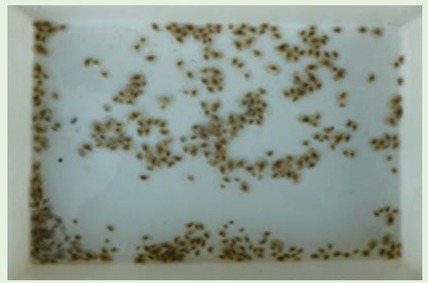

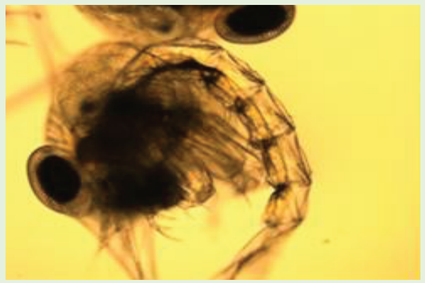

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Sub-Project ID: 029

Program Based Research Grant (PBRG)

Sub-project Completion Report

on

Adoption of Innovative Technology: Seed Production to Fattening of Mud Crab (*Scylla olivacea*) and Health Management in Bangladesh Condition

Sub-project Duration
31 May 2018 to 28 April 2022

Coordinating Organization

Research Planning & Evaluation Division
Bangladesh Fisheries Research Institute
Mymensingh-2201

Project Implementation Unit (PIU)
National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

April 2022

Program Based Research Grant (PBRG)

Sub-project Completion Report

on

Adoption of Innovative Technology: Seed Production to Fattening of Mud Crab (*Scylla olivacea*) and Health Management in Bangladesh Condition

Implementing Organization

Fisheries Division
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

Backishwater Station
Bangladesh Fisheries Research Institute Paikgacha
Khulna-9280

Fisheries and Marine Resource Technology Discipline
Khulna University
Khulna-9208

Project Implementation Unit (PIU)

National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
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April 2022

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The execution of PBRG sub-project has successfully been completed by Bangladesh Fisheries Research Institute, Brackishwater Station, Paikgacha, Khulna and Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna using the research fund of WB, IFAD and GoB through Ministry of Agriculture. We would like to acknowledge the World Bank for arranging the research fund and supervising the PBRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NAPT-2 in respect of field implementation of the sub-project in multiple sites. Preparing the sub-project completion report, required assistance of a number of persons was required for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who have made it possible, deserve heartfelt appreciation. Our thanks are due to the Director PIU-BARC, NATP-2 and his team who have given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

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Abbreviation and acronyms

%	Percent	ICMSF	International Commission on Microbiological Specifications for Foods
@	At the Rate		
<	Less Than	IFAD	International Fund for Agricultural Development
ANOVA	Analysis of Variance	kg	Kilogram
AOAC	Association of Official Analytical Chemists	KU	Khulna University
APHA	American Public Health Association	l	Liter
BAM	Bacteriological Analytical Method	LSI	Larval Stage Index
BARC	Bangladesh Agricultural Research Council	m	Meter
BS	Brackishwater Station	mg	Milligram
BCR	Benefit Cost Ratio	MS	Micro-Soft
CFU	Colony Forming Unit	NATP-2	National Agricultural Technology Program Phase-2
CL	Carapace Length	^{O}C	Degree Celsius
cm	Centimeter	P	Probability
Co-PI	Co Principal Investigator	PBRG	Program Based Research Grant
CW	Carapace Weight	PCR	Polymerase Chain Reaction
DMRT	Duncan's Multiple Range Test	pH	Negative Log Value of Hydrogen Ion Conc.
DoF	Department of Fisheries	PI	Principal Investigator
EMB	Eosin Methylene Blue	PIU	Project Implementation Unit
FGD	Focused Group Discussion	Ppt	Parts Per Thousand
g	Gram	SD	Standard Deviation
GAP	Good Aquaculture Practice	SoE	Statement of Expenditure
GIFT	Genetically Improved Farmed Tilapia	SPSS	Statistical Package for Social Science
GoB	Government of Bangladesh	SWS	South-West Sunderbans
GSP	Glutamate Starch Phenol-red	TBC	Total Bacterial Count
ha	Hectare	TCBS	Thiosulphate Citrate Bite Salt-Sucrose
HACCP	Hazard Analysis Critical Control Point	WB	World Bank

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Executive Summary

The noteworthy contribution of mud crab (*Scylla olivacea*) in foreign exchange earnings which also provide livelihood opportunities for thousands of coastal dwellers made crab culture and its production as one of the priority fishery commodities in Bangladesh. Besides the hard-shell crabs, the recent interventions of soft shell crab shedding have opened a new arena in mud crab aquaculture in Bangladesh. A few numbers of commercial soft shell crab shedding farms are operated in south-east and south-west coastal region. Both hard shells grow out and fattening, as well as, soft shell shedding practices, as economic activities, are spreading exponentially. In spite of the potential role in the national economy and livelihood improvement, mud crab aquaculture is not yet well established in Bangladesh except limited fattening practice. In the present practice, almost cent percent of the cultured and exportable crabs of the country are being caught from natural sources thus caused intense pressure on the natural stock affecting aquatic biodiversity. However, beside aquaculture, brood stock development in captive condition and seed production in hatchery level and disease infection are also the major bottleneck in mud crab production in Bangladesh. Thus, considering the above stated situation, present collaborative research project was undertaken jointly by Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna; and Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University. BFRI covered research on brood stock development, seed production and various aspects of culture and fattening of mud crab. While the Khulna University component dealt with the research on identification and control of bacterial infection/diseases in mud crabs in natural and culture sources.

As per its set objectives, the Brackishwater Station of BFRI conducted the 1st experiment for brood stock development simultaneously in earthen ponds and cemented tanks. Brood stocks were collected from three different locations (Khulna, Satkhira and Bagerhat) and reared for berried brood production. The highest spawning success (61%) and production of viable larvae (2.2±0.2 million) was observed for brood stock collected from Khulna region, compared to other two locations. Findings of this trial suggested collecting the brood stock from nearby locations to reduce stresses. The 2nd bottleneck was improvement of larvae survival. Consecutive experiments were conducted to optimize the larvae feeding and water quality improvement through different water treatment plans. Feeding with live feed like, rotifer and liquid rotifer in early stages, and enriched *Artemia* nauplii and liquid artemia in the later stages improved the survival up to 1.5%. Further enhancement of crablet survival to 5% was achieved by treating the rearing water with both prebiotics and probiotics. Meanwhile, crablet survival triggered to 7% and metamorphosis into crablet within 25 days by treating the culture water with pre-biotic, probiotic and prophylaxis. Result of larvae rearing experiment suggested that appropriate feeds, feeding schedule and water treatment plan may reduce the disease incidence, enhancement of survival and shortening metamorphosis duration in mud crab larvae rearing.

In case of nursery experiments, it was found that lower stocking density 30/m^2 enhanced both survival (68%), weight gain (26.3 g) and intactness (85%) than higher stocking densities. On the other hand adequate shelter along with soil/sediment attachment resulted in higher survival (64%) and intactness (82%) than hapa net nursery of mud crab. In the case of grow out, significantly higher

($p<0.05$) body weight gain (176 ± 6.2g) and survival rate (64 ± 6%) was found in co-feeding of mud crab with natural feeds (trash fish + mud eel) than co-feeding with trash feed and commercial diet. Simultaneous fattening of mud crab in pond bottom and floating cages along with integration of GIFT enhanced the unit area production, total farm output and economic return. All three districts found suitable for crab fattening with applying this modern technology. In the case of soft shell shedding, prophylaxis treatment of premoulty and setting up of aeration exerted positive effect on both survival and shedding performance, whereas, rainy/wet season was been found as the best period for soft shell shedding in respect to survival and moulting.

To gather baseline information on crab disease and causative agents, the KU component collected primary data and information through field sample collection, survey and secondary sources. Crab samples from wild, farms and hatchery, and water, soil, feed samples from farms and the BFRI-BS hatchery were collected. To record bacterial disease incidence, the healthy and unhealthy (apparently) mud crabs were collected from the farms. This study detected *Vibrio, Aeromonas, Pseudomonas, Escherichia, Salmonella, Shigella, Enterococcus, Enterobacter and Klebsiella* species in the samples analyzed. In the mud crabs, *Vibrio* spp. were the most dominant bacteria followed by *Aeromonas*; the *Vibrio* prevalence was found >25%, >50%, and >80 in the wild, hatchery and farmed crabs, respectably. This study has reported that larval feed, particularly *Artemia*, was the foremost source of *Vibrio* contamination; axenic live food culture practice and probiotics use were suggested, and consequently no *Vibrio, Aeromonas, Pseudomonas* species were detected and higher larval survival was observed in the following batch of larval production in the BFRI-BS hatchery. The comparative microbiological analysis showed that chitinolytic bacteria *Vibrio, Aeromonas* and *Pseudomonas,* predominantly were responsible for shell diseases, counts were higher in the unhealthy mud crabs. More than 75% unhealthy samples were infected *with V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus,* whereas <40% healthy samples were infected with at least one of these pathogens. *Aeromonas* spp. and *Pseudomonas* spp. were detected in >65% and >40% unhealthy samples. The present research work demonstrates that *Vibrio* spp. were the most prevalent chitinolytic bacteria in mud crab population of Bangladesh. Accordingly, this study determined the *Vibrio* inoculum of $>10^6$ cfu/g through *in vivo* challenge test, which could cause acute symptoms of shell diseases and mortality in mud crabs. The FGD revealed that 2-7% mortality occurred in hard shell farms and 10-30% in soft-shell farms; 1-4% diseases incidence experienced by hard-shell crab farmers but no experience by soft-shell farms. The farmers found higher mortality from late spring till summer when weather becomes hotter (high temperature) as well as from late rainy season to early autumn when salinity falls. The farms located in Rampal upazilla of Bagerhat district experienced higher mortality of mud crabs, which was likely associated with low salinity in the farms. Salinity variation between the fattening farms and the waters from where mud crabs were caught has been found linked to the sudden mortality of mud crabs in the farms. This study has come across that salinity stress, hot weather, high stocking density and seasonal abundance of particular pathogenic bacteria are likely having compound effect on mud crab health; accordingly, disease and mortality occurrence in the farms are evident. This study addressed the importance of commercial mud crab hatchery establishment, cluster farming practice, GAP and HCCP application along the production channel for the sustainability of mud crab aquaculture in the south-west coastal region of Bangladesh.

Key words: Mud crab, Broodstock, Breeding, Seed production, Soft-shell, Disease incidence.

PBRG Sub-project Completion Report (PCR)

A. Sub-project Description

1. **Title of the PBRG sub-project:** Adoption of Innovative Technology: Seed Production to Fattening of Mud Crab (*Scylla olivacea*) and Health Management in Bangladesh Condition

2. **Implementing organization (s):**

   Bangladesh Fisheries Research Institute, Mymensingh-1602; Bangladesh Agricultural Research Council, Dhaka-1215; Brackishwater Station, Bangladesh Fisheries Research Institute, Paikgacha, Khulna- 9280; and Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208.

3. **Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator and PI/Co-PI (s):**

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4. Sub-project budget (TK.)

4.1 ***Total: 2,62,54,768.00*** (Two crore sixty two lac fifty four thousand seven hundred sixty eight).

Coordination component (BFRI)	: Tk. 1120000.00
BARC component	: Tk. 4855000.00
BFRI-BS component	: Tk. 12254768.00
KU component	: Tk. 8025000.00

4.2. Latest revised (if any):

Total 19256368.00 (One crore ninety two lac fifty six thousand three hundred and sixty eight**)**

Coordination component (BFRI)	: Tk. 1060,000.00
BARC component	: Tk. 960,000.00
BFRI-BS component	: Tk. 9211,368.00
KU component	: Tk. 8025,000.00

5. Duration of the Sub-project

Start date (based on LoA signed)	: 31 May 2018
End date	: 28 April 2022

6. Background of the Sub-project

Mud crab (*Scylla olivacea)*, a non-conventional export oriented aquaculture commodity is being exploited commercially in Bangladesh since early 1980's around the coastal belt (Hasanuzzaman et al., 2014). It is traditionally exploited by fishermen and provides a basic source of income for a number of coastal fishing communities. Mud crab from Bangladesh is being exported mostly in live forms and the soft shell crabs in frozen forms. In 2013-14, Bangladesh earned $22.91 million by exporting 8,520 tons of live crabs. The demand and price of mud crab in the international market are increasing tremendously (Shelley and shelly, 2013). Its noteworthy contribution to raising the foreign exchange earnings and providing

livelihood opportunities made the sector as an industry. The importance of live mud crab as an export commodity has opened up great opportunities for diversification of mud crab aquaculture to enhancement the production and improvement of livelihoods to climatically stressed fishing communities (Huq et al., 2015). Besides the hard shell crabs, the recent interventions of soft shell crab shedding has opened a new arena in global mud crab aquaculture (Quinitio and Lowin, 2009) as well as in Bangladesh. A few number of commercial soft shell crab shedding farms are operated in South-east and South-west coastal Bangladesh. But the soft shell shedding farms are reportedly facing problems with scarcity of shedders, high mortality rate and low shedding performance (*Pers. Communication with soft shell farmers of Satkhira and Cox's Bazar*).

Despite the potential role in the national economy and livelihood improvement (Kamal, 2002; Zafar, 2004; Zafar and Hossain, 2008), mud crab aquaculture is not well established in Bangladesh except fattening (Begum et al., 2010). About cent percent of the exported crabs are being caught from natural source, subsequently fattened the gonadless crabs and exported in live forms (Shelly, 2008; Shelly and Lovatelli, 2011), causing intense pressure on the natural stock. Seed production in hatchery condition and development of nursery management might be the bottleneck for sustainable aquaculture of the species. Brackishwater Station of Bangladesh Fisheries Research Institute has successfully produced the mud crab seed in hatchery condition during 2015-2017, but the survival was low. This demand continuous research and development efforts for increasing the survival of mud crab seed in hatchery condition.

Besides fattening, some potential crab farming practices developed in other countries could be adopted in Bangladesh environment. Continuous research and development of innovative crab fattening, soft shell shedding, integrated seaweed-crab culture, integrated mangrove-crab culture, crab seed production and nursery operations are the key options for increasing mud crab production in a sustainable manner. Besides the enhancement of culture practices like other aquaculture ventures, mud crab farming is at risk from microbial contamination and diseases. There have been increasing reports that infection and/or disease incidence in mud crabs is a global concern (Lavilla-Pitogo et al. 2001; Poornima et al. 2008; Jithendran et al. 2010). Some fragmented reports on disease out breaks from crab fatteners in Bangladesh have also been noticed (*pers. communication with farmers*). In this prospective adoption of new mud crab aquaculture technologies, considering the disease outbreaks and its causative agents and necessary remedies might be considered as research priority. Wide spreading of innovative mud crab aquaculture practices can create alternate livelihoods for the poor coastal communities' dependent on mangrove forest for sustainable management of Sundarbans biodiversity.

Thus to address the situation through establishing strong research support and linkage, as NATP-II thoughts, all research and extension institutes need to make strong footing with team building holistic research culture to achieve desired output. With this consideration, as an effective approach, the program based research grant of NATP-II is particularly aimed to support coordinated research program amongst NARI to jointly combating national agricultural problems and strengthening the research and research management capacities of the institutes. Therefore, under the principal objective of NATP-II, the fisheries division component shall have to play the role to ensure smooth and efficient implementation of sub-project activities to achieve the desired project output through coordination of activities and strong and effective monitoring of research progress under an additional increased research support against each institute.

7. Sub-project general objective (s)

Development and establish a framework of mud crab aquaculture for sustainable production through conducting research on each critical stages of life cycle in accounting the pathogenic (microbial) threats on respective stages.

8. Sub-project specific objectives (component wise)

A. Coordination component (Component 1)

Under the principal objective of the NATP, the coordination component (BFRI) ensured smooth and efficient implementation of the sub-project components activities to achieve desired project outputs within the stipulated timeframe under strengthened capable research management system.

B. Component 2 (BARC)

i) To coordinate project implementation efforts and integration of activities to generate desired information /technology as per methodology of the sub-projects;
ii) Identify operational deviations and addressing constraints/problems (if any) under a process of strong and regular monitoring of the project activities;
iii) To upgrading the level of output of the sub-project through reviewing of yearly technical progress;
iv) Collect and collate project data, finding and observation and production of compiled Project Completion (PCR) ;
v) Finally, to ensure increased safe fish food production technologies under an environment friendly atmosphere with concomitant increase in rural employment, earning and involvement of rural women through effective coordination among the sub projects.

C. Component 3 (BFRI-BS)

i) To domesticate bloodstock and development of breeding, larvae rearing and nursery management protocol for sustainable seed production of mud crab, *S. olivacea*.
ii) To investigate the effect of different rations of natural and commercial diets on grow out and fattening of mud crab, *S. olivacea*.
iii) To adopt and demonstrate innovative mud crab (*S. olivacea*) fattening techniques in different aqua-eco regions of south-west coast.
iv) To find out the regulatory factors on soft shell shedding of mud crab (*S. olivacea*) for sustainable production.

D. Component 4 (KU)

i) To enumerate bacterial load and type/infection level in mud crab hatchery, farms and in wild mud crab, *S. olivacea*.
ii) To record disease incidence in mud crab (*S. olivacea*) population in the south-west coastal region of Bangladesh
iii) To determine the association of other driving factors (i.e. soil and water quality) with infection/diseases occurrence in mud crab, *S. olivacea*.

9. Implementing location (s)

The sub-project was simultaneously implemented in the laboratory, hatchery and in pond complex of Brackishwater Station at Paikgacha of BFRI. Field trials were conducted at Satkhira, Khulna and Bagerhat districts. Samples for bacterial load study were collected from each trial conducted by BFRI and from wild stocks of Khulna, Satkhira and Bagerhat districts. Analysis of bacterial samples was performed in the FMRT discipline laboratory of Khulna University (KU), Khulna.

10. Methodology in brief (*with appropriate pictures*)

10.1. Coordination component (Component 1, BFRI)

With the target to achieve the sub-project goal at its highest through establish strong and efficient coordination within the component activities of the sub project, the coordination component thus arranged several numbers of meetings at different stages of sub-project implementation with the component leaders (PI) and supporting project scientists**.** Recommendations of the various coordination meetings are shown in *Annexure **1*** of the report. In addition, the program coordinator also performed several visits to the research fields and lab/hatcheries for monitoring the sub-project progress of activities physically which has been mentioned in the respective section **(***Section:* I. *Information regarding desk and field monitoring*) of this report**.**

10.2. Component 2 (BARC)

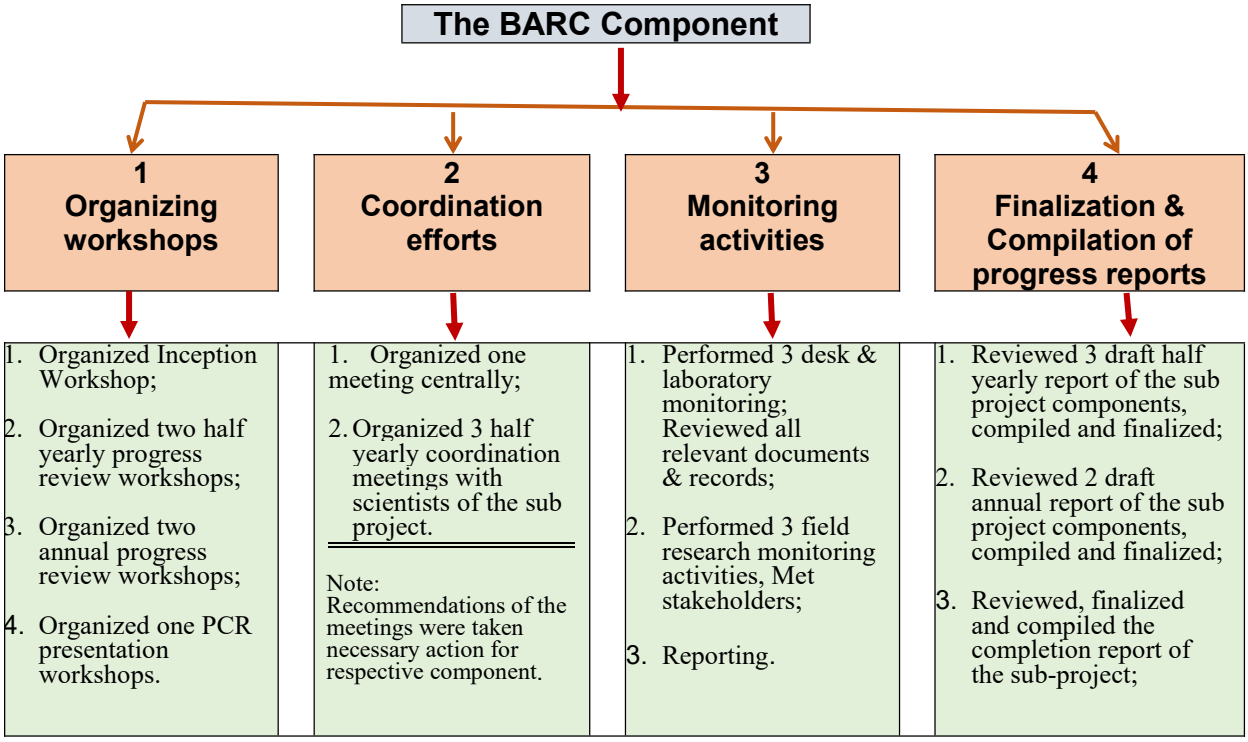
10.2.1. Activity implementation approach of BARC component

The BARC component was responsible to initiate all required efforts in the process of implementation of each component under the sub project so that the general objectives and goal of the sub-project are achieved through smooth and successful completion of each of the specific objectives as per activity time plan of the sub- project. To ensure that, the BARC component, taken into consideration its own activity and objectives and duration of the sub-project, thus accordingly designed its own plan of activity (approach) for the proposed period.

