishmeal in aquaculture production could significantly reduce pollution, reduce the cost of feed as well as the overall cost of fish production. There have been some studies on the acceptability and nutrient digestibility in respect of such waste materials[4,5]. Processing of fish waste for use in aquaculture feed preparation in forms such as fish silage, fish hydrolysate for fortifying fish feeds in the form of offal meal as protein supplement, will require the development of effective linkages between university / R & D establishments and user groups. There is a need to impart training to the

Scales and fins discarded after dressing rohu and catla in a retail market.

Cleaning catla at the market.

Figure 4. Relative proportion of total body weight , net consumable (NC) weight, head and offal generated from Rohu and Catla.



concerned people and fish traders on aspects of processing and storing fish waste and in the development of eco-friendly and responsible aquaculture.

Employment / income generation enterprise

Recycling of fish wastes has the potential to generate employment and additional income, particularly for fishing communities and traders, if suitable processing procedures can be developed for production of animal protein sources and their safe utilization as feed ingredients for the aquaculture industry. Research on utilization of fish wastes with regard to processing and appropriate storage and handling may increase the diversity of fish produce. Research reports from Central Institute of Fisheries Technology (CIFT) at Cochin indicate that excellent fine grade absorbable sutures can be prepared from intestines of Indian major carps[6]. One fish body can yield a long suture up to three meters in length. It has been reported that such fish gut can be processed to very fine grade ophthalmic sutures making the proposition very attractive. The mechanical properties and sterility of the sutures have also been tested by approved testing laboratories in India and found to be comparable with commercially available sutures. The process has also been patented by CIFT. Similarly there is thrust on development of several innovative products from fish wastes for industrial, medical and aesthetic applications, for both domestic and export markets. The huge quantities of fish waste generated in the urban and suburban markets of Kolkata are therefore an unexploited resource with potential for generation of feeds and other valuable products, employment and income generation, if appropriate technologies and utilization strategies can be developed.

References

1. Tripathi, S.D., Aravindakshan, P.K., Ayyappan, S., Jena, J.K., Muduli, H.K., Chandra, Suresh and Pani, K.C. 2000. New high carp production in India through intensive polyculture. Journal of Aquaculture in the Tropics. 15 (2): 119-128.

Continued on page 29.

Peter Edwards writes on

Rural Aquaculture

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Farming carps in leased ponds by groups of poor women in Chandpur, Bangladesh

A leased fish pond.

In a previous column, "Asia Development Bank Study on Aquaculture and Poverty" (Aquaculture Asia, volume X, number 3, pages 6-8), I outlined a recent study to assess channels of effects of aquaculture to generate livelihoods and reduce poverty. In my last column in Aquaculture Asia, volume X, number 4, pages 5-7, I presented a case study from the ADB study of individual small-scale fish pond farmers in Kishoreganj in the Greater Mymensingh Area (GMA), the major area for freshwater aquaculture in Bangladesh. In this column I present details of a case study from the ADB study on farming carps in leased ponds by groups of poor women in Chandpur with little to no previous experience of fish culture, access to ponds or other resources to culture fish.

This case study is based on a suvey of small-scale freshwater aquaculture development in ponds designed to mitigate the adverse environmental impacts on capture fisheries that accompanied the construction of flood embankments in the past. Promotion of poverty-focussed aquaculture was carried out within the framework of the ADB financed Command Area Development Project (CADP) to compensate for the decline in wild fish. The CADP in collaboration with the Department of Fisheries (DOF) promoted a semi-intensive polyculture of carps in ponds in Matlab Upazila of Chandpur District.

Establishing groups of fish farmers

In November 1999, the DOF engaged a non-government organization, Voluntary Organization for Social Development (VOSD), to develop the capacity of the poor in Matlab Upazila and promote fish farming. The development approach was tailored to:

- organize the poor, primarily women, into 175 groups of 10-15 persons;
- equip these groups with fish farming techniques and skills by training and extension;
- provide the groups with access to ponds through leasing of private ponds, and
- provide the groups with microcredit and savings facilities.

The selection criteria gave preference to women, marginal and landless farmers with land of less than 0.2 ha. Other criteria included unemployment, irregular employment and persons with limited access to cultivable land. The project established the groups from 2000-2001, comprising a total of 2,590 members including 2,440 women. Typically, each group acquired access to several fish ponds covering a total of 1 ha of water surface area, with leases ranging from 1-5 years. VOSD helped the groups to lease ponds from individuals or multiple owners. Absentee pond owners found leasing to be a convenient arrangement.

Providing microcredit to the poor

The groups were new, with no previous access to alternative and affordable credit sources. Their own resources were limited, and their assets including savings were not adequate to meet the investment and operating costs of fish farming. VOSD provided the groups with credit for working capital, with a credit limit of Tk 50,000 per group (US $1 = about Tk 58) to pay for fish seed, feed, fertilizers, and hired labor for pond preparation and harvesting. Payments of pond leases were not covered by the credit and group members paid for the costs of pond leases using their

A women's fish farming group with their leased pond behind.

savings and proceeds from the sales of household assets. The credit facility did not require any collateral. The credit terms were based on an interest rate of 15% per annum, with equal quarterly repayments of loan principal. The group members were required to save a minimum of Tk 5 per week.

Technical aspects

The groups obtained carp seed of all farmed species from seed traders who purchased seed from hatcheries located in Chandpur and Comilla. Several households in the villages in which the groups farmed fish also nursed fry to fingerlings in ponds from which the groups purchased seed. The groups farmed a polyculture of carps for 9-11 months a year with a stocking density of 11,250 ha-1 of relatively large (8-11 cm long) fingerlings. Pond fertilization used inorganic fertilizers (urea and triple super-phosphate) and cow manure, and supplementary feed rations consisted of rice bran, mustard oil cake and to a lesser extent grass, banana leaves and wheat bran.

The survey of groups and individual group members

A survey was made in 2003 of 100 of the total of 175 groups, as well as one household from each of the 100 groups surveyed. The groups had been involved in farming fish through the CADP for two fish culture seasons.

