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Standard Operating Procedure for *Artemia* Pond Culture

Introducing Circularity Through Climate-Smart Aquaculture in Bangladesh (Artemia4Bangladesh)

Funded by the European Union

Artemia 4Bangladesh

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Standard Operating Procedure for *Artemia* Pond Culture

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List of acronyms

µm	Micrometer
AHPND	Acute Hepatopancreatic Necrosis Disease
cm	Centimeter
e	Embryo
EHP	Enterocytozoon Heaptopneaeii
EU	European Union
g/l	Gram per Litre
h	Hour
H%	Hatching Percentage
Ha	Hectare
HE	Hatching Efficiency
kg	Kilogram
L	Litre
m	Meter
mg/l	Milligram per Litre
MT	Metric Tonne
n	Nauplii
rpm	Rotations Per Minute
SOP	Standard Operating Procedure
u	Umbrella
WSSV	White Spot Syndrome Virus

Introduction

Artemia is a euryhaline species. It is a non-selective filter feeder and can reproduce two ways either producing cyst (oviparous) or nauplii (ovoviviparous) depending on the environmental condition and strains. *Artemia* production in ponds includes the procedures of site selection, pond design, pond preparation, cyst hatching and nauplii inoculation, salinity management, feed management, harvesting of cysts and biomass, processing, preservation, and quality testing. This Standard Operating Procedure (SOP) describes the production procedure of *Artemia* in ponds considering the experience of Cox's Bazar district in Bangladesh.

1. Proper site selection

- The access and availability of sufficient high saline water is obligatory. Topography, climatic and soil conditions are vital for site selection for *Artemia* production. Flat land, heavy clay soil, favorable climate (dry season, less rainfall, lower humidity) are preferable for *Artemia* pond site.

2. *Artemia* pond culture design

- The integration of *Artemia* culture with salt production and aquaculture are useful culture models for salt farmers. Concurrent and or alternative integration of shrimp and tilapia culture with salt production are useful climate-smart aquaculture models.

3. Pond construction and pond preparation

- *Artemia* pond culture farms require algae production area (reservoir pond or fertilization pond), *Artemia* pond, availability of sea water, and concentrated sea water or crude salt. *Artemia* ponds cover 75-80% of the farm area, and the rest of the area is used for algae production. The required water depth in algae pond is 1.2 meter (m), in *Artemia* ponds 0.4-0.5 m with an inside canal with 0.5 m depth. The pond dikes need to be well

compacted to prevent leakage and seepage. Wave breaker is useful to prevent erosion due to wave action, harvesting *Artemia* cysts and biomass.

- Measure pond soil pH of *Artemia* demonstration ponds and fertilized ponds. Apply lime (calcium hydroxide) @ 1 metric tons (MT) per hectare (ha) before water filling to ensure soil pH range >8.0.

4. Brine preparation

- Optimum salinity for starting *Artemia* culture in ponds is 80-100 g/l. The salinity of *Artemia* ponds can be managed through seawater evaporation, mixing with crude salt or brine stored from last year's salt production.
- The algae ponds and *Artemia* ponds are filled with seawater (salinity 25-35 g/l) and concentrated sea water of 80-90 g/l, respectively. Filtration screens (mesh size of 120 µm) must be used during water intake to prevent predators (for example fish eggs and crustaceans larvae) entering the *Artemia* culture ponds and algae ponds. *Artemia* ponds minimum water level at start need to be at least 20 cm (from the base) and to increase gradually up to 40 cm and higher throughout the culture.
- Do not apply bleaching powder, or herbicide as algae will be killed, but rather filter by a fine mesh screen in the water inlet to ensure that no predators (tilapia, small fishes, shrimps, and crabs) enter into the ponds.
- Both the algae and *Artemia* ponds need to be fertilized (at least 10 days before inoculation) using organic manure (cow dung, chicken manure, kitchen wastes) and inorganic fertilizer. Please consider organic manure particularly chicken manure are not contaminated with antibiotics. The doses of inorganic fertilizers are urea @ 50 kg/ha and triple super phosphate @20 kg/ha.
- Determine water quality parameters (temperature, pH, salinity, dissolved oxygen, transparency and ammonia) to ensure suitable condition of water in *Artemia* pond.