Following are the major activities those were carried out by the BARC component under the plan:

a. Organizing seminars/workshops.
b. Monitoring the sub-project activities (specifically financial and research activities);
c. Coordination activities within the component sub-projects.
d. Review and compilation of half yearly and annual research progress reports;

The implementation approach and activities, under the BARC component of the sub-project, are shown in the following diagram:



Recommendations of the inception workshop, half yearly and annual research progress review workshops and different other meetings are presented in Annexure -2(BARC: A-C).

Following Table presenting the summary statement of achievements performed by the BARC component of the sub project

Summary statement of achievements		
Name of activities	**Performance against each activity**	**Remark**
Inception workshop	Organized centrally at BARC in November' 2018	Attended all PI, Co-PI & expert members.
Revision of PP	Done as per recommendations of Inception Workshop	-
Half yearly progress review workshop	Organized centrally at BARC in March' 2019 and January'2020.	Attended all PI, Co-PI & expert members
Annual progress review workshop	Organized centrally at BARC in July' 2019 & in September' 2020	Attended all PI, Co-PI & expert members.
Coordination meeting (No)	03 *07.02.19, 19.10.19 & 25.06.20*	One Coordination meeting was held centrally.
Monitoring of field and Lab activities	04 *(BFRI, BARC & KU)*	Covered all components under sub-project.
Financial achievement	100% of total released money and 99.78% of total approved budget.	-
Reporting performance	Prepared sub-project inception report, SoE, Half yearly and Annual compiled progress reports of all sub project components as per planned time frame.	Major reports are: • Inception report (1 no) • Compiled half yearly progress report (2 no) • Compiled annual progress report (2 no). • Monitoring reports (3 no).

Plates 1-6: Pictorial views of different workshops, meetings and field monitoring activities

10.3. Component 3 (BFRI-BS)

10.3.1. Baseline information collection

Baseline information collection on status of mud crab resources was done to find out the research gaps for furnishing the proposal as well as augmentation and documentation of information. However, according to literatures and acquired knowledge, crab farming districts were selected as sites. Meanwhile, crab collectors, farmers, *faria's, depots* owners and local transporters were selected as respondents. A questionnaire was initially developed for data collection and it was pre-tested and modified accordingly before final data collection.

i. Purpose: To understand the present status of mud crab resource in Bangladesh, accumulation and documentation of base line information on mud crab.

ii. Location/area covered: Khulna, Bagerhat, Satkhira, Patuakhali, Barguna and Cox's Bazar.
iii. Data were collected through survey and literature review.

10.3.2. Preparation of ponds and tanks for maturation and brood development

For gravid brood stock production, earthen broodstock pond was prepared through drying, liming and fetching with nylon net. Meanwhile, berried brood production was a routine work for continuous operation of mud crab hatchery. Three cemented brood stock tanks were prepared through sand bed, aeration and covering with black nets for production of berried broods in communal basis. Whereas a set of 18 half drums was installed with recirculation water system for individual maturation of berried broods. Incubation tank for berried brood was also installed in the hatchery. Hatchery, larvae rearing tanks and necessary utensils were cleaned and prepared with washing and disinfection. Brine/seawater for brood stock rearing, live feed culture and larvae rearing was collected from brine field and nearby hatchery and stored in storage tanks.

Plate 7: View of Hatchery complex

Plate 8: Brood stock maturation house

Plate 9: Brine/seawater storage tank

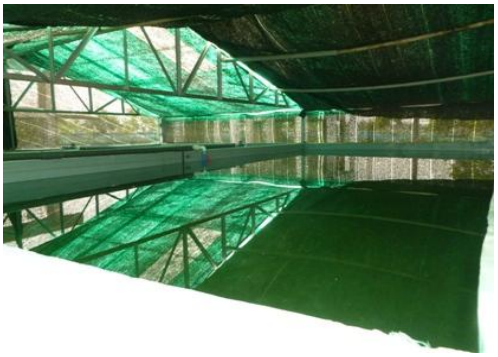


Plate 10: Stored brine/seawater

Plate 11: Biofilter for broodstock maturation

Plate 12: Larvae rearing cemented tanks

Plate 13: View of nursery complex

Plate 14: Newly excavated nursery ponds

Plate 15: Nursery of crablet in hapa net

Plate 16: Grow out and fattening pond

Plate 17: Broodstock maturation pond

Plate 18: Grow out and fattening pond

Plate 19: Hanging substrate/shelter for megalopa and crablet

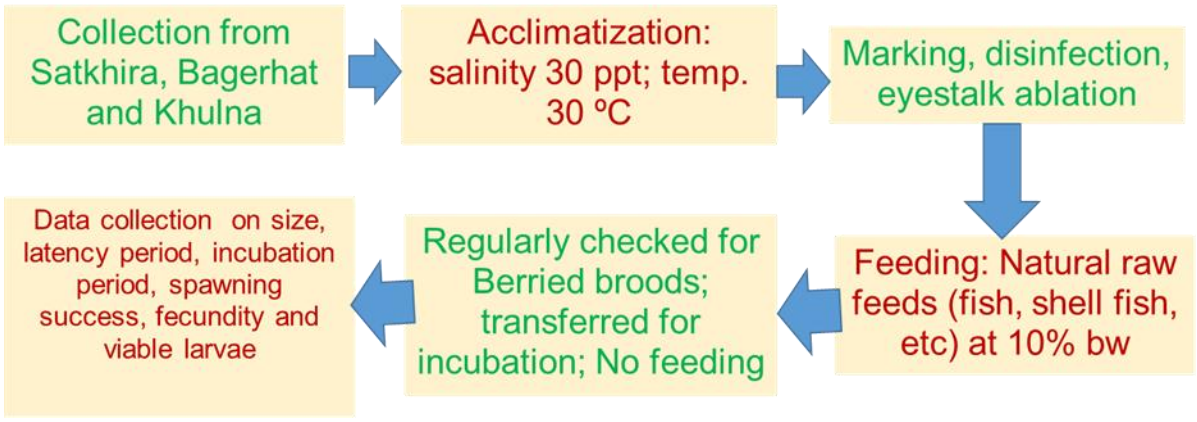
Plate 20: Crablets within hanging substrate/shelter

Plate 21: Individual brood maturation tank

Plate 22: Communal brood maturation tank

10.3.2.1. *Experiment 1:* Brood stock management and production of berried broods

For gravid brood production, semi gravid broods were collected from different locations (Khulna, Satkhira, Bagerhat) and stocked in the earthen ponds. A total of 80 semi gravid broods were collected and stocked in the earthen ponds. The brood stock was fed with chopped trash fish, mussel meat and chopped sea fish @10% of body weight twice in a day. The broods were checked after 15 days of interval for gonad maturation (gravid). Gravid broods were collected; marked properly; eyestalk was ablated unilaterally and stocked into the pre-installed cisterns/drums prepared with sand bed and auto-filter aeration system. A total of 36 gravid broods were collected and stocked into the cemented cisterns as communal basis and 15 broods were individually reared in drums with recirculation water system. Broods were fed with chopped trash fish, mussel meat and chopped sea fish up to satiation level. As the brood became berried (spawned/extruded), they were transferred into the hatching tank with moderate aeration. The berried brood remained unfed until hatching to larvae (zoea-1). After hatching, the viable larvae were collected through scooping by beaker and sub-sample was counted to determine the number of viable larvae produced. Working steps of brood stock management and berried brood production system are shown in the flow diagram below :-



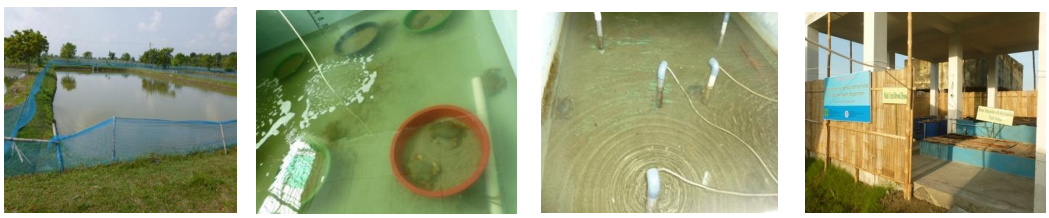


Plate 25: Brood stock pond to produce gravid broods.

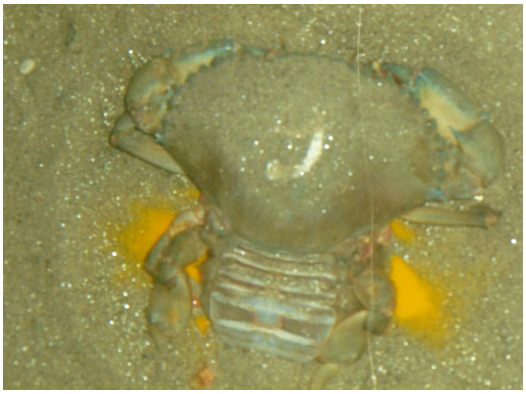


Plate 26: Brood during extrusion of eggs on sand bed.

Plate 27: Berried brood with yellowish egg mass.

Plate 28: Berried brood with yellowish egg mass.

Plate 29: Blackish egg mass ready to hatch the zoea.

10.3.3. Culture of live feed and up scaling

Live feed (green algae and rotifer) production is a simultaneous and routine scheduled activity for maintenance and scaling up of live feed species year round. Live feed laboratory was prepared by cleaning, setting up of aeration line and disinfection of utensils to be used for live feed culture and further scaling up. Live feed (microalgae) were cultured in 2 L glass beakers and up-scaled in 20 L plastic jars under laboratory condition. Further mass scaling was done in 500 L fiber glass tanks. The larval initial feed (rotifer) culture was also done in laboratory condition and up-scaled in 500 L, 1 ton and 2 tons fiber glass tanks under outdoor conditions.

Plate-23. Live feed, microalgae production in laboratory and out door

Plate-24. Live feed; rotifer culture in outdoor

10.3.3.1. *Experiment 2:* Efficacy of different live feeds for the larval rearing of mud crab, *Scylla olivacea.*

To evaluate the efficacy of different live feeds on mud crab larvae rearing, an experiment was conducted with three different treatments (T) and three replications of each. The experiment was conducted in the newly established hatchery complex of Brackishwater Station of BFRI. Detailed experimental design is presented in Table 1.

Table 1. Experimental design for evaluation of efficiency of live feed in mud crab larvae rearing

Treatments	Details	Replications	Feeding schemes	
			Z1-Z2	Z3-M
T1	Live feed (Rotifer + Artemia)	3	Rotifer	Artemia
T2	[1]Liquid diet	3	Liquid rotifer	Liquid Artemia
T3	Live feed + Liquid diet	3	Rotifer + Liquid rotifer	Artemia + Liquid Artemia

[1]Liquid diet, liquid diet is rotifer and *Artemia* based commercial diet developed for crustacean larvae rearing; Liquid rotifer= crude protein- 36%, crude fat- 20%, crude fiber- 3.3%, moisture- 0.0% and phosphorus- 0.7%; [Liquid Artemia= crude protein- 52%, crude fat- 17%, crude fiber- 3.7%, moisture- 0.0% and phosphorus- 0.6%, labeled as dry weight basis].

The larvae rearing trial was done in fiber glass tank (Capacity, 300 L) with 30 ppt saline water and the stocking density was 50 zoea/l. Newly hatched zoea (Z1) of *S. olivacea* was collected within an hour of hatching to prevent microbial attack and stocked to the larvae rearing tank as per design. Feed was supplied four times daily following the design of the experiment. Before each feeding, dead larvae and uneaten feed were siphoned out from the tank. About 50-70% of water was exchanged in alternate days to maintain congenial water quality. Water quality variables, like, temperature, salinity, pH, dissolved oxygen and ammonia was recorded daily following standard method. Daily larvae sampling was done to assess the survival, larval development and any fouling on body of larvae up to metamorphosis into megalopa. Larvae rearing experiment was initiated according to the proposed design of the sub-project following standard larvae rearing protocol.

10.3.4. *Experiment* 3: Impact of different water treatment plan for improvement of water quality and reduce incidence of disease during larval rearing of mud crab, *Scylla olivacea.*

To evaluate the impact of different water treatment protocol for water quality improvement and to reduce disease incidence in mud crab larvae rearing, an experiment was conducted in the hatchery complex of Brackishwater Station of BFRI. The experiment was conducted according to the proposed design of the sub-project component following standard larvae rearing protocol which has been presented in the Table 2.

Table 2. Detailed design of experiment to observe the impact of water treatment in mud crab larvae rearing

Treatments	Details	Replications	Feeding schemes	
			Z1-Z2	Z3-M
T1	Water treated with pre-biotics	3	The best feed in expt.1 was used	The best feed in expt.1 was used
T2	Water treated with pro-biotics	3		
T3	Water treated with pre and probiotics	3		

* Prebiotic= Super TCT Biotic, a *Bacillus subtilis* based prebiotic; Probiotic= Fish Probiotic, a *Bacillus* and *Nitrobacillus* based probiotic.

The larvae rearing cemented tanks of 1 ton capacity were used for this experiment. Tanks were prepared accordingly. Water of T1 was treated with prebiotic @ 10g/ton of water before two days of stocking. For T2, water was treated with fish probiotic @ 1g/ton. For T3 water was treated with both prebiotic (5g/ton) and probiotics (0.5g/ton) of culture water. Stocking density of larvae was 50 zoea/l. Feeding was done with Rotifer + Liquid rotifer for Z1 to Z2 stage followed by Artemia + Liquid Artemia for Z3-megalopa stage (best feed obtained from the 1st experiment). Intermediate application of prebiotic and probiotic was done in each 5 days interval with same doses. About 50-70% of water was exchanged after 5 days interval prior to application of pre and probiotic. Water quality variables, like, temperature, salinity, pH, dissolved oxygen and ammonia was recorded daily following standard methods. Daily larvae sampling was done to assess the survival, larval development and any fouling on body of larvae up to metamorphosis into megalopa. Larvae rearing experiment was initiated according to the proposed design of the sub-project component following standard larvae rearing protocol.

10.3.5. *Experiment* 4: Impact of different water treatment plan for improvement of water quality and reduce incidence of disease during larval rearing of mud crab, *Scylla olivacea* (Repetition and fine tuning of previous experiment with modification)

To evaluate the impact of different water treatment protocol for water quality improvement and to reduce disease incidence in mud crab larvae rearing an experiment was conducted in the hatchery complex of Brackishwater Station of BFRI. The larvae rearing cemented tanks of 1 ton capacity were used for this experiment. Tanks were prepared accordingly. Water of T1 was treated with both Prebiotic (5g/ton) and probiotics (0.5g/ton) of culture water. For T2, water was treated with prophylaxis @0.3 ppm. For T3 water was treated with both prebiotic (5g/ton) and probiotics (0.5g/ton) up to 10 days and with prophylaxis @0.3 ppm from day 14 onwards. Stocking density of larvae was 50 zoea/l. Feeding was done with Rotifer + Liquid rotifer for Z1 to Z2 stage followed by enriched Artemia + Liquid Artemia for Z3-megalopa stage (best feed obtained from 1st year experiment). Intermediate application of prebiotic, probiotic and prophylaxis was done in each 3-4 days interval with same doses. About 50-70% of water was exchanged before application of prebiotic, probiotic and prophylaxis. Water quality variables, like, Temperature, salinity, pH, dissolved oxygen and ammonia was recorded daily following standard methods. Daily larvae sampling was done to assess the survival, larval development and any fouling on body of larvae up to metamorphosis into megalopa. The experiment was conducted according to the proposed design of the sub-project with some modification for fine tuning of 1st year experiment. The experiment was continued following standard larvae rearing protocol and has been presented in the Table 3.

Table 3. Detailed design of experiment to observe the impact of water treatment in mud crab larvae rearing

Treatments	Details	Replications	Feeding schemes	
			Z1-Z2	Z3-M
T1	Water treated with pre and probiotics	3	*Rotifer + Liquid rotifer*	*Enriched Artemia + Liquid Artemia*
T2	Water treated with Prophylaxis	3		
T3	Water treated with both prebiotic, probiotics and prophylaxis	3		

* Prebiotic= Super TCT Biotic, a *Bacillus subtilis* based prebiotic; Probiotic= Fish Probiotic, a *Bacillus* and *Nitrobacillus* based probiotic; Prophylaxis= mixure of 0.25 ppm Treflan and 0.3 ppm Furanidazol.

10.3.6. *Experiment* 5: Evaluation of different habitats for the nursery management of mud crab, *Scylla olivacea*

To evaluate different habitats for nursery of mud crab juveniles, an experiment was conducted for a period of 30 days. The experiment was designed with three different treatments depending on the habitats, *viz.,* T1= nursery in pond bottom without shelter; T2= nursery in hapa net; and T3= nursery in pond bottom with shelters and each of the treatment had three replications. Stocking density of crablet was 50/ m^2 and feeding was done daily with chopped trash fish like small tilapia @10% body weight. Design of the experiment is shown in Table 4.

Table 4. Detailed design of experiment to observe the impact of different habitats on nursery of mud crab

Treatments	Details	Replications	Feeding
T1	Nursery in earthen pond (without shelter) (density 50/m^2)	3	Chopped trash fish
T2	Nursery in hapa net (density 50/m^2)	3	
T3	In pond bottom with different shelter (density 50/m^2)	3	

10.3.7. *Experiment* 6: Optimization of stocking density in nursery of mud crab *S. olivacea*

To observe suitable stocking density of mud crab for nursery, an experiment was conducted for a period of 30 days. The experiment was designed with three different treatments depending on densities, *viz.,* T1= 30 crablet/m^2; T2= 50 crablet/m^2; and T3= 70 crablet/m^2 and three replications were assigned for each treatment. All the treatment had different shelters like, hanging nets, shrinking nets and pieces of plastic pipes. Feeding was done twice daily with chopped trash fish like small tilapia @10% of biomass. Design of the experiment is shown in Table 5.

