

**Analysis of Freshwater Prawn Hatchery
Problems in Bangladesh**

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Executive Summary

The hatchery business for prawns (*Macrobrachium rosenbergii*, known locally as golda) is relatively new in Bangladesh and (until recently) has increased over the past few years due to an increasing demand for hatchery reared postlarvae (PL). This demand has resulted from the expansion and potential profitability of prawn farming, together with a serious lack of PL availability due to government-imposed bans on the capture of wild PL and the importation of foreign seeds into Bangladesh.

Over the past two years, since early 2011 however, serious production problems have affected most of the Bangladeshi prawn hatcheries, forcing them to slow down operations, or in many cases close down, at least temporarily. These problems have thus further reduced the supply of prawn seed to the farmers and caused massive financial losses in all phases of the culture cycle, including broodstock fishermen, the hatcheries themselves, grow-out farmers, processors and exporters.

These hatchery production problems have manifested as a fairly inconsistent set of symptoms which usually lead to mass mortalities of the prawn larvae to as yet unknown causes during the larval rearing cycle. Over the past two years, a number of initiatives have been launched to try and identify the reasons underlying these problems, but to date conflicting results have emerged and none has managed to identify or correct the precise cause(s) of these problems. Thus, most of the remedial measures implemented by the hatchery operators in efforts to mitigate these problems have been unsuccessful.

This consultancy was therefore conducted to further investigate the reasons for these problems and make recommendations to the hatchery operators on how to prevent the future recurrence of these problems.

Interviews were conducted and analysis was made of the previous efforts by researchers from governmental and non-governmental organizations investigating these problems, and the experiences of hatchery operators in trying to cope with these problems.

Due to the fact that all of the hatcheries visited were still dry in preparation for the first stocking of the new year, direct examination of larvae exhibiting the classic symptoms of the problem was not possible, nor could samples be taken for further analysis. Efforts should be made once the new cycle starts to take water and prawn (broodstock and larvae) samples for external analysis in order to better understand the cause of these problems. Which samples are required and what needs to be analysed is detailed within this report.

The classic larval health symptoms observed during these production problems were identified and described. These symptoms include: Larvae with abnormal shape and colour (blue or black), often as early as first hatching (but sometimes later in the cycle), abnormal, usually weak spiral swimming behavior, reduced appetite, degeneration of the tail, with prominent head/eyes, gradual whitening/greying of the body, progressive weakening, disappearance and mortality of the larvae, with up to 100% mortality by the end of the cycle.

An analysis of the known diseases currently affecting prawn larvae, including their symptoms, diagnostic techniques, prevention and treatment strategies are included in this report and compared to the symptoms commonly reported for these production problems. Whilst these current symptoms do not precisely fit any particular known disease, they are closest to known bacterial problems associated with heavy infections

by a number of pathogenic bacterial species including *Vibrio*, *Leucothrix* and possibly *Enterobacter* or *Staphylococcus* sp. Such bacterial infections were indicated as being the most likely cause of the problems by the recent DOF-sponsored analysis of the problem conducted in 2012 and due for dissemination on March 13th 2013. Further analysis is required using specific measures as detailed in this report to fully understand which species are involved, what levels are present and which methods would be best at reducing numbers to non-pathogenic levels.

If bacterial infections were to blame for this syndrome, many researchers and operators believe that the widespread, frequent use of a variety of antibiotic and formalin based treatments should have helped resolve the problem, but these products appear to be incapable of preventing or treating the problem. However, the protocols used for larval treatment (and general husbandry) in many cases are substandard due to a lack of technical expertise on the part of the hatchery operators/owners. In addition, there are questions over the content of many of these products used, so that their use may have exacerbated the problems rather than helped them. Adjustment of the techniques used according to standard protocols are reviewed in this report and should be tested and used to train hatchery operators to assist them in improving their husbandry practices.

Another possible reason for these problems could lie in infections from a viral pathogen called *Macrobrachium rosenbergii* Nodavirus (MrNV) which manifests as the so called White Body Disease (WBD) in larval/PL prawns. This disease is known to have already devastated the prawn hatchery industries of Thailand, China and India recently. Although most of the classic symptoms seen during the current problems do not fit the recognized symptoms of infection by this virus, some symptoms (especially white/opaque tail muscle and rapid mortality) have been seen. Additionally, at least one unconfirmed report has suggested that this virus has already caused mass mortalities and economic losses in some Bangladeshi prawn hatcheries in Chittagong. Whilst previous analysis conducted by DOF and Khulna University has failed to detect this virus in Bangladesh, there may be issues with the techniques used and confirmation using PCR kits developed to detect this virus should be tested in foreign and local laboratories to confirm presence or absence.

Whether or not the ultimate cause of mortalities is due to a bacterial, viral, or any other type of pathogenic organism, this investigation showed that many of the husbandry techniques used in Bangladeshi prawn hatcheries are substandard and operator knowledge and skill is often inadequate. The prawn hatchery industry in Bangladesh is still young and management and husbandry practices have not yet been perfected. The poor management practices noted during the investigation could very well be the direct cause of the problems being seen, and any pathogens present may be of secondary importance, resulting directly from poor management.

Specific management protocols which require improvement include: Lack of screening and poor management of broodstock prawns, lack of biosecurity protocols for all phases of the hatchery cycle, inadequate and uncontrolled treatment protocols for storing, disinfecting and filtering fresh and seawater sources used in the hatchery, inadequate monitoring and control of water quality parameters, poor use of chemical amendments, substandard feeds and feeding regimes, overstocking of both broodstock and larvae, and lack of disease monitoring protocols and tools to monitor and control larval health.

Improved protocols for each of these areas are discussed in this report and implementation of these improvements to basic husbandry techniques may well help maintain water quality, sanitary and health

conditions during the hatchery cycle, thereby reducing stress and hence pathogen infection levels, and in that way resolve the production problems that have recently been plaguing the industry.

Many of these improved techniques have already been documented for use in Bangladeshi shrimp hatcheries and these SOPs are appended to this report. Although many of these SOPs are common for shrimp and prawn hatcheries, the shrimp hatchery SOPs should be modified where required and used as a basis for producing a new prawn hatchery SOP manual, including new sections specific to larval prawn culture. Training courses both in the classroom and through practical demonstrations in selected hatcheries, should then be extended to the hatchery operators in order to train them directly in the implementation and use of these SOPs.

The establishment and/or operation of a number of laboratories including those for bacteriology, virology, chemical quality control and water quality analysis are required to assist disease diagnosis and control of inputs and husbandry in the hatchery sector in order to help underpin adoption of these new SOPs. There are already laboratories in operation in Bangladesh, such as the FIQC bacteriology lab in Khulna, the DOF PCR labs in Dhaka and Khulna and the PCR lab of Khulna University. Approaches should be made to enlist Government support in obtaining access to these labs to help analyse samples and provide support to hatcheries wishing to utilize them.

Stricter Government enforcement of bans on the capture of wild PL and import of foreign (“guest”) PL would be helpful to help reduce the chances of introducing exotic pathogens and to help support and encourage the Bangladeshi prawn hatchery business.

The exact cause(s) of the current hatchery production problems therefore to date remain unknown. A dedicated effort to further analyse the possible causes of these problems (taking on board the results of the recent DOF investigation) is still required and specific sampling and testing procedures required for this analysis are detailed in this report.

It is envisioned that through development, testing and extension of a new set of SOPs for Bangladeshi prawn hatcheries designed to improve husbandry techniques, many of the current production problems can be resolved, even in the absence of the identification of specific pathogens which may be affecting the hatchery stocks.

Introduction

Freshwater prawn farming in Bangladesh has a long history, with freshwater prawns, locally known as golda (*Macrobrachium rosenbergii*) long being part of the production from traditional polyculture systems. The prawn fry were initially either caught from the wild, or came into the ponds naturally with incoming water during tidal exchanges. However, the first hatchery, located in Cox's Bazar, was not built until 1986, after which prawn the prawn hatchery business increased and spread to other parts of Southern Bangladesh (mostly around the Khulna and Barisal divisions).

This was partially a result of a Government-imposed ban in catching wild postlarvae (PL) (in order to avoid possible deleterious environmental problems) in 2000, a ban on importation of foreign "guest" PL from India, and increased demand for PL from the growers. Thus, by 2007, there were estimated to be 81 prawn hatcheries in Bangladesh, although only about half of them were actually operational at this time (Winrock International, 2007). Currently, it is estimated that there remain about 80 prawn hatcheries in Bangladesh, but production problems have become more severe over the past 2 years (especially during 2011-2012).

These problems have resulted in most of the prawn hatcheries closing down (at least temporarily) and most of those that have been operational over the past 2 years have had massive mortalities of larvae and poor production, resulting in unprofitable businesses and a lack of hatchery-reared PL on the market. It is unclear how many hatcheries will operate this year (2013) due to these recent poor results and the financial risks involved in culturing prawn PL in the hatcheries under current conditions.

That there is a market for hatchery reared PL, is clear from a recent 2012 study by Winrock International and KATALYST, which estimated that there was a total demand for at least 1,000 million PL prawns, whilst total supply was just 200 million from the wild (despite the ban) and just 30 million from currently producing hatcheries. It is clear therefore, that the hatcheries will be unable to come close to meeting the demand for seed if these production problems remain unresolved, and heavy illegal capture of wild PL, plus illegal importations are likely to continue in order to satisfy this demand.

With this background, the USAID-funded Feed the Future Aquaculture project (FtF AQ), implemented by the WorldFish Centre, Bangladesh was asked to identify the causes for this poor production and help resolve these problems to reestablish a profitable prawn hatchery sector in Bangladesh and help meet demands for hatchery reared prawn PL.

History of the Production Problems

Over the past two years, since early 2011, many of the Bangladeshi prawn hatcheries have experienced massive mortalities due to unknown causes during the larval rearing cycle, leading to significantly reduced survival and loss of production leading to unprofitable operations. Because of these problems, most of the established hatcheries have been shut down, and of those that remained operational, most lost money due to the poor production obtained.

During this timeframe, there have been a number of initiatives looking into the problems faced by the hatcheries and to identify the reasons underlying these increasing problems. These have included:

1. A committee from Department of Fisheries (DOF) with a multidisciplinary team, mainly from DOF and Dhaka and Mymensingh Agricultural Universities, including Dr. Md. Nazmul Ahsan, Professor and Head of Fisheries and Marine Resource Technology Discipline, Khulna University. The results of the investigation by this committee will be released in a seminar in DOF, Dhaka on 13th March, so remain as yet unknown.
2. Winrock International with consultant Dr. Md. Ayaz Hasan Chisty, professor from Fisheries and Marine Resource Technology Discipline, Khulna University.
3. A project entitled: “Detection of *Macrobrachium rosenbergii* Nodavirus (MrNV) — An emerging threat to the sustainable prawn production in Bangladesh” (October 2010 to September 2013) funded by the Bangladesh Academy of Sciences (BAS) under the BAS-USDA Program in Agricultural and Life Sciences, NMST Bhavan, Agargaon, Dhaka-1207, Bangladesh, by Professor Ghausiatur Reza Banu, Fisheries and Marine Resource Technology Discipline, Khulna University.
4. Bangladesh Fisheries Research Institute (BFRI) investigation into prawn hatchery problems from Shrimp Research Station, Bagerhat, from 2012, run by H. M. Rakibul Islam, Scientific Officer.
5. Previous WorldFish investigations with reports by Sukumar Biswas and Md. Kamruzzaman (National consultants).
6. Baseline water quality study along the shrimp value chain for UNIDO/BQSP/BEST conducted by Stirling University, 2011.
7. Analysis of MrNV and protozoan infections in Bangladesh prawn hatcheries by the Aquaculture Development Network (AQADEN).

Based on the results from the questionnaires and analyses conducted under the above projects, a description of the problems faced by the prawn hatcheries is given below:

Description of Recent Hatchery Problems

Major symptoms

Most of the prawn hatcheries in Bangladesh (and indeed elsewhere, including neighboring India) have been affected over the past two years. From surveys conducted under the various studies mentioned above, the main symptoms of the problem in Bangladesh appeared to vary between hatcheries, together with the time of their appearance during the larval rearing cycle. Some hatcheries report problems immediately on hatching, whilst others stated that symptoms (and subsequent mortality) did not arise until later in the cycle, ranging from 5-8 days, to 10 days, all the way to stage 11 at about 18 days of culture.