Among a sample of 100 individual group members, 64% were not involved in any other aquaculture activity apart from the group-based fish farming. However, 33% farmed fish in household ponds and ditches, and 3% nursed fry. All individual members were attracted to fish farming because of its profitability and the possibility of having fish for household consumption. Other contributing reasons for fish farming included prior knowledge of aquaculture (11%), availability of fish seed (2%), availability of fertilizers (1%), and availability of feed (1%). Fish consumption and income generation were main triggers for getting involved in fish farming. Individual members used pond water for multiple purposes: washing clothes (98%), washing dishes (95%), bathing (93%), livestock (30%), cooking (26%), and watering crops (14%). Despite the multiple water use, there were relatively few water use conflicts. While 86% of the respondents milled their own rice, a majority used their rice bran to feed fish (84%), cattle (15%) and poultry (1%). Only 47% of the respondents claimed to have enough rice bran to feed fish, and 47% reported buying rice bran for fish farming.

Livelihood assets

The respondents comprised households of an average family size of 6.3 persons. Fish farming was neither a full-time occupation nor the sole source of income for the households. Among the household heads, the most important primary occupations were rice farming (32%) and self-employment in micro-business activities (28%), compared to only 9% for fish farming. Fish farming (24%) was a significant secondary occupation for household heads after rice farming (33%). However, 95% of the spouses reported being a homemaker as their primary occupation, and 94% of them reported fish farming as their secondary occupation. Group members had relatively little prior experience in fish farming, with only 4% of the respondents having more than 5 years of experience. Women played a significant shared role with men in pond preparation, procurement of fish seed, fertilizers and feed, as well as in fertilizing ponds and feeding fish. Males were significantly involved in harvesting, marketing, and to a lesser extent grading fish.

The minimum landholdings reported by group members ranged from 0-0.2 ha, and the largest landholdings ranged from 0.1-0.8 ha, indicating that they were landless and marginal farmers. The number of ponds leased by each group ranged from 2-9 ponds with a mean of 3.4 ponds. Among the group members, 90% did not own a pond. In general, the leased ponds had multiple owners, ranging from 1-18 persons with an average of 5 owners per pond. The lease duration ranged from 1-5 years, with renewal options. While the majority of the leased ponds (98%) were more than 10 years old, none was a recently dug pond. All of the leased ponds were previously used for fish farming before the project initiative, but mainly only with fish stocking without fertilization or supplementary feeding. Among the surveyed groups, 84% reported using ground water as their source of water for aquaculture, as well as water from an irrigation canal (40%) and rainwater (16%). Water was not reported (99%) to be a seasonally limiting factor for aquaculture, suggesting that most ponds were perennial. The groups reported no conflicts over water.

All surveyed households had a small plot of homestead land, small ponds or ditches (77%), and a small area of agricultural land (76%). Few households had an orchard (12%) or fallow land (6%), and a significant number of households (35%) leased land. The average area of owned land, excluding leased land, was only 0.21 ha. All households cultivated two to three crops of rice per year, with yields averaging 4.5 t ha-1 per crop. Only 18% of these households reported catching wild fish from their own ponds. However, 79% of these households reported catching wild fish from elsewhere, although the amount of the catch was reported to have decreased over the last 10 years.

Fish culture management and performance

The surveyed groups reported that they harvested fish four times per year with an average yield of 3,700 kg ha-1 year-1, comparable to that achieved among top performers in Bangladesh. Among the surveyed groups, 87% reported that the harvested fish were relatively large (0.5 kg per piece), while the rest reported smaller sizes. Furthermore, 98% of them did not harvest fish on their own, with the majority hiring a local harvesting team, and relying to a small extent on fish buyers. None of the sampled groups experienced any major fish kill in the ponds. The vast majority of fish (94%) were sold, with only 4% consumed by the households of the groups, with the remaining given away and for other use. The surveyed groups reported average gross earnings of Tk 159,000 and net incomes of Tk 83,000 per group, excluding the fish for their own consumption and other disposal.

The groups each borrowed Tk 50,000 per year from VOSD for fish farming but only 2% of these groups experienced difficulties in repaying the loan because of lower than expected fish production during the first 3 months of the growing cycle when fish biomass in the pond was still low and stocked fish were still relatively small. Weekly savings among surveyed groups were a mean of Tk 81, reflecting a savings rate of Tk 5-10 per person per week. Overall, the groups indicated that the credit of Tk 50,000 was not enough to cover all operating expenses, including Tk 25,000-30,000 annually spent for leasing ponds.

None of the groups claimed that they could manage their fish farms without credit. The groups indicated that they needed an average of Tk 77,000 ha-1 of working capital, and that it would take them an average of 87 months to save this sum. However, if sufficient working capital were available to the groups, 99% reported that they would be able to obtain their own fish seed, fertilizers and feeds, indicating the importance of access to credit, without which the groups would have stopped farming fish as financial resources among the groups were generally minimal. Savings from fish farming were used for various purposes, including their children's education (50%), food purchase (44%), house improvement (40%), and health (39%).

A harvesting team showing the large sized fish.

Benefits

The surveyed groups reported overwhelmingly that aquaculture had improved their welfare in the context of food consumption (100%), home improvement (99%), children's education (99%), clothes (87%), sanitation (46%), and increased access to health services (25%) and drinking water (12%). Similar results were observed when the same groups ranked the three most important benefits gained from fish farming.

Compared to 5 years ago, the surveyed group members also overwhelmingly perceived that:

- their overall food and fish consumption had improved,
- they had gained from employment and cash incomes from fish farming,
- the natural resource conditions for fish farming had improved,
- they had acquired means to finance fish farming,
- their housing conditions had improved,
- they had gained access to fish farming technology,
- there had been an increase in the adoption of fish farming technology, and
- their access to credit had improved.

They were also optimistic for the future on these aspects.

Conclusions

Fish farming brought profits, generated cash, and significantly improved households' incomes. By 30 April 2003, the cumulative fish production had reached 1,225 t, with an estimated farmgate value of Tk 55.5 million (US$ 1 million). This scale of production among the 175 groups represented a sizable contribution to the local rural economy. Marketable fish could easily reach as much as 650 t annually with farmgate value of Tk 29.3 million (more than US$ 0.5 million), providing direct employment to 2,590 group members, and spinning off employment benefits to seed traders, small-scale input suppliers, fish harvesters and market intermediaries. Fish marketing activities through various intermediaries to final consumers could add as much as

50% to the farmgate values of fish, and provided significant self-employment opportunities to market intermediaries and their wage laborers.

The project initiatives benefited the poor, primarily disadvantaged women who were not heads of households. Only 7% of the surveyed group members claimed that they could find alternative employment. This condition indicated that the group members, primarily women, could not have obtained their own incomes if they had not been assisted by the project. Major barriers for seeking other employment were cited to include social barriers (45%), household work responsibilities (34%), and the inability to work physically as wage laborers (7%).

