Observing external clinical signs of the idiopathic Myonecrosis (IMN) during production of Pacific white shrimp (*Litopenaeus vannamei*) in Brazil

Gustavo Dominguez[1] and Juan José Alava[2*]

In Brazil, shrimp aquaculture has been developed in the last decade and shows signs of growth. For example, the output of the shrimp production in northeastern Brazil increased from 40,000 to 60,128 t during the period 2001-2002 and the demand for Pacific white shrimp (*Litopenaeus vannamei*) postlarvae went from 0.5 to 11.4 billion in 1994 compared with 2002 (Camara *et al.* 2004). This productive activity is associated with increased demand for land, hatchery stocks and feed. At the same time, shrimp viral diseases have emerged on Brazilian shrimp farms and threaten the country's outstanding production. The importation of non-native shrimps (*L. vannamei* and *L. stylirostris*) during early 1980s introduced several viruses, such as the Infectious Hypodermal and Haematopoietic Necrosis (IHHNV), Taura Syndrome Virus (TSV) and Necrotizing Hepatopancreatitis (NHP) into Brazil (Briggs *et al.* 2004). Another viral disease that has appeared there and, which is causing severe shrimp mortality, is the Idiopathic Muscle Necrosis, recently renamed Infectious Myonecrosis (IMN; Lightner *et al.* 2004). Other names used to describe this disease are white muscle disease, muscle necrosis, spontaneous muscle necrosis, muscle opacity, idiopathic myopathy, white syndrome and milky prawn disease (Rigdon and Baxter 1970, Lakshmim *et al.* 1978, Nash *et al.* 1987, *Tonguthai 1992,* Flegel *et al.*1992). The disease first appeared in a shrimp farm located in the Municipality of Parnaíba, state of Piaui, northeastern Brazil, in September 2002 and it has been identified in other countries where Pacific white shrimp are cultured (Lightner *et al.* 2004).

In Brazil, the economic losses in shrimp production because of the IMN were calculated to be US$20 million in 2003 (Nunes *et al.* 2004). Infectious myonecrosis has been found elsewhere in the world in other penaeid shrimp species (*Penaeus aztecus*, *P. japonicus* and *P. monodon*; Rigdon and Baxter 1970, Lakshmim *et al.* 1978, Momoyama and Matsuzato 1987, Flegel *et al.* 1992), giant freshwater shrimp (*Macrobrachium rosenbergii*; Nash *et al.* 1987, Andserson *et al.*1990), freshwater crayfish (*Cherax terminatus*; Evans *et al.* 1999), swamp crayfish (*Procambarus clarkii*; Lindqvist and Mikkola 1978) and Norway lobsters (*Nephrops norvegicus*; Stentiford and Neil 2000). Lightner *et al.* (2004) recently reported that the etiologic pathogen is a spherical RNA-virus 40 nm in diameter.

Viral IMN (IMNV) has been cataloged as a new disease for cultured Pacific white shrimp, causing necrosis of the skeletal muscle (Lightner *et al.* 2004, Tang *et al.* 2005). This information is similar to the description previously made in black tiger shrimp (*Penaeus monodon*; Flegel *et al*. 1992). More recently, bioassay studies have demonstrated that both *L. stylirostris* and *P. monodon* are also susceptible to IMNV infection (Tang *et al.* 2005). Infectious myonecrosis is characterized as a disease with an acute display of gross signs and high mortalities, followed by a chronic phase with persistent low-level mortality, affecting postlarvae, juveniles and subadult cultured stocks of Pacific white shrimp (Lightner *et al.* 2004). This particular species of shrimp has been found to be the most susceptible to INMV infection when compared to infected *L. stylirostris* and *P. monodon* during bioassays (Tang *et al.* 2005).

In this article, we present an original description of a set of several clinical signs found in Pacific white shrimp from ponds on a shrimp farm located in Camocim, a village located 360 km from Fortaleza, which is the capital city of the state of Ceará, northeastern Brazil, as well as notes on the shrimp culture production during the outbreak of this viral disease in 2003 and 2004.