Often, there are no particular symptoms reported by the hatchery operators, and the larvae merely appear to have low appetite and gradually disappear from the larval rearing tanks, especially after water exchanges. Where there are symptoms, the following are the most common:

- Larvae have abnormal colour and shape often immediately after hatching. Primary differences from normal larvae are black or blueish colour (sometimes in spots or lines on the body) as early as upon first hatching
- Larval movement is abnormal, showing random slow movement or spinning on the water surface and the larvae appear weak and do not move with the water current nor form clusters as is their normal behaviour
- Appetite is low and feed intake is reduced
- The head and eyestalks of the larvae appear large, whilst the tail region appears thin
- Larvae do not grow normally and have problems with moulting
- Over time, larval colour turns whitish or greyish (and sometimes greenish)
- Some larvae develop reddish patches on body surface (possibly expanded chromatophores)
- After 4-5 days, the weaker larvae are broken by the aeration and appear on the walls of the tanks
- After 6-7 days, some larvae move slowly and settle to bottom of the tank
- Larvae die progressively until 9-10 days, after which, rapid mortality and disappearance of larvae is evident over the next 3-4 days
- Ultimately, production is very low, with up to 100% mortality and often complete failure of the production cycle

Remedial actions taken by hatchery operators

These symptoms are not specific to any known disease problem and the cause(s) remain as yet unidentified. It is still unclear whether this originates from pathogen(s) causing disease or to some other reason. In response to these symptoms, all hatcheries take remedial action in trying to avoid larval mortality. In nearly all cases, this has not proved successful and mass mortality (up to 100%) has resulted. In most cases, hatchery operators assume that the problems are caused by a disease which is caused by either viral, bacterial, protozoan or fungal infections, such that the standard response has been to try one (or more) of a range of antibiotics, both prophylactically, every few days (up to 5-6 times) through the cycle, and as a treatment once the problems start. The antibiotics used are usually oxytetracycline (OTC), often in combination with ciprofloxacin, each at 2-3ppm or more for 24 hours, but a host of other antibiotics, including erythromycin, chloramphenicol, furazolidone and whatever else is available in the market, are also being used. In addition, most hatcheries also use 24 hour bath treatments with formalin

(usually at 25-35ppm of the 37-40% stock solution) routinely through the cycle and in response to any disease outbreaks/health problems apparent with the larvae, but particularly in response to supposed protozoan and fungal infections.

However, in some hatcheries, problems have been less, and some have been almost unaffected, producing good numbers of PL. These are unfortunately in the minority though, and analysis of the management differences between successful and unsuccessful hatcheries have so far failed to reveal any consistent differences between them which could account for success or failure. Therefore, without further analysis, the real cause(s) of the problems remain elusive.

Conclusions from previous studies on hatchery problems

Possible diseases

Since most of the treatments applied to try and remediate the problems encountered in the hatcheries have been unsuccessful, the hatchery operators/owners believe that this means that the problems are therefore not likely to be due to bacterial or protozoan/fungal infections, otherwise these treatments would have worked. Alternatively, it is believed that perhaps the chemicals used are sub-standard and ineffective, or that the causes for larval disease and death are due to other reasons, including viruses (which are known to be unaffected by such treatments) or environmental. Thus the conclusion of the Winrock report from 2012 was that whilst the causes remain unidentified, it may be considered most likely a new viral disease (perhaps imported with foreign “guest” PLs from India), or due to environmental problems.

However, this idea appears to be disputed by the findings of the DOF committee which analysed the hatchery production problems last year. Although their report and major conclusions was not available during this consultancy, discussions with one of the researchers indicated that they were unable to find any viral problems (despite checking for 7-8 different viruses), but that they did find high concentrations of potentially pathogenic bacterial species in the larval rearing tanks monitored during their study. Hence, their preliminary conclusions were that bacterial problems probably play an important role in this syndrome.

Although little work has been done testing for different viruses that may affect prawn larvae, there has been some work conducted by two of the above initiatives on *Macrobrachium rosenbergii* nodavirus (MrNV) which is a viral pathogen known to have caused devastating losses to larvae in prawn hatcheries in other countries in the region, including India and Thailand. Both these studies failed to get positive results from their analyses, but further efforts are being made to collect samples and have them double checked in Thailand, or with the use of Thai PCR kits for this virus in Bangladesh. Moreover, the symptoms associated with MrNV infections of larval prawns are not reflected in most of the symptoms as mentioned above occurring in Bangladeshi hatcheries. Which instead are characterized by definite whitening of the tail muscle in the larvae towards the end of the larval rearing cycle. Although some whitening of the larvae has been noticed, it is not the most dominant symptom, which suggests MrNV may not be the primary cause of the current problems in Bangladesh. However, this should be double checked with foreign PCR primers and labs (see later section). Additionally, there is one report from the Bangladeshi Aquaculture Development Network (AQUADEN at www.aquaden.org) which reports up to 100% mortality and massive economic losses due to MrNV from prawn hatcheries in Chittagong, suggesting that this virus may be present in Bangladesh, but they could not yet be reached for confirmation of this finding.

In further support of a bacterial cause, the BFRI report suggests that the direct causative agent may be due to a syndrome recorded in prawn hatcheries in other countries including Malaysia, Thailand, Philippines and Australia, known as Mid Cycle Disease (MCD). The actual pathogen causing this syndrome is currently unknown, but researchers have suggested that it may be considered infectious in nature and may be caused by a bacterial species called *Enterobacter aerogenes*. This suggestion is based on the symptoms of the sick larvae found in Bangladeshi hatcheries, including microscopic symptoms with young larvae (stage IV-XI) exhibiting a blueish colouration, with weak, spiral swimming, loss of appetite, atrophy of hepatopancreas epithelium, cannibalism of weak larvae by healthier ones, disappearance of large numbers of larvae and mass mortalities 16 days post-stocking. However, general bacterial necrosis has similar symptoms (See Annex 3), and no work was done positively identifying this disease and the possibilities of this syndrome being the cause will be discussed in a later section.

Protozoan parasites including *Epistylis*, *Zoothamnium*, *Vorticella*, *Acineta*, and *Ephelota* often attach to the body and gills of larval prawns and can lead to stress and mortality if left untreated. Recent research reported by the Aquaden organization in Bangladesh reported discovery of a severe infestation of an unknown protozoan which has had adverse effects on seed production in Khulna prawn hatcheries. This infection was reported to come from contaminated surface water, which was not treated adequately. Although many hatchery operators discount this possibility (despite not conducting routine microscopic analysis), due to their frequent use of formalin, protozoan parasites commonly have deleterious effects on prawn hatcheries and without proper monitoring and control could cause mass mortality, so this may be a contributory factor in the current problems.

Poor management practices currently in use

In addition to the above suggested direct pathogenic causes of larval mortality, all previous reports on this problem believe that hatchery management is substandard and may be the most important factor causing the current hatchery production problems. These poor management practices are manifested in many ways and may be summarized as follows:

Lack of investment in hatcheries Owners current reluctance to invest in their hatcheries is due to previous problems resulting in increased cost of production, variable and often poor market value for hatchery reared PL and hence operating losses, leading hatchery owners to reduce spending on hatchery infrastructure. This is particularly noticeable in a lack of water treatment equipment to filter the water prior to use, lack of water exchange in order to save money on buying and transporting expensive seawater/brine, a lack of water and larval health monitoring equipment, and in some cases to a lack of water heating capacity. If the nature of this problem can be determined and measures put in place to prevent/treat it, then owners will be more likely to consider re-investing in their hatcheries.

Lack of expertise in hatchery management and rearing protocols Many of the hatchery owners are not technical and thus have to rely of technical staff to run their hatcheries and there is a general lack of suitably qualified staff in Bangladesh. Although there have been small scale training courses run previously, notably, one by Winrock International with a local NGO in 2009-2010, and one by USAID in Shibli hatchery, Patuakhali: the majority of staff have never received technical training in prawn larviculture and most do not have an academic background in either aquaculture or fisheries. Consequently, most of the protocols used in Bangladeshi prawn hatcheries appear to be identical and seem to be based on a 2005 prawn hatchery manual produced by BRAC. Although this manual was not

available at the time of this consultancy visit, some of the protocols which were presumably from that manual and which are still being used by most hatcheries now appear to be inadequate in the face of the current problems and need adjustment, but the current technical ability of the hatchery staff appears inadequate to make the required adjustments without alternative protocols and enhanced training. Therefore, a new SOP manual and training courses based upon it are required to improve the technical ability of the hatchery personnel.

There is general poor adherence to best management practices, code of conduct or SOPs, especially with regards to lack of biosecurity (absence of footbaths, hand washing facilities, larval tank and building cleanliness) etc. This should be remedied by production of an updated SOP manual for Bangladeshi prawn hatcheries, and then this information should be provided to the hatchery operators through training courses conducted in selected hatcheries, to which all operators can come and learn these improved techniques.

Overstocking of broodstock holding and larval rearing tanks (in response to anticipated poor survival), leading to poor water quality, stressful conditions and more frequent disease problems.

Obtaining broodstock from uncertified sources including both wild and pond-reared broodstock without passing through quarantine or disease-checking prior to entry into the hatchery facilities. Currently broodstock banks of genetically improved broodstock are not available.

Poor water quality resulting from poor source water (both fresh and salt/brine) quality, lack of storage and treatment facilities and inadequate disinfection protocols for water. Plus inadequate water exchange and waste siphoning protocols during larval rearing.

Failure to monitor and control water quality during larval rearing due to a lack of water quality monitoring equipment, inadequate water exchange, poor quality feed and sub-optimal feeding regimes.

Although most hatcheries have rudimentary water quality analysis equipment and do not monitor water quality parameters as they should, reports on studies done on water quality and operator experience have suggested that water quality may not be a major cause for the hatchery production problems recently encountered. However, this conclusion may be erroneous and will be discussed in a later section of this report.

Inability to monitor the pathogen status of hatcheries and stocks due to lack of equipment, competent laboratories and expertise in prawn pathogens both within the hatcheries (most hatcheries do not possess a microscope) and within local institutions such that the real causes of this production problem have yet to be identified.

Poor use of hatchery feeds due to the expense and unavailability of good quality artemia cysts (and poor disinfection protocols used), non-enrichment of artemia nauplii, possible under-or overfeeding and the use of egg custard as an artificial diet without additional ingredients normally included to enhance nutritional status of the larvae.

The use of substandard or adulterated chemicals (often made for agricultural use) which may have been repacked and mislabeled in Bangladesh, and without the ability to check quality and active ingredients.

Over-reliance on antibiotics, with ineffective protocols for use which may well have created antibiotic-resistant strains of pathogenic bacteria, which could now be responsible for the current production problems. For example, 100% of the hatcheries visited in previous studies of this problem have reported using at least oxytetracycline (and often other antibiotics as well) as 24 hour bath treatments using a low level of 2-3 ppm every 5-7 days, for a total of 4-6 applications during each cycle. Standard protocols elsewhere would treat sick larvae with higher dosages (5-6ppm) every day over a treatment period lasting 5-7 days, once tests had been conducted to ensure that such doses and application times were capable of killing the problematic pathogens. However, no such testing has been done to identify effective antibiotics, and no bacteriology is done to confirm the bacterial origin of disease problems encountered. Such treatments are thus unlikely to have any positive effects and instead are very likely to promote antibiotic resistance, rendering the use of such antibiotics ineffective. For this reason, the claims of some people that this problem cannot be of bacterial origin due to the non-effectivity of the antibiotics given is probably erroneous. Alternative prevention and treatment protocols using probiotics and disinfectants should be developed to replace the use of antibiotics.

In many cases, particularly with viral disease, and in many instances of disease caused by bacterial, protozoan or fungal parasites, improving management, especially in terms of implementing SOPs including proper disinfection protocols, destruction of infected stocks, followed by disinfection of all facilities, use of separate equipment for each tank, tank covers to reduce disease transmission between tanks, optimizing larval nutrition through good quality, decapsulated, disinfected and enriched artemia, supplemented by high quality artificial diets, improving water quality through increased monitoring, control (with probiotics and disinfectants), water exchange and reducing stocking densities may all be expected to assist maintenance of good water quality and healthy larvae which can promote high survival of quality PLs.

Certainly, attention should immediately be placed on developing protocols and training the hatchery staff in management of these SOPs in order to improve general husbandry of the prawn hatcheries, which should in turn lead to improved production of high quality prawn PLs.

Environmental problems

In addition to management problems, there is a consensus amongst hatchery operators and researchers that environmental problems may be contributing to these production problems in the hatcheries. Specific problems could include the following:

- **Temperature:** During the first cycles over the past 2 years there has been extreme climatic variation from the normal situation which has made it difficult to maintain constant and ideal temperatures in the larval rearing tanks (especially for hatcheries lacking electric water heaters)
- **Salinity:** Most hatcheries take fresh/slightly saline water from the local rivers, which allows them to reduce the amount of expensive brine required. However, over the past 2 years, from March-June (during the first cycle of those years), river water was of very low salinity meaning more brine was required, and also resulting in the low availability of ripe (brown-coloured egg) females from the rivers. This meant many hatcheries were taking immature (orange-coloured egg) bearing females, which require longer maturation in the hatcheries (under stressful conditions) which resulted in poor hatching results

- **Drought:** Especially during the first cycle of 2012, there was a long drought in Bangladesh, meaning that there was insufficient water available to the hatcheries to use their normal sources of rain/well or river water for the hatcheries. This lack of water made it difficult to exchange enough water and therefore impossible to maintain adequate water quality during the larval rearing cycle
- **Geographical location:** Results from last year from the different hatchery locations suggested that some of the hatcheries located in the Barisal district performed relatively better than those in the Khulna division. This is most likely due to the presence better water quality in Barisal, especially in regards to river water quality, which tends to be better with less sediment and pollution than that obtainable from the Khulna area. It is clear that most of the prawn hatcheries in Bangladesh are located in either the Khulna or Barisal divisions, and that neither location is ideal, as both rely of local fresh/slightly saline water supply from the rivers in their respective areas, and mostly on brine water trucked in at great expense either from Cox's Bazar, Koira or Munshigonj. There is some concern that there may have been changes in the quality of brine water from the salt pans in Cox's Bazar, Koira and Munshigonj, and that possibilities of chemical/pesticide contamination exist. Reports have been made that brine water quality was inferior in 2012, with the water being very dirty (high sediment load), with a bad smell. However, there does not appear to have been any analysis of these brine solutions, which is urgently required. What would be preferable is if the hatcheries were located close to the sea, so that good quality seawater could be used to dilute with rain or clean river/bore water for making the ideal 12ppt salinity water required for prawn larval rearing. Recommendations on water quality are made in the following section.