Fish farming significantly emancipated the women group members, providing them with lucrative opportunities in pursuing income generating activities and allowing them to play a significant shared role with men in social and cultural contexts normally dominated by men. Group members gained skills and confidence in operating and maintaining the fish farms, including skills in marketing. The project helped the disadvantaged poor in providing access to and overcoming barriers of opportunities. The key channels by which the poor benefited from fish farming were primarily through accessing livelihood assets, extension/advisory services, and markets.

The CADP developed human and social capital by facilitating skill acquisition, promoting confidence building, establishing groups and motivating them. The project also helped the groups in accessing land and water (fish ponds), natural assets for fish farming, by securing renewable lease arrangements. Without these leases, the disadvantaged poor would not have had access to fish ponds. The poor would not have been able to afford to engage in fish farming on their own because of their limited resources including financial capital to invest in fish ponds and pay for the operating expenses to stock and maintain the fish ponds.

The microcredit operations were instrumental in providing the poor with working capital to complement their meager resources. Without credit and the NGO advisory services, the poor would not have been able to start and sustain their fish farms. Although the group members had started saving a portion of their incomes, their savings alone would not be able to replace their reliance on credit as the groups indicated that it would take them an average of 87 months to save enough working capital, an average of Tk 77,000 ha-1. Thus, the continuation of access to credit with affordable terms and conditions is one of the key channels for enabling the poor to engage in small-scale aquaculture.

Access to markets for input supplies and fish made fish farming feasible and profitable. Rainfall, the use of wells, and water retention in ponds enabled farmers to farm fish all year round. With high demand for fish, the harvested fish had been mostly sold in local markets. Thus, fish farming contributed to local food security.

Considerable project support was required and mobilized to develop the requisite human and social capital of the fish farming groups. The use of a local NGO familiar with the social dimensions of poverty affecting the area was instrumental. Coupled with microfinance services, capacity building with practical training in aquaculture for the groups in the context of feasible income generating initiatives provided a breakthrough in providing self-help employment opportunities for the disadvantaged.

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":

- 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

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Fish wastes in urban and suburban markets

Continued from page 25.

2. Kaviraj, A. 2003. Towards better exploitation of fish environment. Environment & Ecology. 21 (Spl.Pub) : 1-2.
3. Tacon, Albert .G.J. 1995. The potential for fishmeal substitution in aquafeeds. Infofish International, 3: 29-34.
4. Rao, R.J., Mahendrakar, N.S., Chakrabarty , N.M. and Raghavan, S.L. 1996. Utilization of fermented fish and poultry offals in fed for common carp (Cyprinus carpio). Seafood Export Journal. 27(3):17-23.
5. Giri, S.S., Sahoo, S.K., Sahu, A.K. and Mukhopadhyay, P.K. 2000. Nutrient digestibility and intestinal enzyme activity of Clarias batrachus juveniles fed on dried fish and chicken viscera incorporated diets. Bioresource Technology. 71: 97-101.
6. Mukundan , M.K. 1992. Surgical sutures from fish gut collagen. Central Institute of Fisheries Technology, Cochin Publication.

Lymphocystis Disease and Diagnostic Methods in China

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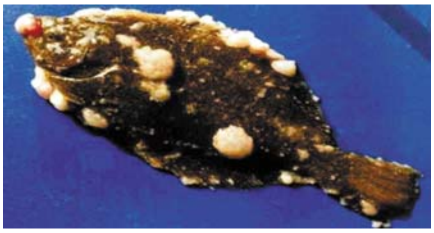
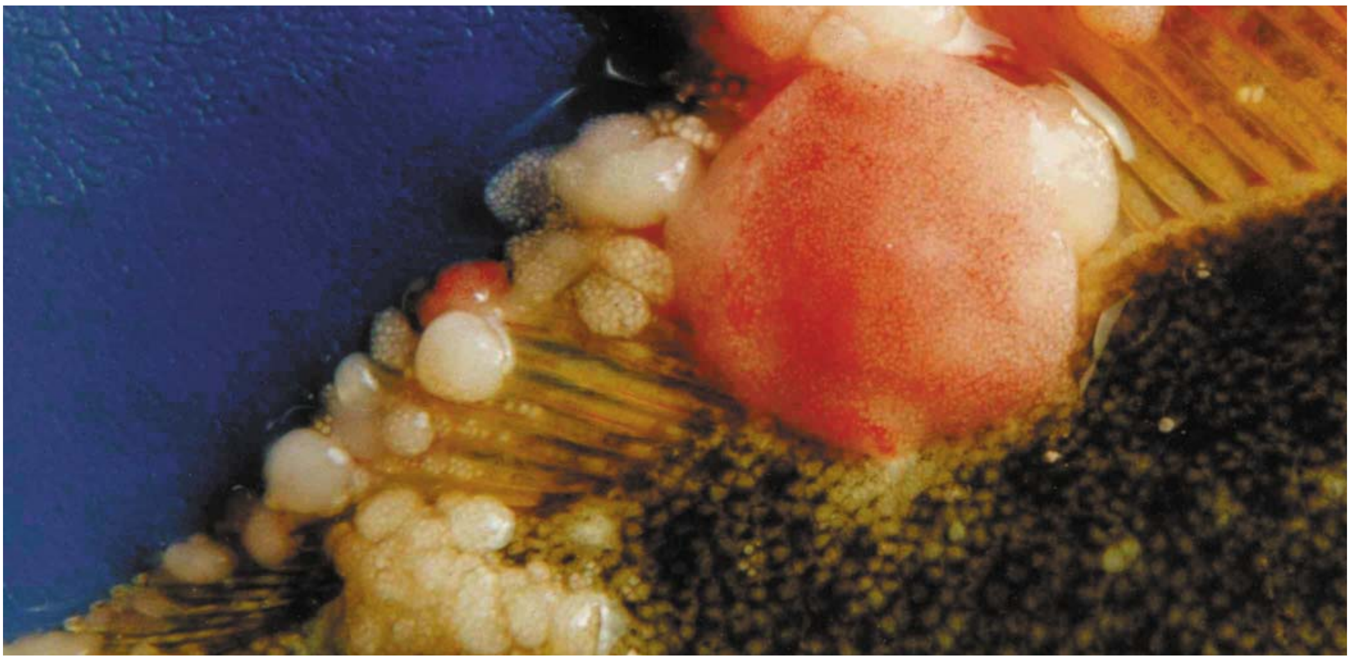
Lymphocystis disease (LCD) is a well-known viral infection of teleost fish species, characterized by the formation of papilloma-like nodules on the skin of body surface. It is highly infectious, common in culture or confined conditions, and is often associated with injury, pollution, rainfall and elevated water temperatures (Sindermann, 1996; Marcogliese et al, 2001), or dense host population during spawning time (Essbauer et al, 2004). The lymphocystis disease virus (LCDV), an iridovirus, is the causative agent. LCDV has a worldwide distribution and possesses a great host range; it can infect, both naturally and experimentally, over 100 species of fish in seawater and freshwater belonging to nine orders and 34 families. It is found in wild and cultured fish populations, including ornamental fishes, and has no known cure.