Table 5. Experimental design on stocking density evaluation of mud crab in nursery phase

Treatments	Stocking density	Replications	Shelters	Feeding
T1	30 crablet/m^2	3	Hanging nets, shrinking nets and pieces of plastic pipes	Chopped trash fish
T2	50 crablet/m^2	3		
T3	70 crablet/m^2	3		

10.3.8. *Experiment* 7: Evaluation of different prepared feeds for the culture of mud crab, *Scylla olivacea* in grow out phase

During the reporting period, two consecutive trials was conducted. The first experiment were conducted to evaluate the effect of different feeds on growth, survival and intactness of mud crab in grow out phase in the earthen ponds of brackishwater station, BFRI. The experiment had three treatments depending on feeding variations (Table 6).

Table 6. Experimental design for testing of different feeds on grow out of mud crab

Treatments	Details	Replications
T1	Chopped trash fish	3
T2	Chopped mud eel	3
T3	Chopped trash fish + chopped mud eel	3

10.3.8.1. *Pond preparation*: For the experiment, the ponds were drained out and sun dried the bottom. Bottom sludge was re-excavated and dykes of ponds were be repaired as to hold up to 1 m water depth. Ponds were encircled with nylon net and bamboo (*Bana*) fencing to check escaping of crabs. Pond soil was treated with lime (agricultural lime: dolomite: 1:1) @250 kg/ha. Ponds were filled with brackishwater from adjacent canal/river system. Water of the ponds was treated with 60 ppm bleaching for disinfection. After 5-7 days, pond water was fertilized with urea and TSP @ 2.5 ppm and 3.0 ppm, respectively. The ponds were left for 7 days for development of primary producers.

10.3.8.2. *Stocking of crabs and management*: Stocking was done with 48.50±3.6 g of juvenile crabs. Stocking density was 4crab/m^2. Feeding ration was maintained at 3-5% of the body weight in daily basis according to the body weight. About 30-50% of pond water was exchanged after 15 days interval.

10.3.8.3. *Water quality and health monitoring*: Water quality variables *viz.,* temperature (°C), salinity (ppt), pH, dissolved oxygen, alkalinity (ppm) and ammonia (ppm) was monitored weekly basis or depending on the needs. Water quality variables were analyzed following standard methods.

10.3.8.4. *Harvest*: The experiment was carried out for a period of 60 days. Then harvesting was accomplished through pond drying and data were computed.

10.3.9. *Experiment* 8: Evaluation of different prepared feeds for the culture of mud crab, *Scylla olivacea* in grow out phase (Repetition of previous experiment with modification of feeding regimen)

In the second trial, the first experiment was repeated with some modification employing five treatments *viz*, T1= Chopped trash fish; T2= Chopped mud eel; T3= Chopped trash fish + chopped mud eel (1:1); T4= Chopped trash fish+ commercial diet (1:1); and T5= 100% commercial diet. Each of the treatment had 3 replications. The experimental design is shown in Table 7.

Table 7. Experimental design for testing of different modified feeds on grow out of mud crab **(**Repetition with different feeding regimen)

Treatments	Details	Replications
T1	100% Chopped trash fish	3
T2	100% Chopped mud eel	3
T3	Chopped trash fish+ chopped mud eel (1:1)	3
T4	Chopped trash fish+ commercial diet (1:1)	3
T5	100% commercial diet	3

Ponds were prepared following the methods as applied in the 1st experiment. Stocking was done with 43.40 ±3.4g of juvenile crabs following the stocking density of 4 crab/m^2. Feeding was done as per experimental design at the rate of 5-7% of body weight. The experiment was continued for a period of 105days, harvesting was done and data were computed.

10.3.10. *Experiment* 9: Impact of fattening of mud crab *S. olivacea* in floating cages and stocking fish along with fattening of mud crab in pond bottom on the productivity of the pond.

The experiment was conducted at field level condition at different selected sites of Khulna, Satkhira and Bagerhat region and pond complex of Brackishwater Station, Paikgacha. The design of the experiment is given in Table 8.

Table 8. Design for fattening of mud crab at different aqua-ecological region

Treatment	Treatment description	Replications	Pond size	Crab density		GIFT density
				Pond	Cages	
T1	Crab fattening in pond bottom	4	300-500 m^2	2/m^2	-	-
T2	Crab fattening in pond bottom + cages	4	300-500 m^2	2/m^2	1/cage	-
T3	Crab fattening in pond bottom + cages + GIFT	4	300-500 m^2	2/m^2	1/cage	2-4/m^2

10.3.10.1. *Pond preparation*: For the experiment, the ponds were sun dried. Bottom soil was re-excavated and dikes of ponds were repaired so that the ponds can hold water up to 1 m. Ponds were encircled with nylon net and bamboo (*Bana*) fencing to check escaping of crabs. Pond soil was treated with lime (agricultural lime: dolomite:: 1:1) @250 kg/ha. Ponds were filled with brackishwater from adjacent canal/river system. Water of the ponds was treated with 60 ppm bleaching for disinfection. After 5-7 days, water was fertilized with urea and TSP @ 2.5 ppm and 3.0 ppm, respectively. The ponds were left for 7 days for development of

primary producers/bio-flocks. Plastic boxes were set on floating frame made of plastic pipes in the ponds following the design.

10.3.10.2. *Stocking of crabs and fishes*: Required number of immature crabs (120-180 g) and fish fingerlings (3-4 g) were stocked in the ponds and cages following the design of the experiment.

10.3.10.3. *Feeding*: Feeding of crabs was done with low cost small fishes (tilapia, silver carps, etc) @ 3-5% body weight and fishes were fed with commercial floating feeds @3-5% body weight daily.

10.3.10.4. *Water quality, soil profile and health monitoring*: Water quality parameters *viz.,* temperature (°C), salinity (ppt), pH, dissolved oxygen, alkalinity (ppm) and ammonia (ppm) was monitored biweekly or depending on the needs following standard methods.

10.3.10.5. *Checking of crabs and fishes*: The stocked crabs in the pond and cages were observed daily for checking the progress of gonad maturation. Growth performances of fishes were monitored in 15 days intervals and feed will be adjusted.

10.3.10.6. *Harvesting*: After 105 days of rearing, all crabs and fishes were harvested and production of fishes and % of gonad maturation in ponds and cages were recorded, and cost benefit analysis was calculated.

10.3.11. *Experiment*-10: Regulatory factors on soft shell shedding of mud crab (*S. olivacea*) for sustainable production

To find out the regulatory factors influencing the overall soft shell crab production, three consecutive trials were conducted in the cemented cisterns (7 m^3 each) in the hatchery complex of Brackishwater Station, BFRI.

10.3.11.1. Effect of prophylaxis treatment on soft shell shedding

The experiment was set with two treatments, T_1: pre-moulty treated with prophylaxis, and T_2: pre-moulty directly stocked without disinfection. Each of the treatment had 3 set of replication with 20 crabs in each replica. Each crab was stocked in plastic boxes floated on a plastic pipe frame. Stocking size of crab was 30-45 g. Collected crabs were washed with clean brackishwater. For treatment-1, collected crabs (pre-moulty) were treated with 0.3-0.5 ppm prophylaxis for 30 minutes, meanwhile crabs were directly stocked in boxes for T_2. Water salinity was maintained between 12-15 ppt. Crabs were fed with chopped tilapia fish in alternate days. About 30% of tank water was exchanged after 5 days interval and the experimental duration was 45 days.

10.3.11.2. Effect of aeration on soft shell shedding

The experiment was conducted with two treatments, T_1: shedding tank aerated with bottom line aeration, and T_2: shedding tank with no aeration. As like as experiment 10.3.2.10.1**, e**ach of the treatments had 3 sets of replication with 20 crabs in each replica. Each crab was stocked in separate plastic boxes floated on a plastic pipe made frame. For treatment-1, aeration was set at the middle line of tank bottom with plastic pipe bearing several holes for air passing and one edge of the pipe was connected to an air compressor whereas the other edge was blocked by an end cap. Stocking size of crab was 30-45 g. Collected crabs were

washed with clean brackishwater and stocked according to the treatments. Water salinity of this experiment was also maintained between 12-15 ppt. Crabs were fed with chopped tilapia fish in alternate days. About 30% of tank water was exchanged after 5 days interval and the experimental duration was 45 days.

10.3.11.3. Seasonal variations on soft shell shedding of mud crab

The experiment was conducted in three seasons viz, S_1: winter, S2: summer and in S3: rainy season. Each of the seasons had 3 replicated set of boxes with 60 crabs in each set. Each crab was stocked in separate plastic boxes floated on a plastic pipe made frame. Stocking size of crab was 30-45 g. Collected crabs were washed with clean brackishwater stocked according to the treatments. Water salinity of this experiment was also maintained between 12-15 ppt. Crabs were fed with chopped tilapia fish in alternate days. About 30% of tank water was exchanged after 5 days interval and the experimental duration was 45 days.

10.3.12. Data analysis and reporting

Collected data in all the experiments were computed and analyzed with the help of MS excels and SPSS (version 20-22). ANOVA was done to compare the biometric parameters among the treatments. DMRT was done for ranking the results among the treatments.

10.4. Component 4 (KU)

10.4.1. Baseline information collection

Relevant information from secondary materials such as books, journal, reports etc. available at library, other organizations (such as DoF, BFRI), and internet were collected. The information on pond/pen preparation, lean crab source, stocking density, feeding regime, water quality management, marketing and economics were collected from mud crab farms through Focus Group Discussion (FGD).


Plate -30: Focus group discussion with mud crab collectors and farmers.

10.4.2. Sample collection and preparation

Samples of crabs, feed, water and soil were collected from mud crab hatchery of BFRI which was selected as per the project proposal, and all the trials were conducted by BFRI-BS. Samples were also collected from the mud crab farms. Mud crab farms were selected taking into account the occurrence of mortality level as well as farming intensity in Satkhira, Khulna and Bagerhat districts, but it was no possible to collect samples as per the plan of the activity

chart due to COVID-19 pandemic situation. Mud crabs were also collected from the waters in and around the South-west Sundarbans (SWS) areas directly from the boats of the fishermen/collectors. Samples were preserved following standard approaches (APHA, 1992; BAM, 2004; Lavilla-Pitogo et al. 2004). Specifically samples were kept on ice immediately after collection in the insulated box during transportation. Sometimes the mud crab samples were kept at -20 °C as well as in water, feed and soil samples were kept in the refrigerator. Water parameters such as temperature, dissolved oxygen, pH, salinity and soil parameters (pH, organic carbon, total nitrogen) were checked using measuring instruments, kit (Hanna), and standard methods (APHA,1992; AOAC, 1997). The samples were prepared and analyzed in the Fish and Shellfish Quality Control and Pathology lab, KU. For microbial analysis, the shell, gill, abdominal muscle, hepatopancrease and gut were dissected from the mud crabs, using sterile dissecting instruments, and thereafter pool samples were prepared by mixing these dissected organs from each crab using tissue homogenizer. Twenty five gram of the pooled sample was homogenized with 225 ml alkaline peptone water (pH 8.6) and the supernatant portion was collected with micropipette and taken into eppendorf tube containing phosphate buffered saline for further analysis, considering as stock. For *Vibrio* count, the samples were further enriched by incubation at 37 ° C for 6-8 hrs.

Plate-31: Healthy mud crabs collected from wild source

Plate-32: Apparently unhealthy mud crabs collected from wild

Plate-33: ventral view of healthy mud crabs Collected from culture farms

Plate-34: Ventral view of unhealthy mud crabs collected from local farms


Plate-35: Selected mud crab farm for samples collection


Plate-36: Soft and hard shell mud crab farms selected for unhealthy samples collection.

Plate- 37: Unhealthy crabs from *hard shell* farms

Plate-38: Unhealthy crabs from *soft-shell* farms

10.4.3. Bacterial count and isolation

Total bacterial count (TBC) was estimated, and several specific bacteria including *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., were enumerated and isolated. In addition, *Escherichia coli*, *Enterobacter* and *Klebsiella*, *Enterococcus*, *Salmonella, Shigella* were also counted. The TBC (cfu/g) was determined using nutrient agar by standard plate count method. For estimation and isolation of specific bacteria, Thiosulfate Citrate Bile Salts Sucrose (TCBS), glutamate starch phenol-red (GSP) agar, *Aeromonas* selective Agar, *Pseudomonas* selective agar, Eosin Methylene Blue (EMB) and MacConkey agar plates were used. All steps for isolation and enumeration have been being performed according to the standard guidelines and the instructions of media manufacturers (Himedia, India; Merck, Germany; Oxoid, Uk). Briefly, the serial dilution was prepared from the stock solution; 0.1ml of stock solution was taken by micropipette and was mixed with 0.9 ml of sterile phosphate buffered saline which

gave 10^{-1} dilution. From 10^{-1} dilution 0.1ml of solution was taken in another test tube containing 0.9ml phosphate buffered saline which gave 10^{-2} dilution. In this way, 10 fold dilutions of the samples were made up to 10^{-5}. Then, 0.1ml suitable dilution of each culture was inoculated in culture plates, and culture plates were incubated.

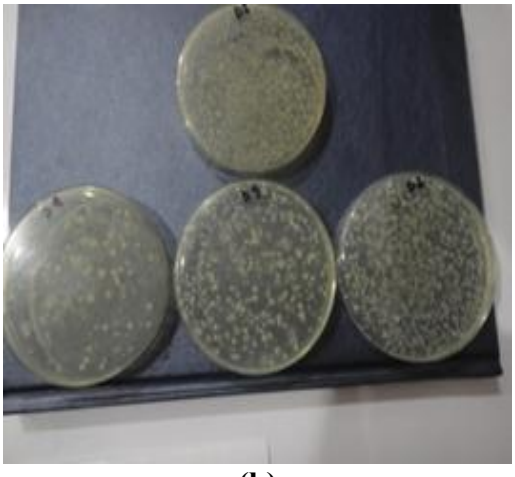
10.4.4. Diagnosis of disease

Due to COVID-19 pandemic situation, most of the farms were no longer in operation, thus, a few samples have recently been collected, and gross observations (i.e. external signs, discolored patches, soft and black exoskeleton, orange or brown spot on shell) were performed for preliminary diagnosis of diseased crabs. For detail histopathology, tissue samples from the gill, exoskeleton and skeletal muscle of healthy and infected crabs were collected and preserved in Davidson's fixative for 48 h and then in 70% ethanol. The histological investigation was done following standard procedures used; briefly the biopsies were processes embedding in paraffin wax and preparing thin sections of 5 µm with rotary microtome (Leica RM2125RTS). The sections were stained with haematoxylin and eosin (H&E), and examined under light microscope.

10.4.5. Identification and confirmation of bacterial isolates

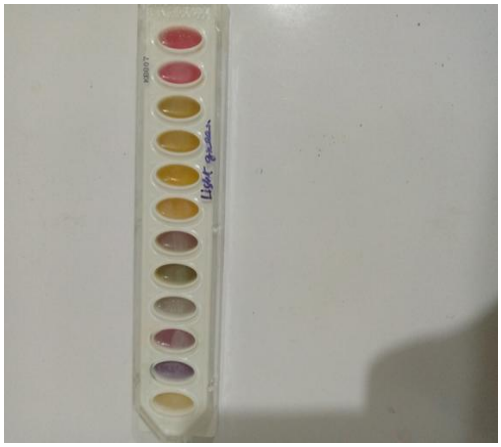
To identify *Vibrio* isolates in crab samples (healthy and unhealthy), biochemical tests including oxidase test, indole test, motility, Voges-Proskauer test, salt tolerance tests were performed (Photo-15-17; Table 10.1). In addition, PCR method (end-point approach) was applied to confirm the identification of pathogenic bacteria such as *Vibrio* spp., in diseased mud crabs. The genomic DNA was was extracted from the selected isolates for PCR amplifications and sequencing of their 16S rRNA genes using universal (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′, 1495R: 5′-CTACGGCTACCTTGTTACGA-3′). For amplifying the gene, PCR reaction was done; each 20 µl PCR reaction mixture contained 1 µl DNA extract (25-65ng/ µl), 10 µl master mix (M7431 Master Mix, Promega), 1 µl forward and reverse primers and 7 µl distilled water. The PCR was carried out in a thermal cyclers (Gene Atlas, Model G2; Astec, Japan). The PCR amplifications were done at initial denaturation temperature of 95 °C for 3 min, and 35 cycles of denaturation at 95 °C for 30 second, annealing at 48 °C for 30 s, and extension at 72 °C for 90 sec with final extension at 72 °C for 5 min. The gene bands were viewed on gel electrophoresis system (Horizontal; CBS Scientific, USA) with the aid of Gel Documentation (Alpha Imager, mini, USA).

(a)



(b)

(c)



(d)

Plates-39 (a-d): Biochemical confirmation test of bacteria isolates

10.4.6. Data management

All data were assembled, analyzed, and presented, using Microsoft Office Word and Excel. In case of sequencing data, the 16 S rRNA sequences of isolates were annotated against reference genomes of NCBI database (16 S rRNA sequences of Bacteria and Archaea) and were identified using BLAST with the program selection optimized for "Highly similar sequences (Mega BLAST)". Phylogenetic relationship was constructed according to UPGMA method, using Molecular Evolutionary Genetics Analysis (MEGA) software.

Table 9. Biochemical confirmatory characteristics of the isolated pure colonies

	V. alginolyticus	V. cholerae	V. fluvialis	V. parahaemolytics	V. harveyi	V. vulnificus	V. mimicus	Aeromonas spp.	Pseudomonas spp.
Oxidase	+	+	+	+	+	+	+	+	+
Glucose fermentation	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	-	-	+	-	+	-	-	+	
Ornithine decarboxylase	+	+	-	+	+	+	+	-	
L-lysine decarboxylase	+	+	-	+	-	+	+	-	
0% Nacl	-	+	-	-	-	-	+	+	
3% Nacl	+	+	+	+	+	+	+	+	
8% Nacl	+	-	+	+	-	-	-	-	
Motility	+	+	v	+	+	v	v	+	+
Indole	+	+	+	+	+	+/-	+	+	-
Voges-Proskauer	+	+/-	-	-	-	-	-	+	-
Urease	-	-	-	+/-	+/-	-	-	+	-

11. Results and discussions

11.1 Component 3 (BFRI-BS)

11.1.1. Study-1: Baseline information

Detailed status of farms and *Depots* has been summarized in Table 10. Majority of the farms are located in the southwest coastal region of Bangladesh. The highest number of farms (1841) is established in Satkhira district followed by Khulna (889) and Bagerhat (780) district. The lowest number of farms (5) is in Barguna district. Similar distribution was also observed for total area of farms. Whereas, highest number of depots (346) was in Bagerhat district followed by the Khulna (191) and Satkhira (146).

Table 10. Status of crab farms and depots in Bangladesh

District	No. of Farms	Farm area (ha)	No. of *Depots*
Satkhira	1841	194	146
Khulna	889	181	191
Bagerhat	780	168	346
Patuakhali	10	1	20
Barguna	5	0.5	10
Cox's Bazar	484	150	85
Total	**4049**	**695**	**798**

Source: BFRI-BS Field Survey (2018-19)

Production records of crabs for 3 successive years have been furnished in Table 11. Production of exportable crabs decreased gradually in all the districts except Cox's Bazar that ultimately lowered down total production of crabs for the successive years.

Table 11. Crab production records (Ton)

District	2015	2016	2017
Satkhira	5375	5080	5100
Khulna	5140	4880	4750
Bagerhat	3725	3520	3508
Patuakhali	1880	1798	1750
Bagerhat	1108	1070	1000
Cox's Bazar	2280	2700	2850
Total	**19508**	**19048**	**18958**

Source: BFRI, BS report on Assessment of mud crab

Distribution of soft shell shedding farms and production has been shown in Table 12. Soft shell shedding is a continuous process for year round except winter. Average moulting rate was estimated 5% daily. However, daily requirement is 1,75,000 crabs thus leaded to (262.5+35) lacs = 297.5 lacs yearly. All these juveniles are being caught from natural sources.

Though the production level was recorded 20 ton/ha/year and profitable, but the investment is out of marginal farmers. Sometimes the farms are operated partially due to lack of required shedders.

