

SHRIMP FARMING

FARMER'S GUIDE



FISHERIES DEVELOPMENT BOARD
MINISTRY OF NATIONAL FOOD SECURITY &
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**SHRIMP FARMING
FARMER'S GUIDE**

TRAINING MANUAL
ON
SHRIMP FARMING

Prepared under Pilot Shrimp Farming and Cluster Development Project

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SHRIMP FARMING FOR WHITELEG SHRIMP (*P. Vannamei*) CULTURE

PREFACE

Shrimp farming is a profitable aquaculture enterprise. The development of shrimp culture during the past 15-20 years has attracted farming communities worldwide and has created mass awareness among the people about the potential of shrimp aquaculture for earning a livelihood and for generating cash surplus. Shrimp farming in inland areas with less salinity is very popular in many countries, especially in Thailand, Bangladesh, and India.

Fisheries development Board (FDB), started shrimp farming trials in 2014 in District Thatha in earthen ponds using seawater from a creek. The farm was consisted of 25 acres having 10 ponds of 2.5 acre each with a reservoir. FDB operated the farm for one year and then the farms were operated by private parties for 3 years. Later, FDB promoted shrimp farming in Punjab where initially *P. vannamei* PL were distributed among some fish farmers, good results were obtained during the initial trial and later farmers started shrimp farming. This manual is prepared based on the experience gained by FDB during these shrimp farming field trials and operations.

As the shrimp farming is a new farming activity in Pakistan, people do not have basic knowledge for shrimp farming. Therefore, this guide will help interested people to venture into shrimp farming by learning the farm development methods and best management practice for farm operation.

This model can easily be replicated in Pakistan if the value chain and infrastructure are developed, technology is transferred, training is imparted and finances are arranged for potential shrimp farmers; and with this, the desired results can be achieved in Pakistan. The goal of this guide is to become a continuous query tool for the aquaculture producers and technical staffs obtain a high quality shrimp production, based on a farming competitive model which meets sanitary requirements demanded by local and international markets.

SITE SUITABILITY/SITE SELECTION CRITERIA

One of the most critical points in any shrimp aquaculture operation is site selection, because doing it correctly, large amount of possible effects on the environment and neighboring communities can be minimized. By the way, it can be identified limitations that affect operation sustainability.

The site selected for the location of the farm, should be in an area where the operation thereof don't induce environmental or social conflicts, according to planning and legal framework and making efficient use of water and soil resources. It should conserve biodiversity, ecologically sensitive habitats, and ecosystem functions, and recognizes other possible uses of ground and that other people and species depend on these same ecosystems. Factors that must be considered when selecting a suitable land for shrimp farming, include:

- Cost-effective and environmental health
- Value of the site where it will be operated a shrimp farm, related to the intrinsic value (cost-opportunity)
- Impact on the local and regional economy
- Changes in the value of other sites within the same ecosystem because of the farm operation

Regarding food safety, the most important features are again water and soil quality. This is the first prevention step for reducing danger of human food consumption risks. In fact, constructed farm on contaminated soil or using polluted water, will not obtain a safe product. It must be known the history of use of the selected land and by laboratory analysis, must be confirmed the absence of hazardous products for shrimp and/or damaging the quality of the final product (harvested shrimp), due to their potential risk to human health.

Likewise, the use of high-quality water for shrimp production, will have a “plus” for producing a quality and safety shrimp for the final consumer. Therefore, it must be ensured that the water is not contaminated or that there is not possibility for water contamination with industrial, mining, agricultural or domestic waste.

Considering production and safety environmental aspects when shrimp farm site selection, soil characteristics are relevant to the production sustainable success. Potentially acid and sulfated soils must be excluded when site selection for the location of a shrimp farm. However, moderately acidic soils can be treated to improve its pH, through the process of liming with calcium carbonate. Flooding risks must be considered when shrimp farm site selection. Preference should be given for the gravity flow of water to facilitate easy pond drying and proper water exchange.



A minimum elevation of 0.45 to 0.6 m is essential to ensure proper drainage as drying of the pond bottom and proper water exchange. Generally, clayey loam soil is the preferred sand soil to avoid because of high water percolation. Soil with a Ph below 5 and a high concentration of heavy metals should be avoided.

1) OPTIMAL SOIL PARAMETER

Properties	Optimal Range
Soil texture	Clayey soil is best suited
PH	7.0-8.0
CaCO ₃ %	>5.0
Available nitrogen (mg/100 g soil)	50-75
Available phosphorus (mg/100 g soil)	4-6
Soil and water salinity	>3 ppt.

- The infrastructure facilities like roads, grid electricity, freshwater source, proximity to market, ice plant, processing plant should be considered while choosing the site for shrimp farm since these play a very important role in the viability of shrimp culture.
- At least 20 m distance between two adjacent farms should be maintained, a minimum distance of 150 -200 meter shall be maintained between the nearest agricultural land.
- Areas where already a large number of shrimp farms are located should be avoided.
- Mangrove, agriculture lands, saltpan lands, ecologically sensitive areas like sanctuaries, marine parks etc. should not be converted for shrimp farm.
- Good quality and adequate amount of brackish- water should be available throughout the culture period. The water source may be from brackish water creek, canal, lagoons, or backwater.

- While locating the farm site, careful study should be made on the source of water, the quantity of water available during the different seasons and the quality of water. The water source should be free from all kind of industrial or agricultural pollution.

2) OPTIMAL WATER QUALITY PARAMETERS FOR SHRIMP CULTURE (L. VANNAMEI)

Properties	Optimal range
Salinity (ppt)	>3 ppt
Temperature (Degree Celsius)	28-31
Ph	7.0-8.5
Dissolved oxygen (ppm)	3 - 7
Alkalinity (ppm)	> 80
Total suspended solids(ppm)	< 100
Transparency (cm)	25-45
Turbidity (NTU)	25-50
Total ammonia N(ppm)	< 1
Nitrite-N (ppm)	< 0.25
Hydrogen sulphide (ppm)	< 0.003
Chemical oxygen demand (ppm)	< 70
Biochemical oxygen demand (ppm)	< 10

POND DESIGN FOR SHRIMP FARMING

Rectangular or square pond are appropriate for shrimp culture. The longest axis of a pond should be parallel to the prevailing wind direction. This facilitates water movement generated by wind action thereby increasing dissolved oxygen in the water and minimizing water temperature fluctuations in summer or warmer months.

The breadth of a pond depends largely on the purpose and the operational system employed. The following are the various sizes recommended:

Nursery pond	500 to 1,000 m ²
Grow-out pond - intensive	0.25 to 1.0 ha
- semi-intensive	0.5 to 2.0 ha
- extensive	1.0 to 10 ha

The rearing pond must have a minimum depth of 1.0 meter. Most traditional brackish water ponds for shrimp farming are relatively shallow. To satisfy depth requirement, a ditch is constructed along the dike or a central canal between two opposite sides of the pond. The average depth is 1.0 – 1.2 meters and depth of the platform is 30–60 cm.

Such pond design with peripheral ditches and central platform affords several benefits:

- a. The ditch provides better living conditions during hot weather.
- b. The shallow centrally located platform serves as growing area for the natural food organisms.
- c. The ditch also serves as harvesting canal.

Pond bottom should be as even as possible, free from projecting rocks and tree stumps. The bottom must have a gradual slope from the inlet gate towards the drainage gate.

Ease of pond preparation should be considered in pond design and construction. Ponds should have individual inlet and exit structures, and bottoms should be sloped to facilitate rapid draining. Gates also should be constructed to accommodate filtration devices. Embankments should be wide enough to accommodate the equipment needed in pond preparation, and there should be a means of easily moving equipment into and out of ponds. Where water supplies have high concentrations of suspended solids, water should be passed through a settling basin for clarification before it enters culture ponds.



BIOSECURITY

- Biosecurity in aquaculture is the sum of all procedures in place to protect the living organism from contracting, carrying, and spreading diseases and other non-desirable health conditions, with biotherapeutic agents like probiotics.
- Biosecurity barriers or fences around the pond, prevention of the carrier/vector including birds, disinfection of intake water, avoidance of cross-contamination.
- Overall hygiene including that of equipment and personal are some of the in-built features of the biosecurity system.
- Provide settling and treatment of source water through reservoir pond.
- Adopt minimally or zero water exchange for pathogen exclusion from the source.
- Adopt a proper multi-level water filtration system with an appropriate mesh size to avoid predator animals.
- The establishment of an effluent treatment system is necessary to bring the shrimp farm waste within the prescribed standards and mitigate any adverse impact on the ecology of the open water.