LCD was first noted in European flounder from British coastal waters and has usually been reported from Europe, southern and northern America in the past. However, in recent years more and more reports on LCD have come from Asia. Japan was the first to find that LCDV can infect the flounder *Paralichthys olivaceus* (Miyazaki and Egusa, 1972). In 1988, infection of LCDV to the cage-cultured kelp bass occurred in Korea. In 1990, LCD was first reported from China in kelp bass (*Epinethelus moara*) in a farm of Guangdong province. The outbreak occurred in September leading to a mortality of 9.2% of one-year old fish and around 2% of two-year old fish, although there were no other reports of LCD in other areas of Guangdong (Zhang et al, 1992; 1997). In 1997, a serious LCD outbreak occurred in cultured *P. olivaceus* in the Weihai region of Shandong province, China, due to introduction of fry and mature fish from Korea by several companies (Qu et al, 2001). From then on, more than ten fish species have been reported to be infected by LCDV in China including red sea bream (*Pagrosomus major*) and common sea bass (*Lateolabrax japonicus*). LCD has resulted in serious economic losses in the Chinese mariculture industry and has become one factor impacting on aquaculture development.



Flounder with severe lymphocystis (photo J. Yulin).

The causative agent of lymphocystis disease in China has been identified as LCDV-C (isolated in China from the flounder *P. olivaceus*) (Sun et al, 2000; Xu et al, 2000). Previous reports concerning LCDV-C described general histopathological features (Zhang et al, 1992, 1997; Qu et al, 1999, 2000), occurrence and development of lymphocystis cells (Sheng et al, 2004), isolation and characterization (Song et al, 2003; Liu et al, 2002; Wang et al, 2004; Xing et al, 2005), pathogenicity and immunogenicity, and diagnostic methods of LCDV (Sun et al, 2000, 2003; Liu et al, 2002; Cheng et al, 2005). Recently, the complete genomic DNA sequence of LCDV-C has been determined, it is 186,250bp, while the LCDV-1 (isolated in the United States from the flounder *Platichthys flesus*) genome is only 102,653bp in length. It



Lymphocystis lesions showing granular particle inclusions. Photo: J. Yulin.

has been suggested that LCDV-C and LCDV-1 should be considered a species different from LCDV-1, because of the unexpected levels of divergence between their genomes in size, gene organization, and gene product identity (Zhang et al, 2004).

The data about the molecular mechanism underlying LCDV infection, replication and pathogenesis is very short, the major obstacle to progress is the lack of an efficient cell-culture system for propagation of this virus (Tidona & Darai 1999). Currently, much interest is being focused on the development of a susceptible cell-culture system. Cell lines established have a susceptibility to LCDV include hirame natural embryo cells (HINAE) (Kasai et al 2001; Iwamoto et al 2002), SAF-1 cell line from fins of gilt-head seabream (*Sparus aurata*) (Bejar J et al, 1997; Perez-Prieto et al, 1999; Garcia-Rosado et al 2002; Alonso et al, 2005), SF cell line from Asian seabass fry (Chang et al, 2001), GCO (grass carp ovary) and GCK (grass carp kidney) derived from the freshwater grass carp *Ctenopharyngodon idellus* (Zhang et al, 2003), and FG from gill of flounder *P. olivaceus* (Tong et al, 1997; Lv et al, 2003; Hu et al, 2004; Sun et al, 2002). In China, Zhang et al (2003) successfully examined the infection and propagation of LCDV-C in a grass carp cell line and demonstrated a complete replication of the virus under electron microscope.

In our laboratory, we explored the epizootiology of LCD occurrences in China and examined the histopathological features and occurrence of lymphocystis cells (Sheng et al, 2004), the infection and propagation of virus in FG cell line (Xing et al, 2005), and developed monoclonal antibody (Cheng et al. In press). For diagnosis, different methods including FAT, immunohistochemical assays (Xu et al. 2004; Sheng et al, 2004), PCR and nested-PCR, immunoblot and in situ hybridization (Wang et al. In press) were applied to detect the virus. We are also conducting research on neutralized antibody and vaccine of LCDV.

Epizootiology surveys

In September 1997, serious LCD outbreaks occurred almost simultaneously in nine farms of Weihai in Shandong province, China, due to the introduction of *P. olivaceus* fry from Korea. The incidence of disease reached 60%, and more than 90% in one or two farms. This disease was not acutely fatal, but the death of fish occurred around one month after the incidence of disease. After three months, mortality increased to 20%. Spontaneous recovery occurred after a long course of disease. In 1998, LCD broke out once again on a large scale in the cultured *P. olivaceus* in north China, resulting in a great economic loss to the arising Chinese marine culture industry. From then on, LCD spread quickly from Shandong to Heibei, Zhejiang province. The infection of LCDV to the cultured fish took place in four seasons from year to year, especially in temperatures of less than 25˚C. Apart from the flounder *P. olivaceus*, the infection has occurred in over 10 fish species, such as the whitespotted puffer *A. hispidus*, sting fish S. schlegeli, and common sea bass *L. japonicus*, etc. In June to July 2003, we found an occurrence of LCD in the cultured sting fish (*S. schlegeli*) in a farm of Weihai, Shandong province; the body length of fish averaged 15cm. In addition, we found another new host for LCDV in April 2003, that is the ornamental aquarium-held whitespotted puffer (*A. hispidus*). The water temperature in the aquarium was about 25˚C all the year round. There are no reports of LCD in wild fish populations in China to date.