Current Visit

Analysis of Hatchery Problems

At the time of this consultants visit to Bangladesh (23rd February-1st March, 2013), unfortunately none of the hatcheries were in operation as they were all just starting preparations for the first cycle of the year, which is due to start in early/mid-March. Therefore, it was difficult to get first-hand knowledge of exact techniques used. Neither was it possible to look at samples of sick larvae nor take samples for analysis in the disease laboratories of Thailand, although one sample of adult prawns taken from a grow-out pond in Bagerhat and one sample of a broodstock prawn with whitish muscle (obtained from Khulna University) were taken and will be analysed for MrNV in Thailand. Results are not available yet, but will be communicated to WorldFish in Bangladesh when obtained.

With the inability to analyse sick shrimp first hand or take samples, this consultancy concentrated on having discussions with national consultants, prawn hatchery owners and operators, and technical specialists who have been working on these recent production problems, and associated with previous efforts at analyzing and resolving this problem as listed above.

A number of hatcheries were visited and their operational protocols reviewed in order to determine any deficiencies based on standard protocols used elsewhere.

It is envisioned that once the new cycle begins in March 2013, the consultant should return to Bangladesh to help provide better operational protocols, analyse any problems and take samples of sick larvae and broodstock for analysis both within Bangladesh and (if necessary) in Thailand.

The main potential problems identified as a result of these discussions and analyses are summarized below:

1. Inadequate technology/protocols for hatchery management

1.1 Water quality

It does appear that poor water quality management may be one of the major causes of these production problems, as lack of control over this critical part of hatchery management can lead to a multitude of problems with larval health and production. Currently poor protocols are being used and there is a lack of proper monitoring and control measures in place.

Brine water: Brine is currently trucked in from the salt pans in either Cox's Bazar, Koira or Munshigonj and used to dilute the locally available freshwater supply to make the 12ppt salinity water required for prawn larval rearing. The salinity of this brine is often very high (at 120-180ppt), meaning that less of it is required to make up 12ppt salinity water used in larval rearing, saving costs of purchase and transportation. However, there may be a number of problems with this brine water:

1. It may be contaminated with chemical/pesticide residues. This possibility needs to be investigated using facilities for pesticide and chemical testing (which exist in Dhaka). If found to be contaminated, then alternative sources of seawater/brine need to be identified, or artificial salt solutions can be prepared and used directly in the hatchery. A salt mixture used elsewhere for prawn hatcheries is included in Annex 1.

2. Because the brine water is such a high salinity, once diluted, there may be an imbalance in the mineral content of the resulting brackish water. This problem is common, and elsewhere is dealt with by taking brine at no more than 90ppt (although this will incur more expense). Commonly, once diluting these high salinity brines, most minerals are present in adequate concentrations and at suitable balance, with the exception of the halogens (iodine and bromine) which tend to be lacking in the diluted brine water. For this reason, addition of compounds such as sodium or potassium bromide at 0.01-0.03 mg/l for 12 ppt salinity water are used elsewhere to support larval health, as it is known that larval prawns can suffer mortalities when their culture water lacks these halogens. It is suggested that mineral levels in the brine (and diluted brackish water) are checked by mass spectrophotometry. If unavailable in Bangladesh, there is a lab in the USA that can do this (www.amtestlab.com) cheaply (\$75/sample), to ascertain whether this may be a cause of the production problems. If this is found to be the case, such supplementation of the lacking elements can be tested to see if this resolves the problems.
3. The brine brought in last year was very dirty (high suspended solids and smelly) so required settlement/disinfection before use. However, the current protocols used by the hatcheries tend not to treat the brine water, but merely to store it until mixing with freshwater, which is then treated (normally by settlement and chlorination). What would be better is if the brine itself is treated by settlement and filtration prior to mixing and disinfection, which would assist proper disinfection of the brackish water used for larval rearing.

Fresh water: The fresh, or slightly brackish water used in the prawn hatcheries is either obtained from rain water (when there is no drought), river/canal water, or from wells dug on site. In most cases, these water sources could be contaminated with chemicals or pesticides used in agriculture or other industries or sometimes with potential prawn pathogens, especially viruses, bacteria, protozoan and fungi. The following precautions should therefore be taken:

1. Conduct analyses of the freshwater sources used for potentially harmful chemicals/pesticides and if found, search for alternative supplies of freshwater.
2. Conduct bacterial analyses of freshwater supplies both before and after treatment to ensure that all potential pathogens are eliminated before use. This can be done using an outside laboratory (i.e. the FIQC lab of the DOF in Khulna), or on site with the help of local bacteriologists. This is something that is done in a crude, but effective manner in most shrimp hatcheries to check water disinfection protocols and determine the sources of contamination within the hatchery – i.e. water sources, algae and artemia cultures, broodstock etc. such protocols should be outlined in a prawn hatchery SOP and then staff trained in how to do this for themselves in the hatchery.
3. As is the case for the brine, the freshwater supplies used in most prawn hatcheries in Bangladesh are also untreated and are usually stored in large open earthen ponds within the hatchery grounds, whether the original freshwater source is from rain, river/canal or bore. No proper treatment of this freshwater is attempted and it is merely mixed with the brine/seawater and then settled and chlorinated. However, it was clear from this visit that the freshwater supplies were often highly turbid, with much phyto- and zoo-plankton, suspended solids and other organic matter present. If this water is not treated prior to mixing with brine and chlorination, this amount of organic matter will utilize much of the active chlorine and render it unable to properly disinfect the water sources (especially with the use of low concentrations of weak chlorine as is the case in Bangladesh). In

addition, any potential pathogens may not be killed, leading to proliferation of them in the larval rearing tanks and hence disease.

Mixed brackish water: Once the supplies of salt (brine) and freshwater have been obtained, the standard practice is for the 2 sources to be mixed in appropriate ratios to derive 12ppt water suitable for prawn larval rearing. Typically, the appropriate quantities of brine and freshwater are pumped direct from their respective holding tanks into a mixing tank. This is then settled and chlorinated by taking 10-12 grammes of 35% calcium hypochlorite, dissolving this in the mixing tank and then aerating for a few minutes, then leaving without aeration for 2-12 hours. This is then usually dechlorinated by adding 7-12ppm or more of sodium thiosulphate. Sometimes (but not always) residual chlorine levels are then checked with a test kit and if clear, the water is pumped through a coarse home-made sand filter into the larval rearing tanks. Often water used for the broodstock holding tanks is not treated like this, with untreated water at the appropriate salinity (often 5-6ppt) being pumped directly into the broodstock holding tanks.

In either case, there is never any attempt made to measure the response to the treatment in the final quality of the treated water. It is necessary to at least occasionally check bacterial concentrations in the treated water as a measure of the level of disinfection obtained during the treatment process. This should be done first as research (perhaps in agreement with the FIQC lab in Khulna) and thereafter included in the SOP manual and taught to the hatchery operators. Alternatively, the hatchery operators should be offered the opportunity to send samples to an existing (or new) laboratory where these analyses can be done and the results given back to the hatcheries in a timely manner.

The currently used chlorination protocol (which appears ubiquitous in Bangladeshi prawn hatcheries) is certainly inadequate to successfully treat these waters for use in larval rearing. The proper protocol is outlined in the shrimp hatchery SOP manual shown in section 1.4 of Annex 5 and should be included in the new SOP manual for prawn hatcheries and taught to the hatchery operators. The main problems with the existing technique are as follows:

1. *Non-filtration of fresh and salt (brine) water:* If the two kinds of source waters are mixed and disinfected without first filtering them to remove all suspended and organic matter, then these impurities will neutralize the chlorine disinfectant used, meaning it is unable to kill the pathogens that it is meant for. Therefore pathogens present in the source waters will be passed onto the larval rearing tanks resulting in the kinds of problems currently seen. Currently, these waters are pumped into the mixing tank and partially settled before chlorination. However, settlement and chlorination should be conducted in separate tanks, as settled solids are largely organic and will render chlorination less effective, so if settlement (rather than filtration of source waters) is done, this should be in a separate tank to that in which the water is subsequently chlorinated, and all water coming from the settlement tank should also be filtered to 1-5 microns prior to chlorination.
2. *Inadequate concentrations of chlorine utilized:* The chlorine normally used in Bangladeshi prawn hatcheries is (supposedly) a 35% active chlorine type of calcium hypochlorite. Usually 10-12 grammes of this powder are added per 1,000 litres of water meaning that, if the powder really is 35% active chlorine, in reality the dose rate of active chlorine is just 3.5-4.2ppm. Standard protocols using **clean, filtered** water are for 10ppm of active chlorine for 24 hours contact time. Thus current practices are probably under-chlorinating by at least 3 times the recommended dose

rates. If possible hatcheries should try to source stronger chlorine (60-70% is readily available and used in shrimp hatcheries in the country, and 90-100% may also be available). The actual chlorine content can be checked with simple techniques and this should be done on each batch of chlorine purchased to ensure that the actual active chlorine concentration is known prior to use. Additionally, in order to optimize chlorination protocols it is essential to monitor bacterial concentrations before and after treatment with different quantities of chlorine to assess the correct (minimum) dose to remove 100% of the bacteria. Only in this way can the hatchery operator be sure that his disinfection process has been successful.

3. *Inadequate time provided for disinfection:* As mentioned above, currently, prawn hatchery operators are turning on the aeration immediately after adding the chlorine in the mixing tank and starting the dechlorination process immediately, which will quickly reduce the concentration of chlorine in the tank to a point where it cannot disinfect the water successfully. Standard protocols elsewhere turn on aeration after adding chlorine for a few minutes, just to disperse the chlorine in the water, then it is left for 12-24 hours without aeration so that the chlorine has time to kill all pathogens at maximum concentration
4. *Over-reliance on sodium thiosulphate for dechlorination:* Standard practice in Bangladesh is to use sodium thiosulphate to neutralize any chlorine left in the mixed water prior to pumping into the larval rearing tanks. Thus, the treatment water is only being chlorinated (with low active chlorine levels in highly organic water) for a few hours, before it is all dissipated, rather than 12-24 hours. Additionally, normally in Bangladeshi prawn hatcheries, 1ppm of thiosulphate is used for every 1ppm of chlorine added into the tank. Thus, with 10g of calcium hypochlorite added, 7-12g of sodium thiosulphate is used for dechlorination¹. The problem with this protocol is twofold:
 1. Excess use of thiosulphate leaves a residual in the water which is toxic to the prawn larvae and
 2. The correct ratio of thiosulphate is 1ppm for every 1ppm of active chlorine left at the time of dechlorination – not 1 ppm of thiosulphate for every 1ppm of total chlorine powder added to the system. Thus, it appears that sodium thiosulphate is being overused and may even be causing toxicity to the larvae (which could explain the immediate poor quality of the larvae on hatching). To be safe, thiosulphate should not be used at all and instead, heavy aeration applied to the tanks 24 hours after chlorination, together with direct sunlight (through transparent roofing tiles over the treatment tanks) so that all chlorine can be blown off without the need to use chemicals. This will take more time than using thiosulphate, but the benefits could be substantial.
5. *Inadequate filtration following chlorination/dechlorination:* The standard protocol used currently is to pass all treated water through a home-made coarse-grained sand filter, comprising whole shells, rocks and rough sand directly into the larval rearing tanks prior to stocking the larvae. There is no use of fine (1-10 micron) cartridge filters or filter bags or UV filters as is standard practice in shrimp hatcheries worldwide. Such fine filtration is required, especially after using low quality chlorine powder as a lot of sediments may remain after chlorination and filtration by sand alone. These particles, if left in the water, can negatively affect larvae due to blocking of gills and adherence of particles onto the larvae body and limbs, resulting in poor mobility and difficulties moulting, as has been noticed in the current problems affecting the hatcheries. Therefore, following chlorination/dechlorination, water should be passed through a sand filter and then through in-line cartridge filters or filter bags (preferably down to 1-5 micron pore size)

¹ In the USAID/PRICE prawn hatchery manual, 100ppm of sodium thiosulphate is recommended to dechlorinate 10ppm of chlorine. This may be a typo, but should not be used under any circumstances

before (ideally and if possible) passing through a UV filter to ensure sterilization of water, before final passage to the larval rearing and broodstock tanks. If possible, an active carbon filter can also be used following chlorination to remove chloramines, which are a byproduct of chlorination and which cannot be removed by mechanical filtration as they are dissolved in the water.