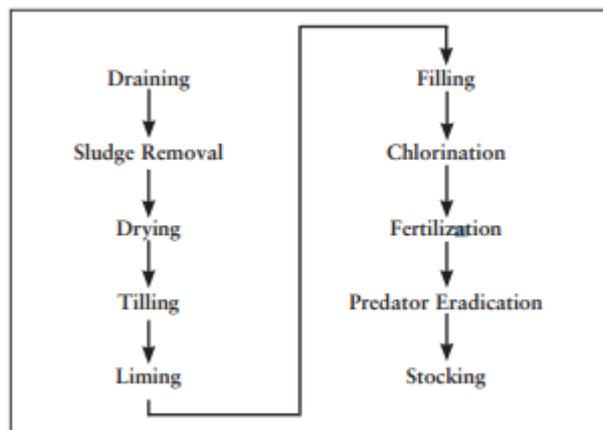
- Wastewater discharge during harvest is usually the most important contribution to overall wastewater loading, comprising over 75% of total load,
- Adopting effluent treatment by individual farmer/farmers, association in an area where the farm is cultured together, will help in the development of sustainable shrimp farming, without causing adverse impact to the environment and the ecosystem.

POND PREPARATION

The basic objectives of pond preparation are to:

1. Oxidize organic wastes and reduced inorganic compounds that accumulate in pond bottoms during the grow out period.
2. Eradicate predators, pathogens, and vectors of pathogens that may be present from the previous culture cycle or enter before the pond is stocked for the next cycle.
3. Improve soil pH and alkalinity of the water.
4. Enhance the availability of natural food organisms before stocking.
5. Remove or redistribute sediment as necessary.

The pond preparation process depicted in Figure below is suggested for use by shrimp farmers worldwide. After harvest, ponds should be completely drained, dried, and tilled to enhance the oxidation of organic wastes and reduced substances, and eradicate disease agents and vectors from the previous culture cycle. Ponds with acidic soil should be treated with agricultural limestone to increase pH to 7.0 or 7.5.



POND BOTTOM PREPARATION

1. DRYING.

The bottom should be thoroughly dried in all areas and cracks should develop in the soil. However, over drying inhibits the natural microbial decomposition of organic matter and should be avoided. When dust is seen when a handful of soil is tossed into the air, the soil is

too dry. If sediment is deep, it may not dry thoroughly. The surface can become dry and inhibit the evaporation of moisture from deeper in the soil mass. Thus, it may be necessary to remove sediment from some areas of pond bottoms.

2. TILLING.

The purpose of tilling is to enhance the oxidation of organic matter by pulverizing the soil to increase the exposure of wastes to sunlight and air. Bottom soil cracks into column blocks that can appear completely dry. However, the soil mass inside the block often is still wet. Tilling breaks the blocks to improve drying and oxidation. Tilling the upper 5 to 10 centimeters of soil is most important, for waste from the previous crop accumulates in this layer.

3. LIMING.

The term liming is used to describe the application of agricultural limestone, marl, burnt lime, hydrated lime, or other basic substances to soils. The primary purpose of liming is to improve the pH and alkalinity of the ponds. But liming also can be used to oxidize wastes and disinfect bottoms. Agricultural limestone, the most common liming material, is made from limestone or quarried and crushed seashells. Agricultural limestone can be calcitic (primarily calcium carbonate), dolomitic (consisting of nearly equal mixtures of calcium and magnesium carbonates), or a mixture of calcium and magnesium carbonates in less than 1:1 proportion. All forms of agricultural limestone are suitable for use in ponds, but the raw material should be finely ground. Agricultural limestone neutralizes acidity and improves the buffering capacity of soils. The application of lime ranges from 500 kilograms of lime per hectare to 1,000 kilograms of agricultural limestone per hectare during pond preparation.

In some cases, pond bottoms may dry except in selected areas. The wet places can be treated with burned or hydrated lime at 0.25 kilogram per meter or saturated chlorine solution at 1 liter per meter to effect disinfection. Treatment of such wet spots with sodium, potassium, or calcium nitrate at 0.1 to 0.2 gram per meter also can enhance oxidation.

Liming recommendations based on soil pH.

Soil pH	Quantity of CaCO ₃ Lime (kg/ha)	Quantity of Ca(OH) ₂ Lime (kg/ha)
Above 7	0	0
6-7	1,000	500
5-6	2,000	1,000
Below 5	3,000	1,500

4. FILL WATER

Precautions to prevent the entry of predators, diseases, and excessive amounts of settleable solids are required while filling ponds with water for a new culture cycle. Conventional precautions include filtering the inlet water through a coarse net to prevent the entry of predators. If incoming water is high in suspended solids, it should first be pumped into a sedimentation pond for solids removal before transfer to culture ponds. Treatment of pond water with chemicals – mainly natural products such as rotenone and tea seed cake – to eliminate predators before stocking is often an integral part of pond preparation. With the advent of serious disease problems, it has also become prudent to pass water through fine filters before introducing it into ponds. Filters with mesh openings as small as 200 microns are used to remove pathogens and their vectors. A series of progressively finer filters installed between

the intake point, reservoir, and ponds is employed at many farms. Sediment ponds also are becoming a regular feature in modern farms to provide initial water treatment, maintain water levels, or effect water exchange.

Chlorine usually is applied in the form of calcium hypochlorite. Chlorination should be done two or three days after filling because of its adverse effect on plankton blooms. The application rate is 15 to 20 milligrams of active chlorine per liter of water, which can be attained using 25 to 30 milligrams of calcium hypochlorite per liter.

WATER TREATMENT AND FERTILIZATION

Ponds then should be filled with water that is filtered through a fine mesh screen to restrict the entry of pathogens and predators. The water can then be tested and treated to eradicate predators and disease agents that may have eluded the screen. Pond waters should be fertilized with inorganic fertilizers, organic fertilizers, or both to encourage the development of plankton blooms. Once a healthy bloom is established, post larvae should be stocked.

It is important to have a healthy bloom of plankton in ponds before stocking so that natural food organisms are available to the newly stocked post larvae. Whether fertilization is required during pond preparation depends on the nutrient concentrations in the water. Excessive concentrations of nutrients result in excessive phytoplankton blooms that crash and cause severe water quality problems.

Fertilizer	Nitrogen (%)	Phosphorus (%)
Urea	45	0
Ammonium nitrate	33-35	0
Ammonium sulfate	20-21	0
Ammonium phosphate	11	48
Diammonium phosphate	18	48
Calcium nitrate	45	0
Sodium nitrate	16	0
Super phosphate	0	18-20
Triple super phosphate	0	44-54

1) NITROGEN FERTILIZATION

Targeted nitrogen concentration for fertilization is 1 to 2 milligrams per liter. The commonly used nitrogen fertilizers and their nitrogen content are listed in Table above. Urea was the top choice of farmers worldwide as a nitrogen source. Other nitrogen sources are ammonium nitrate, diammonium phosphate, calcium nitrate, ammonium sulfate, and sodium nitrate.

2) PHOSPHORUS FERTILIZATION

Targeted phosphorus concentration for fertilization 0.1 to 0.2 milligram phosphorus per liter of water. Table above shows the commonly used phosphorus fertilizers and their phosphorus content. Triple super phosphate was the primary choice of farmers worldwide. Other phosphorus sources are diammonium phosphate and phosphoric acid.

3) ORGANIC FERTILIZATION

Organic fertilizers are used widely in shrimp pond preparation. They have low cost and easy availability and decompose to release nutrients slowly. They provide nutrients other than nitrogen and phosphorus, as well as substrate for the growth of microbial food organisms. These advantages are somewhat offset by a few disadvantages. Organic fertilizers have a low concentration of nutrients and may not be cost-effective. They increase the oxygen demand in ponds by increasing microbial activity and are less predictable in terms of nutrient content and release.

The use of molasses during pond preparation is a relatively recent phenomenon. Molasses provides a readily available source of carbon and is believed to stimulate the growth of beneficial bacteria. Typical application rates range from 50 to 100 kilograms per hectare. Grain residues such as rice bran are also widely used. Initial application rates range from 250 to 750 kilograms per hectare.

4) PREDATOR CONTROL

Filtration used during the filling stage limits the entry of predators into ponds. Screens are usually applied to ponds. Despite the fine filters used in modern shrimp farming, predators may still enter ponds, and additional water treatment may be required.

Organic fish toxins such as rotenone or tea seed cake, and chlorine compounds are widely used to eradicate predators. Tea seed cake was commonly used in the Far East countries, while rotenone was more common in the West. Application rates for tea seed cake ranged from 200 to 300 kilograms per hectare. The cakes are soaked in water overnight, and the resulting liquid is applied uniformly over pond surfaces one week before stocking so there are no residual effects on the post larvae.



- Generally, 20 – 30 ppm chlorine can be used as no microbes or their host could survive such chlorination does however ascertaining the organic load in the source water (chlorine demand) and chlorine % in bleaching powder would be a better indicator to decide the chlorination.

- Ageing of pond water after chlorination will help in removing the free-living viral pathogen after 12 days. The addition of organic fertilization like cow dung or fermented juice is beneficial to enhance the primary productivity.
- Primary productivity is directly related to the temperature and available nutrients in the water and soil nitrogen, phosphorus and potassium are the essential prerequisites for primary productivity of any aquatic system.
- The availability of different forms of nitrogen and their related rates of utilization are important factors contributing to the related success of productivity of different phytoplankton.

