Project ID -779

Competitive Research Grant

Sub-Project Completion Report

on

Improvement of live feed culture for Brackishwater hatchery operation

Project Duration

May 2017 to September 2018

Bangladesh Fisheries Research Institute
Brackishwater Station, Paikgacha, Khulna

Submitted to

Project Implementation Unit-BARC, NATP - 2
Bangladesh Agricultural Research Council
Farmgate. Dhaka-1215

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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
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Acronyms

AV.	Average
BARC	Bangladesh Agriculture Research Council
BFRI	Bangladesh Fisheries Research Institute
CoPI	Co-principal investigator
CRG	Competitive Research Grant
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
eg.	Example
FGT	Fiber Glass Tank
GOB	Government of Bangladesh
HUFA	Highly Unsaturated Fatty Acid
ID	Identity
Ind.	Individual
L	Liter
MFTS	Marine Fisheries and Technology Station
ML	Milliliters
NATP	National Agricultural Technology Program
N: P: K:	Nitrogen: Phosphorus: Potassium
PCR	Project Completion Report
PI	Principal Investigator
PIU	Project Implementation Unit
R	Replication
Sp.	Species
T	Treatment
TK	Taka
USAID	United State American Aid
%	Percentage

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Executive Summary

Brackish water and Marine hatcheries rely upon live feeds as the main source of nutrition for larvae of the species being cultured. Live feed is the basic food source and nutrient security for successful seed production of commercially important fishes, mollusks and crustaceans.

The *Nannochloropsis* sp.; *Nannochlorum* sp. and *Tetraselmis* sp. are rich in relatively high content of essential fatty acids in comparison to other marine algae. The Rotifer *Brachionus* sp. is ideal feed item for brackishwater finfish and mud crab larvae due to the suitable sizes and easy digestibility. However, live feeds are needed to be enriched to enhance the qualitative and quantitative nutrients, especially the essential fatty acids of the HUFA's.

This research was carried out to scaling up of live feed culture in qualitative and quantitatively. Also generate knowledge on live feed culture growth phase, stationary phase and proper harvesting time of different microalgae and to find out enrichment protocol, enrichment media and nutritive value of different microalgae and Rotifer for successful brackish water hatchery operations.

Three microalgae species, *Nannochloropsis, Nannochlorum* and *Tetraselmis* was cultured in Brackish water hatchery in indoor and outdoor condition. All 3 species started cell division immediately after inoculation and reached to peak on 9th day of culture. The stationary phase was observed within 9-15 days and then started to collapse. Indoor trial, in culture vessel (2L conical flask) containing 1ml F2/L filtered sea water (25-30ppt), inoculated with microalgae (0.5×10^6/ml) @ 5-10% of total culture volume showed higher average growths such as 5.08, 3.47 and 6.91 cells/ml×10^6 for *Nannochloropsis, Nannochlorum and Tetraselmis sp* respectively on 14th days of culture then collapsed sharply. Similar growth pattern was observed in case of 60 liter white container using same methodology in indoor condition where average growths were 3.31, 2.63 and 2.64 cells/ml×10^6 for *Nannochloropsis, Nannochlorum and Tetraselmis* sp on day 14th then collapsed sharply.

For mass production of microalgae, 300L tank containing filtered sea water (25-30ppt) fertilized with N+P+K=6g+0.5g+6g, inoculated with microalgae (0.5×10^6/ml) @ 5-10% of total culture volume demonstrated higher average growths; 2.60, 3.90 and 2.09 cells/ml×10^6 for *Nannochloropsis, Nannochlorum and Tetraselmis* sp on 14th days of culture. In proximate analysis, *Tetraselmis* sp was found to demonstrate highest protein content (63%) followed by *Nannochloropsis* sp (62% protein) and *Nannochlorum* spp. (60% protein) enriched with different media.

Rotifer culture (inoculation density 15-20 ind/ml) in 300L of 25-30 ppt salt water for a period of 7-10 days demonstrated higher average growths of Rotifer (*Brachionus plicatilis*) such as 189 ind/ml in yeast+microalgae media followed by Microalgae diet media yielded 158 ind/ml, lower growth rate was observed in baker's yeast media as 132 ind/ml.

The Rotifer, (*Brachionus plicatilis)* was scaled up under outdoor condition in 300 liter plastic jars with 300-400/ml inoculum density using different media. Higher average growths of Rotifer (*Brachionus plicatilis*) were 721 ind/ml in microalgae+fish oil media, Microalgae diet media yielded 398 ind/ml, and lowest growth rate was noticed in commercial diet media, 295 ind/ml and no Rotifers were found in fish oil media during 10 days of culture period.

Enriched Rotifer (*Brachionus plicatilis*) with microalgae+fish oil demonstrated the highest protein content (68.4%), followed by yeast+microalgae 65.5%, Baker's yeast 55.4%, microalgae 34.5% protein and the lowest 30.5% protein content was found in commercial diet after 10 days of culture.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project: Improvement of live feed culture for Brackishwater hatchery** operation
2. **Implementing organization:** Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna- 9280.
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

Principal Investigator:

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4.Sub-project budget (Tk):

4.1 Total: 28,00,000.00
4.2 Revised (if any): None

5. Duration of the sub-project:

5.1 Start date : 8 May 2017
5.2 End date : 30 September 2018

6. Justification of undertaking the sub-project:

Brackishwater and Marine hatcheries rely upon live food as the main source of nutrition for larvae of the target species being cultured. Though micro encapsulated and other inert diets have been developed for some commercial species (e.g. *Penaeus monodon, Scylla olivacea*), there is still a requirement for microalgae in the early stages and a combination of inert diets along with *Artemia*.