Table 12. Status of soft shell crab farms and production

District	No. of Farms	Farm area (ha)	No. of boxes (lacs)	Ave. Production (Ton/ha)
Satkhira	40	50	21	20
Khulna	2	3	4	
Bagerhat	1	2.5	5	
Patuakhali	0	0	0	
Bagerhat	0	0	0	
Cox's Bazar	5	13	5	
Total	**48**	**68.5**	**35**	

Catch composition of crabs during harvest from natural sources has been shown in Fig. 1. A large portion of juveniles are being caught from the natural sources in each month. Sometimes the proportion of juveniles reached to 89% of total harvest. Indiscriminate harvest of juvenile crabs is severely limiting regular recruitment and alarmingly reducing the natural stock size of mud crab in the coastal region of Bangladesh.

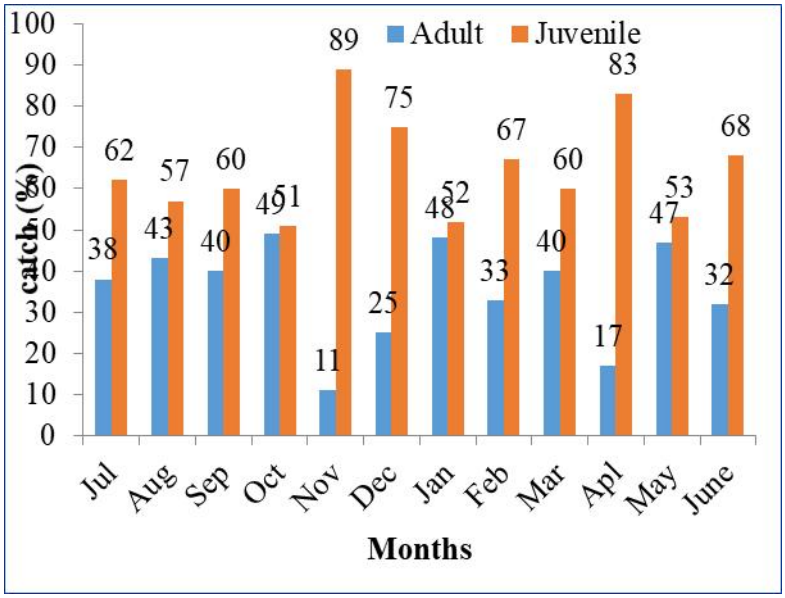


Fig. 1. Catch composition of adult and juvenile crabs

As shown in Fig. 2, among the harvested juvenile crabs, 65% is being used in soft shell farms, 20% died during transport due to rough handling and over packing and 15% injured during transport. Off the 65% used in soft shell farms, 15-35% died further during shedding.

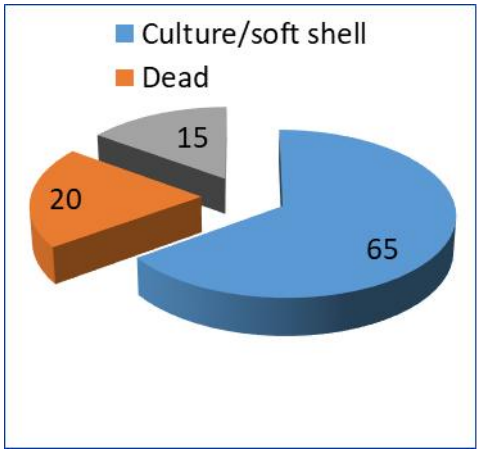


Fig. 2. Status and use of harvested juvenile crabs

11.1.2. Expt. 2: Brood stock management and production of berried broods

In the 1st year, 36 gravid broods were collected from different locations and stocked into the cemented cisterns to produce berried broods in hatchery condition. Average weight of gravid broods ranged between 220.42 to 272.50 g, carapace width between 10.42 to 11.28 cm and carapace length between 7.63 to 7.95 cm (Table 13).

Table 13 . Carapace length (CL), carapace width (CW) and weight (W) of gravid broods stocked in the cemented cisterns

Brood No.	Weight (g)			CW (cm)			CL (cm)		
	T-1	T-2	T-3	T-1	T-2	T-3	T-1	T-2	T-3
1	300	230	440	11.6	10.5	12.8	8.6	7.6	9.0
2	340	250	400	11.8	10.9	12.4	8.7	7.8	8.7
3	250	220	280	11	10.0	11.7	8.2	8.0	8.2
4	200	200	270	10.4	10.4	10.9	7.8	7.5	7.9
5	200	180	300	10.0	10.3	11.5	7.5	7.3	8.2
6	200	220	250	10.0	10.1	10.7	7.4	7.6	8.0
7	170	250	160	10.2	11.0	10.0	7.2	8.3	7.2
8	200	200	260	10.3	10.0	11.3	7.6	7.4	7.8
9	200	210	250	10.2	10.3	1o.7	7.5	7.5	7.4
10	200	205	160	10.4	10.2	10.4	7.4	7.4	7.4
11	200	250	200	10.5	10.6	10.2	7.5	7.5	7.4
12	230	230	300	10.8	10.7	12.2	8.1	7.6	8.2
Mean	**224.17**	**220.42**	**272.50**	**10.60**	**10.42**	**11.28**	**7.79**	**7.63**	**7.95**
SD	**49.44**	**22.61**	**84.00**	**0.59**	**0.33**	**0.93**	**0.49**	**0.28**	**0.55**

In addition, 15 broods were reared in the drum with recirculation water system. Average weight of gravid broods in recirculation system was 271.69±82.87 g, carapace width between 10.54±0.99 cm and carapace length between 7.57±0.70 cm (Table 13).

Table 14. Carapace length (CL), carapace width (CW) and weight (W) of gravid broods stocked in the drum with recirculation water.

Brood No	Total weight (g)	Cw (cm)	CL (cm)
1	315.00	10.80	8.00
2	215.00	9.80	7.30
3	316.20	11.10	8.10
4	278.10	10.70	7.70
5	318.10	11.50	8.00
6	190.00	9.40	6.80
7	192.00	9.50	6.85
8	198.00	9.60	6.75
9	192.00	9.75	7.05
10	475.00	12.70	9.10
11	391.00	12.10	8.60
12	297.00	10.90	7.80
13	233.00	10.20	7.10
14	207.00	9.70	6.90
15	258.00	10.40	7.50
Mean	**271.69**	**10.54**	**7.57**
SD	**82.87**	**0.99**	**0.70**

Performance of brood stock collected from different locations is shown in Table 15. Despite of similarity in size, the highest spawning success (61%) was found for brood stock collected from Khulna region, compared to that from Satkhira and Bagerhat (50%). Production of viable zoea in brood stock of Khulna region (2.2±0.2 million) was also significantly higher than that of Bagethat (1.7±0.2 million), but was similar to Satkhira (1.8±0.2 million). This might happened due to distance of site, time after collection and stress to the brood stock of long distance locations. Result of this study suggested that collecting the brood stock as possible as from nearer places may reduce stress.

Table 15. Performance of brood stock collected from different locations

Particulars	Treatments		
	Khulna region (T1)	Satkhira region (T2)	Bagerhat region (T3)
Ave. body weight (g)	224.17± 49.44 [a]	220.42± 22.61 [a]	272.50± 84.00 [a]
Ave Carapace width (cm)	10.60± 0.59 [a]	10.42± 0.33 [a]	11.28± 0.93 [a]
Number of brood	18	16	16
Total No. spawned	11	8	8
Spawning success (%)	61 [a]	50 [b]	50 [b]
Fecundity (million)	2.4±0.2 [a]	2.3±0.1 [a]	2.5±0.2 [a]
Incubation (days)	12±1 [a]	12±1 [a]	12±1 [a]
Fertilization rate (%)	94.5±4.5 [a]	91.6±2.3 [a]	90.2±4.6 [a]
Hatching rate (%)	93.6±2.6[a]	84.7±5.2[b]	88.8±5.9 [ab]
Number of viable zoea (million)	2.2±0.2 [a]	1.8±0.2[ab]	1.7±0.2 [b]

In the 2nd year, 54 gravid broods were collected from different locations. Average weight of gravid broods ranged between 238.42 to 252.50 g, carapace width ranged from 10.48 cm to 11.18 cm (Table 15). Performance of brood stock collected from different locations is shown in Table 16. Despite of similarity in size, significantly ($p<0.05$) highest spawning success (44%) was found for brood stock collected from Khulna region than Satkhira and Bagerhat (33%). Production of viable zoea in brood stock of Khulna region (2.1±0.2 million) was also significantly higher ($p<0.05$) than that of Bagethat (1.8±0.2 million), but was similar to Satkhira (1.9±0.2 million). Hatching rate was also higher in broods collected from Khulna region (92.6%) was similar to that of broods collected from Bagerhat (89.8%), but differed with broods collected from Satkhira region (84.7%). Fecundity, incubation period and fertilization rate was similar for broods collected from three different locations (Table 16). This might happen due to distance of site, time after collection and stress to the brood stock of long-distance locations. However, highest spawning success (44%) achieved in this year seemed lower than the previous year. Result of this study suggested collecting the brood stock as possible as nearer places to reduce transportation stress.

Table 16. Performance of brood stock collected from different locations

Particulars	Treatments		
	Khulna region (T1)	Satkhira region (T2)	Bagerhat region (T3)
Ave. body weight (g)	244.17± 39.44 [a]	238.42± 32.61 [a]	252.50± 44.00 [a]
Ave Carapace width (cm)	10.66± 0.57 [a]	10.48± 0.34 [a]	11.18± 0.53 [a]
Number of brood	18	18	18
Total No. spawned	8	6	6
Spawning success (%)	44 [a]	33 [b]	33 [b]
Fecundity (million)	2.6±0.2 [a]	2.3±0.1 [a]	2.6±0.2 [a]
Incubation (days)	12±1 [a]	12±1 [a]	12±1 [a]
Fertilization rate (%)	95.5±3.5 [a]	92.6±2.4 [a]	91.2±4.4 [a]
Hatching rate (%)	92.6±2.6[a]	84.7±5.2[b]	89.8±5.9 [ab]
Number of viable zoea (million)	2.1±0.2 [a]	1.9±0.2[ab]	1.8±0.2 [b]

11.1.3. Expt. 3. Efficacy of different live feeds for the larval rearing of mud crab, *Scylla olivacea.*

Efficacy of different live feeds in larvae rearing of mud crab was evaluated with three different feeding treatments, *viz*, T1: larvae fed with live feed only (rotifer+Artemia); T2: larvae fed with liquid diet (liquid rotifer+liquid Artemia); and T3: larvae fed with live feed+liquid diet. The larvae rearing media was 30 ppt sea water and green water. An initial mortality in all treatments was observed due to sudden fall of water temperature to 18ºC. Larvae reared with only liquid diet (T2) dropped at 16 days of culture. Whereas, larvae reared with only live feed reached to the megalopa stage but not to crablet. Larvae in T3 metamorphosed into megalopa stage (M) after 19 days and reached to the crablet (C1) stage after 29 days of hatching. Crablet were harvested after 35 days from Z1 stage. The survival rate at the crablet stage (C1) was 1.5% of the stocked Zoea under live feed+liquid diet feeding treatment (Fig.3. and Plate-g). However, live feed co-feeding with liquid diet

provided better performance than other two treatments. The experiment was repeated for improvement and refinement in the 2nd year.

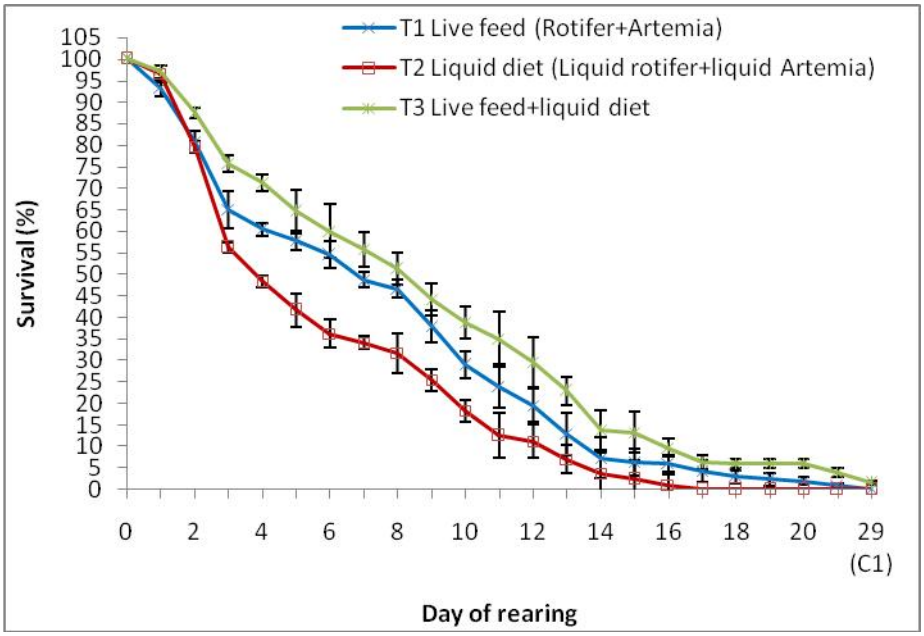


Fig.3. Survival rate of larvae at different rearing days under different treatments

Plate-40: Larvae rearing tanks, ready for larvae culture

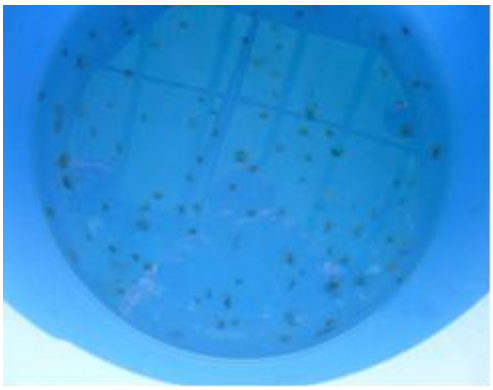


Plate-41: Partial view of produced crablet through larvae rearing

11.1.4. Expt. 4: Impact of different water treatment plan for improvement of water quality and reduce incidence of disease during larval rearing of mud crab, *Scylla olivacea.*

Impact of different water treatment plan on mud crab larvae rearing was assessed with three water treatment protocol, *viz,* water treated with pre-biotics (T1), with probiotics (T2) and with pre and probiotics (T3). Feeding was done with live feed and co-feeding with liquid/commercial diet best feeding derived from previous experiment. Water temperature ranged from 29.1 ºC to 30.4 ºC, salinity from 27.0 ppt to 30.0 ppt, pH from 7.7 to 8.5, Dissolved oxygen from 5.1 mg/l to 6.6 mg/l and NH_4 between 0.00 mg/l and 0.22 mg/l (Table 17). Water quality variables in all the treatments were similar and within the standard ranges of crustacean larvae rearing.

Table 17. Range of water quality variable during larvae rearing with different water treatment protocol

Variables	T-1	T-2	T-3
Water Temperature (ºC)	29.3-30.4	29.2-29.8	29.1-29.7
Salinity (ppt)	27.0-30.0	27.0-30.0	27.0-30.0
pH	7.8-8.3	7.7-8.3	7.8-8.5
Dissolved Oxygen (mg/l)	5.4-6.5	5.5-6.6	5.1-6.5
NH_4 (mg/l)	0.00-0.20	0.00-0.21	0.00-0.22

Larvae growth was monitored as larval stage index (LSI) indicated that for the first four days LSI was similar for all the treatments then started to differ within the treatments (Fig. 4). The larval stage index was observed always higher for T3 than the other treatments, indicating suitability of pre and probiotic together for faster metamorphosis.

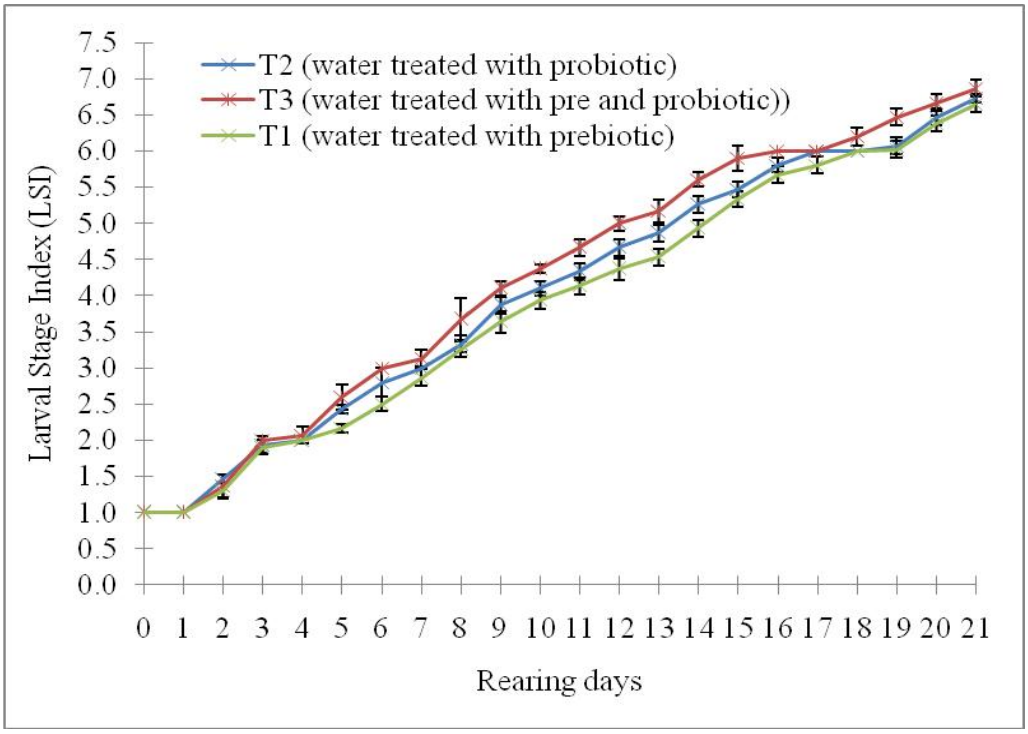


Fig. 4. Larval stage index (LSI) of larvae under different water treatment plan.

Larvae in all the treatments decreased gradually, but with a higher pace in T1 and T2. Except for the first two days, survival of larvae in T3 was always higher for rest of the days with significant difference to other treatments. Larvae in all treatments metamorphosed into megalopa stage (M) at 18 days and reached to the crablet (C1) stage after 25 days after hatching. The highest survival rate at crablet stage was found in T3 (5%) (Fig. 5), compatible to world average. Result of this experiment suggested that pre and probiotics have positive effects to reduce disease incidence, enhancement of survival and promoting of metamorphosis in mud crab larvae rearing.