POND STOCKING

1. POSTLARVAE SOURCES

Local or foreign postlarvae supplier hatchery, must have strict and well-defined biosecurity procedures and it must ensure their real implementation. When postlarvae are going to be imported, they must have a health certificate from their origin country, which includes at least the major pathogens such as: White Spot Syndrome Virus (WSSV), Infectious Hypodermic and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), *Penaeus vannamei* Nodavirus (Pv NV), Baculovirus penaei (BP) and Infectious Mionecrosis Virus (IMNV), and bacteria such as the alpha Proteobacteria causing of NHP disease (*Hepatobacter penaei*) and *Vibrio penaeicida*.and have a good general health status. They should also have good gill development and have a morphological development according to their age (stage vs. length in mm).

2. SEED SELECTION AND QUALITY VARIFICATION

Shrimp hatchery are an important supply source for shrimp fry. Some hatcheries can produce shrimp fry all year round if spawners are available. An added advantage is that hatchery-bred fry of the same age is almost uniform in size and can be produced in large quantities at any one time. At 15–21 days old, the fry is ready for stocking in ponds. The detail of seed selectin and its quality verification is given in Annex-1.

- Selection of disease-free quality shrimp seed is the prerequisite for the success of shrimp farming operation it has a direct relationship with the survival and growth of the cultured shrimp
- Buy SPF (specific pathogen-free) *P.vannamei* seed from approved shrimp hatchery.
-



- A farmer from the shrimp cluster should procure seeds collectively through their association from a reputed and trusted hatchery; they should stay in the hatchery to monitor the seed production process closely.
- General selection characteristics are given below.

Sr. no	Parameters	Standards
1.	Color	Light at least 90% to dark brown
2.	Activity	Very active
3.	Feeding behavior	Readily accept and eat feed
4.	Hepatopancreas	Developed in at least 90% of the sample given and full of oil globules
5.	Body length	12 mm or above
6.	Size variation	Less than 10%
7.	Appendages	Intact without any deformity
8.	PCR screening for viral and bacterial diseases	Negative
9.	Pigmentation	Chromatophore well defined and located along the mid-ventral line
10.	Stress test with survival	100% for salinity reduction and above 90% for formalin test
11.	PL stage	≥ PL 12 and above with adequate gill development will be chosen.
12.	WSSV and EHP	Absent based on real-time PCR-two step nested PCR
13.	Gut	Full gut and with a tail muscle to hind gut ratio of 4:1 or more.
14.	Necrosis	Absent
15.	Fouling	Clean without any fouling organism.



Keys to success

1. Good PL quality
 - reach 10 g. within 60-65 days
 - 17 g. within 90 days
2. Adequate aerators
 - maintain DO > 4 mg/l
 - alkalinity > 120 mg/l - pH 7.5-8.2 - 20 g. within 100 days (yr2010-2011)
clean pond bottom.
 - alkalinity > 120 mg/l - pH 7.5-8.2
3. Good PL quality should come from good genetically bloodstocks
4. Properly rearing and feeding during maturation
5. Water temperature 28-29 °C

3. POSTLARVAE ACCLIMATION

Shrimp postlarvae are one of more expensive inputs in shrimp farming. Handling and management of postlarvae that includes harvest, packaging, shipping, reception at farm, acclimation and pond stocking, are extremely critical to their survival. During acclimation, all efforts of the technical staff must be focused on minimizing postlarvae stress and mortality, while they adjust gradually to new water quality conditions in the ponds. A successful acclimation, helps ensuring economic success of the crop cycle.

The most important variables to monitor during shrimp postlarvae acclimation, are salinity, temperature and dissolved oxygen. Avoid stress and rapid environmental changes are essential during acclimation (Annex 2).

4. POSTLARVAE STOCKING

Before postlarvae stocking, it should be ensured that the pond meets several basic conditions that favor a good crop development. These are framed in a suitable pond water level, good phytoplankton concentration (mainly diatoms), and normal physicochemical parameters. This doesn't exclude monitoring these parameters during acclimation and stocking. It is important that when possible, farm develops its own bacteriological history for each pond (mainly species from genus *Vibrio*, *Pseudomonas*, *Aeromonas*, *Plesiomonas*, *Streptococcus* and *Flavobacterium*), with which farm has established the range of bacteria populations (forming colony units - FCU) that are prevalent during each season of the year (dry and rainy). Based on this, it should be monitored bacterial load of a pond before stocking, to ensure good water microbiological quality that don't be a risk for postlarvae viability.

When possible, it's preferable to stock during the coolest part of the day (6:00 a.m. to 8:00 a.m., or overnight). This is because temperature is lower, and thus reduces postlarvae stress and could become shorter acclimation time. It's recommended to release postlarvae in the pond as soon as possible.

Determining an appropriate stocking density, depends on the size and age (weight) projected to be harvested, water quality, pond design, water exchange rates, possibility of mechanical aeration, staff experience, and farm technical resources and capacity. Each shrimp farmer or company should establish sustainable biomass carrying capacity for each pond, according to the proper and individual conditions and to the pond production history. Based on that and taking into account the economic breakeven point of each farm and market conditions, it can be defined the optimal stocking density for each production system, without affecting projected economic benefits.

Once stocking densities be defined according to the established farming system, and having finished acclimation, postlarvae must be released in the downwind pond side. Thus, waves will help postlarvae to be dispersed along the pond after stocking avoiding overcrowding in the pond corners. It's recommended to monitor postlarvae survival 24 and 48 hours after stocking.

FEEDING

The feed selected must fulfill the known nutritional requirements of the targeted species. It must be fresh and free of mycotoxins and pesticides and have low pollution potential. The feed should have attractants and/or natural foods to improve detection and palatability and stimulate rapid consumption. Attractants can improve feed perception and reduce feeding response times as much as 50% when compared to feeds without attractants.

1) FEED DISTRIBUTION

There are several ways to apply formulated feeds to shrimp production systems. In larger ponds, aquafeeds are applied by manual broadcasting from boats. In small, intensive ponds, tanks, and raceways, automatic feeders with timing mechanisms can be used. Automatic feeders are a useful alternative to manual feed broadcasting in smaller systems but should not replace regular observations by trained personnel. At many farms in several countries, all feed is applied exclusively using feeding trays. As production cycles progress, pond managers must be aware that shrimp react to changing microhabitat variability in the pond, both in space and time. Animals avoid areas where anaerobic sediments accumulate and noxious compounds such as hydrogen sulfide are present. These areas include internal drainage canals and areas close to the drainage structures. This is more relevant toward the end of the grow out cycle when considerable sludge can accumulate.

Shrimp move to deeper areas within ponds during the day to avoid light. Therefore, very shallow areas or those with anaerobic sediments should not receive feed during daylight hours, because it is unlikely shrimp will consume it. However, it is also important to consider that shrimp distribution in ponds is generally not uniform, and that several factors influence how animals distribute themselves.

2) ADJUSTING FEED QUANTITY

Feed amounts are still widely established based on a set schedule that depends on animal weight and estimated biomass/survival in the pond. Feeding based on tables is also widely practiced. These tables can vary by species, geographic area, age of animals, target size, culture intensity, water temperature, dissolved-oxygen level, feed attractability, water stability, frequency, and times of feeding, and other factors. Daily rations are calculated based on estimates of density, mean individual animal weight, survival, and body weight percentage to feed. Each farm can develop its own in-house survival and feeding tables over time but should also properly consider the natural feeding habits of shrimp and their physiological states.

3) FEEDING TRAYS

Feeding trays are used to monitor feed consumption and estimate adjustments to ration size. Especially in large ponds, however, observation of a limited number of trays is not an adequate measure of actual feed consumption. Considerable day-to-day variations in feed consumption occur due to water quality fluctuations, overcast weather, molting activity, light intensity, natural productivity, and other factors.



4) FEEDING REGIME

Feed charts have been widely used as a method for controlling the pond feed supply, based on weekly shrimp sampling for growth and survival determination to estimate the current biomass of the pond. In this manner, it's determined the feed amount needed to provide for each pond, considering shrimp individual weight and percentage of biomass established in the chart used as a guide (Annex 4). Feed trays are a good tool for the estimation of how much are shrimp eating daily. To achieve this tool benefits, results "reading" and interpretation must be done by responsible and well-trained personnel. Proper use of feed trays will help preventing underfeeding and overfeeding. They can be used as control tools (partial pond feeding with feed trays) or they can be used at 100% (pond feeding with feed trays only) for shrimp feeding in the farms. This last practice (100% trays) requires big logistics and enough well-trained personnel, which could be offset by the savings in daily feed optimization (pro-environmental feed management) and eventual production profits having pond water with less organic load.



Check tray observation		
Color of gut	Content in gut	Problem cause
Black dark brown	Benthic detritus, sediments	Under-feeding, inadequate feeding frequency
Light or golden brown	Manufactured feed	Normal
Red, pinkish	Cannibalism body parts from dead shrimp	Disease event pond
Green	Benthic algae	Under-feeding
Pale, whitish	None (disease condition)	Pathogens

WATER QUALITY MANAGEMENT

Shrimp farm effluents: Most shrimp farms cannot be operated without discharge. Water exchange may be necessary at many locations to prevent evaporative losses from causing excessive salinity. Water exchange also is an important water quality management tool at semi-intensive farms for preventing dissolved oxygen depletion when dense phytoplankton blooms occur. Heavy rains can exceed the storage capacity of ponds and overflows cannot be avoided. Shrimp ponds also must be drained to facilitate harvest.