Shrimp Production during the Epidemic

Before IMN appeared, at the beginning of 2003, shrimp production on the farm was generally considered to range from 2,500-3,000 kg/ha, with a stocking density of about 25-30 individuals/m^2. During the period August-December 2003, total shrimp production area was 30.1 ha, divided into five ponds ranging 4.7-8.9 ha, with an average size of 6.0 ha. Routinely, feeding was done four times daily, twice in the morning (0700 and 1030) and in the afternoon (1330 and 1630). In accordance with consumption strategies, feed trays were used to provide food and to monitor feed consumption for feeding rate adjustments and biomass estimations. Water exchange was carried out using a continuous bottom water releasing method. Because of production costs and the lack of appropriate aquaculture fertilizers, urea was used as a nitrogen source and super triple phosphate was used as a phosphorus source.

During the last quarter of 2003, shrimp production was 2,000 kg/ha after 138 days of culture, with a FCR of 2.1. By that time, some shrimp showed grey discoloration in muscle tissues of the abdominal region. Mortality appeared two months before harvesting when the shrimp reached a size of 6 g. Final survival was 65 percent. When there were hypoxic conditions in the ponds, some shrimp displayed a reddish tail that was associated, in most of the cases, with necrosis. During the molting period, shrimp mortality increased dramatically. Normally, the number of dead shrimp encountered daily in ponds or feed trays were 6-10 individuals. In contrast, this number increased to as many as 200 dead shrimp daily in a large pond during molting periods and the acute phase of IMN. To enhance production, stocking was decreased from 25 to 16 individuals/m^2 in ponds with reduced survival. However, this shrimp pond management strategy did not function as expected and the mortality continued to hamper production. The increased mortality persisted until the final cycle. In the final cycle, the maximum mortality registered was approximately 400 individuals per day in a large pond. In general, mean survival was only 53 percent.

Light microscopic examination of shrimp guts revealed the presence of an extreme abundance of bacteria and gregarines, especially during the 2004 rainy season (January-May), as well as broken and evacuated guts. Gregarines were represented by different developmental stages (gamonts, gametocysts and trophozoites) and could have also played a critical role in low shrimp production.

IMN Macroscopic Clinical Signs

The external appearances and features of the affected shrimp showing the different grades of infection generated by the IMNV are as follows:

Initial phase (grade 1 infection). This phase is initiated with a minor, light whitish–pink coloration, opacity, or grey discolorations in focal areas, expanded slightly along the muscle of the abdominal segments from the under parts to the upper parts (Figure 1).

Moderate phase (grade 2 infection). During this stage of the disease, the opacity increases in size, reaching other areas of the abdominal region and becoming more whitish than the coloration found in the initial phase (Figure 2). Here, the muscular necrosis extends more into the abdominal area.

Severe or Pre-Acute phase (grade 3 infection). In this grade of IMN, the focal whitish opacities are more evident and concentrated, mainly on the under parts and sides of all the abdominal segments, including the base of the pleopods (Figures 3 and 4). The extensive opaque white coloration can appear in the cephalothorax.

Acute–Chronic phase (grade 4 infection). This transition of the terminal phase is characterized by complete necrosis of the abdominal striated muscles of the segments. Here, a diffuse milky white opacity can be observed through the entire abdominal area, though it is most evident in segments four through six. The myonecrosis extends from the telson and uropods to the cephalothorax. A reddish-pink necrotic coloration is clearly evident on the tail fan (telson and uropods; Figures 4 and 5), and even in the last segments. Mortality occurs during this phase.

Fig. 1. Individual showing the IMN–initial phase or grade 1. (Credit picture: ®Gustavo Dominguez).

Fig. 2. A shrimp reflecting the IMN–moderated phase or grade 2. (Credit picture: ®Gustavo Dominguez).

Fig. 3. Specimens presenting IMN–pre-acute phase or grade 3. (Credit picture: ®Gustavo Dominguez).

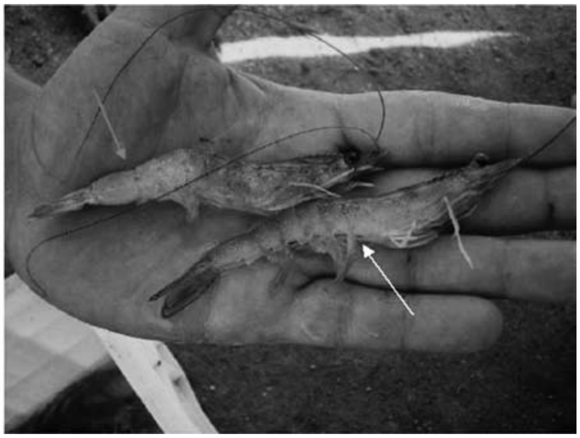

Fig. 4. Unhealthy individuals showing pre-acute or grade 3 (indicated by a white arrow) and acute phase or grade 4 (indicated by arrow) during shrimp production. It is noted almost total necrosis of the abdominal segments (white color) and presence of red uropods during the acute phase. (Credit picture: ®Gustavo Dominguez).