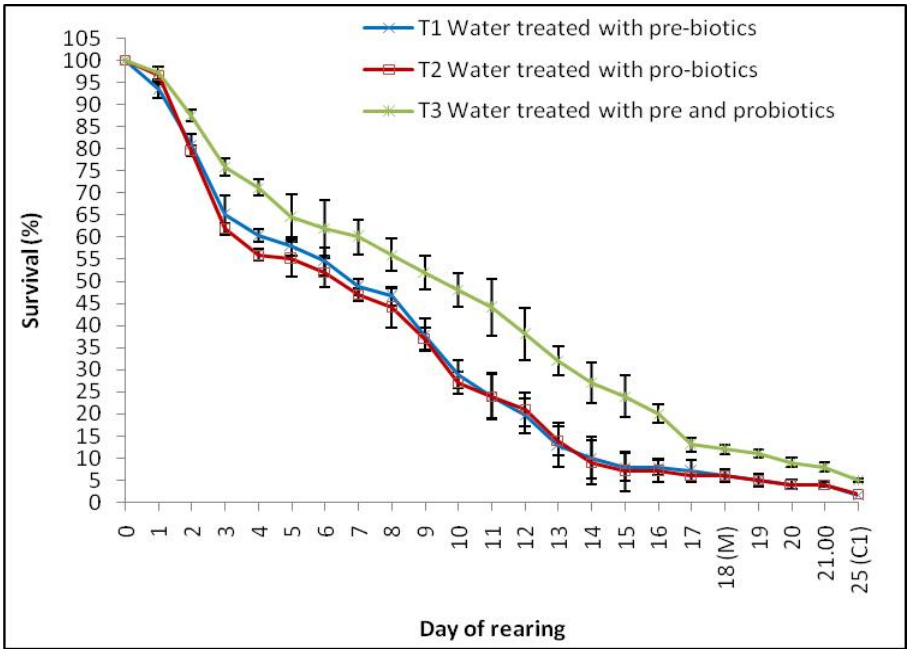


Fig. 5. Survival rate of larvae at different rearing days under different water treatment plan

Plate-42: View of larvae rearing tanks

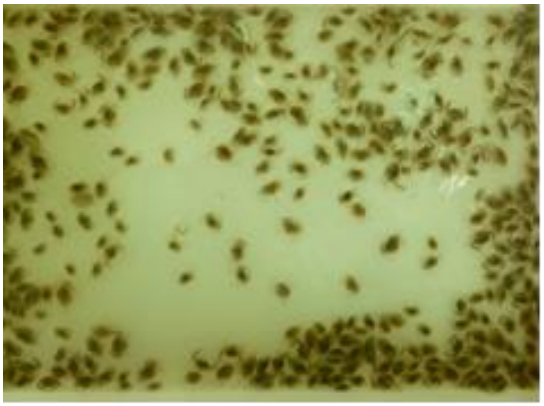


Plate-43: Crablet produced under different water treatment protocol

11.1.5. Expt. 5: Impact of different water treatment plan for improvement of water quality and reduce incidence of disease during larval rearing of mud crab, *Scylla olivacea* (Repetition and fine tuning of previous experiment with modification)

Larval stage index (LSI) indicated that larvae growth was similar for all the treatments for the first four days then started to differ within the treatments due to a-synchronized metamorphosis to the next stage (Fig. 6) and this variation continued for entire rearing period. The larval stage index was observed always higher for T3 (pre, probiotic and prophylaxis) than for other treatments, which indicated that was suitable for faster metamorphosis.

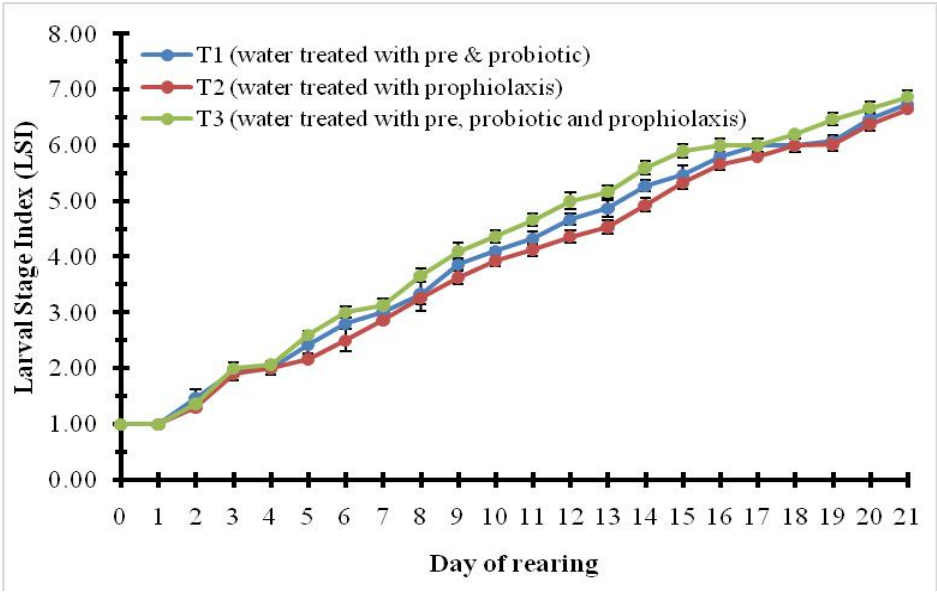


Fig. 6. Larval stage index (LSI) of larvae under different water treatments (repetition).

Larvae in all the treatments sharply decreased up to 4th day of rearing then gradually decreased for rest of the days with a higher pace in T1 and T2 than T3 (Fig. 7). Except for the first two days, survival of larvae in T3 was always higher for rest of the days with significant difference to other treatments. Larvae in all treatments metamorphosis into megalopa stage (M) at 18 days and reached to the crablet (C1) stage at 25 days after hatching. The highest survival at crablet stage was found in T3 (7%) and it was 2% higher than 1st year experiment. Result of this experiment suggested that prebiotic, probiotics and prophylaxis exerted positive effects to reduce disease incidence, enhancement of survival and promoting of metamorphosis in mud crab larvae rearing.

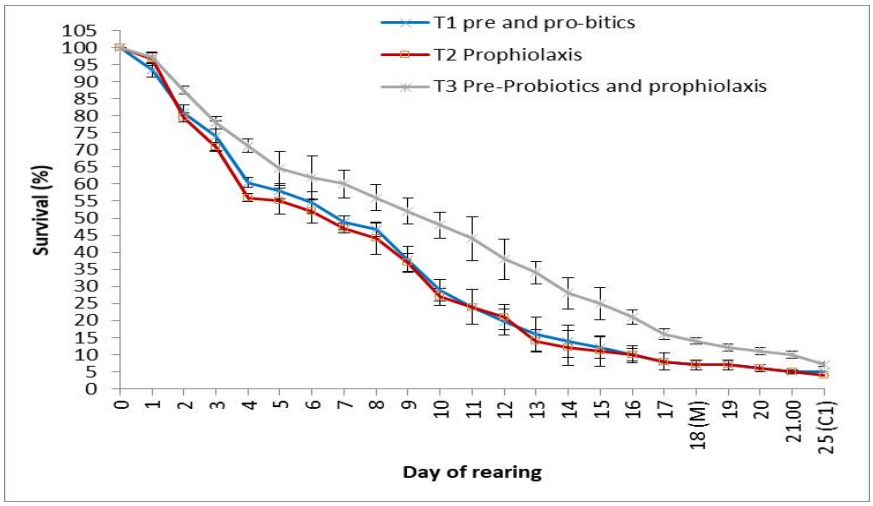


Fig. 7. Survival of larvae at different rearing days under different

Water temperature ranged from 29.1 ºC to 31.0 ºC, salinity from 27.0 ppt to 30.0 ppt, pH from 7.7 to 8.8, Dissolved oxygen from 5.1 mg/l to 6.7 mg/l and NH_4 between 0.00 mg/l and 0.22 mg/l, respectively in all the treatments (Table 18). Water quality variables in all the treatments were similar and within standard crustacean larvae rearing ranges.

Table 18. Range of water quality variables under different water treatment protocols

Variables	T-1 (Pre and probiotics)	T-2 (Prophylaxis)	T-3 (Prebiotic, probiotics and prophylaxis)
Water Temperature (°C)	29.3-31.0	29.3-30.8	29.1-29.9
Salinity (ppt)	27.0-30.0	27.0-30.0	27.0-30.0
pH	7.9-8.3	7.8-8.6	7.7-8.8
D. Oxygen (mg/l)	5.5-6.6	5.5-6.7	5.1-6.5
NH_4 (mg/l)	0.00-0.20	0.00-0.21	0.00-0.22

The lower survival rates of mud crab larvae is a major problem commonly encountered in the hatchery. Like other crustacean species, mud crab also grows through moulting and this process might be affected by many factors *viz.* temperature, stress and scares from predator, lack of shedding/hiding places, improper nutritional feeding, hydrology, disease infection etc. Any interruption in moulting might slower the growth of mud crab larvae. Thus longer time is needed to attain desirable size and even cause death to the victim. The development of metamorphosis in crustacean can be started by a variety of factors such as the availability of the proper water quality, nutrient and energy capable of accelerating the change of metamorphosis from zoea into megalopa. There remain several factors that may affect the survival rate in the larvae rearing process of mud crab such as cannibalism, shedding, temperature and salinity in stabilities, feed, shelter and stocking density. Optimizing the size and nutritional feed as well as feeding schedule improves the survival. In addition, optimizing water treatment strategy for the improvement of water quality, reduces the chance of disease; therefore, minimize mortality and shorten metamorphosis period during the larval rearing of mud crab. However, the survival rate increased to 1.5% when the larvae were fed with live feed like rotifer and Artemia. Larvae survival at crablet stage further increased to 5% as water was treated with both prebiotic and pro-biotic. Meanwhile, larvae survival enhanced to 7% and metamorphosis period shortened to 18 days for megalopa and 25 days for crablet due to water treatment with prebiotic, probiotics and prophylaxis. Therefore, proper feed, feeding schedule and water treatment protocol has been derived from consecutive trials on larvae rearing of mud crab.

11.1.6. Expt. 6: Optimization of stocking density in nursery of mud crab *S. olivacea*

As shown in Table 19, survival (68%), weight gain (26.3 g) and intactness (85%) were higher in T1 (30/m^2) followed by T2 and T3. Result of the experiment focused density dependent survival and weight gain. However, better performance was obtained from the lowest density, thus the experiment needed to be repeated with further lowering the stocking density to achieve specific density for nursery of mud crab juvenile.

Table 19. Details of survival and intactness of crablet under different nursery habitats

Treatment (Stocking density)	Initial weight (g)	Survival (%)	Intactness (%)	Weight (g)
T1 (30/m^2)	0.30±0.01	68±8.0	85±6.0	26.3±6.50
T2 (50/m^2)	0.30±0.01	65±6.0	80±10.0	23.8±5.10
T3 (70/m^2)	0.30±0.01	40±10.0	48±9.0	18.4±5.00

11.1.7. Expt. 7: Evaluation of different habitats for the nursery management of mud crab, *Scylla olivacea*

Growth of crablet under different nursery habitat has been presented in Table 20. Observed Growth increment was always found higher in T3 **(**nursing in pond bottom with different shelter) followed nursing by T1 **(**Nursing in earthen pond without shelter) and T2 **(**Nursing in hapa net).

Table 20. Weight increment (g) of crablet under different nursery habitats

Treatments	Growth (g)				
	initial	***7 day***	***14 day***	***21 day***	***30 day***
T1 (*Nursery in earthen pond without shelter*)	0.3±0.01	1.0±0.12	3.5±0.16	11.9±2.10	20.6±4.10
T2 (*Nursery in hapa net*)	0.3±0.01	0.7±0.11	2.9±0.14	9.8±1.80	16.4±4.00
T3 (*In pond bottom with different shelter*)	0.3±0.01	1.8±0.14	5.3±0.16	14.9±2.90	24.2±6.25

Survival and intactness of crablet under different nursery habitat has been shown in Table 21. Survival of 64% and intactness of 82% in T3 was significantly ($p<0.05$) higher than in T1 and T2, but it was similar in T1 and T2. Result of this experiment indicated that adequate shelter along with soil/sediment attachment is necessary for nursery to achieve better survival and intact crablets.

Table 21. Details of survival and intactness of juvenile crabs under different nursery habitats

Treatment	Initial weight (g)	Survival (%)	Intactness (%)	Weight (g)
T1 (*Nursery in earthen pond without shelter*)	0.30±0.01	48.0±6.0 ^{b}	68.0±9.0 ^{b}	20.6±4.10
T2 (*Nursery in hapa net*)	0.30±0.01	42.0±11.0 ^{cb}	54.0±9.0 ^{cb}	16.4±4.00
T3 (*In pond bottom with different shelter*)	0.30±0.01	64.0±7.0^{a}	82.0±5.0 ^{a}	24.2±6.25

The survival rate of aquatic organisms mainly depends on the culture environment, stocking density and the nature of the cultured organism. The mud crab is mainly found wild,

aggressive and cannibalistic in nature and has a tendency to escape through burrowing. Minimizing stress and fighting among the crab individuals, shelters play a remarkable role by providing refuge for the crabs. In terms of survival rate of crab population, shelter gives a good result by reducing the mortality due to antagonistic effect between rival crabs.

11.1.8. Expt. 8: Evaluation of different prepared feeds for the culture of mud crab, *Scylla olivacea* in grow out phase

Results of 60 days experiment have been summarized in Table 22. From this experiment, it was found that feeding with different natural diets did not affect the growth, survival and intactness of mud crab significantly. However, an apparently better performance in body weight gain (177±7.2g), survival (66±6%) and intactness (68±4%) was noticed in co-feeding of trash fish and chopped mud eel treatment (T3) than the other two single feeding treatments. Chopped trash fish is the preferred feed item than chopped mud eels.

Table 22. Details of survival and intactness of juvenile crabs under different feeding regimen in grow out phase

Parameters	T1 Chopped trash fish	T2 Chopped mud eel	T3 Chopped trash fish + chopped mud eel (1:1)
Initial wt. (g)	48.50 ±3.6 ^{a}	48.50 ±3.6 ^{a}	48.50 ±3.6 ^{a}
Initial CW (cm)	5.6±0.51 ^{a}	5.6±0.51 ^{a}	5.6±0.51 ^{a}
Final wt. (g)	172±6.8 ^{ba}	164±5.8 ^{cba}	177±7.2 ^{a}
Final CW (cm)	8.4±1.1 ^{ba}	7.7±0.9 ^{cba}	8.7±1.1 ^{a}
Survival (%)	64±6 ^{ba}	61±3 ^{cba}	66±6 ^{a}
Intactness (%)	63±6 ^{cba}	65±4 ^{ba}	68±4 ^{a}

11.1.9. Expt. 9: Evaluation of different prepared feeds for the culture of mud crab, *Scylla olivacea* in grow out phase (Repetition of previous experiment with modification of feeding regimen)

Table 23. Details of growth, survival and intactness of juvenile crabs under different feeding regimen in grow out phase (Repetition with different feeding regimen)

Parameters	T1 Chopped trash fish	T2 Chopped mud eel	T3 Chopped trash fish + chopped mud eel (1:1)	T4 Chopped trash fish+ commercial diet (1:1)	T5 100% commercial diet
Initial wt. (g)	43.40 ±3.4 ^{a}	43.40 ±3.4 ^{a}	43.40 ±3.4 ^{a}	43.40 ±3.4 ^{a}	43.40 ±3.4 ^{a}
Initial CW (cm)	5.4±0.42 ^{a}	5.4±0.42 ^{a}	5.4±0.42 ^{a}	5.4±0.42 ^{a}	5.4±0.42 ^{a}
Final wt. (g)	175±5.8 ^{bac}	168±8.8 ^{cba}	176±6.2 ^{abc}	155±10.5^{d}	126±6.4 ^{e}
Final CW (cm)	8.3±1.1 ^{bacde}	7.8±0.9 ^{cabde}	8.5±1.2 ^{abcde}	7.7±1.8 ^{dabce}	7.4±0.8 ^{eabcd}
Survival (%)	64±6 ^{a}	58±3 ^{c}	62±6 ^{b}	54±4 ^{de}	48±3 ^{e}
Intactness (%)	65±5 ^{a}	62±3 ^{a}	67±3 ^{a}	63±6 ^{a}	60±7 ^{a}

In this trial (Table 23), significantly higher ($p<0.05$) body weight gain and survival rate was achieved from co-feeding natural feeds (T1, T2 and T3) than co-feeding with trash feed and commercial diet (T4) and solely feeding commercial diet (T5). Meanwhile, the rations of intactness of harvested crabs were similar within all treatments. The highest body weight gain (176±6.2g) was in T3 and lowest was in T5 (126±6.4g). Whereas, survival rate was highest in T1 (64±6%) and lowest in T5 (48±3%).

Table 24. Ranges of water quality variables under different feeding regimen in grow out phase (Repetition with different feeding regimen)

Variables	T1 Chopped trash fish	T2 Chopped mud eel	T3 Chopped trash fish + chopped mud eel (1:1)	T4 Chopped trash fish+ commercial diet (1:1)	T5 100% commercial diet
Water Temperature (°C)	29.3-31.0	29.3-30.8	29.1-29.9	29.3-31.5	29.3-31.0
Salinity (ppt)	12.0-17.0	12.0-17.0	12.0-17.0	12.0-17.0	12.0-17.0
pH	7.9-8.3	7.8-8.6	7.7-8.8	7.6-8.7	7.7-8.8
D. Oxygen (mg/l)	5.5-6.6	5.5-6.7	5.1-6.5	5.5-6.4	5.5-6.3
NH_4 (mg/l)	0.00-0.20	0.00-0.21	0.00-0.22	0.00-0.22	0.00-0.21

Observed water quality variables for both the experiment were within congenial levels for crustacean culture like mud crab (Table 24). Results of the two consecutive trial suggested that natural feeds with equal ratios (Chopped trash fish: chopped mud eel= 1:1) may be the best suite for grow out culture of mud crab. Whereas, available commercial diet in local market may not be suitable enough for mud crab grow out.

11.1.10. Expt. 10: Impact of fattening of mud crab *S. olivacea* in floating cages and stocking fish along with fattening of mud crab in pond bottom on the productivity of the pond

As stipulated in Table 25, survival rate of crabs was within 95% to 97% in cages, whereas that of crabs varied from 70% to 79% in pond fattening system for all the locations. Average survival rate of fattened crab was 96% in cages, which was significantly higher ($p<0.05$) than that of earthen pond fattening system (75%). The lower survival rate of crabs in earthen areas might be due aggressive and cannibalistic nature in free areas. It might also due to be escaping from ponds due to burrowing habit of crab and escaped from pond.

Table 25. Total and category-wise survival of fattened crab under different treatments at different locations

Treatments	Survival (%)		
	Satkhira	Khulna	Bagerhat
T1 (Crab in ponds)	79±3.0	77±2.0	78±4.0
T2 (Crab in pond + Crab in cage)	85.5±14.85 (c: 96; p: 75)	84.5±17.68 (c: 97; p: 72)	84.5±18.38 (c: 97; p: 72)
T3 (Crab in pond + Crab in cage + GIFT in pond)	83±18.38 (c: 96; p: 70)	86.5±12.02 (c: 95; p: 78)	85.5±13.44 (c: 95; p: 76)

c= crab fattening in cages; p= crab fattening in pond bottom

Growth performance of GIFT has been stipulated in Fig. 8, showing uniform weight increment in all the locations. Details of fattening duration, production of crab, production of GIFT and economic return from integrated mud crab fattening within different locations has been presented in Table 25. Crab fattening in floating cages reduced the fattening duration 2-4 days than that of earthen ponds. Meanwhile, survival of fattened crab was higher in cage fattening than fattening in ponds.

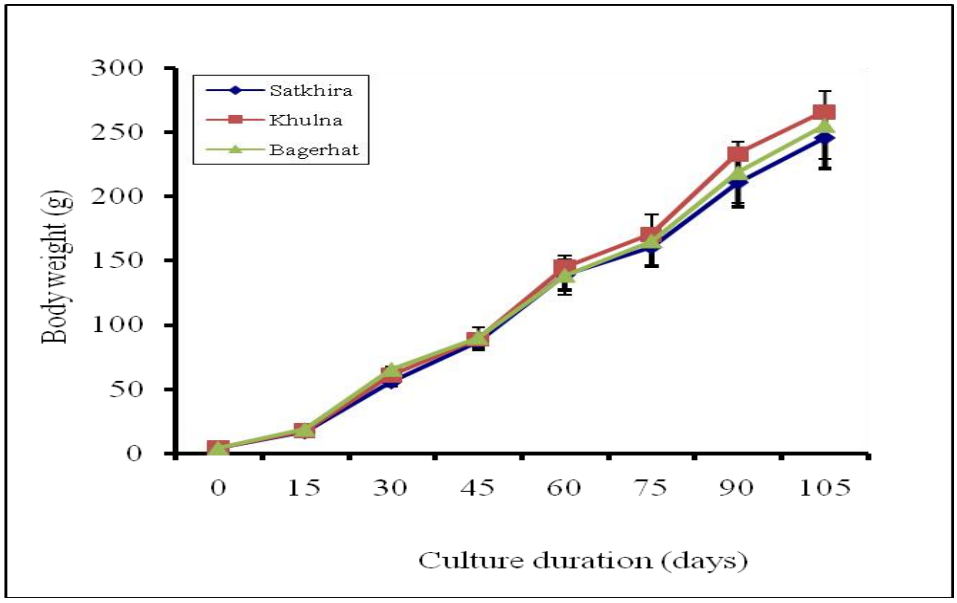


Fig. 8. Growth pattern of GIFT in different locations cultured with crabs fattening.