Nevertheless, shrimp farms should be operated in a manner to minimize effluent volume and avoid adverse effects of the effluents on receiving water bodies. The same water body often serves as both water supply and effluent recipient for shrimp farms. Thus, responsible effluent management protects not only the environment but also the water supply for shrimp farms.

Several procedures are available for developing management strategies that reduce the volume and improve the quality of shrimp farm effluents. These include reducing nutrient inputs and applying erosion control to lessen the suspension of soil particles. With these procedures in place, treatment of effluent before final discharge into natural water bodies may not be necessary.

Pond water quality: Pond water quality is a critical point in production process and must be controlled within the physical, chemical, and biological parameters. These should be suitable and maintained within acceptable ranges for shrimp development. Otherwise, farming shrimp population could have low growth, pathogen proliferation with disease outbreaks, eventual mortality, and low quality of harvested shrimp.

It is important to remember that shrimp farming ponds are dynamic water bodies with close interaction of physico-chemical factors such as pH, salinity, temperature and DO. Organic and inorganic nutrients also participate in that dynamics, affecting pond microbial populations. These are susceptible to change of these factors affecting bacterial number and composition. Some aquatic environment variables as pH, temperature, and salinity, have specific ranges for certain bacteria species. Changes in these factors favor proliferation of certain species, altering the environmental balance with consequent pathogens dominance.

Knowledge of each pond characteristics as behavior of water conditions, will lead to better production results because during shrimp crop, it arises peculiarities that determine actions to be carried out during cycle management.

1. WATER QUALITY MONITORING:

Pond water quality is a critical point in production process and must be controlled within the physical, chemical and biological parameters. These should be suitable and maintained within acceptable ranges for shrimp development. Otherwise, farming shrimp population could have low growth, pathogen proliferation with disease outbreaks, eventual mortality and low quality of harvested shrimp.

Knowledge of each pond characteristics as behavior of water conditions, will lead to better production results because during shrimp crop, it arises peculiarities that determine actions to be carried out during cycle management.

The farm must have a plan for monitoring pond physical, chemical, and biological parameters, in which procedures with each one must be defined (Annex 5). Some water quality parameters can be measured in the farm laboratory.

Water quality parameters for optimum pond management

Parameter	Optimal range	Sampling frequency	How it is done
Dissolved oxygen	>4 ppm; >5 ppm preferably for optimal growth and survival	Twice daily	Field probe
pH	7.5–9.0; <0.5 variation diurnally; optimal is approx. 7.8	Twice daily	Field probe
Temperature	Above 24°C and below 34°C	Maximum and minimum weekly	Field probe
Salinity	10–28 ppt (optimal for growth is 15–25 ppt)	Weekly	Field probe
Total ammonia nitrogen (TAN)	<3 ppm (toxicity is pH dependent)	Daily if necessary	Laboratory analysis
Un-ionised ammonia (NH ₃)	<0.25 ppm (percentage of TAN increases with pH)	Daily if necessary	Laboratory analysis
Nitrite (NO ₂)	<10 ppm at salinities >15 ppt; <5 ppm at salinities <15 ppt	Daily if necessary	Laboratory analysis
Alkalinity	>80 ppm (ideally 120 ppm)	Daily if necessary	Laboratory analysis
Hardness	>2000 ppm	Daily if necessary	Laboratory analysis
Hydrogen sulfide	<0.1 ppm (pH dependent)	Weekly	Laboratory analysis
Secchi disk (turbidity)	20–30 cm	Daily	Field test (see below)



2. AERATION

In semi-intensive farming systems, aerators should be used only if they are strictly necessary to ensure shrimp survival; otherwise, there will be energy waste and an increase in production costs. The decision to use them, must be made based on DO concentration in the pond, which is dependent on the stocking/farming density (biomass), phytoplankton concentration and pond depth.

Intensive shrimp farming systems require aerators that must be turned on almost permanently to keep stable bacterial systems (flocs or "bioflocs") and physicochemical conditions required by shrimp. In these cases, schedule for on-off aerators must be based on the metabolic requirements of the bacterial strains used, in order to maintain optimal pond conditions, although heterotrophic systems usually require continuous aeration.

3. POND WATER EXCHANGE

It is advisable to minimize water exchange without affecting shrimp production and maintaining acceptable levels of physicochemical parameters during the crop. Water exchange must be done just when be clear that it will benefit shrimp production, because incoming water conditions could be worse than pond water conditions. It's recommended to do water exchange only when pond water physicochemical variables are below the minimum acceptable levels. Pond water exchange reduction will help reducing fuel costs, pumping equipment maintenance and nutrients input in farm effluents. Some of the new management practices include water recycling through pond systems that allow water to be purified and reused. Besides reducing organic loads in the farm effluents, this practice is advantageous because it reduces farm inputs from an external ecosystem, helps decreasing the risk of predators and disease spread from other farms or wild shrimp, and loss of natural productivity within the farm ecosystem. It must be avoided the unnecessary use of freshwater as alternative to lower salinity in the pond water because freshwater has become a scarce resource for humans around the world. During dry season (summer), water lost by evaporation must be replaced to avoid high salinity increase and drastically fall of pond operating water level. When extreme high salinity, it is necessary to make pond deep water exchange, decreasing pond water levels and retrieving with water from the reservoir channel if its salinity is lower. When diseases be present in a pond, no water exchange must be done and water level will be kept by replacing when losses by evaporation.

During adverse weather conditions (excessive rainfalls or prolonged drought), it is important to measure physico-chemical parameters of farm water sources before introducing water into the ponds (filling, water exchange or replacement) since they can be altered and produce a negative effect on shrimp during stocking and/or crop. If it happens, farms must avoid or reduce water pumping into the reservoir or pond filling.

BIOSECURITY AND HEALTH MANAGEMENT

Biosecurity literally means ‘life protection’, but in practice it means preventing the introduction, establishment and spread of unwanted biological organisms or agents. In the context of shrimp farming, biosecurity is about managing dangerous disease risks. Biosecurity programs can be applied at several levels, including pond, farm, locality and so on, up to national levels.

Shrimp farm biosecurity involves applying sets of targeted, science-based procedures to eliminate or reduce the risk of a particular pathogen — that is, a disease-causing infectious agent such as a virus — (a) entering the farm, and (b) spreading within a pond, between ponds, to other farms, or to the wider environment.

Farm-level biosecurity programs in many overseas countries typically target very serious pathogens such as White Spot Syndrome Virus (WSSV), Yellowhead Virus (YHD), Taura Syndrome Virus (TSV) or Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV). These viruses are dangerous because they are highly contagious to shrimp, are lethal and untreatable and have a diverse host range. The viruses and the diseases associated with them are relatively well studied and understood. Important epidemiological information about each pathogen — its carriers, its routes of entry and modes of transmission — is generally available. This means that quite focused biosecurity programs can be developed for each pathogen. In those countries where domesticated, ‘specific pathogen free’ (SPF) or ‘specific pathogen resistant’ (SPR) shrimp species such as *Litopenaeus vannamei* or *L. stylirostris* are available and affordable, using such stocks is an important first step in reducing risk.

1) BASIC PRINCIPLES OF BIOSECURITY PROGRAMS

Pathogens can be introduced to, or transmitted between, ponds or farms in several ways. These include the introduction of diseased or apparently normal, but infected, ‘carrier’ shrimp, entry of wild carrier animals such as shrimp or crabs, improper disposal of dead shrimp, contact with contaminated objects, contaminated water such as drainage water from other farms, contaminated feeds, or aerosols from infected ponds.

It is also important to recognize that not all potential causes of disease on shrimp farms can be excluded by the application of a biosecurity program. For example, many *Vibrio* species occur naturally on farms as part of the shrimp’s normal microbial fauna and in the pond environment. Under certain stressful conditions, these bacteria can cause significant losses. Because they cannot be excluded, farmers must use a health management approach to minimize such losses.

An effective biosecurity strategy will use the following approaches to reduce risks of dangerous pathogens entering the farm to acceptable levels.

1. Stock only post larvae that have acceptable test results in terms of pathogen prevalence and load.

Pre-stocking tests to establish specific infection status are essential wherever post larvae are derived from wild-caught spawners. In some cases, it may also be worth testing stock at selected times during grow out to keep track of infection status.

For any pathogen, the infection status of an individual shrimp (such as a spawner) or a population of shrimp (such as a batch of post larvae) can be established only by sophisticated laboratory testing (usually involving PCR test of a sample taken from the shrimp or the population). Ideally, a sample of 150 post larvae is recommended for pre-stocking PCR testing. If possible, select the weakest animals; the assumption is that these are most likely to be infected with the target pathogen. The sample is then divided into five pools (each of 30 post larvae), and the pools are tested separately. You will probably need to discuss the test results with a qualified professional. In particular, you need to recognize the limitations of tests and test results. And even if the test result is negative, there will always be inherent uncertainties because of the possibility of sampling error (only a part of the individual shrimp or a fraction of the population is actually tested) and because of imperfections in the test (no tests are absolutely perfect; they can sometimes produce false positive or false negative results).