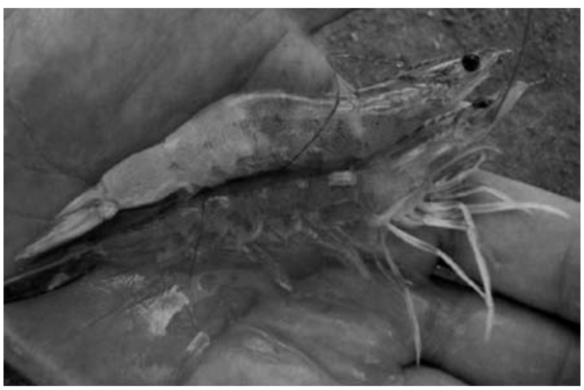

Fig. 5. A clear distinction of the IMN-acute phase (grade 4), when compared to a healthy Pacific white shrimp. (Credit picture: ®Gustavo Dominguez).

Discussion

IMN is a relatively new pathology for *L. vannamei*, though it has already been described in several other crustacean species. Field clinical identification of IMN is an important preliminary step in the management and control of this disease during production, especially when laboratory diagnostic approaches are not available. Even though we have not shown histological or ultrastructural evidence of this viral disease, a prominent existing body of literature, including presentations of well documented and detailed histopathological and electron microscope slides concerning with IMN, can be found in Nash *et al.* (1987), Stentiford and Neil (2000), Lightner *et al.* (2004) and Tang *et al.* (2005). Both histopathologically and ultrastructurally, microscopic diagnosis is characterized by myofibrillar and sarcoplamic necrosis or fibrosis, with hemocytic infiltration during the chronic phase (Nash *et al.* 1987, Lightner *et al.* 2004). Shrimp with acute and chronic IMN showed lesions with coagulative muscle necrosis and coagulative–liquefactive necrosis, respectively (Lightner *et al.* 2004). The external appearance of infected individuals in advanced stages is similar to the morphological alterations previously found in penaeids, showing the myonecrosis in the distal abdominal segments, mainly from four to six (Rigdon and Baxter 1970, Lakshmim *et al.* 1978). Nash *et al.* (1987) pointed out that the myonecrosis is likely to predominate in distal segments inasmuch as this abdominal region presents the highest metabolic activity, most obviously in hypoxic conditions during hyperactive stress.

The chronic advanced stage of myonecrosis and septic form of this disease is reached when the distal region of the abdomen turns red, becoming entirely necrotic and generally linked with shrimp mortality (Lightner 1993). Tang *et al.* (2005) reported that typical IMNV lesions on *L. vannamei*, injected with purified virions, were exhibited after six days, with a mortality of 20 percent. However, diagnosis of IMN based on only clinical signs and histopathological examinations is not sufficient to confirm the disease, so other methods, such as *in situ* hybridizations (ISH) to detect shrimp virus have been found to be effective for definitive IMN diagnosis (Tang *et al.* 2005).

As evidenced by recent bioassays conducted in Brazil, the major route of exposure to this disease appears to be ingestion of contaminated food or infected tissues of shrimp when compared to fecal matter of birds fed with contaminated shrimp, horizontal infection using effluents from cultured contaminated shrimps and biomass of adult *Artemia* fed with contaminated shrimp extracts.[3] From those preliminary experiments, the transmission of the IMN virus by direct consumption of contaminated tissues, 1.6 g of infected material caused a maximum mortality of 35 percent in a population of 25 juvenile shrimp ranging 0.2-0.5g (total biomass = 5-12.5 g) after 24 hr exposure under laboratory conditions (salinity = 35, temperature = 25-28° C; feeding was 5 percent of biomass/day with 35 percent protein; water was exchanged at 50 percent daily).[3]

Additionally, several environmental and intrinsic factors can promote the generation of IMN. For example, dramatic oscillations in salinity and temperature, hypoxia, overstocking, hyperactivity, poor handling and transfer techniques, collection by cast-net, direct solar radiation and low-quality feeds have been identified as stress factors associated with IMN (Rigdon and Baxter 1970, Lakshmim *et al.* 1978, Nash *et al.* 1987, Lightner 1993, Lightner 1988, Lightner *et al.* 2004).