6. *Non-treatment of broodstock water:* As mentioned above, typically, the water prepared for the broodstock holding and hatching tanks (if separate from the larval rearing tanks) is not chlorinated, but used without treatment. This exposes the broodstock and externally held eggs and larvae to all the pathogens found in the raw source waters. Clearly this is undesirable and all water used in the hatchery whether for broodstock or larval rearing must be disinfected as described above and in section 1.4 of Annex 5.

Due to the above reasons, it is highly probable that pathogens are entering the larval rearing system through improper disinfection of water so that just adjustment of this water treatment protocol will improve the production of prawn larvae and reduce disease issues within the hatchery.

Water quality testing and maintenance: Water quality used for larval rearing needs to be checked routinely for important parameters and maintained as follows:

Parameter	Ideal range	Stressful levels	When	Equipment
Temperature	28-30°C	<25, >31°C	Twice daily	Thermometer
Salinity	12ppt	<10, >15ppt	Daily	Refractometer
pH	7.5-8.0	<7, >8.5	Twice daily	pH meter or test kit
Dissolved oxygen	5-8ppm	<5ppm	Daily	DO meter or test kit
Ammonia	<0.1ppm NH ₃	>0.1ppm NH ₃	Every 3 days	Test kit
Nitrite	<0.1 ppm NO ₂	>0.2ppm NO ₂	Every 3 days	Test kit
Hardness	50-100ppm	<40, >150ppm	Weekly	Test kit
Alkalinity	80-130ppm	<50, >200ppm	Weekly	Test kit

Water quality parameters:

- *Temperature:* temperature during larval rearing must be controlled ideally at 28-30°C throughout the larval rearing cycle and with minimum diurnal fluctuations (<1°C difference between day and night) in order to promote optimum growth and survival in the larvae. Especially during the first cycle from March – May, temperatures can often be cool, and during the past 2 years, cold snaps have reduced hatchery temperatures during the first cycle, which may have contributed to the production problems. Most hatcheries have electric heaters to help maintain water temperatures, but often not enough for all tanks, and sometimes only enough for the reservoirs holding the treated water. This inevitably results in big changes in tank water temperatures day to night and especially during water exchanges as cold water is replaced by hotter water. This is a big stress for the larvae and may have contributed too many of the problems encountered over the past 2 years. Treated water at 12ppt should therefore be held in the same environmental conditions as the larval rearing tanks and all water quality parameters adjusted to closely match those in the larval rearing tanks before changing water, so that water exchanges will not result in stressful changes in water quality. Furthermore, each larval rearing tank should have its own heater and temperatures maintained constant and optimal (28-30°C) throughout the cycle. This is aided by

covering each tank with plastic sheet, to help maintain water temperature, which has the additional benefit of reducing contamination between tanks.

- *Salinity*: Salinity must also be controlled at ideal levels (12ppt during larval rearing) and constant throughout the cycle, which again means monitoring and controlling salinity levels especially during water exchanges to avoid stressful salinity changes. On the other hand, the standard protocol for holding and hatching broodstock is to hold and hatch them at 5-6ppt and then slowly bring up the salinity to 12 ppt for larval rearing. In some cases, the broodstock are hatched directly in the larval rearing tanks and water salinity is increased within 3-7 hours to 12ppt for continuing larval rearing once the spent broodstock have been removed. This process is very stressful for the larvae, and should be done more slowly so that the broodstock are hatched in 5-6ppt and then removed, after which the salinity is gradually brought up to 12ppt over at least 24-48 hours, and not more quickly than that to reduce stress due to salinity shock. Rapid salinity acclimation could be a cause of the poor movement of newly hatched larvae and subsequent lack of appetite and mortality.
- *pH*: pH should be controlled to be a constant 7.5-8.0, although hatching of larvae in the broodstock hatching tanks has been found to be better at lower pH of 7.0-7.2, which can be reached through addition of an acid such as hydrochloric acid (HCl) until this pH level is reached. pH levels can be adjusted by adding some form of lime (CaCO₃, CaOH or CaO) to increase, or an acid (HCl) to decrease it.
- *Dissolved oxygen (DO)*: Dissolved oxygen is maintained through proper use of enough airstones in the larval tanks to maintain oxygen saturation or at least 5ppm at all times. Air blowers should never be allowed to stop, so diesel powered generators are required, sufficient to at least continue running the air blowers in order to maintain DO levels. Any break in aeration will cause stress and very quickly larval mortality. Ideally, DO measurements are taken by DO meter, but since this is an expensive item, and DO can be assumed to be adequate as long as the air blowers are constantly working, many hatcheries do not measure DO, which is acceptable if close eye is kept on aeration in the tanks.
- *Ammonia (NH₃) and Nitrite (NO₂)*: Both of these are breakdown products of shrimp metabolism and tend to build up in the water of larval rearing tanks as the cycle progresses and more feed is added to maintain an increasing biomass of prawn larvae. Improper feeding regimes, particularly overfeeding with artificial diets like egg custard, and a failure to adequately siphon the bottom of tanks or change enough water can all lead to rapid increases in ammonia and nitrite levels, which can quickly stress the larvae and lead to disease and mortality. The dangerous form of ammonia, the unionized NH₃ part, is toxic at very low levels and especially at higher pHs, so routine water quality monitoring is required to aid decision making about when and how much water must be changed on a daily basis and whether or not to add probiotics to help control these byproducts. Thus, the absence of test kits for ammonia can quickly lead to water quality problems which can manifest in sick and dying shrimp, whilst the hatchery operator is unaware that there is any problem with his water quality.
- *Hardness and alkalinity*: Hardness is a measure of the divalent magnesium and calcium ions in the water and alkalinity is a measure of the basic substances in the water (bicarbonates, carbonates, phosphates, hydroxides etc.). These must be kept within optimum ranges for prawn larvae, or they may suffer from weak shells and problems with osmoregulation, moulting and swimming. These can be adjusted upwards through addition of lime, dolomite and sodium

bicarbonate to the water until optimal levels are obtained. Routine monitoring and adjustment is required. Some surface fresh waters and rain water can be very low in both measures and thus may require supplementation. On the other hand, some bore waters may be excessively high in both hardness and alkalinity, which may require adjustment downwards through aeration and filtration or the addition of hydrochloric acid or sodium bisulphate or zeolite.

- *Mineral content:* Ensuring that diluted seawater/brine has the correct concentration of minerals at correct ratios is vital to support optimum growth and health of larval prawns. Complete analysis of the mineral content of the water used in larval rearing is required to ensure that it provides a good balance of essential minerals and to identify any treatments or supplements required to optimize the balance. This is particularly important for hatcheries using brine (as mentioned above) which could well be limited in halogen groups, or deep well water (which may have excess heavy metals). The ideal concentration and balance of minerals in 12 ppt water is given in Annex 2.

Water exchange protocols: Data provided to the consultant during this trip suggests inadequate water exchange during the larval rearing cycle, perhaps due to the expense of obtaining, treating and pumping water and perhaps due to lack of training of the operators. In many cases, operators reported no changes for the first 3-4 (or even up to 10) days, followed by a 30-40% change, then no change for another 3-4 days, after which a water treatment with an antibiotic and a disinfectant (typically 2-3ppm oxytetracycline and 20-35ppm formalin) is done, after which 70% of the water is changed, then the process is repeated, with 30-40% exchange after another 3-4 days, followed by another treatment and 70% water exchange 3-4 days later. This represents an average of about 30% water exchange per day after the first 3-4 days. Other hatcheries use even less, changing 60% of the water every 4-5 days, equivalent to just 13% water exchange per day.

This is almost certainly not enough and results in poor water quality, which remains unknown to the operators due to their inability to check most water quality parameters, especially ammonia and nitrite and bacterial concentrations. In order to maintain water quality in open (flow-through) larval rearing systems with heavy use of feed, periodic water exchanges must be carried out. These should start 3-4 days after stocking with the first addition of formulated feeds (with merely addition of water to a 50% full tank on stocking done over the first few days). Thereafter, and in increasing quantities, water exchanges amounting to roughly 50% of tank volume per day (or every other day) in the middle of the cycle up to 50-100%/day by the end of the cycle are usually required to maintain ideal water quality. The amount of water exchange will depend on the stocking density and amount and type of feed fed, plus the stage of the larvae and results of water monitoring. The routine use of probiotics can also help reduce water exchange requirements as the bacterial strains in good probiotics can reduce concentrations of ammonia, nitrite and bacteria and protozoans in the water, thereby reducing the requirement to change water. In addition, and daily (after 3-4 days) as a matter of routine, the aeration should be stopped and any settled material and dead larvae should be siphoned from the larval rearing tanks in order to help maintain water quality. Many operators reported omitting this practice or using it infrequently. This should be incorporated into the SOP manual and training of operators conducted on the importance, timing and extent of water exchange and siphoning.

Alternatives to this water exchange system would be to introduce either green water (algal-based) closed water systems or biofiltration, limited exchange systems, both of which are used elsewhere in the world,

with consequent savings in costs associated with purchase and shipping of water, and improvements in water quality maintenance. However, this technology is largely unknown and untested in Bangladesh, so should only be investigated as a long term solution to these problems.

1.2 Chemical use

Use of unchecked and ineffective (and in some cases illegal) chemicals could be a contributory factor to the current production problems. Testing of the products used for content, efficacy and legality is required. Use of other products (like probiotics, disinfectants and EDTA) instead of antibiotics may help resolve some of the problems currently being experienced.

Antibiotic abuse: It appears that 100% of prawn hatcheries use a wide range of antibiotics to try to resolve the production difficulties that they are currently facing. This can create problems for three main reasons:

1. All of these antibiotics (with the exception of oxytetracycline) are banned from use in prawn culture due to continuing problems with antibiotic residues in prawns exported from Bangladesh
2. None of them appear to help prevent the problems being faced, at least using the current application protocols, and may in fact lead to reduced growth
3. Antibiotics can be dangerous for the staff using them

It has been reported and many hatchery operators believe that due to the non-effectiveness of nearly all antibiotics used (including oxytetracycline OTC, furazolidone, chloramphenicol, erythromycin and others), that the production problems cannot therefore have a bacterial origin. This impression is probably false for the following reasons:

1. The current protocols for using antibiotics are mostly prophylactic, where low doses (24 hour baths using 2-3ppm typically of OTC) are given routinely every 5-7 days over the larval rearing cycle, with a 4-6 total applications. However, higher doses are given using the same application in case of disease outbreaks. This use of antibiotics is sure to create antibiotic resistance in the potentially pathogenic bacterial strains found in the hatchery, especially with constant use of the same antibiotics over the past few years. Thus, without first checking for sensitivity of these bacteria to the various antibiotics used and at what concentration, it is unlikely that this type of approach would ever be able to control any pathogenic bacteria present.
2. One hatchery visited had a complete failure (with the classic symptoms mentioned above) in the first cycle of 2012 using their standard prophylactic treatment regime with OTC, so switched to using a new antibiotic for them, of 98% furazolidone in the second cycle which resulted in good production, indicating that their problems may have been due to OTC-resistant bacteria.
3. No hatcheries (or outside institutions) are conducting bacteriological analysis of the infection levels of bacteria within the hatchery process. Thus, there is complete unawareness of bacterial loading in the hatcheries. The only known analysis of bacterial counts in the hatcheries conducted last year by the DOF committee appears to have found very high levels of various pathogenic bacteria in the affected larval rearing tanks, suggesting that these strains were indeed present and could therefore be assumed to be causing problems during larval rearing².

² Final results of this analysis are not available until 13th March, 2013

4. From analysis of the hatchery management protocols it would appear that there are multiple potential avenues for bacteria to enter the larval tanks, multiply and cause problems – particularly with substandard protocols for water disinfection, lack of effective broodstock/egg disinfection protocols, lack of effective artemia nauplius disinfection, poor biosecurity, inadequate water exchange and overstocking of hatchery tanks. This suggests that even if water disinfection were successful, pathogenic bacteria could still enter the systems through one of the many other potential routes of infection.
5. Since the antibiotic products available in Bangladesh are often illegal, there is no control over which products are sold and their quality in terms of purity and content, so many of the antibiotics used may not be what is claimed on the package and may be ineffective for that reason.

If antibiotics are used in prawn hatcheries, they must be used according to strict SOPs, which have already been developed for Bangladeshi shrimp hatcheries (See section 1.6 of Annex 5) and these should be modified for inclusion into new SOPs for prawn hatcheries.

What is essential now is a more thorough investigation into the possible bacterial origin of the hatchery production problems. Some of this work may have been done by the DOF-led committee who will release their findings of their investigation on 13th March. However, if there are gaps in this report, what is required is that a local laboratory is identified (ideally asking permission of the DG Fisheries to use the excellent FIQC lab in Khulna) where bacterial analysis of some representative hatcheries can be done (see section 4.4 and recommendations).