Both plant and animal originated aquatic microscopic organisms are generally termed as live food. Live feed is the basic food source and nutrient security for successful seed production of any commercially important aquaculture species of fishes, mollusks and crustaceans. They are the basic food items in early stages (larval stage) of life cycle due to small sizes, easy digestibility and enriched in nutrients. Plant originated live food are known as phytoplankton (microalgae) are primary producers in the food web. Whereas, animal originated microorganism are the secondary producer those grazes on phytoplankton.

Hence, the live food supports for better survival and growth of fin fish, and crustacean larvae. They are also considered as water purifiers since they consume soluble nutrients, bacteria and detritus. The culture and production of adequate nutritive live food organisms is considered as the heart of the hatchery for sustainable seed production. However, production of available nutritive live foods is a challenge for the operation of hatchery in a sustainable manner, particularly in under developed countries.

The *Nannochloropsis* sp., *Nannochlorum* sp. and *Tetraselmis* sp. are considered rich with relatively high content of essential fatty acids in comparison to other marine algae. Likelihood, the Rotifer *Brachionus* sp. is also considered as an ideal feed item for brackishwater finfish and mud crab larvae rearing due to suitable sizes, nutrient quality and easy digestibility of the newly hatched larvae. Very often, naturally grown and available live feeds did not in most cases contain available nutrients to support considerable level of survival and growth of larvae, especially for the crustaceans. In this regard as an alternative live feeds are needed to be enriched to enhance the qualitative and quantitative nutrients, especially the essential fatty acids of the HUFA's to meet the larval requirement.

Gradual shipment of aquaculture practice towards selected marine species from the freshwater finfish, particularly in coastal water also demanding to ensuring of high quality such of the target species like, mullet (*Mugil cephalus*), parse fish (*Chellon subviridis*), mud crab (*Scylla* sp.) and shrimps. Therefore scaling up of live feed culture and production has become a prime requirement for the last few years. Under the stated condition, the present research highlighted the production of selected nicroalgae for enhancing production of Rotifers. So that better survival and growth can be attained by the hatchery operators.

7. Sub-project goal:

Promotion of qualitative and quantitative live feed culture protocol for sustainable brackishwater and marine hatchery and nursery operations through uninterrupted supply of larval feeds for mass seed production of fish and crustacean species.

8. Sub-project objective (s):

i. To up-scale the production of different microalgae (*Nannochloropsis* sp.; *Nannochlorum* and *Tetraselmis* sp.) for brackishwater and marine hatchery operations;

ii. To enhance the production of Rotifers (*Brachionus* sp.) for the improvement of larval rearing of fish and crustacean species; and

iii. To observe the nutritive values (proximate contents and fatty acids) of microalgae and Rotifers grown under different protocols.

9. Implementing location (s):

The proposed research was carried out in the laboratory of Brackishwater station, BFRI, Paikgacha, Khulna. Indoor culture trials of microalgae were performed in the laboratory but the outdoor culture experiments were done in the hatchery complex.

10. Methodology in brief:

10.1 Growth performance of three live feed (microalgae)

Three live feed microalgae (*Nannochloropsis* sp*, Nannochlorum* sp and *Tetraselmis* sp) species were cultured under indoor and outdoor condition in order to compare their growth in F2 media and

inorganic fertilizer. F_2 media is a stock solution prepared by mixing the following chemicals in 1L distilled water.

F_2 media=

A= 75 gm Sodium Nitrate+ 5 gm of Sodium Phosphate

+

B= 180 gm Manganese Chloride + 22 gm Zinc Sulphate+ 10 gm Copper Sulphate+ 10 gm Cobalt Chloride+ 6gm Sodium Molybdate)= 1ml+4.36 gm EDTA+3.15 gm Ferric Chloride.

+

C= 20gmThiamineHydrochloride+100gmD-Biotine+100gm Cianocobalamine)

For indoor culture, 1ml F_2medium/L of filtered seawater (25-30 ppt) was inoculated with microalgae (0.5×10^6/ml) @ 5-10% of total culture volume. Light intensity was maintained from 1500 to 2000 Lux for 24 hours with a constant temperature of 20-25 °C with duration of 6- 14 days. For outdoor culture of microalgae, 0.5 ml F_2 medium/20L filtered seawater (25-30 ppt), inoculated with microalgae (0.5×10^6/ml) @ 5-10% of total culture volume. Light intensity was maintained from 1500 to 2000 Lux for 24 hours with a constant temperature of 20-25 °C. Duration of culture was 6-14 days. For mass culture, microalgae (0.5×10^6/ml) @ 10-30% of total culture volume was inoculated into 300L , 25-30ppt salt water fertilized with N+P+K=6g+5g+6g and cultured for 5-14 days under day-light photoperiod condition. Experimental design is presented in table 1.

Table 1. Experimental design for culture of live feed (microalgae)

Treatment	Replication	Species	Protocol	Culture vessel	Inoculum density	Media
T_1	3	*Nannochloropsis* sp	Indoor	2 liter conical flask	0.5×10^6/ml @ 5-10% of total culture volume	F_2 medium
T_2	3	*Nannochlorum* sp				
T_3	3	*Tetraselmis* sp				
T_1	3	*Nannochloropsis* sp	Outdoor	2L / 60 L flask /300L	0.5×10^6/ml @ 5-10% or @10-30% of total culture volume	F_2 medium or inorganic fertilizer (N+P+K= 6g+0.5g+6g)/ 300L
T_2	3	*Nannochlorum* sp				
T_3	3	*Tetraselmis* sp				

The experiment was repeated for thrice. Performance of microalgae was evaluated from the cell density and nutrient content especially proximate composition and levels of essential fatty acid contents such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

The experiment was repeated for thrice. Performances of microalgae were evaluated from the cell density and longevity of log phase, stationary phase and nutrient content especially proximate composition.