Etiology

Gross symptoms and light microscopy

Lmphocystis nodules occur in a variety of sizes and shapes. They may be grey, white or pink in colour corresponding to different stages, occurring mainly on the skin, dorsal and caudal fins and mouth. Histopathological examination has shown that lymphocystis cells appear in the gill of *P. olivaceus* and *L. japonicus* and the spleen of *P. olivaceus*. Hypertrophied cells are present in the spleen of *L. japonicu*s.

Electron microscopy

The fibroblast in the connective tissue beneath the epidermis of the fish engulf virus particles with the pseudopod forming phagocytosis vacuole in the cytoplasm. Infected cells proliferate, become round and hypertrophied, and then formed lymphocystis cells.

In mature lymphocystis cells high electron density inclusion bodies were generally located at the cell periphery with virions budding from the surface. Mature or immature virus particles and empty capsids, 200-220nm in diameter, were observed within the cytoplasm but not in nucleus. Particularly, moderate electron density particles, 70-80nm in diameter, filled out the perinuclear cisterna, arranged in crystalloid arrays in the nucleus and released into the cytoplasm by the rupture of the nuclear membrane. These features were present in *P. olivaceus* and *S. schlegeli*. Therefore, DNA replication processes of lymphocystis virus were involved both nuclear and cytoplasmic phases.

Virus particles were present in somewhere in the hyaline capsule in some lymphocystis cells, but elsewhere of the capsule in the same cell no virus particles were observed. The senile

Table 1. Lymphocystis disease virus (LCDV) supernatant dilutions and cytopathic effect (CPE) of flounder gill (FG) cells at post inoculation (PI).

Virus dilution PI	2-0	2-1	2-2	2-3	2-4	2-5	2-6	2-7	2-8	2-9	2-10	PBS
24 h	+++	+++	++	+	-	-	-	-	-	-	-	-
48 h	+++	+++	++	++	+	-	-	-	-	-	-	-
72 h	+++	+++	+++	++	+	-	-	-	-	-	-	-
96 h	+++	+++	+++	+++	+	-	-	-	-	-	-	-
120 h	+++	+++	+++	+++	+	-	-	-	-	-	-	-

+++: 80% FG cells showed CPE; ++: 20-80% FG cells showed CPE; +: few FG cells showed CPE; -: no CPE.

lymphocystis cells sloughed, or terminally ruptured and release the virus particles into the connective tissue or surrounding environment.

No virus particles were observed in inflammatory cells and red blood cells infiltrating in the connective tissue or the blood cells in the capillaries.

Virus purification

Virions of LCDV were isolated directly on discontinuous gradient sucrose. Two virus bands appeared between 40% and 47% of sucrose, and many virions were observed through electron microscopy. Negative staining of viral particles showed important variations in shape and dimensions. They were constantly larger in size than in ultrathin section, with a mean diameter of approximately 250nm. Geometric structures with sharp angles were rarely observed. Most of the particles appeared swollen with more or less hexagonal profile, with a dense center of 200 nm in diameter.

Cell-culture for propagation of virus

LCDV supernatant was inoculated onto flounder gill (FG) monolayer cells, and cells were incubated at 20°C in 2% CO2 incubator. Cytopathic effect (CPE) appeared on monolayer FG cell after 1-2 days post-infection (PI), the cytopathic cells became corrugativus, aggregated and had no adherence. As cytopathic effects progressed, the cells fragmented, and a plaque formed on monolayer cells at 96h PI. (Table.1) Calculated by the Reed-Muench Method, half tissue culture infection dosage (TCID50) of virus supernatants is 40µl 22.57; that means 40µl virus supernatants of 6 times dilution is 1 TCID50.

Diagnostic Methods

Production of monoclonal antibodies

Four monoclonal antibodies (Mabs) against LCDV from *P. olivaceus* were produced by immunization of Balb/c mice with purified virus preparations. An indirect fluorescence antibody test was standardized to evaluate the Mabs for their usefulness as a diagnostic tool for the identification of LCDV in cryosection and as tool for further study of the virus. For IFAT analysis, positive signals appeared in the cytoplasm of lymphocystis cells, especially in the peripheral zone where inclusion bodies of LCDV were located. Results of isotype revealed that the Mabs belonged to immunoglobulin G (IgG) class, and immunoglobulin M (IgM) class, all with kappa light chains. Immunoelectron microscopy was carried out to identify the epitope specificity of these Mabs, immunogold labeling provided visualized evidence that these four Mabs were anti-LCDV and the epitopes recognized by these Mabs were on the envelope of virus.

Indirect fluorescent antibody test

Cryosections were prepared from tissues of naturally diseased Japanese flounder *P. olivaceus*, and from the tissues of healthy fish as control. Using FAT methods, the specific fluorescence was observed in gill, epidermis, stomach and intestine of the diseased Japanese flounder but not in the healthy fish.

Immunohistochemical assay

Cryosections were prepared from tissues of naturally diseased Japanese flounder *P. olivaceus*, and from the tissues of healthy fish as control. The culture fluids of four strains Mabs were used as primary antibody, and secondary antibody was the goat anti-mouse Ig serum, conjugated to alkaline phosphatase (AP), nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyphosphate p-toluidine salt (BCIP) served as substrate. Mabs reactivity with LCDV was detected as brown deposits in stomach, intestine and epidermis of the diseased Japanese flounder but not in the healthy fish.

Immunoblot

The purified LCDV virus, 20 µl, was spotted on nitrocellulose membrane, as a positive control. The gill, stomach and epidermis tissue of naturally infected fish, *P. olivaceus*, were used as samples. The tissues were homogenized in 10% (w/w) with TNE, and centrifuged; the supernatants were spotted on the nitrocellulose membrane, the tissue of healthy fish, processed similarly as the diseased one, served as a negative control. The culture fluids of four strains of Mabs were used as primary antibody, and secondary antibody was the goat anti-mouse Ig serum, conjugated to AP, NBT/BCIP served as substrate. The purification of the virus and samples from the infected fish showed positive. No colour appeared in samples from healthy fish.

Polymerase chain reaction (PCR)

The primers sets for the PCR were designed from the published sequence of the major capsid protein gene of LCDV from *P. olivaceus* (GeneBank: AF126405). For the one-step PCR assay, the primer sequences of P1 and P2 generated a 348 base pair (bp) product. For the nested PCR assay, another primer set, P3/P4, was designed and amplified as a 173bp product. The amplification results demonstrated the specificity of the PCR assays. The one-step PCR product was detected with concentrations as low as 1×10-4 µg of LCDV DNA by agarose electrophoresis and ethidium bromide staining. In the nested PCR amplification, the sensitivity was 105 times higher than one-step amplification, which corresponds to about 1×10-9 µg of viral DNA.