Underuse of probiotics: Very few hatcheries reported use of probiotics to help control water quality and disease in prawn hatcheries in Bangladesh. However, elsewhere in the world, in both shrimp and prawn hatcheries, traditional use of antibiotics has been almost entirely replaced by the use of probiotics. There are a number of reasons for this:

1. Probiotics are not banned by governments or shrimp/prawn importers, so can be safely used without the danger of leaving residuals in the shrimp
2. Bacterial pathogens cannot gain resistance to probiotics despite long term use of low amounts, so effectivity is not compromised over the longer term
3. Probiotics used prophylactically, are capable of improving water quality (ammonia, nitrite, organic matter and bacterial concentrations) which reduces stress and helps prevent disease in larval shrimp/prawns. In contrast, antibiotics do not improve the environment, but merely kill bacteria (when effective), without remediation of the causes of stress which permit expression of disease, thus doing nothing to prevent future reinfections
4. Probiotics can also be used as a treatment for some disease problems and as a tool in remediating poor water quality and gut health, thereby improving larval health and disease resistance

Probiotics are new to prawn hatcheries in Bangladesh, but considerable experience has been gained in Bangladeshi shrimp hatcheries and various products are already available in the market. Care must be taken to select appropriate probiotics for use in brackish water prawn hatcheries and ideally, each potential product should be screened first to ensure its quality and content. Discussions with the BFRI lab in Bagerhat revealed that they are capable of performing this task, so they should be contracted for this purpose.

Protocols for use and confirmation of efficiency of probiotics are given in the SOP manual for shrimp hatcheries shown in section 3.3 of Annex 5. This should serve as the basis of new SOPs developed for probiotic use in Bangladeshi prawn hatcheries.

Testing and use of disinfectants: In addition to probiotics, other legal chemicals including a range of second-generation disinfectants can be used during hatchery preparation and larval rearing instead of relying on only antibiotics and formalin to resolve bacterial contamination. These products are rarely used in Bangladeshi prawn hatcheries currently and should be tested for quality and efficacy and if successful, their use detailed in the SOP manual and extended to the hatchery operators to help them maintain biosecurity and hygiene during the hatchery operations. Such products include disinfectants such as povidone iodine, Virkon-S, PUR and chloramine-T. The use of these chemicals is detailed in the shrimp hatchery SOP manual included in section 3.2 of Annex 5.

Another crucial area where disinfectants are required is in treating broodstock on arrival, berried broodstock or first stage larvae on stocking of the larval rearing tanks and artemia nauplii prior to feeding. At present, a standard disinfection of female broodstock is done just once on introduction to the broodstock holding tanks of the hatchery. This is commonly done by using a bath treatment for 15-30 minutes with as little as 30ppm up to 200-250ppm of formalin (actually a 37% formalin solution) meaning that the effective dose of active ingredient can be as little as 11 ppm or as much as 92 ppm of formaldehyde. The normal recommended dose rate would be 25-30ppm formalin for 24 hours or 200-250ppm formalin for 30 minutes. In both cases, the operator must ensure that aeration levels are maintained full and that after the bath treatment, the water is exchanged at up to 100% to dilute out the excess formalin. After hatching, (preferably in separate broodstock tanks), the larvae should be collected in a fine mesh net and then re-disinfected using one treatment with 200-250ppm formalin for 30 seconds, washed then a second treatment with 50-100ppm povidone iodine for 1 minute (this protocol is included in the shrimp hatchery SOP manual in section 2.4 of Annex 5). This protocol is essential to prevent the spread of diseases from the mother to the larval prawns. However, it is seldom done at present, with even females being spawned directly inside the larval rearing tanks, offering no chance to disinfect the larvae/females prior to stocking. This could be a significant source of contamination which could lead to the production problems currently being experienced. Artemia nauplius disinfection will be dealt with in the next section.

No quality control of chemicals used: None of the chemical and drug products currently used in Bangladeshi prawn hatcheries (including those currently used unsuccessfully to control the current production problems) have been checked for quality and effectivity by competent authorities. It is suspected that many of these products are substandard, do not contain the active ingredients as on the label, may have been repacked and adulterated in Bangladesh, and in many cases are either out of date or otherwise ineffective. It would be very helpful to establish/utilize a government laboratory where such analyses could be done, to ensure use of high quality, efficacious products within the hatcheries (for all aquaculture in the country).

Poor quality products in market: As mentioned above, many chemicals and drugs may be substandard and useless or worse, potentially hazardous. In addition, some products, particularly feeds, such as artemia nauplii may also be substandard and there are suspicions that particularly artemia cysts, which are increasing dramatically in cost are being repacked and adulterated in Bangladesh as hatch rates are

inferior to previously. This is causing problems with underfeeding and overspending for the hatcheries. This also requires routine monitoring and certification of such products by a competent authority, so that hatchery operators can be sure of the products that they are using. It may be that the artemia nauplii being shipped from the USA are simply lower quality than previously due to a lack of supply of high grade cysts on the world market, but this requires confirmation to know for sure.

Underuse of EDTA: Very few of the hatcheries report using the chelating agent EDTA to help chelate heavy metals and control bacterial populations in the water used in their hatcheries. Heavy metal toxicity may be one of the underlying causes of the hatchery production problems. Typically 10ppm of EDTA is added to all water used in the hatchery for this purpose, and this is particularly important when using high salinity brine and potentially contaminated fresh/brackish water containing industrial effluent or from deep bores, which may contain high loads of heavy metals. Once water samples from the various water sources used in the hatcheries have been analysed by mass spectrophotometry (see water quality section), the requirement for EDTA will be clear, but without this, there is no current way of knowing if heavy metal toxicity is a problem for the larvae. This is vital information.

1.3 Feeding regimes

Correct feeding of prawn larvae to maximize intake of high quality diets whilst minimizing the potentially negative effects of feed residues left in the system is crucial in maintaining a clean environment that can support the growth and health of the prawn broodstock and larvae whilst in the hatchery. Feeds comprise of live artemia nauplii, supplemented with artificial diet (typically a home-made egg custard) and occasionally other artificial diets for the larvae and either compounded diets or fresh feeds fed to the broodstock.

Artemia: Since artemia nauplii are the main feed used during larval rearing of prawns, are a significant fraction of the cost of operating a prawn hatchery and are a potential vector for disease, it is vital to make sure that they are used according to the strictest protocols. A complete set of SOPs for artemia decapsulation, hatching and disinfection are included in the shrimp hatchery SOPs included in sections 3.6-3.10 of Annex 5. These protocols should be included in the new SOP for prawn hatcheries, together with a feeding chart specifically for prawn larval rearing, as feeding protocols differ from those used in shrimp hatcheries.

Artemia cysts of the best quality are becoming scarce and increasingly expensive, and over the past two years have almost tripled in cost from Tk 18-22,000 per case (\$46/kg) to currently Tk 50-60,000 (\$130) per case of 5.4 kg. This has led to hatcheries trying to cut back on feeding artemia, which may well leave the larvae weak and susceptible to disease. In these situations, it is imperative to follow the best protocols for optimizing the use of artemia. Currently most hatcheries conduct decapsulation of the artemia cysts which helps improve hatching rate and partially disinfect the artemia nauplii. However, it is not enough to just decapsulate, and disinfection of hatched nauplii is also required to prevent contaminating larval prawns with infected artemia. Currently, artemia nauplius disinfection is not done, or done crudely with 30-200ppm formalin for 30 minutes. This is not enough and disinfection with hydrogen peroxide (as in section 3.10 of Annex 5) is far more effective at disinfecting the nauplii and separating and removing the dirty, unhatched cysts (left after decapsulation) from the edible nauplii.

Having a government certified QC laboratory available to test batches of artemia to inform hatcheries as to the relative quality of the artemia available in the market, and ensure suppliers were providing suitable products would also be beneficial.

Artemia cyst hatching protocols also require optimization, and currently, hatching is often done with insufficient light and using 30ppt salinity water, which is often untreated and therefore possibly contaminated. This should be changed (as detailed in the SOPs in section 3.8 of annex 5) to provide sufficient light (2 * 60 watt bulbs/tank), maintaining temperatures at 28°C, adding 1-2g/l sodium bicarbonate to buffer the water to pH 8-8.5, and utilizing chlorine-disinfected brackish-water (5-25ppt, optimum 10-20ppt) rather than salt-water (30-35ppt), and hatching a maximum of 1 g cysts/litre to optimize hatching rate.

With reduced availability of high quality artemia cysts, lower quality cysts in particular may be nutritionally incomplete and better nutrition can be derived by enriching the artemia prior to feeding them. This is not currently done in most Bangladeshi prawn hatcheries (but is sometimes done in shrimp hatcheries). Techniques for enrichment are well known and should be put into the SOP manual. Basically, this process involves feeding 6-12 hour old artemia nauplii with 0.25 ml/litre of a home-made lipid (cod liver or krill oil) emulsion³, or enrichment product such as INVEs selco for a further 6-12 hours to allow accumulation of lipid droplets within the artemias' digestive tract, thus enhancing their nutritional value to the larval prawns. On harvest from the enrichment tank, the artemia nauplii are washed, re-disinfected and then fed.

Artificial diets: Currently all hatcheries make some version of egg custard to supplement artemia use as feed for the developing larvae. A typical formulation used in Bangladesh would comprise whole eggs, milk powder, ground prawn flesh, water and a vitamin B supplement. If using this formulation, it is possible to pass disease from the prawn flesh in the diet to the larvae, so it would be better to substitute finely ground and sieved fish meal, fish flesh or bivalve (mussel) flesh instead of prawn flesh to reduce the chances of this. In addition, various useful additives can be incorporated into the mixture including Stabilized vitamin C (at 0.2-2g/kg), and cod liver or krill oil at about 10% of the mixture to improve nutrition of the larvae. In addition, adding red food dye to the mixture has been shown to improve feeding behaviour of the larvae.

Feeding protocols: Feeding protocols currently being used in the Bangladeshi hatcheries may be suboptimal, with underfeeding of artemia and overfeeding with egg custard being common, combined with a lack of monitoring larval health and feeding status, leading to poor nutrition of the larvae and often deterioration of water quality and disease. Hatchery operators require training into SOPs for feeding regimes. Recommendations are as follows: First feeding of live, unenriched artemia nauplii should start in the evening of the day of hatching into stage 1 larvae, then continued as the only food source until day 3-4. At this point, egg custard, sieved to a suitable size should be added 2-3 times per day between 7am and 3 pm for days 4-5 then 4-5 times per day (also between 7am and 3pm) every day thereafter until harvest. Meanwhile, live artemia nauplii (preferably enriched) are fed 4-5 times/day (including an additional feed just before dark) for days 2-5, then artemia feedings are gradually reduced to just once per day (plus always an additional feeding just before dark) by the time the larvae reach the first PL stage.

³ Home-made emulsion comprises: 200ml of fish/krill oil + 200ml of hot freshwater blended with 10 ml of emulsifier (liquid soap) + 20g stay-C

After PL1 stage, artemia is only fed once per day as the last feed of the day to allow the larvae to feed at night without suffering problems with water quality.

Feed amounts will depend on stocking density, larval survival and appetite and health status and must be adjusted daily to always have availability of feed in the tank and ensure that larvae are filling their digestive tracts with feed, whilst being careful not to overfeed. At any sign of overfeeding siphon and/or change water in the larval rearing tank to prevent pollution.

In general, for artemia, there should be about 5 artemia nauplii per ml of tank water immediately after feeding, and just 1/ml immediately before the next feeding. Prey density in the water and the level of larval gut fullness (i.e. lipid droplets in the hepatopancreas and gut) is best checked by microscope if available. For egg custard, the basic rule is that each larva should be seen carrying one piece of egg custard immediately after each feeding with egg custard. This should equate to a daily ration of about <10% of the total larval biomass in the tank (which can be estimated by periodic weighing of samples of about 80 larvae per tank and multiplying individual weight by total surviving population). Do not overfeed with this or the water quality will quickly suffer. Ensure that aeration is always sufficient to maintain these inert feeds in suspension and that routine daily siphoning is done to remove uneaten feed before the next feeding. The size of the egg custard particles increases as the larvae grow from 0.3 mm on first feeding on day 3-4 up to 1mm by metamorphosis into PL stage.

Due to the recent rapid price increases for high quality artemia cysts, alternative artificial feeds could be tried, including EZ artemia from Zeigler (already available in Bangladesh as it is used in the shrimp hatcheries), which has been shown elsewhere to reduce or even eliminate artemia requirements in shrimp hatcheries.

1.4 Stocking

Many hatcheries are stocking their larval rearing tanks at 100-170 larvae per litre, which is higher than recommended stocking densities for freshwater prawns in single-phase, flow-through rearing systems (50 up to a maximum of 100 larvae per litre). In some cases, effective stocking densities are even higher, as larvae are stocked into tanks held at low water levels for a few days, as the tanks are gradually filled, resulting in effective stocking densities of up to 250-500 larvae per litre at the start of the cycle in some hatcheries.