10.2 Growth performance of Rotifer under different medium

Growth performances of live feed, Rotifer (Brachionus sp.) were evaluated for growing in different medium. For culture of Rotifer, 15-20 ind/ml of culture volume were inoculated in 300 L of 25-30 ppt salt water for a period of 7-10 days. Rotifers were fed as per experimental design or with baker's yeast 0.5-1 g/millions of Rotifers/day. Harvesting of Rotifer was done with 50-65 µm plankton net. Thirty percent (30%) of harvest was used for subsequent culture and 70% was used as feed of larvae or for experimental purposes.

Experimental design was according to the table below:

Table 2. Experimental design for culture of live feed (*Brachionus* sp.)

Treatment	Replication	Media/feeding	Protocol	Culture vessel	Inoculum Density
T_1	3	Baker's yeast	outdoor	300 liter plastic jars	20/ml
T_2	3	Microalgae			
T_3	3	Yeast + microalgae			

Consecutive three experiments were conducted for a period of 10 days each. Performance of Rotifer was evaluated from the cell density and nutrient content especially proximate composition and levels of essential fatty acid contents of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) at the end of each experiment.

10.3 Nutritive values of microalgae and Rotifers grown under different protocols.

Nutritive value of Rotifer (*Brachionus* sp.) enriched with different enrichments media were evaluated as per design described in Table 3. Rotifer was cultured in baker's yeast, was harvested and enriched with different enrichment as in Table 3. After 6-8 hours of enrichment, Rotifers were harvested further and the nutritive value (proximate composition and fatty acid contents) was investigated. Experimental design for enrichment of Rotifers was according to the table below:

Table 3. Experimental design for enrichment of Rotifers (*Brachionus* sp.)

Treatment	Replication	Enrichment media	Protocol	Culture vessel	Inoculums Density
T_1	3	Microalgae	outdoor	300 liter plastic jars	300-400/ml
T_2	3	Commercial diet			
T_3	3	Fish oil (SELCO)			
T_4	3	Microalgae + fish oil			

Consecutive three experiments were conducted for a period of 10 days each. Suitability of Rotifer was evaluated from the levels of nutrient content especially proximate composition and levels of essential fatty acid contents of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) at the end of the experiment.

11. Results and discussion:

11.1. Growth performance and nutritive value of three live feed (microalgae) species

Indoor Trials-2L conical flask

1st trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under indoor condition in 2 liter conical flask. All 3 species started cell division immediately after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In indoor condition highest average growths 6.87 cells/ml×10^6 and 5.0cells/ml×10^6 were observed for *Tetraselmis* sp and *Nannochlorum* sp, and lowest average growth 3.0cells/ml×10^6 was observed for *Nannochloropsis* sp at 14th days of culture then collapsed sharply. Growth performance of *Tetraselmis* sp was highest in the 1st trial (Table 4).

Table 4. Growth Performance of three live feed (microalgae) species under indoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	3.66×10^6	F_2
		R_2	4.0×10^6	
		R_3	1.33×10^6	
		Av.	**3.0×10^6**	
Nannochlorum sp	T_2	R_1	4.33×10^6	F_2
		R_2	3.33×10^6	
		R_3	7.33×10^6	
		Av.	**5.0×10^6**	
Tetraselmis sp	T_3	R_1	5.28×10^6	F_2
		R_2	6.33×10^6	
		R_3	9×10^6	
		Av.	**6.87**×10^6	

2nd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under indoor condition in 2 liter conical flask.

Table 5. Growth Performance of three live feed (microalgae) species under indoor culture condition.

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	4.23×10^6	F_2
		R_2	3.85×10^6	
		R_3	2.13×10^6	
		Av.	**3.40×10^6**	
Nannochlorum sp	T_2	R_1	3.95×10^6	F_2
		R_2	4.13×10^6	
		R_3	6.95×10^6	
		Av.	**5.01×10^6**	
Tetraselmis sp	T_3	R_1	6.13×10^6	F_2
		R_2	5.75×10^6	
		R_3	8.85×10^6	
		Av.	**6.91×10^6**	

All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. Lim (1991) reported that, the culture grew from 5.00 x 10^6 cells/ ml to 6.70 x 10^7 cells/ ml in six days, and to 2.13 x 10^8 cells/ ml in 20 days. In indoor condition, highest average growth 6.91 and 5.01 cells/ml $\times 10^6$ were observed for *Tetraselmis* sp and *Nannochlorum* sp, and lowest average growth 3.40 cells/ml $\times 10^6$ was observed for *Nannochloropsis* sp at 14th days of culture then collapsed sharply. Growth performance of *Tetraselmis* sp was highest in the 2nd trial (Table 5).

3rd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under indoor condition in 2 liter conical flask. All 3 species started cell division immediately after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In indoor condition highest average growth 6.23 and 5.08 cells/ml$\times 10^6$ were observed for *Tetraselmis* sp and *Nannochlorum* sp, lowest average growth 3.47 cells/ml$\times 10^6$ was observed for *Nannochloropsis* sp at 14th days of culture then collapsed sharply. Growth performance of *Tetraselmis* sp was highest in the 3rd trial. Average growth performance was higher under outdoor culture condition than indoor (Table 6)

Table 6. Growth Performance of three live feed (microalgae) species under indoor culture condition.