In situ hybridization

The DNA fragment of the P1/P2 PCR products was used for the DNA probe. The DIG-labeled probe was generated with the PCR DIG Labeling Mixplus that could direct labeled amplification products with digoxigenin-dUTP in the one-step PCR. The hybridization and coloration procedures were as described by Lightner (1996). After coloration, the slides were counterstained with 0.5% neutral red for 1 min and then followed by dehydration. Positive signals characterized by blue precipitate were presented in lymphocystis cells, spleen, intestine and gill from infected flounder *P. olivaceus*. No reaction was found in the healthy fish tissues.

Conclusion

The causative agent of lymphocystis disease in China has been identified as LCDV-C. The negative-stained virus particles isolated from *P. olivaceus* showed important variations in shape and dimension (approximately 250nm). Geometric structures with sharp angles were rarely observed. Most of the virions appeared swollen with more or less hexagonal profile, with a dense center of 200 nm in diameter. The monoclonal antibodies produce anti-LCDV and have specificity; belong to IgM and IgG class and have the epitopes on envelope. Furthermore, diagnostic methods such as IFAT, immunohistochemical assays, immunoblot, PCR and nested-PCR and in situ hybridization were developed.

Acknowledgements

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References

Alonso MC, Cano I, Garcia-Rosado e, Castro D, Lamas J, Barja JL, Borrego JJ, 2005. Isolation of lymphocystis disease virus from sole, Solea senegalensis Kaup, and blackspot sea bream, Pagellus bogaraveo (Brunnich). Journal of fish diseases, 28(4): 221-8

Bejar J., Borrego JJ, Alvarez M C, 1997. A continuous cell line from the cultured marine fish gilt-head seabream (Sparus aurata L.). Aquaculture 150, 143-153

Chang S F, Ngoh G H, Kueh L F S, Qin Q W, Chen C L, Lam T J, Sin Y M, 2001. Development of a tropical marine fish cell line from Asian seabass (Lates calcarifer) for virus isolation. Aquaculture, 192: 133-145.

Chen S F, Zhan W B, Xing J, Production of monoclonal antibodies against lymphocystis disease virus of Japanese flounder Paralichthys Olivaceus.Journal of General Virolgy. In press

Essbauer S., Fischer U., Bergmann S., Ahne W., 2004. Investigations on the ORF 167L of Lymphocystis Disease Virus (Iridoviridae). Virus gene, 28(1): 19-39,

García-Rosado E. , Castro D, Cano I, Sara I. Pérez-Prieto b, Juan J. B, 2002. Serological techniques for detection of lymphocystis virus in fish, Aquat. Living Resour. 15: 179–185.

Hu G B, Cong R S, Fan T J, Mei X G, 2004. Induction of apoptosis in a flounder gill cell line by lymphocystis disease virus infection. Journal of fish diseases, 27(11): 57-62.

Iwamoto R, Haseqawa O, Lapatra S, Yoshimizu M. 2002, Isolation and characterization of the Japanese flounder (Paralichthys olivaceus) lymphocystis disease Virus, Journal of Aquatic Animal Health, 14, 2: 114-123.

Kasai Hisae, Yosimizu Mamoru, 2001. Establishment of two Japanese flounder embryo cell lines. Bull. Fish. Sci. Hokkaido Univ., 52(2): 67-70.

Lightner D.V., 1996. A handbook of shrimp pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquaculture Society, Baton Rouge, LA.

Liu Y K, Sun X Q, Huang J, 2002. Diagnosis of lymphocystis disease of cultured Japanese flounder Paralichthys olivaceus. High Technol. Letters, 6: 92-95.

Lv H X, Wang M, Li H Y, Guo H R, Zhang S C, Bao Z M, Zhou L, 2003. Isolation and Cell Culture of Lymphocystic Disease Virus (LDV) by Flounder Gill Cell Line. Journal of Ocean University of Qingdao, 33(2): 233-239.

Miyazaki, T., and S. Egusa. 1972. A histopathological observation on lymphocystis disease in the sea bass (Lateolabrax japonicus, Cuvier and Valenciennes). Fish Pathology, 6: 83–89.

Marcogliese D J, Fournier M, Lacroix A, Cyr DG, 2001. Non-specific immune response associated with infection of lymphocystis disease virus in American plaice, Hippoglossoides platessoides (Fabricius). Journal of Fish Diseases, 24(2); 121-124

Perez-Prieto SI, Rodriguez-Saint-Jean S, Garcia-Rosado E, Castro D, Alvarez MC, Borrega JJ. , 1999. Virus susceptibility of the fish cell line SAF-1 derived from gilt-head seabream. Diseases of Aquatic Organisms, 35 (2): 149-153.

Qu J, Shen H P, Li X G, 2001. Epidemiological investigation of lymphocystis disease in cultured flounder Paralichthys olivaceus. Test and Quarantine Science, 11(6); 34-35.

Qu L Y, Sun X Q, Zhang J X, 2000. Electron ultramicroscopic observation on lymphocystis disease of the left-eyed flounder Paralichthys olivaceus. J Ocean Univ. Qingdao, 30(2); 105-110.

Qu L Y, Zhang J X, Sun X Q, 1999. Epidemiological and histopathological study on the lymphocystis disease of cultured left-eyed flounder Paralichthys olivaceus. Journal of Oceanography of Huanghai & Bohai Seas, 17(2): 43-47.

Sindermann C J, 1996. Ocean pollution. Effects on living resources and humans. CRC Press, Boca Raton, FL, p 1–275

Sheng X Z, Xing J, Cheng S F, Zhan W B.2004. Polyethylene glycol embedding method for detection of lymphocystis disease virus in Paralichthys olivaceus.Journal of fisheries of China. 28:106-111

Sheng X Z, Zhan W B, 2004. Occurrence, Development and Histochemical Characteristics of Lymphocystis in Cultured Japanese Flounder (Paralichthys olivaceus). High Technol. Letters, 10(2): 92-96.

Sheng X Z, Zhan W B, 2005. Lymphocystis disease occurred in cultured sting fish (Sebastes schlegeli) in China, Aquatic Living Resources. In press.