2. Do not exceed optimal stocking densities.

Excessively high stocking densities can stress shrimp, and increase opportunities for cross-infections, making disease-related losses more likely.

3. Eliminate or reduce risk from potential ‘vectors’ (infection carrying agents) on the farm.

In general, vectors are living or non-living agents that can carry a disease agent from one susceptible host animal to another. Some living vectors, such as crabs, may not become sick themselves; others, such as birds carrying infected carcasses between ponds, may not even become infected. Inanimate vectors are vehicles, nets, and feed utensils, harvesting equipment, packaging, and wastewater. Remember also that some shrimp viruses can survive for some time in the environment (say, in the damp soil of an empty pond) and remain infectious to healthy shrimp.

Many of the dangerous shrimp viruses, particularly WSSV, can infect vectors such as crabs, which can then serve as sources of infection for farmed shrimp. Overseas, crusticides (including chlorine compounds and organophosphates) are often used to remove these animals from reservoirs before filling ponds. During pond filling and water exchange, water is filtered through twin bag filters (300-micron mesh size) to reduce the chance of other crustaceans entering the pond.

4. Use water management practices that prevent or reduce contamination by the pathogen.

In many overseas countries, the high densities and proximity of shrimp farms can increase the frequency of dangerous disease outbreaks. Farmers have been forced to adopt careful water management practices such as minimal or even zero water exchange, together with mandatory use of reservoirs that combine storage and treatment of intake water. In very high-risk areas, farmers with disease problems notify their neighbours to ensure that neighbours do not pump water while infected water from diseased crops is discharged.

However, farmers should pay careful attention to movements of water within the farm to ensure that it does not carry disease between ponds via pathogens suspended in the water, via infected shrimp or via other carrier crustaceans. They should ensure that, if their outlet and

intake points are relatively close, they are not pumping water onto the farm while water is being released from other ponds, particularly diseased, or even suspect, ponds.

5. Reduce the risk of spreading infection between ponds by restricting movements of people, equipment, and other possible agents.

Movement control entails controlling movement on to your farm, and movement within the farm, of people, vehicles, equipment, water, and other vectors. Control of movement within the farm is aimed at stopping or minimizing contamination of shrimp, feed, water, and equipment used. If there are concerns about a disease on the farm you may need separate equipment in each different production unit (for example, cast nets used for sampling shrimp). Feed trays that are used for monitoring feed consumption should be kept in the same pond throughout a crop. Visitors' vehicles should be restricted to the perimeter of the property. It is also advisable that a hatchery be established on a location separate from a farm.

6. Implement a health management program that aims to minimize stress to shrimp by optimizing the pond environment.

Minimizing stress to the shrimp is a primary aim in pond and water quality management

Managing vectors and biosecurity risk

Vector	Biosecurity risk	Possible remedies
Birds feeding on dead prawns	Viruses if present may be brought on to the farm from outside (other farms) or spread from infected ponds within the farm	Contact your state Parks and Wildlife Service about bird management plans www.epa.gov.au . Bird netting or bird control management plan
Crabs feeding on dead prawns	Viruses if present may be brought on to the farm from outside (other farms or the wild)	Mesh covers for inflow and discharge pipes. Consider removing crabs from canals and reservoir
Packaging and wastewater of new postlarvae arrivals from hatchery	If there is disease infection in the new arrivals you do not want to spread it to other ponds through contaminated water and packaging	Discard. Do not reuse on farm
Vehicles	A vehicle that has visited another farm may carry dirt and moisture onto your farm	Restrict external vehicle movement within the farm and have regular wash-down disinfection for all vehicles on the farm. Have a disinfectant sump at the entrance for all vehicles arriving and departing the pond area
Harvesting and feed equipment, such as feed trays	If there is a diseased pond, transfer to clean ponds may occur	Disinfect after use in the diseased pond, or have separate set for a diseased pond
Wastewater	Infectious water flowing into the environment or to other ponds (if it is a waterborne disease)	Disinfect or treat before discharge

2) HEALTH MANAGEMENT

As many farmers have recognized, the frequency and severity of disease outbreaks in ponds seem to depend on a number of factors, not all of them well understood. The first point to appreciate is that not all shrimp diseases are infectious. For example, some are caused by toxins, others by nutritional imbalances. The following section focuses on infectious diseases because their causes are usually complex, and control and prevention can be more challenging. Infectious disease outbreaks in ponds depend on interactions between the host (shrimp), the pathogen and the pond environment.

3) Shrimp Health Monitoring

Shrimp should be sampled once in a week by cast netting and should be checked for their general health conditions, like external appearance (body color, missing appendages, external/gill fouling, black gills or gill choking, etc.), gut condition, and growth in terms of weight or length. Shrimp behavior and feeding trends should be monitored. These observations should be recorded at regular intervals.

By maintaining recording sheets, it is easy to review the sequence of changes in water and soil conditions, feeding and shrimp health and the management practices followed. A first sign of disease is when diseased or weak shrimp swim at the water surface or come to the edges of the pond. If many shrimps are affected by the disease, then feeding may be reduced. During this period, looking back at the farm records for sharp changes in water quality, bottom soil conditions or shrimp appearance in net sampling and feeding trays will give an idea on the possible causes of disease. With regular monitoring and review of important parameters it is easier to understand and rectify the environmental conditions at an early stage and prevent the spread of disease to another shrimp.

4) Handling Shrimp Disease Outbreak

Despite all the precautions, farmers may still suffer some shrimp disease problems. Prompt action is essential in such circumstances to rectify the problems, reduce the losses and minimize the impacts on neighboring farms.

Times of concern:

- If any sick shrimp has been found in any of the surrounding farms, this is an indication of stressful conditions in the area.
- Weather changes, particularly cloudy and rainy weather for prolonged periods.
- Changes in the color of pond water.
- Sudden blackening of pond bottom.
- Shrimp coming to pond edges and increasing number of dead or weak shrimp.

Under such circumstances, the following actions should be taken:

- Check any abnormalities in water and soil condition and take immediate action to correct the problem.
- Remove dead animals and bury them away from the ponds.
- If the mortality rate is increasing rapidly, and shrimp are not feeding, an emergency harvest can be carried out preferably using cast netting to avoid discharge of infected water into the main water source.
- Before releasing the water to the drainage, treat the pond water with bleaching powder (calcium hypochlorite) and leave for 5-7 days before discharging.

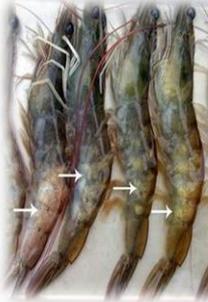
- To avoid spread of disease to other ponds in the area, the neighboring farmers should be kept well informed about shrimp disease problems, emergency harvest and the time and date of water discharge.
- The pond water should be treated in an effluent treatment system (ETS) (as per the guidelines given by Aquaculture Authority, Government of India) before discharging to a common water source. This system must be followed in shrimp farms of 5 hectares water spread area (WSA) and above located within the CRZ and 10 hectares WSA and above located outside the CRZ. In the case of smaller farms farmers should form a group and construct a common ETS to manage disease problems and ensure sustainability of the environment.
- During periods of disease outbreak, surrounding farmers should try to avoid water exchange and should not use any equipment (nets, tanks, pumps, boat, *etc.*) from affected farms. The aim of this practice is to avoid any risk of cross contamination of the virus or other disease-causing agents.
- To maintain water quality in the pond during such periods, feeding may be reduced. This practice reduces the necessity for water exchange. Liming may be necessary to maintain the pH above 7.5.

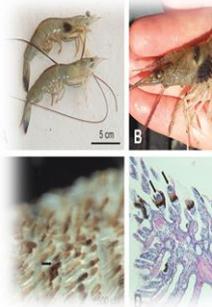
Finally, farmer groups are encouraged to discuss common actions that can be taken during disease outbreaks on a priority basis, to avoid spreading of disease from one farm to another.