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	4.85×10^6	F_2
		R_2	3.45×10^6	
		R_3	2.13×10^6	
		Av.	**3.47×10^6**	
Nannochlorum sp	T_2	R_1	3.76×10^6	F_2
		R_2	4.59×10^6	
		R_3	6.90×10^6	
		Av.	**5.08×10^6**	
Tetraselmis sp	T_3	R_1	4.56×10^6	F_2
		R_2	5.18×10^6	
		R_3	8.95×10^6	
		Av.	**6.23×10^6**	

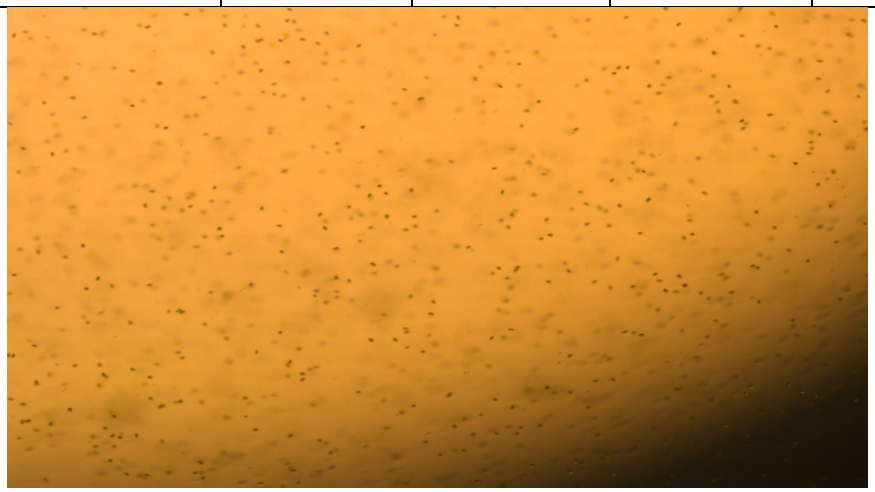

Plate 1: Microscopic view of *Tetraselmis* sp in 2 L conical flask

Outdoor Trials-60L conical flask

1st trial: Three microalgae species*, Nannochloropsis, Nannochlorum and Tetraselmis were* cultured under outdoor condition in 60 Liter white container. All 3 species started cell division immediately after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 2.87 and 2.45 cells/ml $\times 10^6$ was observed for *Nannochloropsis* sp and *Tetraselmis* sp, lowest average growth 2.11 cells/ml $\times 10^6$ was observed for *Nannochlorum* sp at 14th days of culture then collapsed sharply. Growth performance of *Nannochloropsis* sp was highest in the 1st trial (Table 7).

Table 7. Growth Performance of three live feed (microalgae) species under outdoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	4.16×10^6	F_2
		R_2	1.13×10^6	
		R_3	3.33×10^6	
		Av.	**2.87×10^6**	
Nannochlorum sp	T_2	R_1	3.33×10^6	F_2
		R_2	1.46×10^6	
		R_3	1.56×10^6	
		Av.	**2.11×10^6**	
Tetraselmis sp	T_3	R_1	3×10^6	F_2
		R_2	2.9×10^6	
		R_3	1.46×10^6	
		Av.	**2.45×10^6**	

2nd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured in outdoor condition in 60 Liter white container. All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 3.31 and 2.63 cells/ml $\times 10^6$ was observed for *Nannochloropsis* sp and *Nannochlorum* sp, lowest average growth 2.55 cells/ml $\times 10^6$ was observed for *Tetraselmis* sp at 14th days of culture then collapsed sharply. Growth performance of *Nannochloropsis* sp was highest in the 2nd trial (Table 8).

Table 8. Growth Performance of three live feed (microalgae) species under outdoor culture condition.

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	4.65×10^6	F_2
		R_2	2.43×10^6	
		R_3	2.85×10^6	
		Av.	**3.31×10^6**	
Nannochlorum sp	T_2	R_1	3.75×10^6	F_2
		R_2	2.20×10^6	
		R_3	1.95×10^6	
		Av.	**2.63×10^6**	
Tetraselmis sp	T_3	R_1	2.67×10^6	F_2
		R_2	2.85×10^6	
		R_3	2.13×10^6	
		Av.	**2.55×10^6**	

3rd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under outdoor condition in 60 Liter white container. All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 2.91 and 2.64 cells/ml $\times 10^6$ was observed for *Nannochloropsis* sp and *Tetraselmis* sp, lowest average growth 2.16 cells/ml $\times 10^6$ was observed for *Nannochlorum* sp at 14^{th} days of culture then collapsed sharply. Growth performance of *Nannochloropsis* sp was highest in the 3^{rd} trial (Table 9)

Table 9. Growth Performance of three live feed (microalgae) species under outdoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	4.18×10^6	F_2
		R_2	1.15×10^6	
		R_3	3.42×10^6	
		Av.	**2.91×10^6**	
Nannochlorum sp	T_2	R_1	3.43×10^6	F_2
		R_2	1.42×10^6	
		R_3	1.65×10^6	
		Av.	**2.16×10^6**	
Tetraselmis sp	T_3	R_1	3.12×10^6	F_2
		R_2	2.85×10^6	
		R_3	1.97×10^6	
		Av.	**2.64×10^6**	