Song X L, Huang J, Yang B, Shi C Y, Zhang L J, 2003. Pathology of Lymphocystis disease of flounder Paralichthys olivaceus and isolation of its pathogen. Journal of fishery sciences of China.10 (2): 117-120

Sun X Q, Qu L Y, Zhang J X, 2000. Pathogenicity and immunogenicity of lymphocystis virus of Japanese flounder (Paralichthys olivaceus). High Technol. Letters, 9: 19-21.

Sun X Q, Hong X G, Zhang J X, Zhang S C, Wang M, 2002. Propagation in Continuous Fish Cell line FG29307 from Gill of Flounder Paralichthys Olivaceus. High Technol. Letters, 12: 94-96.

Sun X Q, Huang J, Liu Y K, Qu L Y, Hong X G, Zhang J X, 2003. The studies on diagnositic Method of Dot-blot and in situ hybridization for Lymphocystis disease of cultured Paralichthys olivaceus. High Technol. Letters 1: 89-94

Tidona CA, Daral G, 1999. Lymphocystis disease virus (Iridoviridae). In: Granoff A, Wevster RG (eds) Encyclopedia virology. Academic Press, New York, p 908–911.

Tong S L, Hong L, Hong Z M, 1997. The establishment and partial characterization of a continuous fish cell line FG-9307 from the gill of flounder Paralichthys olivaceus. Aquculture, 156: 327.

Wang Y, Zhan W B, Cheng S F. Detection of fish lymphocystis disease virus by polymerase chain reaction and in situ hybridization. Aquaculture. In press

Xing J, Zhan W B, Zeng X H, 2005. Detection of lymphocystis disease virus infection to flounder gill cells in-vitro by monoclonal antibodies. High Technol. Letters. In press

Xu H T, Piao C A, Jiang Z L, Wang W X, 2000. Study on the causatiove agent of lymphocystic disease in cultured flounder Paralichthys olivaceus, in China. China J. Virol. 16: 223-226.

Xu S J, Xing J, Cheng S F, Zhan W B, 2004. Detection of lymphocystis disease virus in flounder tissue by immune technique. J. fish. China. 28: 101-105

Zhang Q Y, Ruan H M, Li Z Q, Yuan X P, Gui J F, 2003. Infection and propagation of lymphocystis virus isolated from the cultured flounder Paralichthys olivaceus in grass carp cell lines. Dis..Aquat. Organ.., 57(1-2): 27-34.

Zhang Q Y, Xiao F, Xie J, Li Z Q, Gui J F, 2004. Complete genome sequence of lymphocystis disease virus isolated from China. J Virol. 78(13): 6982-6994.

Zhang Y J, 1992. Light and electron microscopic study on lymphocystis of kelp bass, Epinethelus moara. Acta Oceanologica Sinica, 14(6): 97-102.

Zhang Y J, Guo Q, Wu Z Y, 1997. Ultrastructural histopathological study on lymphocystis of kelp bass, Epinethelus moara. Oceanol. Et Limnol. Sinica, 28(4): 406-410.

Asia-Pacific Marine Finfish Aquaculture Network

Magazine

SKRETTING

Mesocosm technology advances grouper culture in northern Australia

Elizabeth Cox, Peter Fry and Anjanette Johnston

The Queensland Department of Primary Industries and Fisheries (DPI&F) researchers in Cairns, northern Australia, have succeeded in producing juvenile flowery cod (*Epinephelus fuscoguttatus*) for the first time in Australia. The flowery cod, known as 'tiger grouper' in Asia, is a species in great demand in the live reef food fish trade. Demand for flowery cod, and other reef fish species such as gold-spot cod (*Epinephelus coioides*), is focussed on the live fish markets of Hong Kong and China. Flowery cod retails for AU$55 – 75 per kilogram in these markets (US$75-100). While flowery cod is farmed in many Asian countries, many of the fingerlings used are caught as undersize juveniles in the wild. Hatchery production of fingerlings, as developed by DPI&F researchers, is essential to ensure the long-term sustainability of flowery cod aquaculture.

The Reef Fish Aquaculture Project is based at Northern Fisheries Centre (NFC), Cairns. The recent upgrading of NFC, through the construction of a new Aquaculture and Stock Enhancement Facility, provides a purpose-built, marine aquaculture research facility that supports the development of tropical aquaculture in Queensland. Finfish research at this centre is currently focussed on the production of high-value reef fish species including barramundi cod (*Cromileptes altivelis*), flowery cod and gold-spot cod. Research focus is on the development of methodologies for rearing larvae of these species, including the production of novel prey species such as copepods. The Reef Fish Aquaculture Project is funded by the Queensland Government. Associated projects, funded by the Australian Centre for International Agricultural Research (ACIAR) have been valuable in providing opportunities for collaborative research in the Asia-Pacific region, particularly Indonesia and the Philippines.

Australian Government
Australian Centre for International Agricultural Research

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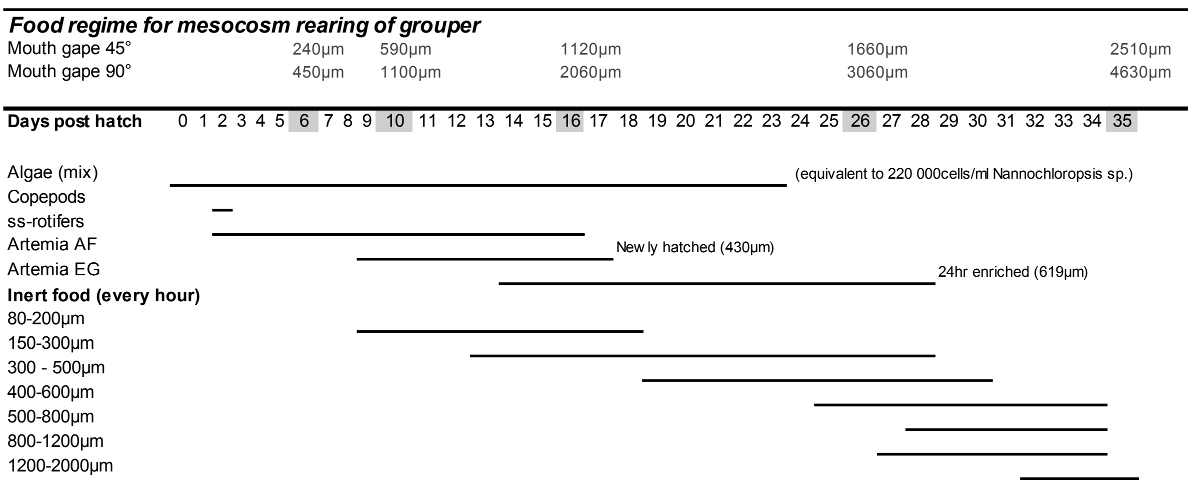
Figure 1. Succession of feed types used to rear larval flowery cod and gold-spot cod.