All these ultimately reduced production cost as a whole. Production of crab ranged between 0.54 to 0.65 kg/m^2 in earthen ponds. Whereas, it was 2.82 to 3.04 kg/m^2 in floating cages. Total production was significantly higher ($p<0.05$) in Satkhira (3.04 kg/m^2) than Khulna (2.98 kg/m^2) followed by Bagerhat. Due to incorporation of floating cages in the same watershed crab production increased 3.66 to 4.42%. Survival of GIFT ranged between 43.25 to 51.54% and that of the production ranged between 0.46 to 0.58 kg/m^2. BCR obtained in the present trial ranged between 1:1.1 to 1:1.3. The BCR values seemed lower than previous authors due low market price during COVID-19 pandemic. However, simultaneous fattening of crabs in pond bottom and floating cages; and integration of GIFT has promoted the production than fattening only in the pond bottom.

Table 26. Production of fattened crab and GIFT at different aqua-ecological locations

Locations	Fattening duration (days)	Production in pond (kg/m^2)	Production in cage (kg/m^2)	Total production (kg/m^2)	GIFT		BCR
					Survival (%)	Production (kg/m^2)	
Khulna	cages 9-12; ponds 13-16	0.64±0.05^a	2.34±0.04^b	2.98±0.01^b	48.22±6.8^a	0.51±0.02	1:1.2^b
Satkhira		0.65±0.03^a	2.39±0.06^b	3.04±0.06^c	43.25±9.6^a	0.46±0.02	1:1.3^c
Bagerhat		0.54±0.05^a	2.28±0.04^b	2.82±0.06^a	51.54±9.5^a	0.58±0.02	1:1.1^a

Note: $p<0.05$ and c>b>a

11.1.11. *Experiment*-11: Regulatory factors on soft shell shedding of mud crab (*S. olivacea*) for sustainable production

Results on three major factors affecting the performance of soft shell shedding have been presented in Fig. 9(A-F).

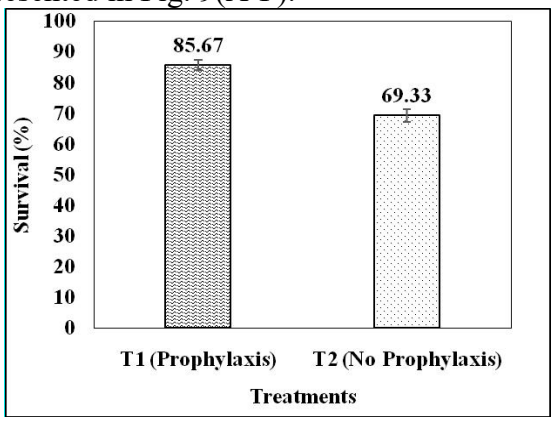


Fig. 9.A. Survival of soft shell crab under different treatments

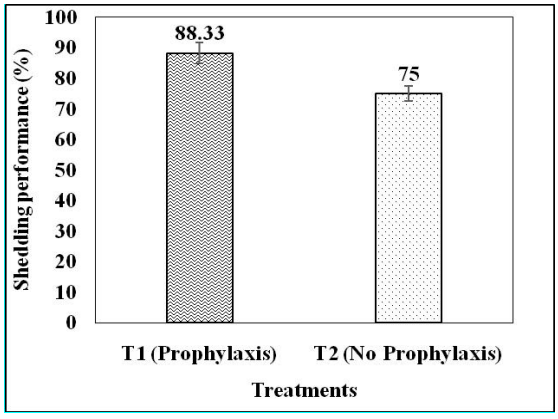


Fig. 9.B. Shedding performance under different treatments

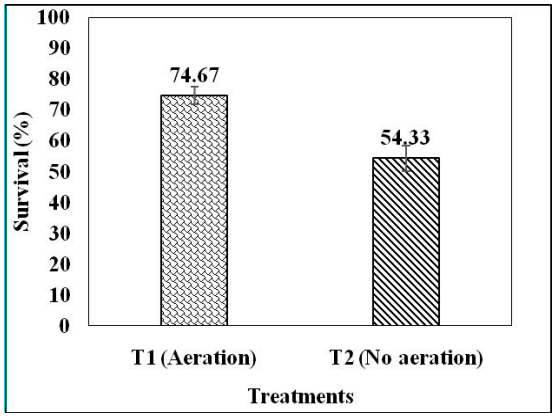


Fig. 9.C. Survival of soft shell crab under different treatments

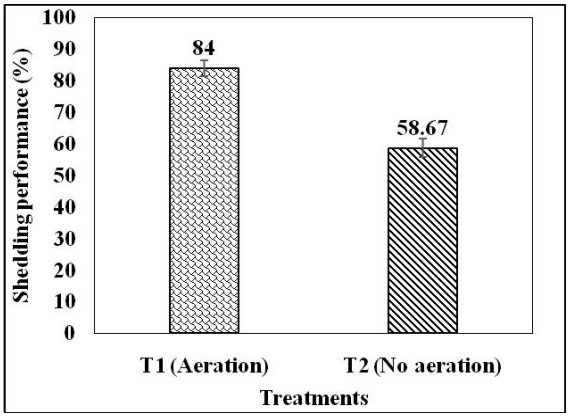


Fig. 9.D. Shedding performance under different treatments

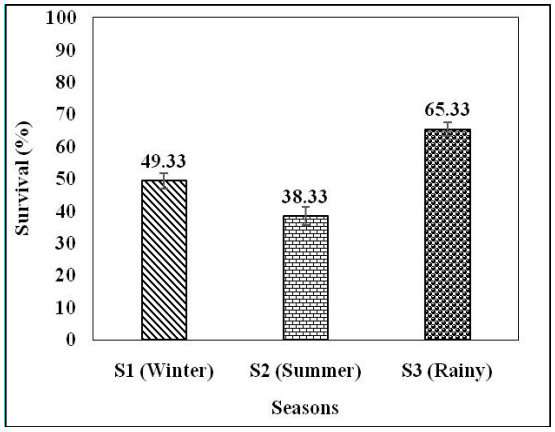


Fig. 9.E. Survival of soft shell crab under different treatments

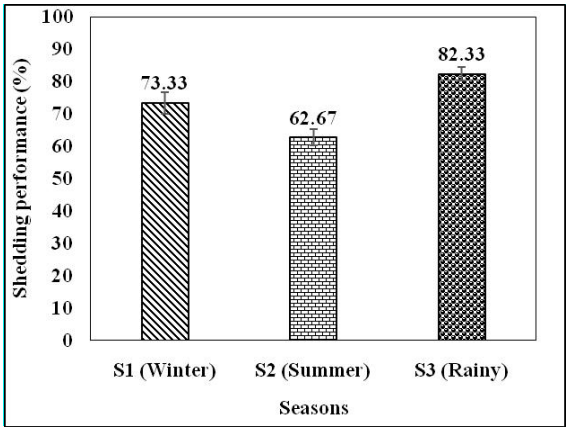


Fig. 9.F. Shedding performance under different treatments

Fig. 9. Observed regulatory factors on soft shell shedding; 9.A & B: Effect of prophylaxis treatment; 9.C & D: Effect of aeration in shedding water; 9.E & F: Effect of seasons on soft shell shedding.

11.1.11.A. Effect of prophylaxis treatment on soft shell shedding

As has been shown in Fig. 9.A, survival of soft shell crab was 85.67% for the prophylaxis (T1) treated premoult crabs. In contrast, the survival rate was 69.33% for the premoulty crabs were not treated with prophylaxis (T2). Moulting performance was 83.33% and 75% in T1 and T2, respectively (Fig. 9.B). However, prophylaxis treatment enhanced the survival and moulting efficiency, might be by minimizing the disease carried out by the crab from the source.

11.1.11.B. Effect of aeration on soft shell shedding

Results on effect of aeration in shedding tank have been presented in Fig. 9. C & D. It has been found that aeration exerted positive effects on survival and shedding. Survival was 74.67% and 54.33% in T1 and T2, respectively (Fig. 9.C). Meanwhile, shedding performance was 84% and 58.77% in T1 and T2, respectively (Fig. 9.D). The reasons behind this might be the requirement of high level of oxygen during moulting as moulting is therefore, regarded as the rebirth of crustaceans including mud crab.

11.1.11.C. Seasonal variations on soft shell shedding of mud crab

Figure 9. E & F showing the seasonal variations on survival and moulting efficiency of mud crab. Recorded survival of crab was 49.33, 38.33 and 65.33% in S1 (winter), S2 (summer) and S3 (rainy/wet) seasons, respectively (Fig. 9.E). On the other hand moulting rate was 73.33, 62.67 and 82.33% in S1 (winter), S2 (summer) and S3 (rainy/wet) seasons, respectively (Fig. 9.F). However, both survival and moulting rate was higher in S3 (rainy/wet) than S1 (winter) followed by S2 (summer). This might be happened due to congenial temperature and salinity during rainy season. Though, crabs are in stressed during the late rainy season due to heavy rain fall and subsequent sudden fall in salinity and fluctuation in water pH. Generally soft shell shedding cages has been floated in the surface water, and the height of the cage is only 15 cm; thus the crabs in the boxes are seriously stressed during summer by higher water temperature. Meanwhile, mortality was observed during winter due to cold shock as the water temperature felt down below 22 °C. High mortality was observed when the water temperature raised above 34 °C and felt down below 22 °C.

11.2 Component 4 (KU)

11.2.1. Baseline information

Traditionally, mud crabs are collected from the sea, coastal rivers, mangrove forests, aquaculture systems including shrimp and other fin fish farms. Since 100 years ago, the collected crabs are stocked in the mud crab cultivation systems. Hitherto, mud crabs are reared in a grow-out system for extended period of few month, and fattened in farms for short period of time. China is the top-most producing country of mud crab; apart from China, Philippines, Indonesia, Vietnam, Malaysia, Thailand, India, Bangladesh also produces mud crabs in farms. Recently, increasing soft-shell mud crab production has been observed in different countries including China, Singapore, Indonesia, USA, Philippines, Thailand, Vietnam, Taiwan, India and Bangladesh.

In Bangladesh, historically the mud crab aquaculture was developed by the low-income fisher-folks and later it became popular among the shrimp farmers as an alternative to shrimp industry, which was affected severely by white spot disease in 1990s. At present, fisher-folks in Bangladesh practice mud crab cultivation in monoculture pen, in composite system with fin-fish and shrimp, fattening in pen, and cage, soft-shell shedding.

The baseline studies provided the information on the mud crab aquaculture system currently practiced in Bangladesh (Table 27). These existing farming patters are found to be similar to the mud crab aquaculture practice in other countries of the world (Table 28).

Table 27. Mud crab farming pattern in Bangladesh

Unit	System	Stocking density	Feeding	Survival	Cycle duration	References
Grow-out	Pond monoculture in Pen	10-20 individual (>180)/decimal ; 3-15kg (4-12 individual/kg)/ decimal	25-30kg/decimal	40-85%	2.5 months	Kamal et al., 2002; Begum et al, 2006; Zafar and Hossain, 2008; Hasanuzzaman et al, 2014; Huq et al., 2015;
	Composite culture	0.5-6kg (4-20 individual/kg)/ decimal	Feed given for main crop shrimp; 2-3 % BW (body weight) by some farmers	20-60%	1-2 months	Personal communication; field survey
Fattening	Pen	12-641 g/ m^2	3-10 % BW (body weight)/day	45-95 %	10-45 days	
	Cage	50 lean crabs (>180 g) in 2 m^2 cage	8-10% BW/day	85-98%	7-21 days	
Soft shell crab shedding	In small plastic cage (LxWxH=0.23m x0.2m x0.15 m)	1 premoultis crab (40-200 g)/box; 9kg (10-25 individual/k)/ decimal	5-10 % BW; 7 g in each box; daily or once within 3 days	> 97 %	22-25 days	

Table 28. Mud crab farming system in the world.

Unit	System	country	Stocking density	Feeding	Survival	Cycle duration	References
Grow-out	pond Monoculture	Viet Nam	0.5–1.5 crabs/m^2	3-15% BW/day	67%	More than 3 months	Allan and Fielder, 2003; Shelley, 2008; FAO, 2011, and references therein.
		Philippines	1–3 crabs/m^2		40-60%		
			0.5–1.5/m^2		45-70%		
	pond Polyculture with finfish and shrimp	Viet Nam	0.01–0.2 crabs/m2		15-30%		
		Philippines	5 000–10 000 per hectare; 1.0–1.5/m^2	3-5 g/ individual	45 to 70%		
		China	500 per hectare	5-10%			
		Indonesia	1- 5/m^2	3%	33-80%		
	Pen		2.5 to 5 crabs/m^2		40-60%		
	Mangrove pen		1–1.5 tonnes/ha		20-80%		
		Philippines	0.5–1.5/m^2		45 to 70%		
	silviculture		0.05 crabs/m2				
Fattening	Pen		3–5 crabs/m^2	7-15%	70-90%	14 to 60 days; usually within 10 to 20 days	
	Cage	Vietnam	35 crabs (200-400g)/ m^2	3-7%		15-40 days	
Soft shell crab shedding			1 premoultis crab/box or case-cell	8% BW once every other 2-3 day	More than 85 %	A few weeks	

Since, mud crab farming is increasingly practiced with intensification through technology (e.g. cage and pen) and farming management (e.g. feeding); like other aquaculture ventures, mud crab farming is at risk from microbial contamination and diseases

In the present study, the focus group discussion FGD revealed that very few diseased crabs (about 2-3%) were found in the wild. In the mud crab farming system, there have been few farms facing mortality problem, especially in Mongla, Bagerhat and Botaighata, Khulna districts. The fig. 10 shows that high mortality occurred in mud crab farms of Bagerhat district; but the causes were not studied and identified. Farmers thought that such mortality was due to viral infection and/or diseases. Across the world, there have been increasing reports on infection and/or disease incidence in mud crabs (Table 29) as reported by Lavilla-Pitogo et al. 2001b; Poornima et al. 2008; Jithendran et al. 2010).

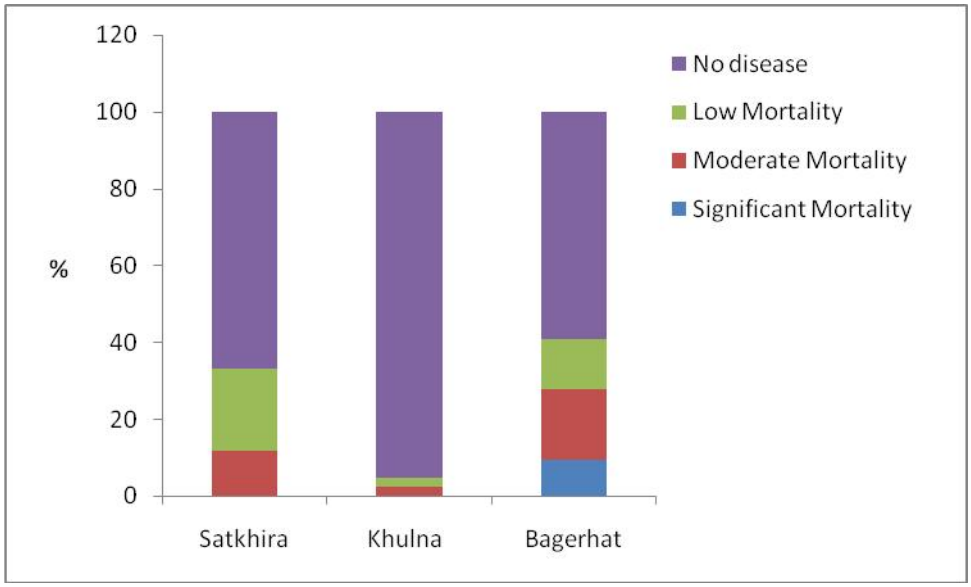


Fig. 10. Mortality occurrence in mud crab farms in three districts of Bangladesh (source: KU-FMRTD baseline study in 2018-2019)

Table 29. Diseases in mud crab population.

Pathogen group	Causative agents	Diseases	Description	Mud crab population
Virus		White spot syndrome virus (WSSV) disease		Wild and farmed
	muscle necrosis virus	muscle necrosis virus disease	White muscle, muscle necrosis	farmed
	Rreovirus	Rreovirus disease	high mortalities; sleeping disease	farmed
	Baculovirus	Baculovirus disease		Wild
Bacteria	: *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., and *Spirillium* spp	black spot', 'brown spot', 'burnt spot', 'shell disease' or chitinolytic bacterial disease.	Bacteria led to damage of shells of mud crab; break down the chitin of the exoskeleton, leading to erosion and melanisation (dark brown to black pigmentation) at the site of infection.	farmed
		Red sternum syndrome	Red legs, joints and discoloured haemolymph	Wild
	Vibrio spp.	Orange crab disease	Septicaemia, mortality	Wild
	V. harveyi and *V. alginolyticus*	Vibriosis	loss of appetite, reduced growth, dark hepatopancreas and	In the hatchery

Pathogen group	Causative agents	Diseases	Description	Mud crab population
			morality	
	Leucothrix mucor, Thriothrix spp. and *Flexibacter* spp	*Filamentous bacterial disease*	Mortalities; discolouration of gills	
		Yellow body disease		
Fungi	*Lagenidium*			Egg and larvae
	Haliphthoros	abortion/resorption of eggs mass; saprotrophs on the surface of egg mass		
	Fusarium spp., *Lagenidium* spp. and *Sirolpidium* spp	*Larval mycosis*		
parasite	*Zoothamnium, Vorticella* and *Epistylis; Acineta* sp		cotton wool like growth attaches to the body as well as appendages and disrupt mobility and feeding.	hatchery phase
	Cirripedians: *Sacculina* sp. and *Loxothylacus* sp		extensive shell erosion	
	Hematodinium	Milky disease", "bitter crab disease" (BCD), "pink crab disease" (PCD), "yellow water disease"	white muscle and milky liquid in mud crab; moribund behaviour, opaquely discoloured carapace, cooked appearance, unpalatable flavour and high mortality	

Plate-44: Composite culture of mud crabs in shrimp gher

Plate-45:: Mud crab fattening in pen

Plate-46: Mud crab fattening in bamboo-cage

Plate-47: Soft-shell mud crab production in Burigoalini, Shyamnagar, Khulna

11.2.2. Quantification of bacterial load and type of infection level in mud crabs

11.2.2.1 Bacterial infection and load in mud crabs collected from the wild sources

Mud crabs collected from wild sources (e.g. the South-west Sundarbans; water bodies adjacent to the South-west Sundarbans) were found infected with bacteria. The present study identified the prevalence of major chitinolytic bacteria such as *Vibrio, Aeromonas, Pseudomonas* (Fig.11). Similar bacterial infection incidence has been reported by Nijiah et al. (2010); *Aeromonas* and *Vibrio* were most frequently isolated from the wild mud crab *S. serrata*. As shown in the Table 28, *Vibrio* spp. are the predominant bacteria casing diseases in wild mud crabs, to which similar infection types were found in this study.