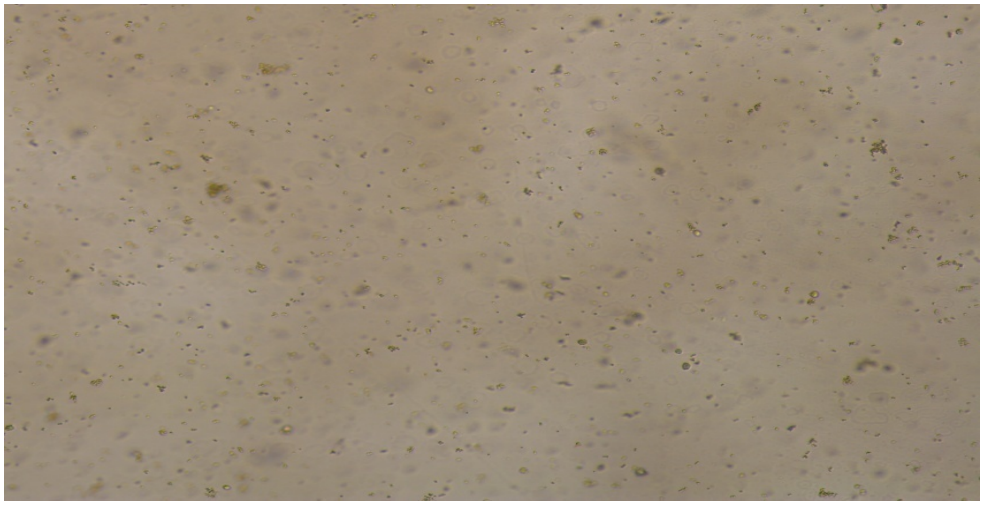

Plate 2: Microscopic view of Nannochloropsis sp in 60 L container

Outdoor trial-300L conical flask

1st trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under outdoor condition in 300 liter white fibre glass tank. All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 3.90 and 2.04 cells/ml $\times 10^6$ were cells/ml$\times 10^6$ observed for *Nannochlorum* sp and *Tetraselmis* sp, lowest average

growth 1.93 cells/ml $\times 10^6$ was observed for *Nannochloropsis* sp at 14th days of culture then collapsed sharply. Growth performance of *Nannochlorum* sp was highest in the 1st trial (Table 10).

Table 10. Growth Performance of three live feed (microalgae) species under outdoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	1.70×10^6	F_2
		R_2	2.52×10^6	
		R_3	1.58×10^6	
		Av.	**1.93×10^6**	
Nannochlorum sp	T_2	R_1	1.46×10^6	F_2
		R_2	3.98×10^6	
		R_3	6.25×10^6	
		Av.	**3.90×10^6**	
Tetraselmis sp	T_3	R_1	1.38×10^6	F_2
		R_2	2.34×10^6	
		R_3	2.33×10^6	
		Av.	**2.02×10^6**	

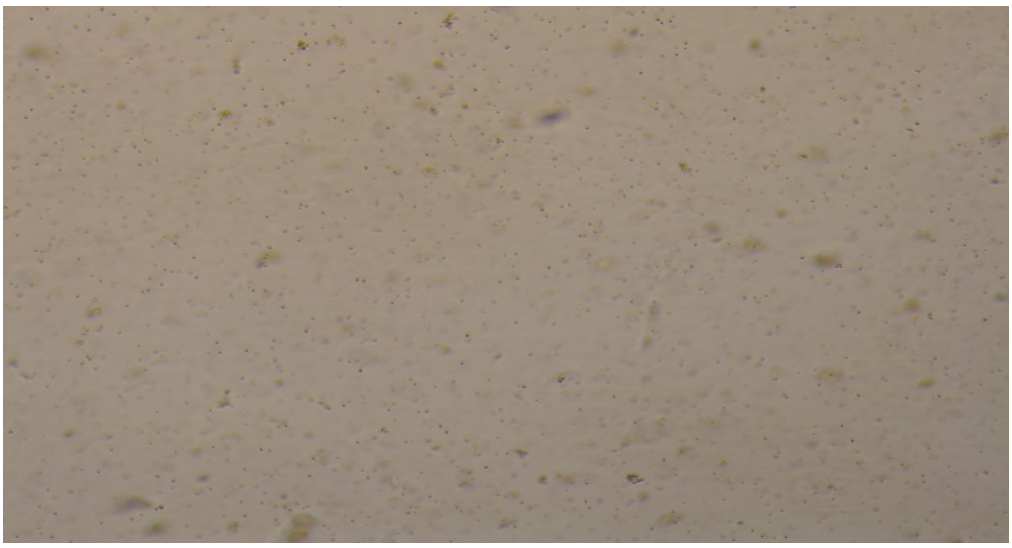

Plate 3: Microscopic view of *Nannochlorum* sp in 300 L tank

2nd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under outdoor condition in 300 liter white fibre glass tank. All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 3.57 and 2.13 cells/ml $\times 10^6$ were observed for *Nannochlorum* sp and *Nannochloropsis* sp, lowest average growth 1.84 cells/ml $\times 10^6$ was observed for *Tetraselmis* sp at 14th days of culture then collapsed sharply. Growth performance of *Nannochlorum* sp was highest in the 2nd trial (Table 11).

Table 11. Growth Performance of three live feed (microalgae) species under outdoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	1.95×10^6	F_2
		R_2	2.58×10^6	
		R_3	1.88×10^6	
		Av.	**2.13×10^6**	
Nannochlorum sp	T_2	R_1	1.16×10^6	F_2
		R_2	3.51×10^6	
		R_3	6.05×10^6	
		Av.	**3.57×10^6**	
Tetraselmis sp	T_3	R_1	1.18×10^6	F_2
		R_2	2.12×10^6	
		R_3	2.23×10^6	
		Av.	**1.84×10^6**	

3rd trial: Three microalgae species, *Nannochloropsis, Nannochlorum and Tetraselmis* were cultured under outdoor condition in 300 liter white fibre glass tank. All 3 species started cell division immediate after inoculation and reached to the peak on 9th day of culture. The stationary phase was observed for 9-15 days and then started to collapse. In outdoor condition highest average growth 2.68 and 2.60 cells/ml $\times10^6$ were observed for *Nannochlorum* sp and *Nannochloropsis* sp, lowest average growth 2.09 cells/ml $\times10^6$ was observed for *Tetraselmis* sp at 14^{th} days of culture then collapsed sharply. Growth performance of *Nannochlorum* sp was highest in the 3^{rd} trial (Table 12).