5) MAIN SHRIMP DISEASES ITS CAUSES, SYMPTOMS, AND PREVENTION

Disease	Cause(s)	Symptoms	Prevention/managment	Identification
1-White spot disease	WSSV	*Reddened body *Broken antennae *white spot on carapace *Accumulation of water in head portion *Animal becomes lethargic *Animal coming to the side of pond	Stocking of SPF quality seed after PCR screening from CAA approved *Adaptation of BMPs and biosecurity	

<p>2-Running mortality syndromes</p>	<p>Unknown</p>	<p>*Antennae and tail portion become red in color *Distinct color of chromatophores on pereopods *Hepatopancreas become yellow and red in color. *Yellowish white feces *Regular mortality of few shrimp or few kg per day</p>	<p>*Optimum stocking density as per the carrying capacity of the pond *BMPs</p>	
<p>3-Infectious hypodermal hematopoietic necrosis</p>	<p>IHHNV</p>	<p>*Bent rostrum *Uneven growth and size different *Shape of animal body become uneven *Broken antennae</p>	<p>*Proper drying of pond bottom *Stocking of SPF quality seed *BMPs and biosecurity</p>	
<p>4-Vibriosis (Bacterial septicemia)</p>	<p>Vibrio sp.</p>	<p>*Reddening of the body *Flared up exoskeleton * Broken antennae *Hepatopancreas size increase or decreases *Black spots or blisters on the body of animal *Florescence appearance during night</p>	<p>*Application of water probiotics at periodic interval *BMPs</p>	

<p>5-Body cramps/ white muscle</p>	<p>Mineral deficiency high diurnal temperature variation</p>	<p>*Animal body cramp/bend *At the place of cramp body become white *Juvenile are more susceptible *Ratio difference between important minerals (Na, K, ca & mg)</p>	<p>*Periodic monitoring of minerals composition in the pond water *Maintenance of proper ratio of important minerals nutrients</p>	
<p>6-White fecal matter</p>	<p>Bacteria/ protozoan algal toxicity due to GBA</p>	<p>*Shrimp gut become white *White feces floating on the water *Hepatopancreas become white and accumulation of fat in HP</p>	<p>*Gut probiotics *BMPs</p>	
<p>7-Brown gill disease</p>	<p>Protozoan – zoothamnium/ water with more iron content / bacterial infection</p>	<p>*Presence of dianoflagellates in pond water *Gills turn to brown color *Shrimp comes to the water surface though DO is at optimum level *Mortality in the morning *More mortality if DO drop</p>	<p>BMPs</p>	

8-Black gill	Poor pond management and H ₂ S formation/deficiency in vitamin-C.	*Gills turn to black color *Severe fungal/bacterial infection. * Shrimp comes to the water surface though DO is at optimum level *Mortality in severe cases.	BMPs	
9-Microsporidiosis (EHP)	Enterocytozo on hepatopenaei (microsporidian parasite)	*It severely damage Hepatopancreas and shrimp gut, causing growth retardation	*A systematic pond preparation is very essential *PCR screening of the seed for presence of EHP is essential *Maintain the strict biosecurity to prevent unwanted animal into farm	

SHRIMP HARVESTING

1) PREPARING FOR A HARVEST

Simple planning and preparation for a harvest can make the work much easier and prevent costly mistakes that may result in wastage or deterioration of the product that you have worked so hard to produce. Make sure you have all the equipment on hand and make sure you have arranged a buyer or market and have booked the transport to get the product there. Essential items that must be ready for a drain harvest include:

- bins for storing chilled shrimp pond-side or at the processing room — more than enough to hold the estimated quantity of shrimp
- bins for storing ice pond-side — more than enough required to chill the estimated quantity of shrimp
- ice delivered or made on site in an ice-making machine
- scoops, hoist or lifting arrangement, pallets, chemicals, salt etc.

2) HARVESTING THE CROP

a) WING NET HARVESTING

Wing or ‘trap’ nets are good for harvesting live shrimp in small quantities. They also have the advantage of enabling a thinning of the crop (reducing the overall biomass) and taking out the larger shrimp in each pond with minimal disturbance to the pond. Thinning can improve the

eventual yield from the pond by providing the smaller shrimp remaining with more room and access to feed. Unlike drain harvesting, the wing nets do not collect sludge and sticks from the pond floor. Using wing nets is an easy way to harvest live medium to large shrimp, usually those with hard skins (because they are more active).

Generally, the pond water level is lowered to one-third to half and 4–8 wing nets per pond are set at right angles to the pond sides in a strong current area 2–4 hours before collection. Depending on density and conditions, wing net hole sizes are chosen according to the preferred shrimp size to be harvested. Lifting the shrimp from the pond to the top of the pond wall and into a vehicle can be strenuous work and should be planned properly to avoid spillage and work-related injuries. Two methods used are:

1. placing shrimp from the cod end in net-covered baskets while standing in the pond, then lifting the baskets up the side of the pond wall
2. using a crane to lift the cod ends and nets directly into the tubs or tanks on the back of a vehicle used to transport shrimp to the processing area.

Collecting shrimp regularly from the nets (approximately each hour) and transporting them quickly in small baskets to the processing area may mean that you will not need to use ice if it is a short distance to the processing room.

b) DRAIN HARVESTING

Drain harvesting can be used for either thinning out a crop (by partial drain) or harvesting an entire crop in one hit. It is generally a lot faster than using wing nets in terms of workload per kilogram of shrimp harvested but can often lead to a significant proportion of the product being harvested with a soft shell (if the shrimp have moulted recently). Because all of the pond water and its contents have to pass through the harvest cod end, the drain method can also cause damage to your shrimp in the very last stage of your farming work: broken shrimp squashed in the net, and sludge or dirt deposited in the gills and around the appendages. You will also have to sort out various kinds of rubbish that may accumulate in the pond during the crop: sticks, filamentous algae and trash fish that may have entered the pond despite the use of filter socks. You need to have a good look at the shrimp before the harvest to minimize the problems of soft shell and quality issues.

Drain most of the pond water through the screens on the monks to prevent escapees, before starting the actual harvest process. This is best done from the bottom of the pond (by screening monk boards to drain from the floor of the pond). When the pond water level has been lowered by approximately half, you can remove the screens and start collecting shrimp in the cod end or cage set up in the harvest bay of the drain. A harvest box with a diverter to two cod ends will allow you to keep the water flowing out and enable uninterrupted collection of the shrimp (it also will minimize the damage to shrimp in each net). Given that quantities collected each time can be significant (50–150 kg per net), the lifting up to a pondside bin or basket (usually on a vehicle) should be by hoist or crane

3) PROCESSING SHRIMP AFTER THE HARVEST

The quality of a shrimp starts to deteriorate immediately after it dies. The digestive organ, or hepatopancreas, contains a range of enzymes that cause changes to the shrimp's appearance and texture because they remain active after death. While not feeding the shrimp for 24 hours before harvest may reduce the number of digestive enzymes (mainly proteases) present in the stomach,

the hepatopancreas still retains plenty of activity. Any longer than 24 hours may make them hungry enough to eat the bottom detritus and spoil the quality of the cooked product. These enzymes digest through the surrounding tissue and attack the ligaments that connect the head (cephalothorax) to the tail meat (abdomen). These weakened tissues will then result in a significant amount of weight loss and head loss during storage and cooking. Initially the only visible defect will be a discoloration of the organ, but this will spread through the head and into the tail meat.

If you want to sell them as green product (fresh chilled or frozen), you will need to handle them differently. As all enzymes operate faster in warmer conditions it is imperative that shrimp be chilled immediately after capture. This can be in an ice slurry, in refrigerated water or under ice.

Another enzyme, polyphenol oxidase, will cause the dark pigment known as black spot to develop on the gills, shell of the head and shell of the tail. Unfortunately, unless the shrimp are frozen immediately, a chemical treatment will be required to prevent black spot. A range of chemicals can be used on shrimp, but the most commonly used is 4-hexylresorcinol. Citric acid can be used with green shrimp in the ice brine to remove any algae fouling on the shell. All chemicals can be applied as an initial quick dip, but if there is subsequent storage in chilled water some of these chemicals will wash off, leaving the shrimp more vulnerable to developing black spot during processing and transport. It is often more convenient to apply chemicals during a holding period. This allows a reduction in the amount of chemical used and better penetration of the chemical into the flesh. If the shrimp are prone to black spot — say, because they have recently moulted — a combination of initial dip and soaking may be required. Remember, the presence of any chemical additives will need to be documented on packaging.



ANNEX-1

POST LARVAE QUALITY EVALUATION AND STRESS TESTS

Microscopic and molecular post larvae assessment

- Hatcheries must have biosecurity procedures, applied during post larvae batches reviews by farm technicians. The hatchery director must inform to the farm about the packing water quality characteristics for post larvae shipping (salinity, temperature, pH, etc.), to set-up acclimation units on the farm.
- Few days before the purchase, the farm technician must go to the hatchery and monitor post larvae sampling for further evaluation in the diagnosis laboratory
- To accept or reject the post larvae shipment, it must be assessed mainly post larvae activity, phototropism, fecal cord, presence/absence of bioluminescence, size uniformity and intestinal contents
- Then technicians must continue with a microscopic evaluation to determine the presence or absence of lipid vacuoles in the hepatopancreas, intestinal contents, deformities, appendages, or gills melanization, presence of epibionts, white balls syndrome, BP (*Baculovirus penaei*) and gill development
- The molecular detection (by Chain Reaction Polymerase - PCR) of important pathogens, is a mandatory step to complete a comprehensive assessment of the quality of a post larvae batch
- Before buying post larvae, the farm technician responsible for their acquisition, should know the results of previous examinations and be fully satisfied with them
- Not all post larvae batches are equal, so it's necessary to perform the

- analysis above to each group of animals that are thought to be acquired by the farm, batch
- quality results don't apply necessarily to other batch because they are different post larvae populations.