IMN induced by environmental stressors has been reported in marine shrimp, such as *P. aztecus*, *P. japonicus* and *P. californiensis* (Rigdon and Baxter 1970, Lakshmim *et al.* 1978, Momoyama and Matsuzato 1987). If predisposing environmental stressors are removed preior to development of the advanced grades of infections, IMN signs can be arrested (Rigdon and Baxter 1970, Lakshmim *et al.* 1978). Nash *et al.* (1987) reported that reduction of stocking density by transferring Asian freshwater shrimp postlarvae affected by IMN to different and lower density ponds apparently helps mitigate the mortality. Management health strategies, such as total cleaning and aseptic conditions associated with tanks and equipment, avoiding overcrowding and maintaining optimal aeration have been used successfully in preventing IMN outbreaks (Nash *et al.* 1987). During the outbreak described here, shrimp stocking was reduced (from 25 to 16 individuals/m^2), but no sign of recovery was noticed. This suggests that in addition to the persistent mortality that characterizes IMN through the culture cycle, the gregarines and bacterial infection have also contributed to the mortality.

Although gregarines were not taxonomically identified in this study, it is likely that the species involved was either in the genus *Nematopsis* or *Cephalobus*. These two genera of gregarines have global distribution in penaeid shrimp aquaculture (Lotz and Overstreet 1990). Generally, gregarines are linked to impaired shrimp health and bacterial infections, such as hemocytic enteritis, that cause reduced growth and causes mortality in cultured pacific white shrimp in Ecuador (Jiménez *et al.* 2002) and Mexico (Fajer–Avila *et al.* 2005). The high abundance of gregarines and intestinal bacteria might have exacerbated the reaction of the shrimp to the viral agent and enhanced mortality. The sporozoites, gamonts and gametocysts of several species of *Nematopsis* (*N. penaeus*, *N vannamei*, *N. marinus*) have been found infecting *L. vannamei* cultured in Ecuador (Jiménez 1992, Lightner 1993, Jiménez *et al.* 2002), where infections have been mitigated by removing such intermediate carriers as the polychaete *Polydora cirrhosa*, which were common benthic pond dwellers (Lightner 1993). Recently, food medicated with sodium monensin (Elancobank) and sulfachloropyrazine (Avimix-STk) has been shown to remove and control *Nematopsis* gametocysts from the intestine of naturally infested cultured Pacific white shrimp (Fajer–Avila *et al.* 2005).

Best management practices such as maintaining a high dissolved-oxygen concentration; keeping stable temperature, pH and salinity levels; and controlling shrimp feeding are important strategies in pond management. Moreover, according to Horowitz and Horowitz (2001), minimization of waste, removal of sludge and organic matter and maintaining optimal water quality, including the reduction of excess ammonia and nitrite, the generation of both specific pathogen free and specific pathogen-resistant shrimp stocks, stimulation of shrimp immune systems with stimulants and enhancement of immnotolerance to viruses by using tolerins are priority issues of biosecurity and shrimp health management that will help the manager avoid the occurrence of various shrimp diseases.

Notes

[1]Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, 800 Sumter Street, Columbia, SC 29208, USA. E-mail: gusadoca@gmail.com

[2]Center for Coastal Environmental Health and Biomolecular Research (CCEHBR)/ National Oceanic and Atmospheric Administration (NOAA)/National Ocean Service (NOS)/NCCOS; 219 Ft. Johnson Road, Charleston, South Carolina 29412-9110, USA. *Current address: Environmental Toxicology Research Group, School of Resource & Environmental Management, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia V5A 1S6, CANADA. E-mail: jalavasa@sfu.ca

[3]Graf, Ch., N. Gervais, M.C. Fernandes, and J.C Ayala.2003. Transmissão da Síndrome da Necrose Idiopática Muscular (NIM) em *Litopenaeus vannamei*. (Technical manuscript unpublished).5p. (Available from the first author).

Acknowledgments

The author thanks Dr. Victoria Otton for her valuable review and corrections on this contribution.

References

Anderson, I.G., G. Nash and M. Shariff. 1990. Mass larval mortality in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in Malaysian modified static ‘green water’ system. Journal of Fish Diseases 13: 127-134.