Table 12. Growth Performance of three live feed (microalgae) species under outdoor culture condition

Species	Treatments	Replications	Growth (cell/ml)	Media
Nannochloropsis sp	T_1	R_1	2.96×10^6	F_2
		R_2	2.64×10^6	
		R_3	2.20×10^6	
		Av.	**2.60×10^6**	
Nannochlorum sp	T_2	R_1	1.38×10^6	F_2
		R_2	2.98×10^6	
		R_3	3.68×10^6	
		Av.	**2.68×10^6**	
Tetraselmis sp	T_3	R_1	1.45×10^6	F_2
		R_2	2.38×10^6	
		R_3	2.46×10^6	
		Av.	**2.09×10^6**	

The proximate composition of three microalgae (*Nannochloropsis, Nannochlorum and Tetraselmis* sp) was done from the Science Laboratory, Dhaka. From the above report we have shown that *Tetraselmis*

sp contain highest 63% protein and *Nannochloropsis* sp contain 62% protein, lowest 60% protein contain with *Nannochlorum* species (Table 13).

Table 13. Proximate composition of three microalgae (*Nannochloropsis, Nannochlorum and Tetraselmis* sp*)*

Parameters	*Nannochloropsis*	*Nannochlorum*	*Tetraselmis*
Dry Weight	18.2 %	18.0%	18.2%
Calories (100g dry wt algae)	450	385	390
Protein	62%	60%	63%
Lipid (Total)	18%	10%	11%
Carbohydrate	9%	12%	11%
Ash	10%	12%	15%
Vitamin C	0.85 %	0.20%	0.25 %
Chlorophyl A	0.89 %	1.40 %	1.42 %

Inoculating live feed

Counting live feed

11.2 Growth performance of Rotifer (*Brachionus* sp.) in different medium

Trials in 300 L Jar

1st trial: Rotifer, Brachionus plicatilis was scaled up under outdoor in 300 liter plastic jars in 20 /ml inocululation density using different media. Higher average growth of Rotifer (*Brachionus plicatilis*) was obtained 189 ind/ml in yeast+ microalgae media. Microalgae diet media yielded 158 ind/ml, lowest growth rate was noticed with Baker's yeast media 124 ind/ml for 6 day of culture. Rotifers (*Brachionus plicatilis*) grow well at the temperature above 26 °C and water salinity of 25 ppt (FAO, 1996). In 300 liter plastic jars in 20 /ml inoculum density, the growth performance of Rotifer in yeast+ microalgae media was better than Microalgae diet and Baker's yeast media (Table 14).

Table 14. Growth performance of live feed, Rotifer (*Brachionus* sp.) under different medium/feedings.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Baker's yeast	T_1	R_1	108
		R_2	160
		R_3	106
		Av.	**124**
Microalgae	T_2	R_1	140
		R_2	165
		R_3	170
		Av.	**158**
Yeast + microalgae	T_3	R_1	150
		R_2	193
		R_3	226
		Av.	**189**

2nd trial: Rotifer, *Brachionus plicatilis* was scaled up under outdoor in 300 liter plastic jars in 20 /ml inoculum density with different media. Average growth of Rotifer (*Brachionus plicatilis*) was higher as 180 ind/ml in yeast+microalgae media. Microalgae diet media yielded 148 ind/ml, lowest growth rate was noticed with baker's yeast 111 ind/ml for 6 day of culture. In 300 liter plastic jars in 20 /ml inoculum density, the growth performance of Rotifer in yeast+ microalgae media was better than Microalgae diet and baker's yeast media. (Table 15).

Table 15. Growth performance of live feed, Rotifer (*Brachionus* sp.) under different medium/feedings.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Baker's yeast	T_1	R_1	115
		R_2	125
		R_3	95
		Av.	**111**
Microalgae	T_2	R_1	120
		R_2	145
		R_3	180
		Av.	**148**
Yeast + microalgae	T_3	R_1	165
		R_2	180
		R_3	195
		Av.	**180**

3rd trial: Rotifer, *Brachionus plicatilis* was scaled up under outdoor condition in 300 liter plastic jars with 20/ml inoculum density using different media. The highest average growth of Rotifer (*Brachionus plicatilis*) was 172 ind/ml in yeast+ microalgae media. For mass scale culture, Rotifers could be stocked @20/ml and fed with 0.5 mg of Baker's yeast for a million of Rotifers twice a day (FAO, 1996).

Microalgae diet media yielded 144 ind/ml, the lowest growth rate was noticed in Baker's yeast 132 ind/ml after 6 days of culture. In 300 liter plastic jars with 20 /ml inoculum density, the growth performance of Rotifer in yeast+ microalgae media was better than those of Microalgae diet and Baker's yeast media (Table 16).

Table 16. Growth performance of live feed, Rotifer (*Brachionus* sp.) under different medium/feedings.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Baker's yeast	T_1	R_1	105
		R_2	102
		R_3	190
		Av.	**132**
Microalgae	T_2	R_1	116
		R_2	140
		R_3	178
		Av.	**144**
Yeast + microalgae	T_3	R_1	154
		R_2	176
		R_3	188
		Av.	**172**