5. Incubation and stocking

- Apply standard conditions for *Artemia* cyst incubation (temperature, salinity, pH, light, density). The standard conditions of *Artemia* cyst hatching are below -

  Water temperature: 28 - 30^{0}C
  Salinity: 25 - 35 g/l
  Dissolved oxygen: >5 mg/l
  Light intensity (illumination): 2000 lux
  pH: 8.0 - 8.5
  Density: 2 g cysts/l
  Hatching time: 18-20 hour (h)

- Calculate the amount of *Artemia* nauplii required for inoculation considering water volume and inoculation density per litre in the pond.
- Estimate the number of nauplii in the hatching tank considering cysts per g, hatching rate, incubation density and amount of cysts used for hatching.
- Harvest *Artemia* nauplii at Instar -I stage from the hatching tank using 125 µm filter net and transferred to clean sea water (30-35 g/l) and then ready for inoculation. It is very crucial to inoculate *Artemia* in the Instar -1 stage. In case of long distance between the hatching place and *Artemia* pond, nauplii can be transported after packing in polybag/s, oxygenated, stored in a styrofoam box together with an ice pack.
- Keep the volume as per incubation and deliver directly to the *Artemia* pond, if the distance from hatching site to pond within 30 minutes.
- The protocol for transportation of shrimp PLs should be applied (i.e. washing newly hatched nauplii, density, oxygen and temperature cooling down), if the travel time is over 30 minutes. Approximately, 5 million nauplii in 3 l seawater per poly bag can be transported for 2-3 h distance.

- After 6-7 days of fertilization, observe growth, density and composition algae in *Artemia* ponds to determine optimum condition of *Artemia* inoculation.
- Inoculation of *Artemia franciscana* Vinh Chau (Vietnam) or Macau (Brazil) strain at Instar-I in *Artemia* ponds @ 100 nauplii/l.
- Estimate the initial survivors after 24 h of inoculation (i.e. 5 randomized sampling points in a pond).
- Acclimatize *Artemia* nauplii with the pond water temperature and salinity during inoculation, and distribute the nauplii throughout the pond surface.
- Inoculate *Artemia* nauplii in the morning or late evening considering optimum water temperature (<30^{o}C) of the pond.

6. Pond maintenance and management

- Life cycle of *Artemia* is 40-60 days depending on water temperature (≤35^{0}C) in the pond and availability of sufficient food (algae and supplemental feed). *Artemia* pond yield largely depends on optimum management of the ponds.
- *Artemia* pond management includes proper maintenance of water level, salinity, water colour, feed, population composition, density, nutritional and health status of female, ratio female carrying cysts and larvae. Weather conditions are particularly critical for prolonged rainfall, out of season rain, low or too high temperature.
- Maintain deep water level (>0.5 m) to prevent bottom algae development, reduce temperature fluctuation, maintenance of salinity for the prolonged period, and enhance availability of algae.
- The feed management in *Artemia* ponds is dependent on the observation of presence of food in *Artemia* gut, uniformity of *Artemia* population, swimming behaviour, time required to adult size, female reproductive efficiency, dead *Artemia* gather at corner.

- Application of organic fertilizers, supply of algae can reduce clay turbidity.
- The presence of all life stages of *Artemia*, females producing cysts and biomass is the ideal condition.

7. Enhancement of algae production

Microalgae is the best food for *Artemia*. Suitable algal species for *Artemia* are *Tetraselmis*, *Dunaliella*, *Chaetoceros*, *Navicula*, *Nitzschia* and *Thalassiosira*.

- Combination of organic and inorganic fertilizer with N: P ratio ≥ 3:1, salinity ≤ 50 g/l is suitable for diatom and green algae to develop.
- Approximately, 2-5 cm of green water (expressed as increase of water level) from algae ponds need to be added every 2 days to the *Artemia* ponds to supply green algae and also compensate for the loss of water due to evaporation and seepage. Optimal range of secchi disc reading is 25-35 cm. The parameters to maintain algae production in *Artemia* ponds are summarized in Table 1.