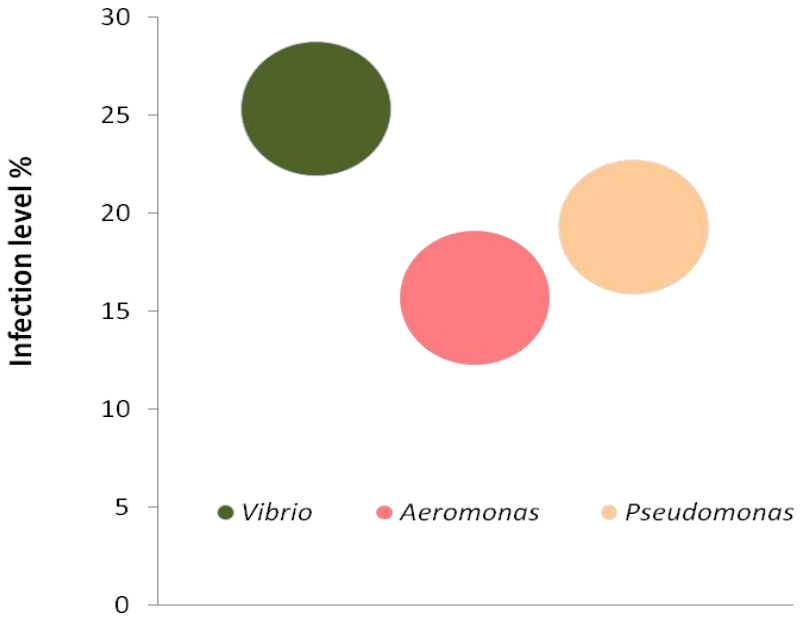


Fig. 11. Prevelance of major chitinolytic bacteria infection in the wild mud crabs.

The Total bacterial count (TBC) that was found in the wild samples ranged between 2.2 x 10^5 to 4.8 x 10^6 CFU/gm. Regarding specific bacterial load, *Vibrio* spp. ranged from 3.0 x 10^2 to

1.2 x 10^5 CFU/gm, *Aeromonas* of $3.6x10^2$ – $4.9x10^2$, *Pseudomonas* of $1.8x10^2$ – $2.7x10^3$, *Escherichia coli* of$1.3x10^3$ - $4x10^3$, *Enterobacter* and *Klebsiella* of $2x10^3$- $1.2x10^4$, *Enterococcus* of $1.7x10^3$ - $6x10^3$, *Salmonella* and *Shigella* of $1.2x10^3$ - $6.0x10^3$ CFU/gm in Mud crabs collected from the wild sources. Several *Vibrio* species were identified (Fig. 12). Similar *Vibrio* species have been reported in the Mud crab *S. serrata* samples from the Chakoria coast of Bangladesh (Uddin et al. 2013). *Vibrio, Aeromonas, Pseudomonas, E. coli* have also been found in the wild mud crabs (Nijiah et al. 2010). The mud crab *S. serrata* samples from the Chakoria coast of Bangladesh had *Vibrio* count ranged between $1.1x10^1$ and $2.6x10^4$ CFU/gm (Uddin et al. 2013). Mahalaxmi et al. (2013) also reported that the shell of the edible crab *Portunus* had 1.4 – 3.6 x 10^5 *P. aeruginosa* and 4.2-7.6 $x10^5$ *E. coli* load. Compared to the previous reports, such variation in specific bacterial load estimated by the present study is likely associated with species as well as environmental condition from where the crabs were collected.

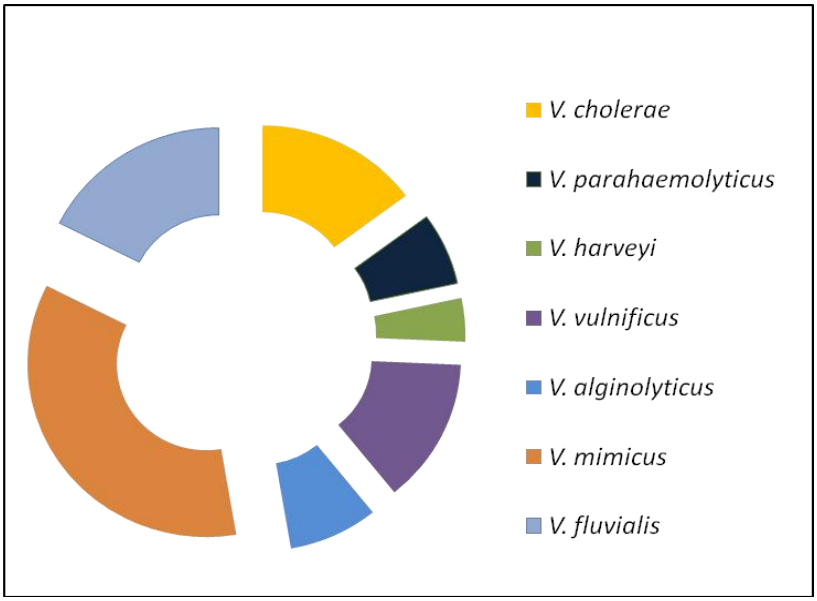


Fig.12. *Vibrio* species isolated from the wild mud crabs.

The wild mud crabs had more frequent bacterial infection in the period of January-June compared to the July-December period (Fig.13). Higher infection rate with *Pseudomonas* spp. were reported in the winter months while the *Vibrio* spp. were found more in Mud crab in the warmer months. Though prevalence rate with *Aeromonas* spp. was mostly higher in January and February period, this species was found to contaminate wild mud crab all the year round in moderate rate. The overall findings indicate more bacterial infection in the warmer months as most of the bacterial species in tropical and sub-tropical region proliferate quickly at high environmental temperature.

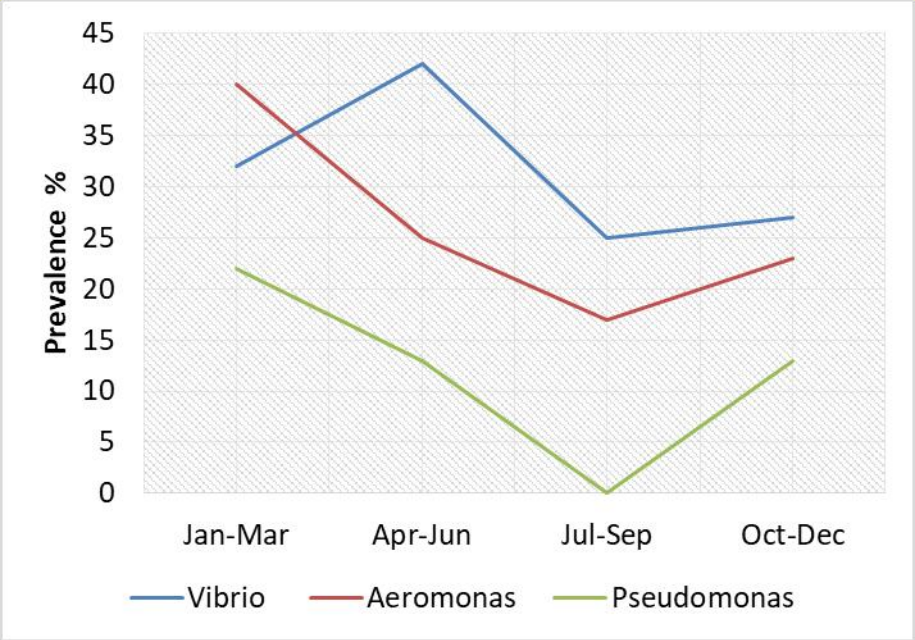


Fig.13. Seasonal trend of bacterial prevalence in the wild mud crabs

11.2.2.2. Bacterial infection and load in mud crab from BFRI-BS Hatchery

The samples from different units of the mud crab hatchery were found to be infected with chitinolytic bacteria pathogenic to mud crabs (Fig.14); *Vibrio* spp. were detected in most of the units, *Aeromons* spp. in some units, while *Pseudomonas* spp. was only detected in the brood crabs. Out of four different batches (summer, wet, autumn, winter), brood sample of three batches, larval sample of two batches and larval feed sample from all batches were found to be infected with pathogenic *Vibrio* spp and *Aeromonas* spp. In case of brood feed sample, one batch was infected with *Vibrio* spp., but no *Vibrio, Aeromonas* and *Pseudomonas* bacteria was reported in the crablet and crablet feed samples.

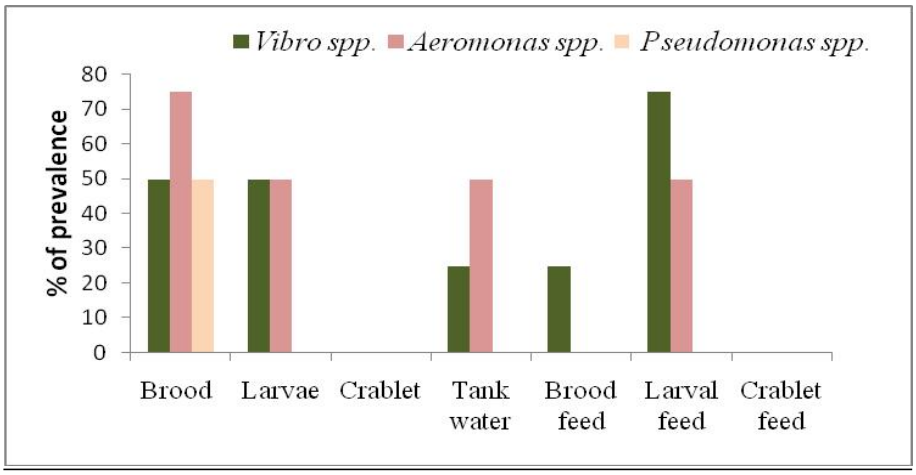


Fig 14. Prevelance of major chitinolytic bacteria infection in the BFRI-BS mud crab hatchery units.

11.2.3. Total bacterial and some chitinolytic bacteria in the brood

The brood crabs spawned at different batches had the TBC of $6.1x10^5$ – $6.8x10^6$ CFU/g (Table 30); the highest TBC was recorded in the 3rd batch. Regarding chitinolytic bacteria infection, *Vibrio* spp., *Aeromonas* spp. and *Pseudomonas* sp. were detected in the brood samples. It is of note that among 4 batches, 3 batches were infected with *Aeromonas* spp. Among *Vibrio* spp., from the brood mud crab, grown in the TCBS plates, the yellow colonies were dominant. Most of the yellow colonies were *V. alginoolyticus* and most of the green colonies were *V. parahaemolyticus*.

Table 30. Total and pathogenic bacterial load in the brood samples

	Total bacterial count (CFU/g)	*Vibrio spp. count (CFU/g)*	*Aeromonas Spp. count (CFU/g)*	*Pseudomonasspp. count (CFU/g)*
Batch 1	*4.3 - 4.8 x10^6*	*3.4 - 3.8 x10^3*	*2.0 - 3.4 x10^2*	*5.0 - 5.2 x10^2*
Batch 2	*6.1 - 6.6 x10^5*	*ND*	*3.0 x10^3*	*ND*
Batch 3	*5.7 - 6.8 x10^6*	*5.0 - 4.6 x10^2*	*2.0 - 2.6 x10^2*	*3.8 - 4.8 x10^2*
Batch 4	*0.96 - 4.4 x10^6*	*ND*	*ND*	*ND*

ND-not detected.

11.2.4. Total bacterial and some chitinolytic bacteria in mud crab larvae

The TBC of the mud crab larvae was between 6.4x 10^4 to 3.2x 10^5 cfu/g; the maximum load was found in the Zoea 3 stage while the minimum was detected in zoea 1 stage (Table 31). In case of chitinolytic bacterial infection, *Vibrio* spp. were detected in all the zoea stages (zoea1 to zoea 5). Among *Vibrio* spp. isolated from the larvae samples, *V. alginolyticus*, *V. harveyi* and *V. parahaemolyticus* were identified as dominant species. *Aeromonas* spp. were also detected in larvae in the period of the batch-1 and batch-2. There was no *Pseudomonas* spp. infection detected in the larval production units.

Table 31. Total pathogenic bacterial load in the zoea stages of crab larvae

Batch	***Bacterial count (CFU/g)***	***Larval stage***				
		Zoea 1	*Zoea 2*	*Zoea 3*	*Zoea 4*	*Zoea 5*
Batch-1	*TVC*	*ND*	*4.8- 6.4 x10^2*	*7.6-8.8 x10^2*	*1.3- 1.6 x10^2*	*1.4-1.7 x10^3*
	TAC	*ND*	*ND*	*ND*	*6.4- 4.4 x10^2*	*9.6- 8.0x10^2*
	TPC	*ND*	*ND*	*ND*	*ND*	*ND*
Batch-2	*TVC*	*ND*	*ND*	*ND*	*ND*	*ND*
	TAC	*ND*	*ND*	*2.3-2.8 x10^3*	*2.8-3.2 x10^3*	*ND*
	TPC	*ND*	*ND*	*ND*	*ND*	*ND*
Batch-3	*TVC*	*3.8- 4.6 x10^2*	*ND*	*ND*	*ND*	*ND*
	TAC	*ND*	*ND*	*ND*	*ND*	*ND*
	TPC	*ND*	*ND*	*ND*	*ND*	*ND*
Batch-4	*TVC*	*ND*	*ND*	*ND*	*ND*	*ND*
	TAC	*ND*	*ND*	*ND*	*ND*	*ND*
	TPC	*ND*	*ND*	*ND*	*ND*	*ND*

TVC-Total *Vibrio* spp. count; TAC-Total *Aeromonas* spp. count; TPC- Total *Pseudomonas* spp. count; ND-Not Detected

11.2.5. Total bacterial and some chitinolytic bacteria in crablet

The TBC of the crablet samples was between $8.6x10^4$ - $8.2x10^5$ CFU/g. The crablet feed had the TBC of 1.6 x 10^4 - $2.2x10^4$ CFU/g. There was no *Vibro spp., Aeromonas spp.*and *Pseudomonas spp.* infection in crablet samples collected from BFRI-BS crablet nursery units.

The present study determined the prevalence of *Vibrio* spp. *Aeromonas* spp. and *Pseudomonas* spp. in the mud crab hatchery units (brood crab, larvae, larval tank water, larval feed and crablet). *Vibrio* spp. *Aeromonas* spp. and *Pseudomonas* spp. were dominant in the brood rearing tanks, which is in accordance with Lavilla-Pitogo et al. (2001) reporting 50-70% of bacteria isolates as chitinoclastic bacteria including *V. vulnificus*. The tank held mud crab broodstock had the TBC of $2.2x10^3$ – $8.0x10^5$ cfu/g and the TVC of $5.0x10^2$ – $7.0x10^4$ cfu/g (Lavilla-Pitogo et al. 2001); similar results were found in our study. Though broods are regularly cleaned in the hatchery, sometimes microbial aggregates, injury from handling and sand abrasion may favour these chitinolytic bacteria causing severe shell damages on the dorsal region of the body.
In mud crab hatchery, Vibriosis is a major concern causing high mortality (Lavilla-Pitogo & De la Peña, 2004), and inconsistent survival of mud crab larvae (Mann et al. 1998; Liessmann, 2005). *V. hraveyi, V. parahaemolyticus, V. alginolyticus* are pathogenic to mud crab zoea (Boer et al. 1993; Parenrengi et al. 1993). The present study identified *Vibrio* spp. infection in the hatchery units; among *Vibrio* spp. isolated, *V. alginolyticus, V. fluvialis, V. harveyi, V. parahaemolyticus* and *V. vulnificus* were predominant, and these *Vibrio* spp. are considered as pathogenic to adult and larval crustacesan (Hisbi et al. 2000); Talpul et al. 2011, Gopal et al. 2005, Liu et al. 2016, Lavitha and Thampuran, 2012). Because of ubiquitous nature, higher multiplication rates and well adaptation to environmental changes, *Vibrio* spp. infection is a very frequent phenomenon in aquaculture systems. *V. harveyi, V. alginolyticus and V. parahaemolyticus* have been frequently reported in association with shell disease outbreaks in shrimps (Esteve and Herrera, 2000; Soto-Rodriguez et al. 2010; Soto-Rodriguez et al., 2012). Gunasekaran, et al. (2019) reported *V. alginolytigus* involvement in mortality of *S. serrata* culture farms. *V. harveyi* has also been isolated from infected *S. tranquebarica* (Poornima et al. 2012). *V. harveyi* is often an opportunistic bacteria (Karunasgar et al. 1998).
It is of note that the crablets had no infection with *Vibrio, Aeromonas and Pseudomonas* which were detected in brood and larval rearing units. Continuous changing of tank water, use of probiotics and antibiotics might reduce pathogenic load gradually in the later stages of mud crab larvae.

11.2.6. Bacterial infection and load in mud crab farms

Vibrio spp., *Aeromonas* spp., and *Pseudomonas* spp. were identified in the crabs collected from the mud cabs farms. The highest prevalence rate (81.57%) was found in case of *Vibrio* spp. followed by *Aeromonas* and *Pseudomonas* (Fig.15). According to the suggestions from the 1st annual workshop, some specific bacteria including *Salmonella* and *Shigella* which are human pathogen and can be horizontally and/or vertically transmitted from crabs. The bacterial analyses show that the total bacterial count ranged between 1.6 -2.0 x 10^5, $1.6x10^6$ - 3.0 x 10^8 , 3 $.8x10^5$ – $1.0x10^7$ CFU/gm in mud crabs from Satkhira, Khulna and Bagerhat areas, respectively.

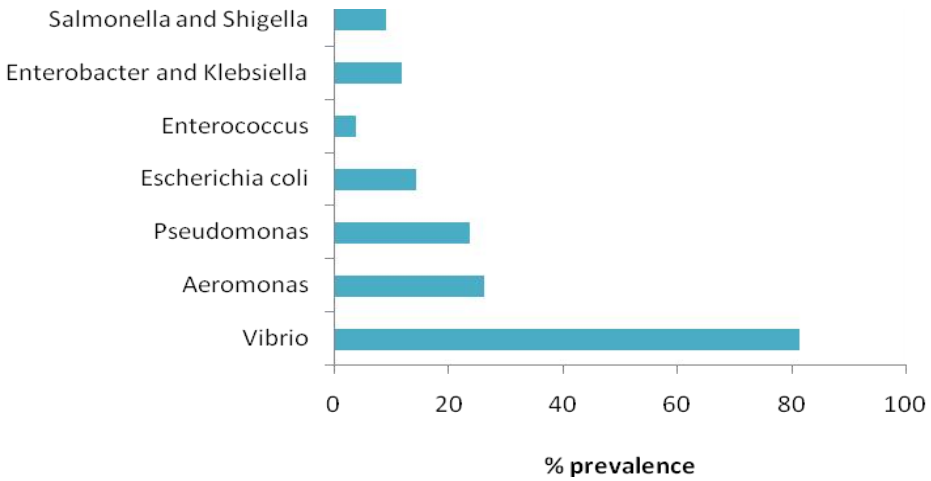


Fig. 15. Prevalence % of specific bacteria in mud crabs collected from farms.

Table 32. shows the load of specific bacteria in farmed mud crabs. Among *Vibrio* isolates, *V. parahaemolyticus* (22.5%), *V. alginolyticus* (17.45%), *V. harveyi* (12.05%), *V. vulnificus* (6.5%), *V. cholera* (28.06%), *V. mimicus* (7.3%) and *V. fluvialis* (6.14%) were identified. Similarly, there are reports of chitinolytic *vibrios* in mud crabs: *Vibrio vulnificus, V. parahemolyticus, V. splendidus,* and *V. orientalis* in Philippines (Lavilla-Pitogo et al., 2004); *V. harveyi, V. alginolyticus* in India (Poornima et al., 2012; Gunasekaran et al., 2019) and in Indonesia (Sarjito et al. 2018).

Table 32. Specific bacterial load (CFU/gm) in mud crabs collected from farms.