The reasons given by the hatchery operators for such overstocking were that since they expected survival rates to be low, they deliberately overstocked at the beginning. This technique should be discouraged since this will result in large numbers of sick and dying larvae in the tanks, which will infect the healthy ones, leading to poor survival. It also places more pressure on broodstock supplies, since much more broodstock are required for such overstocking, resulting in overfishing for broodstock⁴, more expense for buying higher numbers of broodstock, and more difficulties maintaining the large stocks of broodstock required (often 500-600 animals per cycle) to stock the hatcheries. A much better technique is to stock at low densities 50-80 larvae per litre, which will give the hatchery operators the best chance of maintaining water quality and shrimp health and hence obtaining good production. Often, the production of PL prawns from hatcheries is almost independent of stocking densities and a maximum productivity of around 50

⁴ A reduction in the catch (but not quality) of wild broodstock has been noted by broodstock fishermen who report 50-60% lower catches over the past 2 years in the Khulna/Sathkhira/Bagerhat area.

PL/litre is often seen, indicating that aiming for higher survival of larvae stocked at lower densities would be the most cost effective and repeatable way of running these hatcheries.

To stock at these high densities requires very close control over all water quality parameters and strict adherence to standard waste siphoning and water exchange procedures. In many cases, this is not done, leading to water quality problems due to addition of excess feed to support such high populations and thus elevated levels of toxic metabolites (ammonia and nitrite) and bacterial and protozoan/fungal pathogens within the larval rearing tanks. This inevitably leads to disease outbreaks and the kinds of mass mortalities characteristic of the current production problems.

Obtaining broodstock from either wild or farm reared sources without first disease-screening or quarantining them prior to introduction into the hatchery could also be a major source of infection. Currently broodstock are merely surface disinfected by giving them a bath with formalin at 200-250ppm for 30 minutes on arrival in the hatchery then added directly to the broodstock tanks which are often prepared with untreated water. Better disinfection, quarantining in a separate facility using fully treated and disinfected water, and then screening them for pathogens by virology, bacteriology and microscopic analysis should be practiced (See section 4). Production might be improved if a broodstock bank of genetically selected and improved (i.e. Pathogen free SPF or resistant SPR) prawns could be developed and held (especially in greenhouses) separate to the hatchery to prepare them for an early start to the larval rearing season.

Broodstock are also being overstocked into broodstock holding tanks and then given unfiltered, unchlorinated water, which may be leading to poor water quality and excess stress on the broodstock. In addition, without better disinfection protocols for the broodstock and larvae on transfer to the larval rearing tanks, this environment leads to immediate contamination of the water in the larval rearing tanks on stocking, and from there to disease and mortality later in the cycle (as is seen in the current problem). Broodstock should be stocked at no more than one individual per 30-40 litres of water, and all water must be chlorinated according to standard protocols and filtered prior to use, then water must be exchanged at a minimum of 50-100% per day (depending on observations) to maintain optimum environmental conditions and health of the sensitive berried females.

Another potential problem with stocking the larval rearing tanks is in hatching the larvae and acclimating them to conditions in the larval rearing tanks. The first problem is that broodstock are usually held at 5-6ppt for hatching, then the first stage larvae are placed into the larval rearing tanks and acclimated to normal larval rearing salinities of 12ppt. This process is stressful and if done too quickly, can cause larval disease and mortality. Current techniques take only a few hours (typically 3-7 hours) to acclimate from 6-12ppt, whereas this process should take at least 24-48 hours. There should be no hurry to increase the salinity as stage I and II larvae are well able to tolerate low salinities. In some cases, no separate hatching tank is used, and ripe broodstock are placed directly into the larval rearing tanks and the larvae allowed to hatch their before increasing salinity after removal of the broodstock. This technique is undesirable as it leads to contamination of larval rearing tanks from the broodstock and does not permit disinfection of larvae prior to larval rearing.

1.5 Biosecurity

General levels of biosecurity are poor in the majority of Bangladeshi prawn hatcheries. This means that inadequate precautions are taken to prevent the introduction of diseases into the facilities and their spread

around the facilities. A section in the new SOP manual needs to elucidate effective biosecurity protocols to help reduce problems in the hatcheries. Some recommendations from a shrimp hatchery SOP manual could be adapted for use in prawn hatcheries, and are shown in section 1.5 of Annex 5. Specific measures required include the following:

- Repair, clean and disinfect all hatchery infrastructure
- Disease screening of broodstock held in a separate quarantine section of the hatchery prior to use
- Use of footbaths and hand-washing facilities for staff/visitors entering each separate area of the hatchery
- Implementation of proper disinfection protocols for broodstock, eggs/first stage larvae, live feeds, water, buildings and tanks, equipment and personnel
- Use of separate broodstock hatching facilities to separate broodstock holding from larval rearing
- Routine monitoring and maintenance of ideal water quality parameters throughout the hatchery process
- Use of plastic covers over larval rearing tanks during the rearing cycles
- Use of separate equipment for each tank unit in the hatchery to prevent spread of disease tank to tank
- Establish routine for monitoring and analysis of pathogen levels at each stage of the larval rearing cycle and in each input (water, fed, stocks etc) to understand health status of animals at all times
- Screening of chemicals used to ensure content and efficacy
- Thorough disinfection of entire facility between cycles to break pathogen infection cycles
- Establishment of contingency plans detailing actions to be taken when disease outbreaks occur in the facility
- Follow protocols for disposal of sick stocks and water effluents from the facility to prevent re-infection

2. Lack of skilled manpower

In reviewing the comments of previous reports made into these hatchery production problems and interviewing hatchery and research staff during the visit, it was clear that the technical ability of most hatchery operators is substandard. Most of the hatchery owners do not have technical knowledge of prawn hatchery culture and most of the technicians employed have no academic background in aquaculture or fisheries, let alone prawn hatcheries. They have also received little training for hatchery operation, cannot trouble shoot problems and do not have the experience or knowledge on how to test water quality and health of their stocks or prevent or treat any diseases that may arise. Most of the techniques used are remarkably similar, apparently having originated from the 2005 hatchery manual produced by BRAC. Some of the techniques that are being used are clearly inappropriate and inadequate to prevent or treat the current production problems being faced.

The number one priority is thus for a new SOP hatchery manual to be produced and then the hatchery operators trained in the use of these SOPs. This project should take the initiative to develop this manual and then organize training courses to extend the techniques to the hatchery operators and preferably conduct on-site demonstrations with selected partner hatcheries (which will accept visits from other hatchery operators) to help develop, prove and extend these new techniques.

3. Climate change/Environmental problems

Due to the sudden onset of the hatchery production problems in just the past two years, coincident with some environmental changes experienced during that time frame, many operators and experts consider that such environmental changes may have triggered the onset of these problems. Although during this visit, the likelihood of this was impossible to determine, and it is not possible for the hatcheries to control the climate change issues themselves, there are some remedial measures that can be taken to try to mitigate the recent environmental changes observed.

Unusually cold weather during the first cycle of each year can be controlled somewhat through better heating systems, providing electric heaters to each larval rearing tank in each hatchery and by using plastic covers over each individual tank to help maintain ideal temperatures throughout the larval rearing cycles.

Excessively low salinity in the river water usually used to make up the 12ppt water supply for larval rearing can be compensated for by either using more brine or making up salt solutions in the laboratory (See Annex 1) to increase salinities when required.

If ripe (brown-egg carrying females) are not available during low salinity times, then immature (orange-egg carrying females) can be held and matured in the hatchery, but they must be held in treated water, at appropriate densities and with good feeding to optimize broodstock (and therefore larval) health. This may mean building bigger broodstock holding facilities in a separate area, away from the larval rearing tanks to avoid potential contamination from broodstock to larval stages and permit disinfection of eggs/first stage larvae prior to stocking the larval rearing tanks.

Low availability of fresh water during periods of drought can be compensated for through sinking deep wells, or installing rain water capture devices or building bigger freshwater holding ponds to tide the hatchery over during periods of drought.

Problems with the quality of brine, perhaps resulting from contamination of salt pans, must be subjected to analysis of the quality of brine from the different available locations (i.e. Cox's Bazar, Koira and Munshigonj), checking for both pesticide and mineral levels to make informed decisions on which is the better source for purchase. Additionally, artificial seawater can be made if brine quality remains poor. Finally, those hatcheries located far from good supplies of sea (or fresh) water may have to be shut down and/or relocated to better areas with access to higher quality waters.

Although all of these measures involve some degree of investment, thereby increasing operational costs, it must be remembered that mass mortalities increase cost of production much more, and if such mortality can be (at least partially) mediated through the use of these techniques, then they will be cost effective to implement.

Additionally, some people have speculated that two large cyclones, Sidr and Aila in 2007 and 2009 may have caused deleterious changes to the environment, but the mechanism for this is unknown and as yet, nobody has been able to demonstrate what these changes might have been. Thus, remedial measure to counteract these changes are not possible at this time.

Other environment-related problems which may have had an impact on the Bangladeshi prawn hatcheries include the siting of industrial and agricultural businesses within 1 km of some prawn hatcheries

(especially around Khulna and Sathkhira). Whilst these may have had an effect through toxic residue leakages to those particular hatcheries, it seems unlikely that they can be responsible for the consistent production problems recorded over the past two years all over Bangladesh.

4. Disease issues

As mentioned previously, since none of the hatcheries were working at the time of this visit and no broodstock or larvae were available for inspection or sampling, it was difficult to identify any causative agent for these problems during this visit. However, it appears likely that poor management may be playing a significant role in the recent hatchery production problems, and that resolving these technical problems should help improve production levels. Whilst the possibility remains that a particular disease (possibly new to Bangladesh) may also be involved in these problems, and may even be the ultimate cause of them, improving management techniques should still improve hatchery performance and help to prevent the worst effects of any disease present.

In searching for diseases, neither the investigations conducted to date nor the hatchery owners and operators themselves have yet been able to identify any new disease or occurrence of an existing known disease, and so far nearly all efforts at prevention and control utilizing the currently available technology and protocols has failed to resolve the problems.

The set of symptoms recorded as associated with these problems (although quite varied and inconsistent) appear to be new, which does suggest a new disease agent, but which may also be due to other problems including changed environmental conditions, some new toxicity from a chemical or product used or a new more pathogenic strain of an existing disease agent. A summary of the potential disease agents and their symptoms which may have a role in these production problems is given in Annex 3, whilst possible prevention methods and treatments for prawn larval diseases are given in Annex 4.

4.1 Bacterial diseases

The most thorough analysis yet conducted on this problem by the Bangladeshi DOF committee appears to have indicate the likelihood of a bacterial pathogen (See Annexes 3 and 4), whilst ruling out known viruses, but the full results of their study are still unavailable until 13th March this year.

The tentative conclusions of the BFRI study suggest that due to the symptoms observed, the problems may be caused by the so-called Larval Mid-Cycle Disease (MCD), about which very little is known, although there has been some speculation that it may be caused by the bacterium, *Enterobacter aerogenes* (See Annex 3) although this was not confirmed by their study. Additionally, normal larval bacterial necrosis, often caused by a combination of *Vibrio*, *Leucothrix* and other bacterial species can have similar symptoms (See Annex 3), and should not be discounted. These two bacterial diseases certainly present the symptoms which most closely match the most common symptoms observed with these hatchery production problems. However, further research is needed to positively attribute the current problems to any specific disease.

If bacterial pathogens are to blame, then the best remedy would be to attempt prevention of bacterial contamination through utilization of all the biosecurity and improved disinfection and management protocols suggested in this report, and to conduct sensitivity analyses on the pathogenic bacteria with legal, certified antibiotics and probiotics to determine optimal prevention and treatment chemical and

protocols. The hatcheries themselves, with a set of SOPs (as shown in section 6.2 of Annex 5) and some training can carry out some basic bacteriology themselves to assist this process.

The BFRI study meanwhile, all but discounts a bacterial pathogen, due to the non-effectiveness of almost every antibiotic tried, and instead opined that a new virus or environmental problems may be to blame, but without any evidence for this theory.

4.2 Viral diseases

In other Asian countries including China (from 2003) India (2004), Thailand (2006), Australia (2008) and Taiwan (2008), a new viral disease known as White Tail Disease (WTD) caused by the *Macrobrachium rosenbergii* nodavirus MrNV (usually in conjunction with an associated virus called the extra small virus or XSV). This disease has been proven to be responsible for mass mortalities of larvae and postlarvae in these other countries (See Annex 3). There is a lot of concern that this viral disease may be responsible for the problems in the Bangladeshi prawn hatcheries. This is especially so considering the fact that many prawn PL are being imported from India and that these PL may well have been infected with this virus and thus served as a vector for introducing it into Bangladesh, resulting in the problems seen recently.