Table 1: Water quality parameters, optimum range and frequency to maintain algae production in *Artemia* ponds

Parameters	Frequency	Optimum range
Water temperature (°C)	Twice per day (7 AM, 2 PM)	20-30°C
Salinity (g/ litre)	Once per day	80-100 g/l
Turbidity (Secchi disk)	Every day 2 PM	20-35 cm
Dissolved oxygen	Twice per day (7 AM, 2 PM)	Minimum 2.5 mg/l
Water pH	Every other day	7.8-8.2
Nitrogen (mg/l)	Every week	0.5-1
Phosphorous (mg/l)	Every week	0.1-0.3

a. Organic fertilization

a.1. Cow dung application

- Raw cow dung needs to be fermented for 48 h at low-salinity (0-25 g/l) water. After fermentation, cow dung filled into the bags and hung into *Artemia* and algae ponds @ 200 kg/ha. Two or three bags can be used for each pond depending on the pond's condition and algae production.
- The bags need to be refilled at least every 15 days interval and replaced after 45 days with new bags.

a.2. Chicken manure application

- Raw chicken manure needs to be fermented for 72 h at low salinity water (0-25 g/l) and hung in both *Artemia* and algae ponds @ 200 kg/ha.
- The bags need to be refilled at least every 15 days and replaced after 45 days with new bags.

b. Inorganic fertilization

- Apply urea @ 30 kg/ha and triple super phosphate @ 10 kg/ha per week in both algae and *Artemia* ponds during culture period depending on water transparency measured through secchi disk. Ensure sufficient sea water intake in algae ponds using the proper filtration before fertilization. Pond racking 2-3 times per week is useful to avoid filamentous algal growth, stir up the particulate matter settled at the pond bottom to re-suspend in the water column and improve availability for filtering by the *Artemia*.

8. Processed feed and supplementary feeding

- Supplementary feeds for *Artemia* pond culture are rice bran, wheat bran, molasses, ami-ami (waste products of mono sodium glutamate factory), fish meal, shrimp feed.

- During dense *Artemia* population, with pale and empty gut and lack of sufficient algae in the ponds, fermented rice bran and soyabean (particle sizes≤50 µm) can be supplemented @ 4-6 kg/ha/day. Rice bran and/ or soyabean meal with molasses needs to be diluted and fermented for 24 h and then applied to *Artemia* ponds as supplementary feeds.

9. Diseases and health management

- Diseases can cause mass mortality of the *Artemia* population in ponds. The common diseases are fouling with filamentous bacteria, black spot in appendages, long faeces traits and white abdomen. The health management includes optimizing rearing conditions, changes in salinity, suitable algae production, supplementary feeding, probiotics and biofloc culture.
- *Artemia* population can be checked for the presence major shrimp pathogens such as White Spot Syndrome Virus (WSSV), Acute Hepatopancreatic Necrosis Disease (AHPND), Enterocytozoon Hepatopenaei (EHP) and nutritional quality (protein, lipid, fatty acid and amino acid).

10. Harvesting cysts and biomass

- After 21 days of *Artemia* inoculation, *Artemia* cysts and biomass harvesting can be started depending on the preference of culture (cyst harvest, biomass harvest or both cyst and biomass harvest).
- Cysts need to be collected daily and cleaned through fine nets using pond water. Then, stored in saturated salt water (300 g/l) to remove water and stop cyst metabolism. Cysts can be stored in saturated brine for several months without losing viability. They can be used for nauplii production in hatcheries or be further processed (drying, packaging). The protocol of *Artemia* cyst processing and preservation is described below:

a. The protocol for *Artemia* cyst processing and preservation

- Store freshly harvested *Artemia* cyst in saturated brine with crude salt at bottom.
- Freshly harvested cysts transferred to brine reservoirs (need regular mixing a few times within first hours and then once per day for a few days) to ensure homogenous dehydration, otherwise the ones on top will keep the hydration level as in the pond water.
- Remove large debris using 500 µm net and small debris will sink to the bottom.
- Removal of heavy debris using density separation in a conical shaped funnel with clean saturated brine water (300 g/l) for a couple of hours (2-3 h). Saturated brine prepared using sodium chloride (NaCl) or crude salt). Cysts are already dehydrated, now it is a matter of removing the dirt. Heavy debris will sink at the bottom and cysts (full and empty) will float.
- Collection of cysts via 120 µm scoop net, wash thoroughly with freshwater to remove brine, place in another conical shaped funnel and maintain low temperature (using ice pack) in freshwater for density separation for maximum 30 minutes. Normally full cysts will sink to the bottom and empty shells and light debris will float in the freshwater.
- In case cysts need to be preserved for a long period (months), cysts are collected via scoop net (120 µm) and placed again in clean saturated brine. The cysts need to be mixed several times in the first few hours in the saturated brine to ensure homogenous dehydration. Check under a microscope for a few days.
- Put the cysts in centrifuge for 30 minutes at 1800 rpm (rotations per minute) before being placed in zip bags (each bag 500g wet weight) in case of commercial processing.
- Put the dehydrated cysts in a net and squeeze out as much brine as possible before packing in zip bags; no need to add crude salt, just make sure that bags are closed air-tight.