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Larval rearing

Rearing methodologies were initially developed from small-scale replicated experiments used to address issues during the early larval stages. However, larval survival in small-scale recirculation systems is poor with 100% mortality common by day 10. Physical parameters identified during small-scale trials were transferred to a larger pilot scale rearing trial using mesocosm technology. The same protocol has been used to produce fingerlings of two grouper species with difficult early life stages.

Tank management

Newly fertilised eggs (day 0) were stocked at a density of 30/litre into a 6m³ fibreglass mesocosm system. Water was exchanged during days 1-2 at 5% tank volume per hour during the day. No water was exchanged on days 3-4 to prevent the removal of prey, particularly copepod nauplii, from the tank during the critical first feeding period. From day 5, water was exchanged overnight starting at 5% per hour, increasing to 11% per hour with continuous flow from day 17 post-hatching.

Squid oil was added to the water surface twice daily from day 1 to 6 post-hatching to prevent larvae from becoming caught in the water surface tension. A photophase of 12 hours of light and 12 hours of dark was supplied by two overhead daylight fluorescent tubes supplemented by a low level of natural light. Light intensity ranged between 300 and 700 lux across the tank water surface.

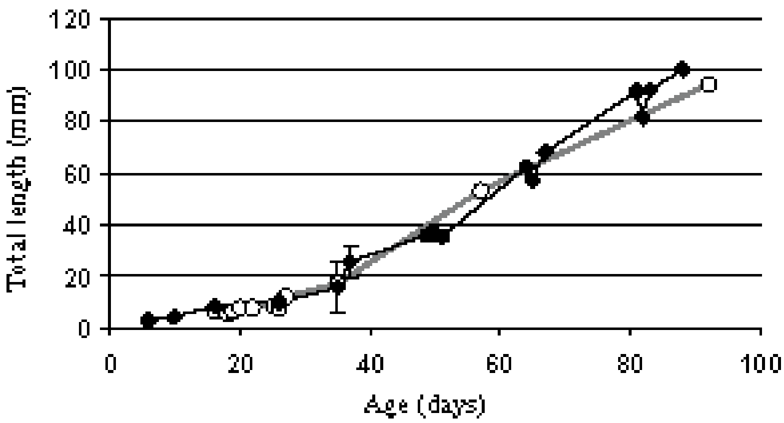
Feeding schedule

Four microalgal species (*Tetraselmis* sp., *Cryptomonad* sp., *Isochrysis* sp. (T.ISO) and *Nannochloropsis oculata*) were added daily from day 0 to 22. They were added on an equal ration (organic weight) basis to maintain an algal concentration equivalent to 2.2 x 105 *N. oculata* cells/ml^{-1}. On day 2 post-hatching, copepods (*Acartia sinjiensis*) and super-small strain rotifers (*B. rotundiformis*) were added at densities of 1.25/ml^{-1} and 10/ml^{-1}, respectively. Enriched rotifers (Algamac 2000) were added from day 6 until day 16 to maintain a density of 15 to 20/ml^{-1}. *Artemia* nauplii were introduced from day 9 - 13 and enriched (Algamac 3050) meta-nauplii from day 13 - 28. Artificial diets were introduced from day 9 onwards as detailed in Figure 1.

Metamorphosis/ cannibalism

The first metamorphosis of larvae to juveniles was noted on day 29-30 for both species and the majority had metamorphosed by day 40. Growth rates were similar for both species during the larval phase with a slight increase in gold-spot cod growth rates compared to that of flowery cod during the juvenile phase. Cannibalism coincided with the start of metamorphosis and was the major cause of mortality. Grouper larvae are very sensitive to handling stress

Figure 2. Total length (mean value and standard error) of flowery cod (open circles) and gold-spot cod (closed squares).



prior to metamorphosis and grading is not possible. This results in very high levels of cannibalism of larvae during the metamorphosis window. On day 43, surviving juveniles were transferred into a raceway tank where they were graded into three size classes (<5.5, 5.5 – 6.5 and >6.5 mm bar width). Grading reduced cannibalism from 26.9 to 13.7% and cannibalism ceased altogether from day 65 onwards when the total length of juveniles averaged 5.7 to 6.3cm.

Behaviour

During metamorphosis (around day 30-35), larval behaviour changed from an active swimming, surface-oriented feeding pattern to a less active and benthic feeding behaviour. Artificial hides were introduced to correspond with this behavioural change and they were used predominantly by juveniles rather than larvae that had not yet metamorphosed.

Juveniles were increasingly prone to startling in response to stimuli such as activity around the tank. They began to display behaviour similar to that of the adult fish around day 95 – 100. Territorial and dominant behaviour involved individuals displaying a pale underside to other fish or head to tail pushing and mouthing. The provision of adequate hides remained an important factor in reducing this aggressive behaviour.

Future application

This initial success in rearing of two grouper species indicates the beneficial application of mesocosm technology to rear multiple marine finfish species that have sensitive early life phases. Mesocosm systems provide a broader physical parameter range for the sensitive early larval phases reducing the need for strict control over conditions essential in small-scale intensive systems. The system can be changed to more intensive management once this early phase has passed.

Research into production of juvenile grouper at NFC continues to focus strongly on resolving problems occurring during the early larval feeding stages. The current success represents a breakthrough that will allow the concurrent development of techniques for production of juveniles. The recent progress in this area forms the basis of the next project phase, where the more active transfer of outcomes to industry proponents can commence.

Epinephelus fuscoguttatus fingerlings. Photo Sim Sih Yang.

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Natural Alga Nutritional Additive

Decrease mastitis rate, improve conception rate, prolong lactation period;
Improves animals' immunity and resistance to irritability;
Prevents and cures animals' deficiency symptom caused by lack of vitamin and mineral;
Inhibits the growth of penicillium, staphylococcus aureus and salmonella etc.

LEILINATURE Fucoidan

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Effectively prevents and cures infectious disease caused by virus and bacterium for aquatic animals, especially white spot syndrome virus for shrimp;
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