Preliminary investigations looking for this virus in Bangladesh have been largely negative, with studies from DOF and the University of Khulna being unable to find positive results. However, there is a question mark over the techniques they used and independent confirmation is required to double check these results. This should be done either by sending samples of larvae (and broodstock) showing the characteristic symptoms to overseas labs using the PCR kits already developed for detecting this virus, or by buying these kits and importing them into Bangladesh and testing samples in a PCR lab locally⁵. This is urgent, especially considering an unconfirmed report from the webpages of the Chittagong-based Aquatic Development Network (Aquaden) who claim to have found positive results of MrNV and mass mortalities of late larvae and PL in a few prawn hatcheries in Chittagong. This report could not be verified during this visit, but should be verified as soon as possible.

One concern with this theory of MrNV causing the current problems however, is that the typical symptoms of WTD are of white discolouration of the tail muscle of infected larvae, which is not the typical symptom of the production problems as described by previous research into these problems, but which has been seen by a number of hatchery operators, indicating that this virus may at least be partially responsible for some of the problems being faced. This requires confirmation by more analysis of infected samples with the PCR kits designed for this virus either abroad or in Bangladeshi labs provided with these kits.

WSSV and IHHNV are also known to infect freshwater prawns, and while it is believed that negative results were obtained by the DOF committee testing samples of larvae for these viruses, confirmation using other samples sent for analysis to foreign laboratories is still required. There are also a number of other largely unidentified viruses, which could be causing these problems and very little is known about these and no PCR kits have been developed as yet to confirm their presence. What information is available about these other viruses as included in Annexes 3 and 4.

⁵ For example the lab in the Fisheries and Marine Resource Technology Discipline of the University of Khulna, or the DOF labs in Khulna or Dhaka, with support from WorldFish

If it is found that a virus is responsible for these production problems, there would not be any treatment possible, and prevention of viral disease through exclusion (screening of potential viral vectors, especially broodstock and other crustaceans⁶) and maintenance of environmental conditions to reduce stress would be the best techniques for minimizing problems due to viruses. In addition, the government ban on importing foreign (“guest”) PL should be more strictly enforced.

4.3 Protozoan diseases

Protozoan parasites (i.e. *Epistylis*, *Zoothamnium*, *Vorticella*, *Acineta*, and *Ephelota Spp.*) and fungal problems (i.e. *Lagenidium Sp.*) also commonly affect production in prawn hatcheries and they may be responsible for some of the problems encountered (See Annex 3). There is another unconfirmed report by Aquaden reported discovery of a severe infestation of an unknown protozoan which has had adverse effects on seed production in Khulna prawn hatcheries, originating from contaminated surface waters, underlying the importance of proper treatment and disinfection of all source waters as discussed previously.

Most hatchery operators and researchers do not consider protozoans a likely source of the current production problems, as they use frequent formalin (a known treatment for protozoa), baths to treat them, but without the routine use of microscopes, it is difficult to be sure, so better microscopic analysis should be made during the new production season to discount this possibility. If found, trials with different doses of appropriate disinfectants (i.e. formalin, chloramine T, virkon, PUR or povidone iodine), together with the use of improved water exchange and siphoning protocols, and possibly probiotics should be made to find a protocol which can eliminate these protozoan parasites.

4.4 Identification of pathogens

In order to identify whether there are pathogens causing these production problems, and what these pathogen(s) may be, a series of tests will need to be conducted aimed specifically at identifying the causative agent. Some of these may have been done by the DOF committee, but any gaps left will still need filling.

The tests required will involve the following:

Bacteriology: In order to determine whether one or more bacterial species are responsible for the current problems⁷, a thorough comparative analysis of bacterial species and abundance should be performed on selected samples of infected (those animals showing the classic symptoms of the production problems) and non-infected healthy control larvae to test for differences in bacterial infection levels between these larvae. A second set of tests should also be done, looking at the presence of bacteria in the hatchery environment and at the effects of the various treatment/disinfection protocols. These tests should be done in a reputable laboratory and the FIQC lab in Khulna appears perfect for this purpose. Permission should be asked from the DG of Fisheries for use of this laboratory. Specific tests which should be done are included in the later section on recommendations.

⁶ At least for MrNV, WSSV and IHNV using PCR

⁷ In Vietnamese prawn hatcheries, researchers identified 4 main *Vibrio Sp.* bacterial pathogens as being the most common in the water, the larvae and artemia eggs: *V. cholera* (62%), *V. alginolyticus* (20%), *V. carchariae* (10%) and *V. mimicus* (8%), whilst others have found *V. harveyi* and *V. parahaemolyticus* especially common in cases of luminous vibriosis. *V. alginolyticus* was also found in Thai prawn hatcheries, but did not appear to cause disease

Analyses of these results will be able to provide a clearer picture on what bacteria species (groups) are present, in what concentrations they occur, what are the major sources of contamination, whether the disinfection/treatment protocols used are effective or not at removing them, and finally, what are the patterns of bacterial concentration through the larval rearing cycle, and whether this can be correlated with known disease outbreaks in the hatchery.

Virology: There are 5 viruses that have been detected previously in *Macrobrachium* which could be affecting Bangladeshi hatcheries currently, with an unknown virus also a possibility. Of the 5 known viruses, there are only PCR kits for detection of three of them: WSSV, IHHNV and MrNV, so samples of infected broodstock and larvae (and artemia) should be tested (against uninfected controls) for these three as described in the section on recommendations.

Protozoology: Comparative microscopy looking at infected larvae showing the classic symptoms of these current problems against non-infected larvae growing and surviving normally to look for any signs of any protozoan parasites. These are most commonly seen on the external surfaces of the larvae, especially gills, pleopods and on the exoskeleton and can be clearly seen in fresh larvae by microscopy by a trained specialist.

Fungology: Comparative microscopy looking at infected larvae showing the classic symptoms of these current problems against non-infected larvae growing and surviving normally to look for any signs of any fungal hyphae/mycelium growing inside the larval bodies. In addition, yeast infections are known to cause problems in cooler temperatures, especially in combination with high levels of organic matter. Such yeast infections can cause the muscles to change colour to yellow, blue or grey, which can also be checked by microscope by a trained specialist.

Conclusions

This consultancy was conducted in order to investigate the reasons for the current prawn hatchery problems which have been affecting Bangladesh over the past two years, and make recommendations to the hatchery operators on how to prevent the future recurrence of these problems.

Interviews were conducted and analysis was made of the previous efforts by researchers from governmental and non-governmental organizations investigating these problems, and the experiences of hatchery operators in trying to cope with these problems.

The classic larval health symptoms observed during these production problems were identified and described, but do not precisely fit any existing model for shrimp disease. However, they are closest to known bacterial problems associated with heavy infections by a number of pathogenic bacterial species including *Vibrio*, *Leucothrix* and possibly *Enterobacter* or *Staphylococcus sp.* Such bacterial infections were indicated as being the most likely cause of the problems by the recent DOF-sponsored analysis of the problem conducted in 2012 and due for dissemination on March 13th 2013. Further analysis is required using specific measures as detailed in this report to fully understand which species are involved, what levels are present and which methods would be best at reducing numbers to non-pathogenic levels.

Due to the fact that all hatcheries visited were still dry in preparation for the first stocking of the New Year, direct examination of larvae exhibiting the classic symptoms of the problem was not possible, nor could samples be taken for further analysis. Efforts should be made once the new cycle starts to take water and prawn (broodstock and larvae) samples for external analysis in order to better understand the cause of these problems. Which samples are required and what needs to be analysed is detailed in the next section of this report.

Another possible reason for these problems could lie in infections from a viral pathogen called MrNV which manifests as the so called White Body Disease (WBD), which is known to have devastated the prawn hatchery industry of Thailand, China and India recently. Although most of the classic symptoms seen during the current problems do not fit the recognized symptoms due to infection by this virus, some symptoms (especially white/opaque tail muscle and rapid mortality) have been seen and at least one unconfirmed report has suggested that this virus has caused already mass mortality in some Bangladeshi hatcheries. Whilst previous analysis conducted by DOF and Khulna University has failed to detect this virus in Bangladesh, there may be issues with the techniques used and confirmation using PCR kits developed to detect this virus should be tried in foreign and local laboratories to confirm presence or absence.

Whether or not the ultimate cause of mortalities is due to a bacterial, viral, or any other type of pathogenic organism, this investigation showed that many of the husbandry techniques used in Bangladeshi prawn hatcheries are substandard and operator knowledge and skill is often inadequate. The prawn hatchery industry in Bangladesh is still young and management and husbandry practices have not yet been perfected. The poor management practices (especially in terms of water quality maintenance) noted during the investigation could very well be the direct cause of the problems being seen, and any pathogens present may be of secondary importance, resulting directly from poor management. Therefore, improvements to these basic husbandry techniques (even without identification of any pathogenic agents)

may well help maintain water quality, sanitary and health conditions during the hatchery cycle, thereby reducing stress and hence pathogen infection levels, and in that way resolve the production problems that have recently been plaguing the industry.

The husbandry techniques used in the hatcheries and the technical ability of the staff therefore require upgrading in order to ensure proper protocols are used to give the best chance of success. A new SOP hatchery manual is urgently required and many of the SOPs required are detailed in this report (See Annex 5). Training courses both in the classroom and through practical demonstrations in selected hatcheries, should then be extended to the hatchery operators in order to train them directly in the implementation and use of these SOPs.

The establishment and operation of a number of laboratories including those for bacteriology, virology, chemical quality control and water quality analysis are required to assist disease diagnosis and control of inputs and husbandry in the hatchery sector in order to help underpin adoption of these new SOPs.

Stricter Government enforcement of bans on the capture of wild PL and import of foreign (“guest”) PL would be helpful to help reduce the chances of introducing exotic pathogens and to help support and encourage the Bangladeshi prawn hatchery business.

The exact cause(s) of the current hatchery production problems therefore remain unknown. A dedicated effort to further analyse the possible causes of these problems (taking on board the results of the recent DOF investigation) is still required and specific sampling and testing procedures required for this analysis are detailed in this report.

Recommendations

Revise old 2005 BRAC hatchery manual to update and correct techniques where appropriate.

Produce a new SOP manual for prawn hatchery management in Bangladesh (modified from the shrimp hatchery SOP manual included in Annex 5), aimed at preventing and eliminating the production problems encountered. Particular emphasis should be placed on developing new SOPs for the following problematic areas:

- Biosecurity planning and implementation
- Disinfection techniques for hatchery facilities, water, feeds, seed and workers
- Handling, disinfection and filtration of fresh and salt (brine) water supplies to the hatcheries
- Formulation of brackish water using salts (to replace the use of the possibly contaminated or mineral-deficient brine) as in Annex 1
- Sourcing, handling, screening and disinfection of broodstock and acclimation of larvae
- Maintenance of good water quality through not overstocking, monitoring and controlling water quality parameters, use of suitable water exchange and siphoning techniques, use of suitable feeds and feeding protocols, control and use of chemical additions and treatments, use of alternatives to antibiotics (including probiotics, disinfectants and EDTA)
- Routine health monitoring (including basic bacteriology) in the hatcheries to understand health status of animals and environment throughout production cycles.

Organize training courses for hatchery technicians in prawn hatchery management, including seminars (where ideas and experiences can be discussed with hatchery operators) and cycle-long demonstrations in partner hatcheries where practical training in the new SOPs can be provided.

Request governmental support for the industry by stricter enforcement of the ban on imported (“guest”) PL from India and the wild capture fishery for prawn seed to help reduce the chances of disease introduction and help encourage reinvestment in the hatcheries.

Lobby for establishment of new, or earmark existing laboratories to help analyse water quality, prawn diseases and quality of chemical and other inputs into the prawn hatcheries to ensure health and safety of prawns and workers in the hatcheries and support hatchery production.

Enlist the help of the Golda Hatchery Association of Bangladesh (GHAB) to help coordinate activities and liaise between private and public sectors to help support these goals.

Liaise with Government DOF to request collaboration of FIQC laboratory in Khulna to help conduct bacteriological examination of hatchery samples from the new production cycle starting in March. The sampling protocol should be aimed at identifying bacterial species and prevalence in samples of larvae and broodstock, both infected (those showing symptoms characteristic of current production problems) and healthy (those without symptoms) to make comparisons between the two to help identify the causative agents of disease.

Specific analysis using TCBS agar should be done for total, yellow and green and luminous *Vibrio sp.*, with nutrient agar, marine agar (Zobell's), or TSA for total bacterial counts, with blood agar or Vogel-

Johnson agar for *Staphylococcus aerogenes* (often associated with WTD) and MacConkey Agar or EMB agar for *Enterobacter sp.* (possibly involved in cases of MCD).

A second set of tests should also be done, looking at the presence of bacteria in the hatchery environment (water and other inputs) and at the effects of the various treatment/disinfection protocols. This should be done by analyzing samples from the following locations: source waters (fresh and brine/saline river water), mixed 12ppt water both before and after chlorination, berried broodstock before and after disinfection, artemia nauplii before and after decapsulation and disinfection, water in the broodstock holding tanks and water in larval rearing tanks periodically through the rearing cycle (i.e. every 1-3 days), larval rearing water before and after application of antibiotics, probiotics and disinfectants, to ascertain quantitative and qualitative differences in bacterial loads between them. Sensitivity analysis of the potentially pathogenic strains of bacteria identified through these analyses should also be conducted on the various treatment chemicals available in order to establish effective treatment regimens for these pathogens.