b. *Artemia* cyst hatching quality can be determined following the protocol below -

- *Artemia* cyst hatching quality tests include hatching under standard hatching condition, count number of nauplii, umbrella stage and unhatched cysts, determine the conversion factor of wet cysts to dry cysts and determine the hatching percentage and hatching efficiency.
- Incubate 500 mg cysts in 1 litre cone with sea water (salinity 30-35 g/l, water temperature 28°C, 2000 lux illumination), provide gentle aeration from bottom. Run the test in duplicate.
- Collect 6 sub samples of 250 µl out of each bottle after 24 h incubation.
- Nauplii of each subsample fixed by lugol solution and placed in microscope slide .
- Count nauplii and umbrella stage under a microscope for 6 sub-samples of two cones, and calculate the mean value.
- Decapsulate unhatched cysts and dissolve empty cysts shells by adding NaOCL of each cone. Count unhatched (orange coloured after decapsulation) embryos and calculate mean value.
- Wet *Artemia* cysts need to be dried under standard drying conditions. Conversion factor will be known after drying under standard laboratory conditions.

  Hatching percentage: Number of nauplii that can be produced under standard hatching conditions from 100 full cysts.

  H% = {(n*100)/(n+u+e)}

  n= nauplii, u = umbrella, e = embryo

  Calculate H% per cone and calculate mean value and standard deviation.

  Hatching efficiency: Number of nauplii that can be produced from 1 g dry cyst under standard hatching conditions. HE = (sum of number of nauplii in three count/3)*2000.

c. Live *Artemia* biomass transportation

- Harvest live *Artemia* biomass and store in a 100-liter tank with 70-80 g/l salinity for 2 hours for gut cleaning of *Artemia* biomass. In a polybag 500 g live *Artemia* biomass packed with fresh 3 l seawater (salinity 70-80 g/l), oxygenated and covered with styrofoam box.

d. Frozen *Artemia* biomass processing and preservation

- Clean sea water at salinity 70-80 g/l prepared through mixing with crude salt or brine. *Artemia* biomass collected from the pond acclimatized in the clean sea water and kept for 2-3 hours with aeration for gut cleaning. The cleaned live *Artemia* biomass dip into saturated brine (300 g/l) for one minute. 500 g biomass weighed and placed into each zip lock bag (12 inches x 8 inches) and preserved in a deep freezer for future use.

About WorldFish

WorldFish is a nonprofit research and innovation institution that creates, advances and translates scientific research on aquatic food systems into scalable solutions with transformational impact on human well-being and the environment. Our research data, evidence and insights shape better practices, policies and investment decisions for sustainable development in low- and middle-income countries. We have a global presence across 20 countries in Asia, Africa and the Pacific with 460 sta of 30 nationalities deployed where the greatest sustainable development challenges can be addressed through holistic aquatic food systems solutions. Our research and innovation work spans climate change, food security and nutrition, sustainable fisheries and aquaculture, the blue economy and ocean governance, One Health, genetics and AgriTech, and it integrates evidence and perspectives on gender, youth and social inclusion. Our approach empowers people for change over the long term: research excellence and engagement with national and international partners are at the heart of our e orts to set new agendas, build capacities and support better decision-making on the critical issues of our times. WorldFish is part of One CGIAR, the world's largest agricultural innovation network.

About Artemia4Bangladesh

WorldFish Bangladesh is implementing the European Union (EU) funded "Artemia4Bangladesh" project in Cox's Bazar district to improve livelihood of the salt farmers. The overall objective of the Artemia4Bangladesh project is to enhance agricultural and food systems in the Cox´s Bazar area of Bangladesh. The specific objective of the project is Increased productivity of salt producers and aquaculture farmers linked to Artemia-related innovative initiatives in the Cox´s Bazar area.

For detailed information

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