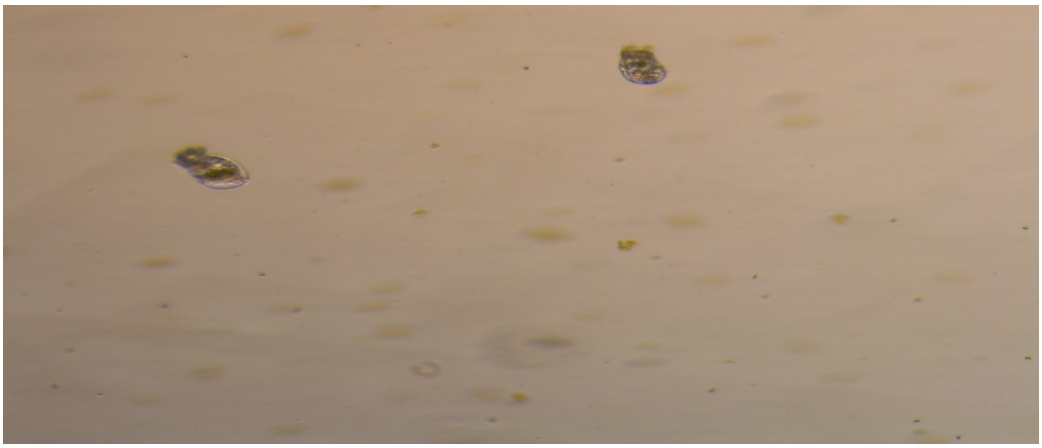

Plate 4: Microscopic view of Rotiers *Brachionus plicatilis* in Different Media

11.3 Evaluation of nutritive value of Rotifer (*Brachionus* sp.) enriched with different medium.

Outdoor trials – 300L jar

1st trial: Rotifer*, Brachionus plicatilis* was scaled up under outdoor condition in 300 liter plastic jars in 300-400/ml inoculum density with different media. Average growth of Rotifer (*Brachionus plicatilis*) was higher as 527 ind/ml in microalgae+ fish oil media. Microalgae diet media yielded 353 ind/ml, lowest growth rate was noticed with Commercial diet media 231 ind/ml for 6 day of culture and no Rotifers found in fish oil media. In 300 liter plastic jars in 300-400/ml inoculum density, the growth performance

of microalgae+fish oil media showed better growth performance than those of microalgae diet media and commercial diet media (Table 17).

Table 17. Evaluation of nutritive value of Rotifer (*Brachionus* sp.) enriched with different enrichments.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Microalgae	T_1	R_1	438
		R_2	336
		R_3	285
		Av.	**353**
Commercial diet	T_2	R_1	279
		R_2	159
		R_3	256
		Av.	**231**
Fish oil (SELCO)	T_3	R_1	Nil
		R_2	Nil
		R_3	Nil
		Av.	**NIL**
Microalgae + fish oil	T_4	R_1	780
		R_2	693
		R_3	609
		Av.	**527**

2nd trial: Rotifer, *Brachionus plicatilis* was scaled up under outdoor condition in 300 liter plastic jars in 300-400/ml inoculum density with different media. Average growth of Rotifer (*Brachionus plicatilis*) was higher 721 ind/ml in microalgae+fish oil media. Microalgae diet media yielded 398 and ind/ml, lowest growth rate was noticed with Commercial diet media 218 ind/ml for 6 day of culture and no Rotifers found in fish oil media. In 300 liter plastic jars in 300-400/ml inoculum density, the growth performance of microalgae+ fish oil media was better than microalgae diet media and commercial diet media (Table 18).

Table 18. Evaluation of nutritive value of Rotifer (*Brachionus* sp.) enriched with different enrichments.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Microalgae	T_1	R_1	490
		R_2	380
		R_3	325
		Av.	**398**
Commercial diet	T_2	R_1	260
		R_2	145
		R_3	250
		Av.	**218**
Fish oil (SELCO)	T_3	R_1	**Nil**
		R_2	Nil
		R_3	Nil
		Av.	Nil
Microalgae + fish oil	T_4	R_1	810
		R_2	715
		R_3	640
		Av.	**721**

3rd trial: Rotifer, *Brachionus plicatilis* was scaled up under outdoor condition in 300 liter plastic jars in 300-400/ml inoculum density with different media. Average growth of Rotifer (*Brachionus plicatilis*) was higher as 641 ind/ml in microalgae+ fish oil media. Microalgae diet media yielded 379 ind/ml and lowest growth rate was noticed with Commercial diet media 295 ind/ml for 6 day of culture and no Rotifers found in fish oil media. In 300 liter plastic jars in 300-400/ml inoculum density, the growth performance of microalgae+ fish oil media is better than microalgae diet media and commercial diet media (Table 19).

Table 19. Evaluation of nutritive value of Rotifer (*Brachionus* sp.) enriched with different enrichments.

Media/feeding	Treatments	Replications	Growth (ind/ml)
Microalgae	T_1	R_1	435
		R_2	382
		R_3	320
		Av.	**379**
Commercial diet	T_2	R_1	205
		R_2	132
		R_3	248
		Av.	**295**
Fish oil (SELCO)	T_3	R_1	**Nil**
		R_2	Nil
		R_3	Nil

Media/feeding	Treatments	Replications	Growth (ind/ml)
		Av.	Nil
Microalgae + fish oil	T_4	R_1	658
		R_2	580
		R_3	686
		Av.	**641**

The Nutritive value of Rotifer, *Brachionus plicatilis* was done from the Science Laboratory, Dhaka. From the above report, enriched Rotifer with Microalgae + fish oil contained highest 68.4% protein followed by Yeast + microalgae 65.5%, Baker's yeast 55.4%, Microalgae 34.5% protein and lowest 30.5% protein contained with Commercial diet for 10 days of culture period (Table 20).

Table 20. Nutritive value of Rotifer (*Brachionus* sp.) enriched with different enrichments.