Analyses of these results will help to provide a clearer picture on what bacterial species (groups) are present, in what concentrations they occur, what are the major sources of contamination, whether the disinfection/treatment protocols used are effective or not at removing them, and finally, what are the patterns of bacterial concentration through the larval rearing cycle, and whether this can be correlated with known disease outbreaks in the hatchery.

To test for viruses, samples of infected (those animals showing classic symptoms) broodstock and larvae/PL, should be preserved in alcohol and sent to certified laboratories abroad (i.e. SBBU in Thailand <http://www.biotec.or.th>) to test with the full range of PCR kits for all known viral problems (especially those known to infect prawn larvae – i.e. WSSV, IHHNV and MrNV).

To date, MrNV analysis has been done using locally made primers, and these should be validated through side by side tests with commercially available PCR kits (including positive controls) that can be obtained from either Thailand (<http://www.biotec.or.th>) or Taiwan (<http://www.iq2000kit.com>) and supplied to the working PCR laboratories in Bangladesh (i.e. the one in Khulna University and the DOF facilities in Dhaka/Khulna). These laboratories should then be used as reference centres to help screen broodstock and check samples of infected prawns from future outbreaks in the hatcheries.

To analyse for the presence of as yet unknown viruses (without specific primers), prawn samples should be sent to disease laboratories with electron microscopy capability to search for any unknown viral particles in infected prawn tissues.

Encourage the use of microscopes in the prawn hatcheries, including training of the hatchery operators in their use to serve as a tool for assessing larval health and nutrition throughout the rearing cycles.

In cooperation with government/private laboratories, or failing that by sending to foreign laboratories: samples of the freshwater and salt or brine water supplies used in the prawn hatcheries should be analysed for contaminants (chemicals/pesticides) and their mineral balances checked to ensure that they are not contaminated and that they contain the proper concentrations and balance of minerals required for rearing larval prawns (as given in Annex 2).

Annexes

Annex 1: Artificial brackishwater (12ppt) mixture for prawn hatcheries

Salt	Quantity (g/m ³)
Sodium chloride (NaCl)	9,200
Magnesium sulphate (MgSO ₄ .7H ₂ O)	2,300
Magnesium chloride (MgCl ₂ .6H ₂ O)	1,800
Calcium chloride (CaCl ₂ .H ₂ O)	467
Potassium chloride (KCl)	200
Sodium bicarbonate (NaHCO ₃)	67
Potassium bromide (KBr)	9

Note: Weigh and dilute the salts individually with previously filtered freshwater. Add the resulting solutions to a tank in the order shown above, and mix thoroughly using a PVC stirrer. Then add freshwater until the salinity is reduced to 12ppt. Maintain the final solution under strong aeration for 24 hours and adjust the salinity again to 12ppt, if necessary, before transfer to the larval rearing tanks.

The salts do not have to be pure reagent grade, lab or technical grade is adequate.

Annex 2: Normal mineral concentrations (ppm or mg/l) in seawater (32ppt) and brackish water at 12ppt for larval rearing of prawn larvae

Mineral	32 ppt	12 ppt
	Seawater	brackishwater
Magnesium	1,248	468
Calcium	384	144
Potassium	352	132
Sodium	9,760	3,660
Chloride	17,632	6,612
silver	0.0003	0.0001
aluminium	0.01	0.004
arsenic	0.003	0.0011
boron	4.6	1.7
barium	0.03	0.011
beryllium	0.01	0.004
bromine	67	25
cadmium	0.00011	0.00004
cobalt	0.0004	0.0002
chromium	0.00005	0.00002
copper	0.003	0.0011
flourine	1.3	0.5
iodine	0.05	0.019
iron	0.01	0.004
mercury	0.0002	0.00008
lithium	0.17	0.06
Manganese	0.002	0.0008
Molybdunum	0.01	0.004
nickel	0.007	0.003
phosphorus	0.07	0.03
lead	0.00003	0.00001
sulfur	885	332
antimony	0.0003	0.0001
selenium	0.00009	0.00003
silicon	3	1.1
tin	0.0008	0.0003
stontium	8	3
titanium	0.001	0.0004
thallium	0.000001	0.0000003
vanadium	0.002	0.0008
yttrium	0.0000002	0.0000001
zinc	0.01	0.004

Annex 3: Diseases, symptoms and diagnosis of known larval prawn diseases

Disease	Symptoms	Diagnosis
White Body Disease (WTD) caused by Macrobrachium rosenbergii Nodavirus (MrNV)	Opaque, whitish muscles in abdomen (starting at tail, spreading to head) of late larvae/PL, lethargy, anorexia and telson/uropod degradation, followed by severe mortalities up to 100% within 2-3 days of first signs. Can be spread vertically from broodstock and horizontally from prawns, shrimp, artemia and even aquatic insects. Infected larvae also infected by <i>Staphylococcus sp.</i> Bacteria	RT-PCR and LAMP test kits, Elisa and dot-blot and in-situ hybridization
White Spot Syndrome Virus (WSSV)	Lesions in cuticular epidermis, stomach, gills and hepatopancreas. Prawns may be carriers but possibly unaffected by disease. Shrimp and most other crustaceans are carriers	PCR and LAMP test kits
Macrobrachium Muscle Virus (MMV)	Muscle tissue becomes opaque, followed by necrosis. Reduced feeding and swimming. Usually occurs in ponds shortly after stocking causing up to 50% mortality	No kit, only by electron microscopy (looking for icosahedral viral particles in cytoplasm and inclusion bodies)
Macrobrachium Hepatopancreatic Parvo-like Virus (MHPV)	None, not associated with significant morbidity or mortality	No kit, only by electron microscopy
Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)	Eosinic intranuclear inclusion bodies in hepatopancreas of infected PL which can suffer up to 100% mortality	PCR and LAMP test kits
Bacterial Necrosis	Necrosis, melanization (blackening) and breakage of appendages, particularly antennae, pleopods and uropods. Affected larvae do not eat and become blueish, often associated with external infestations of <i>Leucothrix</i> filamentous bacteria, plus internal infections with <i>Vibrio harveyi</i> and others, plus non-filamentous bacilli and cocci. Can be just local, but if systemic, becomes fatal causing mass mortality within 48 hours, especially in younger stage IV-V larvae	Microscopic examination, bacterial plate counting and species identification
Midcycle Disease (MCD)	Bluish/grey colour, reduced food consumption, epibiont fouling, weak spiral swimming behavior, cannibalism, atrophy of hepatopancreatic epithelium and coccobacilli in the digestive tract. Reduced growth rates, disappearance of larvae and high mortality (90-95%) during second third of larval cycle from stage IV-XI	Microscopic examination, bacterial plate counting and species identification looking specifically for <i>Enterobacter aerogenes</i>
Internal Bacterial Infections	Cessation of feeding, body changes colour to pale and white, larvae appear listless, slow swimming and can be fouled. Can result in mass mortality, especially if infected by luminous vibrios (<i>V. harveyi</i>) which exhibit a continuous greenish luminescence when observed in total darkness. When viewed under the microscope, the internal tissues of these larvae are densely packed with highly motile bacteria.	Microscopic examination, bacterial plate counting and species identification looking specifically for luminous <i>Vibrio</i> and <i>Aeromonas Sp.</i>
Rickettsial Disease	Whitening of larvae throughout bodies, become weak and inactive, followed by significant mortalities	Microscopic examination, bacterial plate counting and species identification
Protozoan Diseases	Ectocommensal ciliated protozoans (<i>Zoothamnium</i> , <i>Epistylis</i> and <i>vorticella</i> etc.) attach onto shell, appendages and gills of larvae giving fuzzy appearance of larvae, moults and tank surfaces to the naked eye, weak swimming and reduced feeding behaviour. Endoparasites including gregarine worms in the oesophagus and microsporidians in the female ovary. Occasional high mortality	Microscopic examination of external and internal surfaces of larvae and broodstock
Fungal Diseases	Extensive fungal mycelium network observed through exoskeleton inside whitish or reddish larval body, occasional necrosis and melanization. Larvae weak and move abnormally. Can lead to mass mortality within 24 hours. Caused by <i>Lagenidium</i> , <i>Fusarium</i> , <i>sirolopidium</i> and <i>Saprolegnia sp.</i> Can also effect gills and eggs of females, turning them white and unable to hatch	Microscopic examination
Yeast Infections	Muscles appear yellow, blue or grey, can cause mass mortality especially in low temperatures or with high levels of organic matter	Microscopic examination
Exuvia Entrapment Disease (EED) or Moulting-Death Syndrome (MDS)	Larvae become trapped in the old exoskeleton by eyes or rostrum during moulting, especially at surface of water. Mostly occurs at end of cycle as larvae moult into PL	Microscopic examination

Annex 4: Known prevention and treatment of larval prawn diseases

Disease	Prevention Methods	Treatment Methods
All Viral Diseases	Screen all broodstock for viruses by PCR if available, pass all broodstock through quarantine before introduction into larval rearing unit, screen feed sources and other potential disease vectors (especially other crustaceans), test and implement effective biosecurity and disinfection protocols, maintain optimum environmental conditions throughout hatchery cycle, disinfect first stage larvae before stocking into larval rearing tanks to stop transmission from resistant but infected broodstock. Don't feed larvae prawn flesh in egg custard	For light infections, can only minimize environmental stress. No known treatment for advanced epizootics. Kill all stocks and dispose using sanitary methods, completely disinfect and dry facility and start again. Do not allow importation of prawn stocks (brood or PL) from abroad, especially without prior screening for known viruses
Bacterial Necrosis	Exchange treated (chlorinated, filtered and UV'd) water at 80-90% per day after siphoning wastes to maintain water quality, use checked probiotics and strict biosecurity and sanitation protocols, disinfect first stage larvae before stocking, avoid physical damage due to handling, maintain constant temperature, reduce stocking densities and stress and use plastic covers over tanks	Use of 3-5ppm of sensitivity-tested antibiotics as bath treatments every day for 5-7 days straight. Can also use formalin (50ppm) or other disinfectants like virkon (1ppm), chloramine-T (2-5ppm) and PUR (1ppm) as 24 hour bath treatments. If unsuccessful, disinfect entire hatchery, clean and dry and start again
Midcycle Disease (MCD)	Cleaning, disinfecting and drying out all hatchery equipment and facilities between cycles, take care of general hygiene through larval rearing cycle, use of separate equipment for each tank, plastic covers over tanks, reduce stocking density and use enriched feeds especially good quality artemia	No known treatment for advanced epizootics. Kill all stocks and dispose using sanitary methods, completely disinfect and dry facility and start again
Internal Bacterial Infections	Maintain water quality as for bacterial necrosis, add probiotics to diet of larvae as well as in water	Use of sensitivity-tested antibiotics as bath treatments every day for 5-7 days straight and disinfectants as for bacterial necrosis
Rickettsial Disease	Screen and use disease-free stock, good management, application of quicklime (CaO) to tanks and equipment before stocking	Antibiotic treatments with 10ppm OTC (possibly combined with 10ppm furazolidone if permitted) daily for 5-7 days until cured
Protozoan Diseases	Regular monitoring of larval health, routine 24 hour aerated bath treatments with 25-50ppm formalin (40% formaldehyde) followed by 100% water exchange up to every 2-4 days during the cycle. Maintenance of water quality by increased water exchange of chlorinated, filtered water, disinfection of first stage larvae before stocking decapsulation of artemia cysts and disinfection of artemia nauplii with H ₂ O ₂ .	50-100ppm of 40% formalin for 24 hours in well aerated bath treatment followed by 100% water exchange, or 200-250ppm formalin dip for 30 minutes, 2 ppt of acetic acid as a 1 minute dip (repeated as required), 0.4-0.5ppm copper sulphate every 6 hours every day until cleared, Malachite green at 0.2ppm for 30 minutes every day until cleared
Fungal Diseases	Ensure feeding regimes are optimized without allowing overfeeding, improve water quality through reduced stocking density, more water exchange (with chlorinated and filtered water), siphoning of sediments/dead larvae and addition of probiotics, avoid physical damage due to handling, care with artemia as above	0.05-0.1ppm treflan or 20ppm merthiolate added to water on a daily basis, 0.2ppm malachite green for 30 minute bath, 5ppm malachite green for 5 minute bath or 200-250ppm formalin for 30 minute aerated bath. After treatment exchange 100% of the water
Yeast Infections	Optimize management to reduce stress, maintain optimum temperatures with individual heaters in each tank, maintain water quality through increased water exchange etc.	None known
Exuvia Entrapment Disease (EED) or Moulting-Death Syndrome (MDS)	Improve feeding of larvae with enriched artemia and artificial feeds, maintain good water quality, especially with regards to mineral balance and hardness, alkalinity and pH levels	None known

Annex 5: Bangladeshi shrimp hatchery SOP manual