Macroscopic and microscopic assessment of post larvae quality

It should be taken a random sample of 20 post larvae and evaluate individually the following features:

- Activity: at least 95% of the post larvae must be active. Healthy post larvae swim actively against the water flow produced by aeration in the acclimation tank or produced manually
- Presence of deformities: don't accept post larvae with deformed or bent rostrum, damage in appendages caused by bacteria, molting problems, and appendages loss, etc. The presence of twisted bodies is evidence of the use of various medications. The rostrum and appendices must have a normal shape, without erosions or deformities. It must not be accepted post larvae showing more than 5% of deformities
- Homogeneous size: more developed post larvae have better disease resistance, full gill development, and ability to tolerate abrupt salinity and temperature changes. Stocking size for *L. vannamei* and *L. stylirostris* are usually PL-9 to PL-11 (post larvae from nine to eleven days). It's not advisable to receive for stocking post larvae older than PL-11 as this will require considerably lower packaging densities (# post larvae per liter in each bag), increasing shipping costs. Otherwise, there is an important risk of high mortalities during the shipping.
- Intestinal contents: healthy post larvae usually feed continuously and

aggressively, and they should display a full gut. Post larvae under stress usually stop eating.

- Gut movements (peristalsis): rhythmic gut movements indicate good performance of post larvae digestive system. Similarly, dark color of the hepatopancreas is a good sign that post larvae have been feeding properly
- Presence of epibionts: healthy post larvae when are observed under the microscopy, don't display organisms attached to the exoskeleton. Post larvae with abundant attached epibionts, are indication of the existence of poor water quality. Under these conditions, post larvae usually don't molt regularly and exhibit a poor general health status. It is advised not to accept post larvae shipments greater than 5% of epibionts
- Muscle opacity: the presence of shrimp with muscle opacity is indicative of stress caused by poor environmental conditions. Post larvae shipments with over 10% of animals presenting this condition are unacceptable
- Gill development: a well gill-development is observed when the post larvae gill lamellae look like a branched Christmas tree. Post larvae usually reach this gill development on day 9 and 10 (PL-9, PL-10); a well gill-developed allows post larvae to tolerate more easily rapid changes in salinity and other parameters during acclimation
- Color changes and melanization: reddish color of post larvae can be caused by poor nutrition, improper handling, infections, and stress. The melanization (dark spots) indicates infections bacterial. In unhealthy post larvae, pigment cells (chromatophores) expand displaying continue pigment bands. Chromatophores status inspection should be done immediately after the samples have been taken out

of the water, due to chromatophores tend to expand still in healthy post larvae when stress is produced by excessive handling

Stress tests

Post larvae quality can be assessed by a stress test, which measures the tolerance of animals to well-known extreme parameter. To perform this test, 100 - 200 post larvae are exposed to a thermal, osmotic and/or chemical shock and then surviving post larvae are determined. A widely used test is to expose postulate to a temperature reduction of 10 - 12°C for 1 - 2 hours, or salinity of 0 - 5 parts per thousand for 30 minutes. The following are procedures for the performance of one of these tests.

Stress test at low salinity:

- Prepare water (500 mL) at salinity of 5 parts per thousand (ppt)
 - Take 100 random post larvae from the farming tank and put in the container with water with salinity of 5 ppt
 - Wait for 30 minutes
 - Take post larvae back to original salinity
 - Leave them for another 30 minutes
- Counting live and dead post larvae, the result is expressed in percentage for the evaluation of the stress test, it must be considered the following survival percentage values:
- 90 - 100%: excellent
 - 85%: acceptable
 - 80%: regular
 - <80%: no acceptable

It is important to ensure a shipping density (# PL/Liter) appropriate to the size or age of post larvae to ensure high survival at the time of their arrival to the farm.

ANNEX 2

POST LARVAE ACCLIMATION: TECHNICAL DETAILS TO PROCEED

The following tips will help to get better results during post larvae acclimatization:

- Acclimation facilities: they should provide shade, air, filtered water and hygienic conditions. Densities of 500 post larvae per liter are suitable during acclimation. If the plan is to keep post larvae for more than 24 hours, this density should be reduced. Similarly, PL-8 to PL-12 must be acclimated to lower densities even if they will not be kept up to 24 hours
- Preparation of acclimation tanks: whole acclimation facility must be washed and disinfected several days before post larvae arrival. Tanks, surfaces, and pipes must be washed and disinfected with chlorine. Then they must be rinsed with abundant top water and allow to dry ensuring removal of any remaining chlorine. The reservoir tank must be filled with pond water from source that will be stocked. Filter the water to be used in the acclimation through a filter of 500 microns (0.5 mm). Place about 200 L of seawater from reservoir tank in the acclimation tank and use ice in plastic bags to chill until 26-27°C. Acclimation water tank should be adjusted to the salinity and temperature of the water used for post larvae shipping.
- Post larvae shipping-bags opening when post larvae arrive to the farm, temperature and dissolved oxygen concentration must be measured and recorded. Shipping water must be smelled, and it must be observed post larvae activity and percentage of mortality. If dead post larvae are seen in the bags, annotate the approximate percentage. If oxygen is below saturation level (>15 mg/L), inject pure oxygen immediately in shipping water until reaching a minimum of 12 mg/L.
- Post larvae transfer to acclimation tanks: once post larvae have been transferred to acclimation tanks; oxygen must be gently pumped to the water column to reduce ammonia levels. Spread on water surface approximately 50 g of activated charcoal pellets in each tank setting the quantity depending on tank size. Using a glass vessel of 500 - 1000 mL to assess at a glance the status of post larvae. Observe and record gut fullness, molt signs, cannibalism signs, presence of dead shrimp and tail opacity. Hatchery personnel should perform volumetric counts to estimate mortality occurred during transport, which in turn will help to determine the alive post larvae amounts at the beginning of acclimation. This count should be done before adding pond water to acclimation tanks.
- Oxygen management during acclimation: during first hours of acclimation, ammonia levels are high, so oxygen levels should be kept above the saturation level (12 mg/L – 15 mg/L). During acclimation it must be held optimal oxygen levels from 8 - 12 mg/L. Throughout acclimation, oxygen levels should not decrease below 6 mg/L. The oxygen level must be raised to 10 mg/L in acclimation tanks just before post larvae stocking to offset the loss during transport.
- Acclimation procedure and program for post larvae 5 to 11 (PL-5 to PL-11): immediately after Finishing post larvae transfer, it must be slowly added water from reservoir tanks through a continuous flow system so that the post larvae water tank volume doesn't change. Salinity change must be

carefully monitored. It must be measured temperature, oxygen and salinity every 30 minutes and pH each hour. Record the results in the acclimation form sheet.

- To acclimate the temperature, it's recommended a change rate of 1°C/h. A good strategy is to keep the temperature constant at 25°C for the first 75% of acclimation time (while adjusting salinity) and then adjusting the temperature slowly towards the end of the acclimation period. Acclimation speed must decrease if post larvae

show signs of molting or stress. The opaque or whitish color, erratic swimming, empty gut or increasing cannibalism, are all indicators of stress.

- Feeding during acclimation: providing feed during acclimation will help post larvae to have more energy to withstand the stress caused by acclimation. To get it, it's recommend the use of live Artemia nauplii, egg yolk (cooked) finely sifted, commercial flakes or frozen Artemia.

ANNEX 3

FEEDING MANAGEMENT BASED ON MOLTING CYCLE

A proven fact is that in a shrimp pond there will be always shrimp in different molting stage. Therefore, a significant proportion of these shrimp will be in pre-molt, molt and post-molt stages, some in which they don't eat feed. The cost of this overfeeding can be very expensive. As an example, let's consider that in a pond shrimp farming system 30% of the population is in late pre-molt, molt and early post-molt stages (unable to eat); then 30% of the daily feed is being supplied with no reason; this feed amount multiplied by feeding days and number of ponds, will represent a big amount of money that is being applied with no-need. Moreover, the environmental cost both internal and external adding this organic matter into the ponds, must be considered. To the extent that not usable organic matter within the production system increases, oxygen (chemical) demand increases and it will increase the chance for these nutrients to be used as a broth for growth of bacteria and parasites, which will increase the oxygen (biochemical) demand, favor shrimp diseases, reduce the yield, and in extreme cases lead to shrimp death. The objective of this topic is to know the molt cycle and use it to optimize the feed management in the shrimp farming. It has been reported a reduction in FCR from 2.1:1 using feeding table versus 1.2:1 obtained by supplying the amount of feed based on shrimp molt stage. To achieve such results, it was required to perform the identification of molt stage in each week of the study.