Diet	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
Baker's yeast	55.4	4.5	28	_
Microalgae	34.5	21.1	_	_
Yeast + microalgae	65.5	22	_	6
Commercial diet	30.5	24.4	16	
Microalgae + fish oil	68.4	22.6	_	4

12. Research highlight/findings:

- Under indoor culture condition in 2liter conical flask using F_2 medium, inoculated with microalgae (0.5×10^6/ml) @ 5-10% of total culture volume demonstrated the highest average growth 6.91 cells/ml$\times10^6$ for *Tetraselmis* sp on 14th days of culture.
- In 60 liter white container under outdoor condition, the highest average growth 3.31 cells/ ml$\times10^6$ was observed for *Nannochloropsis* sp on 14th days of culture.
- Under outdoor condition In 300 liter white fibre glass tank with F_2 medium the highest average growth 3.57 cells/ ml$\times10^6$ was observed for *Nannochlorum* sp on 14th days of culture.
- *Tetraselmis* sp demonstrated the highest protein content (63%) among three microalgae under investigation after 6 days of culture.
- The Rotifer, (*Brachionus plicatilis)* was scaled up under outdoor condition in 300 liter plastic jars in 20 ind./ml inoculum density with different media, the highest average growth was 189 ind/ml in yeast+ microalgae media after 10 days of culture.
- Under outdoor trial, the Rotifer, (*Brachionus plicatilis)* was scaled up in 300 liter plastic jars in 300-400/ml inoculum density using different media, the highest average growth was 721 ind/ml in microalgae+fish oil media after 10 days of culture.
- Rotifer (*Brachionus plicatilis*) enriched with Microalgae+fish oil revealed the highest protein content (68.4%) among the media under trial after 10 days of culture period.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	04	200000	04	196000	
(b) Lab &field equipment	28	500000	28	497000	
(c) Other capital items	-	-	-	-	

2. Establishment/renovation facilities: Not applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
-	-	-	-	-	
-	-	-	-	-	

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	50	10	60	01 days	A one day training program titled "Live feed culture management" was implemented for sixty hatchery technicians and farmers on live feed culture.

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	238800	237100	237056	44	99.98	-
B. Field research/lab expenses and supplies	1227340	1079500	1079474	26	100.00	-
C. Operating expenses	280163	281200	281152	48	99.98	-
D. Vehicle hire and fuel, oil & maintenance	116125	121000	120926	74	99.94	-
E.Training/workshop/ seminar etc.	132000	131200	131140	60	99.95	-
F. Publications and printing	70000	0	0	0	0.00	-
G. Miscellaneous	35572	42100	41756	344	99.18	-
H. Capital expenses	700000	690089	689753	336	99.95	-
TOTAL	**2800000**	**2582189**	**2581257**	**932**	99.96	-

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To up-scale the production of different microalgae (*Nannochloropsis* sp.; *Nannochlorum* and *Tetraselmis* sp.) for brackishwater and marine hatchery operations.	Culture of three microalgae species (*Nannochloropsis* sp*, Nannochlorum* sp and *Tetraselmis* sp) was done under both indoor and outdoor culture condition with F2 and inorganic fertilizer medium	Among three live feed (microalgae) species under indoor culture condition highest average density 6.87×10^6 cells/ml was observed in *Nannochloropsis* sp. Among three live feed (microalgae) species under outdoor culture condition highest average density 2.87×10^6 cells/ml was also observed in *Nannochloropsis* sp.	Increased availability of Live feed and marine hatchery operation.
To enhances the production of Rotifers (*Brachionus* sp.) for improvement of larval rearing of fish and crustacean species.	Culture of live feed, Rotifer (*Brachionus plicatilis* and *B. rotundiformis*) was done with different feedings	Among three media (Baker's yeast, Microalgae and Yeast + microalgae) Average growth of Rotifer (*Brachionus plicatilis*) was higher, 189 ind/ml in yeast+microalgae media.	Increased availability of Rotifer facilitate better survival and growth of fin fish and crustacean larvae at hatchery -level
To observes the nutritive values (proximate contents and fatty acids) of microalgae and Rotifers grown under different protocols.	The proximate composition of three microalgae (*Nannochloropsis, Nannochlorum and Tetraselmis* sp) was done from the Science Laboratory, Dhaka.	*Tetraselmis* sp contain highest 63% protein and *Nannochloropsis* sp contain 62% protein, lowest 60% protein contain with *Nannochlorum* species	Use of microalgae as an idle source of protein will help as important feed ingredient.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	✓		
Journal publication	✓		
Information development			
Other publications, if any	✓		

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Development of live feed culture technique

ii. **Generation of new knowledge that help in developing more technology in future**

Present knowledge of live feed development can be a baseline for further research in developing live feed for several other valuable fish species.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Trained farmers and hatchery operators may adopt this technology in their own hatchery and enhance

iv. Policy Support

Knowledge acquired on live feed development could be useful in formulating future guidelines for live feed development for other valuable fish and crustaceans.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring

Sl. No.	Workshop title	Date	Remarks
1.	Progress review workshop on CRG Sub project	11 April 2018	Satisfactory
2.	Monitoring workshop on CRG Sub project	16 May 2018	Satisfactory
3.	Annual review workshop on CRG Sub project	19 September 2018	Satisfactory

ii) Field Monitoring (time & No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No.)	Remarks
Technical Division, BARC 1. Dr. Md. Monirul Islam, Director (Nutrition), BARC, Dhaka	13 February 2018	1	Satisfactory
PIU-BARC, NATP-2	8 April 2018	1	Satisfactory

1. Mr. Dipok Kumar, PIU-BARC, NATP-2, Dhaka 2. Munshi Mamunur Rahman, PIU-BARC, NATP-2, Dhaka			
Internal Monitoring 1. Dr. Md. Enamul Hoq, PSO, BFRI 2. Dr. Anuradha Bhadra, PSO, BFRI	22-23 June 2018	1	Satisfactory
Others Visitors: 1. Dr. Md. Yahia Mahmud, DG, BFRI 2. Mr. Asim Kumar Bala, Joint Secretary, MoFL, Dhaka 3. Mr. Muhamadullah, Deputy Secretary of MoFL, Dhaka	22-23 June 2018	1	Satisfactory

H. Lesson Learned/Challenges (if any)

Experiment that improper brine water treatment and less attention in maintaining bio security aspect during inoculation may drastically hamper the total production process.

I. Challenges (if any)

Collection of appropriate quality brine water in time was a challenge for the researchers.

Signature of the Principal Investigator
Date …….
Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
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