Briggs, M., S. Funge-Smith, R. Subasinghe and M. Phillips. 2004. Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. FAO–RAP PUBLICATION 2004/10. Food and Agricultural Organization of the United Nations, Regional Office for Asia and the Pacific. Bangkok, Thailand.

Camara, M.R., P.A.Monteiro, L.G. Reis and M.F. Costa. 2004. Farming Artemia in a multi–cycle culture system in Northeastern Brazil. World Aquaculture 35(2):40-42.

Evans L.H., S. Fotedar, A. Fan and B Jones.1999. Investigation of idiopathic muscle necrosis and circulating hemocytes in the freshwater crayfish *Cherax tenuimanus* exposed to acute and chronic stressors. Freshwater Crayfish 12:356-370.

Fajer-Avila, E.J., M. S. Morales, Covarrubiasa, S. Abad-Rosales, A. Roque, P. Meza-Bojorquez and C. Hernandez-Gonzalez. 2005. Effectiveness of oral Elancobank and Avimix-STk against *Nematopsis* (Apicomplexa: Porosporidae) gametocyts infecting the shrimp *Litopenaeus vannamei*. Aquaculture 244:11-18.

Flegel, T.W., D.F. Fegan, S. Kongsom, S. Vuthikornudomkit, S. Sriurairatana, S. Boonyaratpalin, C. Chantanachookhin, J.E. Vickers and O.D. Macdonald. 1992. Occurrence, diagnosis and treatment of shrimp diseases in Thailand. Pages 57-112 *In* W. Fulks and K.L. Main, editors. Diseases of culture penaeid shrimp in Asia and the United States. Oceanic Institute, Honolulu, HA. USA

Horowitz A. and S.Horowitz. 2001. Biosecurity basics for shrimp aquaculture. Global Aquaculture Advocate 4:12-14.

Jiménez, R. 1992. Analisis de gregarinas asociado al detenimiento de crecimiento en camarones *P. vannamei* (Part II). Acuacultura del Ecuador 17:17-25.

Jiménez, R., L. de Barniol and M. Machuca.2002. *Nematopsis marinus* n.sp., a new septate gregarine from cultured penaeiod shrimp *Litopenaeus vannameis* (Boone), in Ecuador. Aquaculture Research 33:231-240.

Lakshmim, G. J., A. Venkataramiah and H. D. Howse.1978. Effect of salinity and temperature changes on spontaneous muscle necrosis in *Penaeus aztecus* Ives. Aquaculture 13(1): 35-43

Lightner, D.V.1988. Muscle necrosis of penaeid shrimp. Pages 122–124 *In* C.J. Sindermann and D.V. Lightner, editors. Disease diagnosis and. control in North America marine aquaculture. Developments in aquaculture and fisheries science, Vol. 17. Elsevier Press, New York, NY. USA

Lightner, D.V. 1993. Diseases of cultured penaeid shrimp. Pages 393-485 *In* J.P. McVey, editor. CRC Handbook of Mariculture, 2nd. ed. Crustacean Aquaculture Vol.1. CRC Press, Boca Raton.

Lightner, D. V., C. R. Pantoja, B. T. Poulos, K. F. J. Tang, R. M. Redman, T. Pasos-de-Andrade and J. R. Bonami. 2004. Infectious Myonecrosis–New Disease in Pacific White Shrimp. Global Aquaculture Advocate 7(5):85

Lindqvist O.V. and H. Mikkola.1978. On the etiology of the muscle wasting disease in *Procambarus clarkii* in Kenya. Freshwater Crayfish 4:363-372.

Lotz, J.M.E. and R.M.Overstreet. 1990. Parasites and predators. Page 92-121 *In* J.C. Chavez and N. O. Sosa, editors. The aquaculture of shrimps, prawns, and crawfish in the world: basics and technology. Midori Shobo, Ikebukuro, Toshima-ku, Tokyo.

(Continued on page 70)

Farming Bath Sponges

(Continued from page 22)

years of growth would guarantee a reliable and sufficient source of sponges to the market.

Survival of farmed *Coscinoderma* sp. and *R. odorabile* varied among the six farming sites (Figure 6), being highest at Pelorus 2 with 100 percent and 97 percent alive after 15 months. These survival results are outstanding and again highlight the potential of commercially farming bath sponges at the Palm Islands. At the remaining five sites, most sponge mortality occurred during the first few months. During that period, sponges are healing their cut surfaces so some mortality may result from pathogens infecting their exposed tissues.