Techniques have been developed that allow calculation of the feed amount to

be added in the pond based on shrimp populations molt stages of farmed *Penaeus schmitti* as follows:

- 1) Sampling of 100 animals every 2 days in five pond points (four at each end and one in the center)
- 2) Determine the shrimp count in early post-molt and late pre-molt, using as reference phenotypic patterns of the distal portion of the uropods under the stereoscope. When farm technician become familiar with this procedure, observations could be done at a glance
- 3) Determine the actual biomass to be fed (in kg) Example: total biomass= 4.504 kg, molting= 15% (stage B0, B1, D3, D4), actual biomass to be fed=3.828 kg
- 4) Refer to the reference tables for shrimp feeding
- 5) Feeding according to actual biomass (following the example above). For actual biomass 3.282 kg (15% molting), 4.5%, weight= 10.5 g, then there will be needed 172.26 kg of feed (regardless molt determining it would be needed 202.68 kg)
- 6) Feeding according to established schedules for the species; for *L. schmitti* circadian rhythms of digestive enzymatic activity are: 10 a.m., 6 p.m. and 2 a.m.
- 7) Determination of the FCR each week.
- 8) Because shrimp behavior during molting is cyclical, it can be followed two complete molting cycles (about 10 days depending on the weight) and then continue giving the same feed ratio per day until weight change. Operational strategies for the daily

feed management based on molting cycle for a population of *L. vannamei* in ponds, suggest that management should be as follows:

- Conduct shrimp surveys twice a week. When feeding be carried out by using feeding trays, shrimp should never be collected from these trays because their physiological status does not necessarily correspond to the pond population
- Determine the molting stage in not less than 100 shrimp per pond, based on identifying inter-molt, pre-molt and post-molt patterns in uropods of sampled shrimp Calculate the percentage of animals that are not eating (physiological fasting)
- Adjust feed ration based on the percentage of biomass that it is conditions to be fed
- Feeding under shrimp feeding schedule based on circadian peaks of digestive enzymatic activity (10 a.m. - 12 p.m. and 6 p.m. - 8 p.m.)

To implement these ration adjustment strategies, it's required trained personnel. Variations in pond dimensions and total area of the farm require particular sampling strategies. It must also be considered human resources spending estimating the cost-benefit of this strategy particularly on farms with large farming areas. Given the shrimp physiological synchronization in farming ponds, sampling can be reduced by grouping ponds depending on stocking date, sampling one pond per group rotating each pond per week. It's important to remember that day-night patterns are crucial to trigger molting and are constant and stable during different seasons of the year. Therefore, in large farm it's suggested

sampling just few representative ponds and then extrapolate the results to the entire farm. For example: on a farm with 20 ponds (> 5 ha), initial sampling is done in all of them to determine shrimp molt stages. If most of the ponds have shrimp with similar percentages for the different molt stages, then there will be selected just 5 ponds to adjust feeding rate.

It has been said that feed has an important role in shrimp farming, especially ration, frequency and feeding schedule.

There have been evaluated several frequencies and feeding schedules on shrimp growing. The results of these studies indicate a considerable variation in this regard and suggest the importance of considering biotic factors (enzymatic activity) and abiotic (photoperiod) as effectors in shrimp feeding behavior. Recent studies show that feeding schedules can be adjusted considering the shrimp circadian activity. It was already mentioned the cyclical effect of photoperiod on feed intake. In particular, the variation of digestive enzymes has been recognized as important part of shrimp physiology and feeding behavior. For this reason, the determination of circadian variation of digestive enzymes and induction time of enzymatic activity is important in setting frequencies and feeding times. The application of the technology of setting feeding times in white shrimp farming, has demonstrated a positive effect on increasing growth rate up to 35%. This section allows knowing techniques to establish the circadian variation of the digestive proteases and determining schedules for shrimp nutrition in semi-intensive farming systems.

ANNEX 4

Population sampling in a shrimp farm: use of feeding charts and trays

Feed trays are one of the main tools for feeding management in shrimp farms, among other reasons due to its usefulness for the evaluation of shrimp biomass present into the ponds. To determine shrimp population into the pond requires knowing the average weight, amount of feed supplied with feed trays during highest shrimp activity (not under molting and after rotation) and percentage of bodyweight represented by feed. For this it's required to have a guide chart.

Weekly shrimp growth can be known from caught animals from feed trays and/or taking samples through cast nets once a week. Example: in a 4 Ha shrimp pond, the weekly shrimp weight average is 12 g, percentage of biomass in feed corresponding to that weight is approximately 1.8% based on the Table below; maximum total feed supply and consumption per day by controlling feed trays is 120 kg. The data is enough to obtain shrimp biomass from the result of dividing feed consumed by percent of biomass and then the result multiplied by 100%, so:

$$\frac{(120 \text{ kg} \div 1.8\%) \times 100\%}{1} = 6,666.66 \text{ Kg of shrimp biomass}$$

Then, to obtain the number of individuals in the pond population, obtained biomass is converted from kg to grams and then is divided by the average weekly weight, as follows:

$$\frac{6,666.66 \text{ Kg} \times 1000 \text{ gr.Kg.-1} \div 12 \text{ gr.shrimp-1}}{1} = 555,555 \text{ shrimp}$$

Shrimp density per hectare is obtained dividing number of shrimp in the pond by the pond area as follows:

$$\frac{555,555 \text{ shrimp}}{4 \text{ ha}} = 138,888 \text{ shrimp per hectare}$$

Data must be confirmed with the pond crop results, determining an adjustment factor in %. Using this method, population sampling data must should be analyzed frequently as feed consumption can vary seasonally (higher in summer than in winter), and the contribution of natural productivity of the pond, the feed quality and consumption control in feed trays by responsible personnel.

Feeding chart for *Penaeus (Litopenaeus) vannamei* in percent of biomass, fed daily under semi-intensive conditions.

Shrimp weight (g)	% of body weight
1	10.0
2	6.0
3	4.5
4	3.5
5	3.0
6	2.5
7	2.3
8	2.0
9	2.0
10	2.0
11	1.8
12	1.8
13	1.8
14	1.8
15	1.7
16	1.7
17	1.7
18	1.5
19	1.5
20	1.5
21	1.3
22	1.3

ANNEX-5

PARAMETER MONITORING IN SHRIMP PONDS

- Dissolved Oxygen

It's recommended to measure oxygen levels in the pond water in the morning at sunrise (6 a.m.) and afternoon between 2 and 4 p.m. It's important to measure DO in the evening, in case that afternoon concentrations are below 6 mg/L; in this way, it can be implemented corrective actions to avoid episodes of hypoxia, such as deep water exchange and application of oxygenating inputs (ammonium or calcium nitrate and potassium permanganate). In order to maintain consistency in oxygen monitoring, it's recommended to measure in each pond always in the same order and at the same time and deep (1 foot from the bottom) every day. Each time the OD is determined in a water body, measurement equipment must be calibrated according to manufacturer's instructions. The oxymeter must be calibrated before and after performing a series of measurements.

OD Concentration	Effect
Less than 1 - 2 mg/L	Mortal if exposure occurs within hours
2 - 4 mg/L	Slow growth if low dissolved oxygen continues
4 - 12 mg/L	Best condition for optimal growth
> 12 mg/L	Supersaturation: risk of "gas-bubble disease", may suggest high microalgae concentration

- pH measurement in ponds

Since pH measurements change rapidly, this parameter must be measured directly in the field. To avoid damage to the pH meter probe, once measurements be completed probe must be flushed with distilled water. Inside the probe protective cover it's recommended to put a piece of cotton or sponge impregnated with

calibration solution pH 4. This solution prevents the growth of bacteria on the surface of the probe and will keep it wet while not in use.

- Temperature

Water temperature is measured directly in the pond water using a common thermometer or through probes incorporated in oxygen meters, pH and other similar equipment.

The thermometer is placed in the pond such the end be submerged few inches into the water or, it must be take a water sample in a container and measure the temperature therein. It must wait for a moment the thermometer to stabilize before recording the measurement.

In addition to the obtained value, it must be recorded also measurement time. Be sure to use the same thermometer in order to get consistent measurements.

- Secchi Desk

Secchi disk measurement consists in the depth in centimeters at which the disc is no longer visible when submerged in the pond water.

Usually there is an inverse relationship between disk visibility and phytoplankton abundance. As the plankton increases, visibility decreases. Management decisions based on Secchi disk data, require to be sure that turbidity is actually produced by phytoplankton rather than suspended materials in the water column such as clay, sludge or organic debris.

Strong waves, strong winds or sunlight can affect Secchi disk measurements. It's advisable to take measurements on calmed days, and on sunny to partially-cloudy

days. If Secchi disk measurements are going to be taken from a boat, it must be anchored to a solid structure to prevent the wind moves the boat when making the measurement. Farm personnel must evaluate if the site has the right conditions for this measurement. Usually the most suitable time for this measurement is between 9 and 11 a.m.

Secchi disk must be immersed in the shadowed side of the boat, so the person who will perform the measurement be partially back to the sun, the boat shadow doesn't cover the

disk and the sunshine don't affect the observer visibility. Additional weight must be added to the disk in order to a quickly immersion during measurement. Secchi disk readings are subjective as they vary based on the observer visual acuity and weather conditions. For this reason, these measurements must be performed by the same person every day.

Depth (cm)	Phytoplankton concentration
< 25 cm (Pond too shady)	If turbidity is by phytoplankton, there will be low dissolved oxygen concentration problems in the evening or before sunrise. When turbidity is by suspended particles, productivity will be low
25-30 cm	Turbidity is high and it's advisable to low Phytoplankton concentration
30-45 cm	If turbidity is by phytoplankton, the pond is in good condition
45-60 cm	Phytoplankton is scarce
> 60 cm	> 60 cm The water is too clear. Productivity is inadequate and can grow aquatic plants on the pond bottom