Indigenous Australians

Besides developing the best farming method and selecting good farming sites, current research is addressing a range of other issues critical to underpinning the establishment of commercial sponge farms in Torres Strait and the Palm Islands. These include determining the optimal explant size for best farm production, investigating the abundance and size frequency patterns of wild populations to establish seed-stock harvest regimes, determining the genetic structure of sponge populations and connectedness for setting appropriate translocation protocols and establishing exactly what sponges are removing from the water column for food. Thus, as a complete package, research will support the establishment and regulation of sponge farming with a knowledge base for best practice farm production, as well as sustainable environmental management.

Fig. 7. A Torres Strait Islander sponge diver, monitoring the farming experiments.

While regulatory approvals from environmental managers are yet to be granted for either location, the data look positive. The farming response in both locations has been great, with sponges growing quickly and showing high survival in the best treatments. Indigenous Australians at the Palm Islands have ground truthed the experimental data with market analysis and development of a business plan. They have also developed a commercially viable model that will provide employment for 32 people in a community that currently endures over 90 percent unemployment. In Torres Strait the community has begun a similar process to arrive at a commercial production model that will work for them. Sponge farming has the potential to become more than a new sustainable marine industry for Australia. It could also present a platform for training, employment and economic development in communities that have limited opportunities for commerce and enterprise (Figure 7).

Notes

[1]Australian Institute of Marine Science, PMB No 3, Townsville, QLD 4810 Australia

*Corresponding author. Phone: +61 7 47534444; Email: a.duckworth@aims.gov.au

Signs of IMF

(Continued from page 15)

Monoyama K. and T. Matsuzato. 1987. Muscle necrosis in culture kuruma shrimp (*Penaeus japonicus*). Fish Pathology 22:69-75.

Nash G., S. Chinabut and C. Limsuwan. 1987. Idiopathic Muscle Necrosis in the freshwater prawn, *Macrobrachium rosenbergii* (de Man), Cultured in Thailand. Journal of Fish Diseases 10:109-120.

Nunes, A.J.P., P.C.C. Martins and T.C.V. Gesteira. 2004. Produtores sofrem com as mortalidades decorrentes do virus da mionecrose infeccisa (IMNV). Panorama da Aqüicultura (May/June 2004) 83:37–51.

Rigdon R.H. and K.N. Baxter. 1970. Spontaneous necroses in muscles of brown shrimp, *Penaeus aztecus* Ives. Transactions of the American Fisheries Society 99:583-587

Stentiford, G.D. and D. M. Neil.2000. A rapid onset, post-capture muscle necrosis in the Norway lobster, *Nephrops norvegicus* (L.), from the West coast of Scotland. Journal of Fish Diseases 23:251-263.

Tang, K.F.J., C. R. Pantoja, B. T. Poulos, R. M. Redman and D. V. Lightner. 2005. *In situ* hybridization demonstrates that *Litopenaeus vannamei*, *L. stylirostris* and *Penaeus monodon* are susceptible to experimental infection with infectious myonecrosis virus (IMNV). Diseases of Aquatic Organisms 63:261-265.

Tonguthai, K. 1992. Diseases of the freshwater prawn *Macrobrachium rosenbergii* in Thailand. Pages 89-95 *In* M. Shariff, R.P. Subasinghe and J.R. Arthur, editors. Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society. Manila, Philippines.

Prawn-Fish-Rice Farming

(Continued from page 41)

New, M.B. 1995. Status of freshwater prawn farming: a review. Aquaculture Research 26: 1-44.

Rutherford, S. 1994. An investigation of how freshwater prawn cultivation is financed. Bangladesh Aquaculture and Fisheries Resource Unit (BAFRU), Dhaka – 1212.

Uddin, M.J., S. Dewan, M. Ashrafuzzaman and M.M. Haque. 2001. Growth and yield of GIFT (*Oreochromis niloticus*) and Thai silver barb (*Barbodes gonionotus* Bleeker) in rice fields and their effects on the yield of rice. Bangladesh Journal of Fisheries Research 5(1):29-35.

Williams, D. 2003. Freshwater prawn farming in Bangladesh. Fish Farmer 26(5):24-27.