Bacteria	Satkhira	Khulna	Bagerhat
Vibrio spp.	$6.5 \times 10^2 - 3 \times 10^4$	$3.5 \times 10^3 - 6.0 \times 10^5$	$4.6 \times 10^3 - 3.2 \times 10^4$
Aeromonas	ND	ND	$1.3 \times 10^3 - 2.0 \times 10^3$
Escherichia coli	$2 \times 10^2 - 1.6 \times 10^4$	$4.0 \times 10^2 - 1.0 \times 10^5$	$8.0 \times 10^3 - 2.3 \times 10^4$
Enterococcus	$6.0 - 7.0 \times 10^2$	$1.5\text{-}2.5 \times 10^4$	1.1×10^4
Enterobacter and *Klebsiella*	$2 \times 10^2 - 1.6 \times 10^4$	$1.0 \times 10^4 - 4.5 \times 10^4$	$6 \times 10^3 - 3.5 \times 10^4$
Salmonella and *Shigella*	$2.5 \times 10^2 - 2.8 \times 10^4$	$3.0 \times 10^4 - 1.0 \times 10^5$	$5.0 \times 10^3 - 2.2 \times 10^4$
Pseudomonas	ND	ND	$5.0 \times 10^2 - 4.5 \times 10^3$

Fish and shellfish are not only themselves prone to microbial diseases but also are sources of disease transmission to human population. Crabs harbor a diverse amount of bacterial flora, and accordingly food safety and quality of mud crabs for human consumption are matter of concerns. The present study found some farmed crabs contaminated with *E. coli, Salmonella* and *Shigella*; but their contamination was relatively low (14% *E. coli* and 9% *Salmonella* and *Shigella*). Moreover, the *E. coli* load was found similar to Karim et al. (2015) reporting the total *E. coli* counts ranged from 1.25 to 4.33×10^4 cfu/g, which is within the acceptable limit for meat crab recommended by International Commission of Microbiological Specification for Foods (ICMSF, 1988). However, the *Vibrio*, *salmonella* and *Shigella* counts in the present study exceeded the acceptable limit; Karim et al. (2015) has also reported similar counts exceeding the acceptable limit for *Vibrio*, *salmonella* and *Shigella* in crabs. The counts of

these specific bacteria indicate that farming practice has not been scientifically adequate, and poor hygiene and sanitation prevailed; good aquaculture practice (GAP) and hazard analysis and critical control point (HACCP) systems are to be applied to avoid such contamination level in mud crab farms.

11.2.7. Bacterial disease incidence in mud crab (*S. olivacea*) farms

In the third year of the project period (2020-21), mud crab farms were visited to detect disease incidence in mud crab farms. But due to COVID-19 pandemic situation and lock-down, the export of mud crabs were closed for months, and thus most of the farms were not in the operation; very few samples were collected, which were no enough to have robust data on disease incidence in mud crab farms. However, FGD with hard-shell and soft-shell farmers, and *in situ* observations were made. Most of the farmers addressed low mortality in hard-shell farms but high mortality in soft-shell farms (Table 33; Fig.16); the mortality ranged between 2-7% in hard shell farms and 10-30% in soft-shell farms.

Table 33. Mortality and disease incidence in soft- and hard-shell mud crab farms in the South-west coastal region of Bangladesh

Practice	**Level of mortality**		**Level of disease incidence (%)**	
	Hard-shell farms	Soft-shell farms	Hard-shell farms	Soft-shell farms
Fattening	Few; occasionally	-	1-2%; shell perforation, leg broken	No experience
Monoculture	Low	-	1-3%; shell perforation, leg broken; gill fouling; green color mats on gills	No experience
Mixed culture	Low; sometimes moderate	-	3-4%; shell perforation, leg broken; gill fouling; green color mats on gills; insects (~parasites) in gills	No experience
Cage culture	Few; occasionally	High; 10-30%;	Leg broken, sudden death	No experience

Source: KU-FMRTD FGD 2020-21

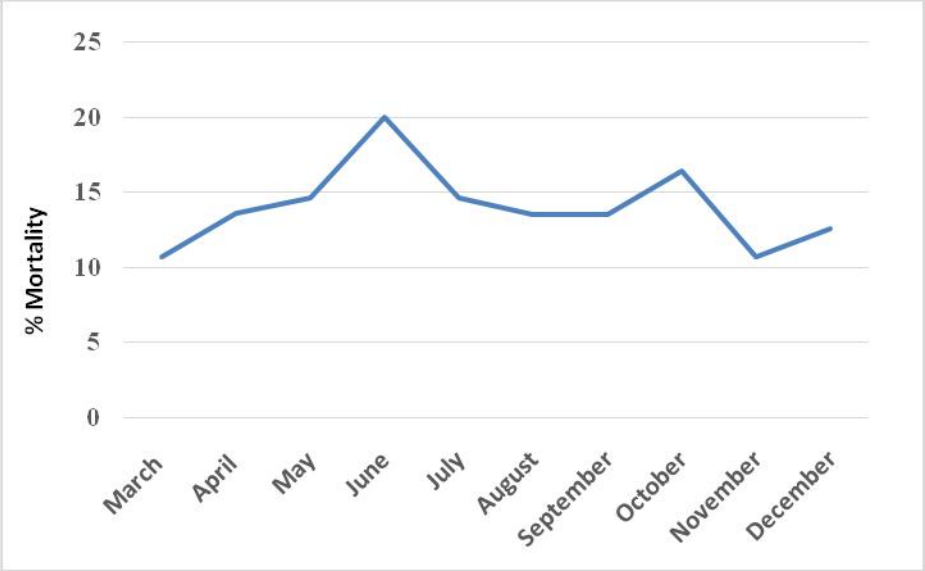


Fig.16. Monthly trend of mortality (%) in soft-shell farms.

The analysis of few samples available in some farms showed that a few samples were found apparently unhealthy mud crabs with discolored patches, soft and black exoskeleton compared with healthy mud crabs from the same ponds/pens. Table 34 shows the load of different chitinolytic bacteria identified from the healthy and unhealthy crabs in the present study; these bacteria causes chitin degradation of exoskeleton as reported by Lavilla-Pitogo et al. (2004).

Table 34. Chitonolytic bacteria load (cfu/g) of healthy and apparently unhealthy mud crabs collected from farms

Bacteria Count	Hard-shell		Soft-shell	
	Healthy Mud crab	Unhealthy Mud crab	Healthy Mud crab	Unhealthy Mud crab
Total Bacteria	$1.2x10^6$ - $3.0x10^7$	$3.0x10^6$ – $2.2x10^8$	$1.7x10^5$ - $4.0x10^7$	$2.5x10^7$ - $1.6x10^8$
Vibrio spp.	$4.4x10^2$ - $2.8x10^4$	$9.0x10^2$ – $1.0x10^5$	$3.6\text{-}4.0x10^3$	$8.6x10^4$ - $2.2x10^5$
Aeromonas	$1.7\text{-}8.2x10^2$	$7.6\text{-}8.2x10^3$	$4.0\text{-}4.5x10^3$	$1.4x10^3$ – $1.3x10^4$
Pseudomonas	ND	$2.3\text{-}2.4x10^3$	$2.3\text{-}2.4x10^3$	$5.5\text{-}6.0x10^3$

The biochemical tests identified *V. parahaemolyticus*, *V. cholera*, *V. mimicus*, *V. vulnificus*, *V. fluvialis* isolates from the healthy crabs while *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. cholera*, *V. mimicus*, *V. vulnificus*, *V. fluvialis* from the unhealthy crabs. The end-point confirmation test for 5 bacterial isolates using 16S rRNA gene sequence produced the expected 1465-bp PCR amplification product as shown in Fig 17. The phylogenetic analysis found close homology of the isolates' sequences with reference *Vibrio, Aeromonas* and *Pseudomonas* (Fig.18).

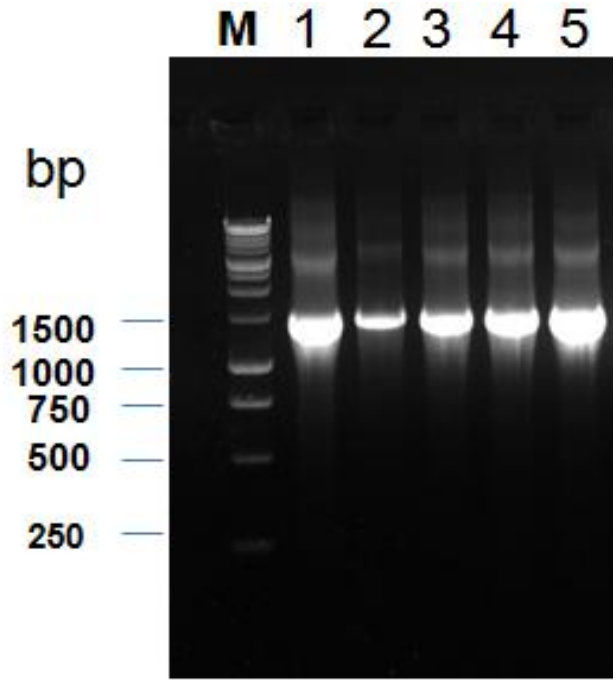


Fig.17. Gel electrophoresis image of PCR product of 5 bacterial isolates. M: 1 kb DNA marker, 1-5: all bacterial isolates with the expected ~1500-bp PCR amplification product.

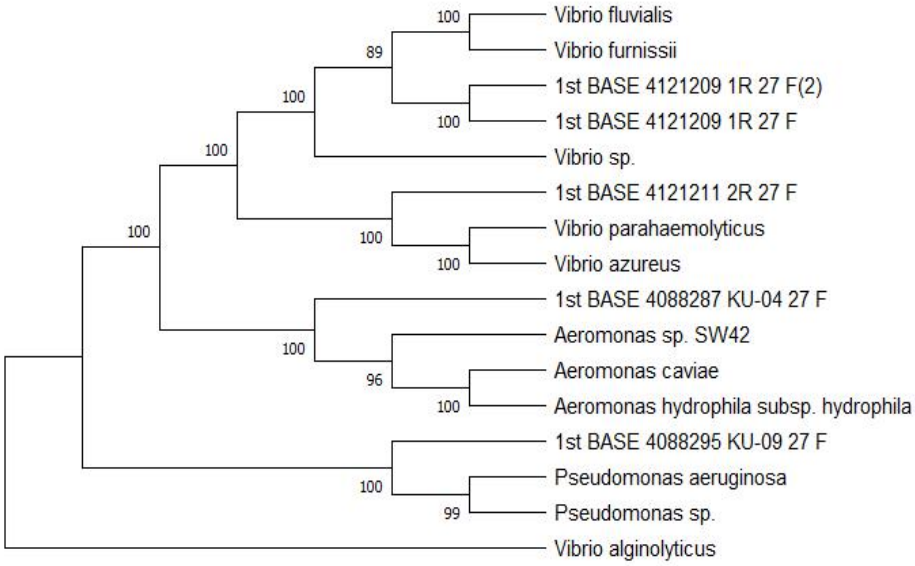


Fig. 18. Phylogram showing the relationship between bacteria isolated in this study (red rectangles) with related species retrieved from the NCBI database. Bootstrap supported value based on 1000 replicates.

More than75% unhealthy samples were infected *with V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus* where as < 40% healthy samples were infected with at least one or more of these pathogens. Most of the unhealthy crabs were also infected with *Aeromonas* spp. (>65%) and *Pseudomonas* spp. (>40%) while in healthy crabs, *Aeromonas* spp. was detected in <40% and *Pseudomonas* spp. was detected in <25% samples.

The histopathological studies revealed that the healthy crabs had intact exoskeleton but the unhealthy (i.e. diseased) crabs had degenerated shell; the membranous layer and epithelium were ruptured (Fig.19 A, B). In case of severe erosion of the carapace, there was degenerated skeletal muscle and accumulation of eosinophilic granular cells (Fig.19 C, D) while the skeletal muscle of the healthy crabs was integral. The gill of the healthy crabs had normal lamellae but the diseased crab had swollen and club shaped lamellae (Fig.19 E, F).

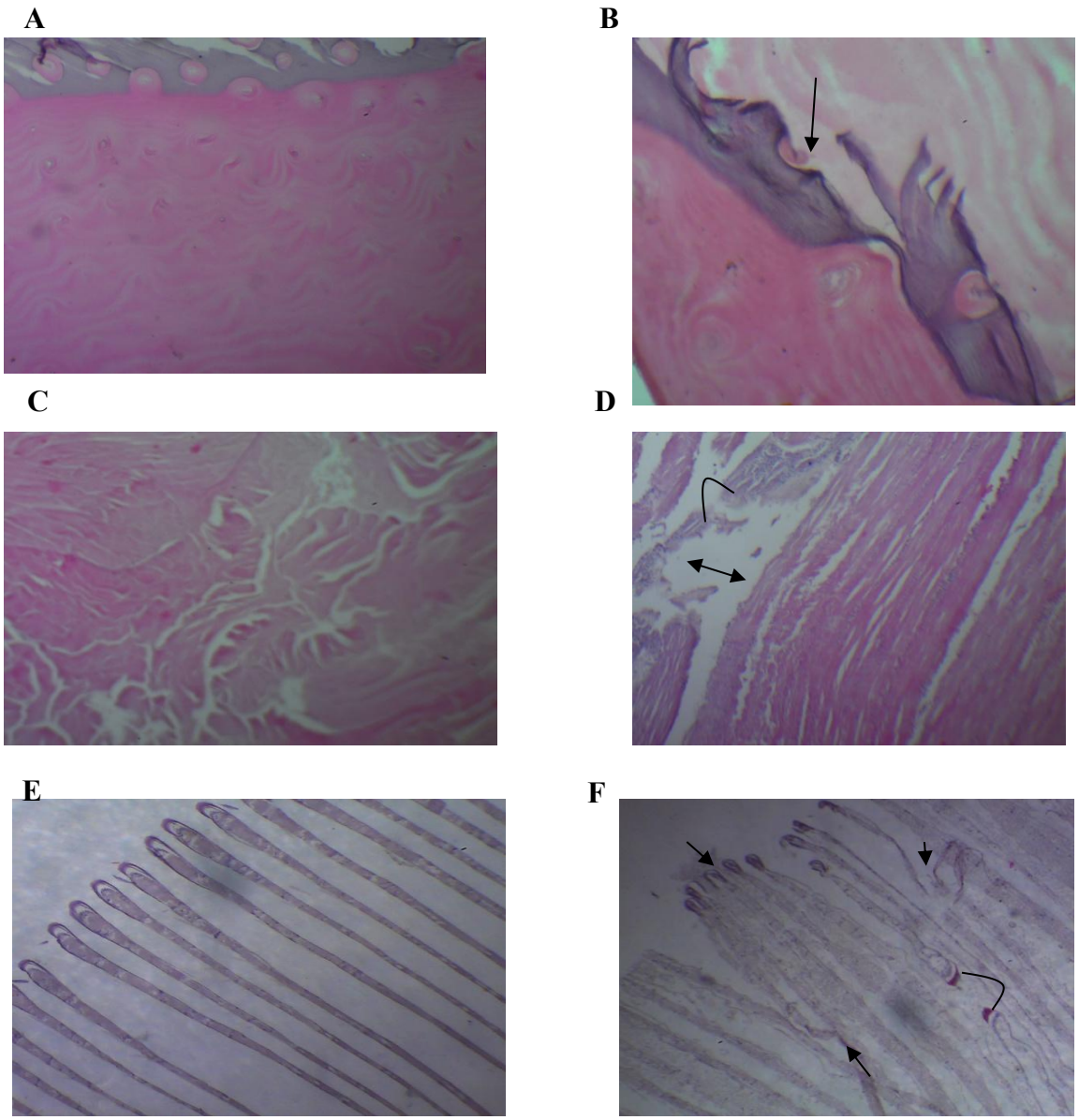
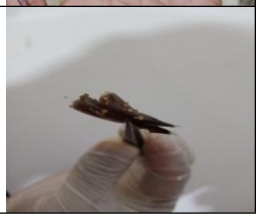


Fig. 19. Histological section of healthy and diseased mud crab S. olivacea. (A) Normal carapace exoskeleton, (B) erosion of carapace skeleton (arrow), (C) Normal skeletal muscle, (D) severe damage of membrane layer (double arrow) and eosinophilic granular haemocytes (curve), (E) Normal gill lamellae, (F) Infected mud crab gill with swollen lamellae (arrow) and haemocyte infiltration (curve).

The present study characterized the diseases occurred in the mud crabs collected from the wild and farms (Table 35).

Table 35. Disease occurrences in mud crab farms

Diseases occurrences	Characteristics /external feature/symptoms	Incidence level/rate (%)	Major isolated bacteria	Mud crab origin	Pictorial views
Shell disease	Spotted shell, shell erosion and perforation, discolor patches on carapace	12-16	Vibrio spp, Aeromonas spp. Pseudomonas spp.	Wild, farm	
Vibriosis	Moribund, blackish gill, loss of appendages	15-20	V. harveyi V. parahaemolyticus V. mimicus Aeromonas spp.	Wild, farm	
Brown spot	Small to medium brwon spot on shell and abdominal site	6-8	V. harveyi Pseudomonas spp.	Wild, farm	
Orange crab disease	Weak/moribund	6-10	V. harveyi V. parahaemolyticus	Wild, farm	
Red sternum syndrome	Deep brown abdominal portion	4-5	V. parahaemolyticus V. mimicus	Wild	
Gill Fouling	Fouling inside gills with herbs like filaments	10-15	V. harveyi V. parahaemolyticus V. mimicus V. fluvialis Aeromonas spp. Flexibacter spp.	Farm	

11.2.8. Association of other driving factors (i.e. feed, soil and water quality) with infection/diseases occurrence in mud crab, *S. olivacea*

In accordance with the activities of the second year, water, soil, and feed samples collected from the BFRI-BS mud crab hatchery and different farms located in the Satkhira, Khulna and Bagerhat districs. The TBC of brood feed was between $6.0x10^4$ - $2.5x10^5$ CFU/g, and the mean TVB of $1.3\pm0.51 \times 10^3$ CFU/g; most of *Vibrio* spp. were *V. cholera* (24%), *V. parahaemolyticus* (16%), *V. mimicus* (30%). The TBC of the sand from the brood tank was between 4.32- $8.32x10^2$ CFU/g; but there was no infection with *Vibrio, Aeromonas* and *Pseudomonas* bacteria.

The larval tank water had the TBC of 0.5 – 7.4 $x10^5$CFU/g, and the highest TBC was found in the 1st batch (Table 36). The TBC was from $7.3x10^3$ to $4.0x10^6$ in larval feed *Artemia*, and from 8.4x10 to $3.6x10^6$ in rotifer (Table 37). The highest TBC was also recorded in both larval feed *Artemia* and rotifer used in the 1st batch. The larval feed had also *Vibrio* spp.

Table 36. Total and pathogenic bacterial load in tank water

Larval Batches	*Total bacterial count (CFU/g)*	*Total Vibrio spp. count (CFU/g)*	*Aeromonas spp. count (CFU/g)*	*Pseudomonas spp. count (CFU/g)*
Batch-1	*7.4- 6.6 x10^5*	*7.3- 7.8 x10^2*	*7.2- 8.4 x10^2*	*ND*
Batch-2	*1.6-1.8 x10^5*	*ND*	*2.2- 3.4 x10^3*	*ND*
Batch-3	*7.2- 8.2 x10^4*	*ND*	*ND*	*ND*
Batch-4	*0.55- 1.2 x10^5*	*ND*	*ND*	*ND*

Table 37. Total and pathogenic bacterial load in larval feed

Larval Batches	*Sample ID.*	*Total bacterial count (CFU/g)*	*Total Vibrio spp. count (CFU/g)*	*Aeromonas spp. count (CFU/g)*	*Pseudomonas spp. count (CFU/g)*
Batch-1	*Artemia*	*3.8-4.0 x10^6*	*7.6-8.4 x10^2*	*7.2-7.5 x10^2*	*ND*
	Rotifer	*3.2-3.6 x10^6*	*1.3-1.4 x10^3*	*4.2-5.5 x10^2*	*ND*
Batch-2	*Artemia*	*1.6 -2.2 x10^4*	*ND*	*ND*	*ND*
	Rotifer	*0.95-1.8 x10^6*	*6.6-8.2 x10^2*	*5.0-6.2 x10^2*	*ND*
Batch-3	*Artemia*	*3.3 - 4.5 x10^4*	*7.6-8.2 x10^2*	*ND*	*ND*
	Rotifer	*8.4 -8.8 x10^4*	*4.6-6.4 x10^2*	*ND*	*ND*
Batch-4	*Artemia*	*7.3- 8.7 x10^3*	*ND*	*ND*	*ND*
	Rotifer	*2.7 - 3.0 x10^5*	*1.0-1.4 x10^3*	*ND*	*ND*

The prevalence of *Vibrio* spp. were frequently detected in larvae, tank water and feed during the 1st batch larval production. The tank water was predominantly contaminated with *V. fluvialis* (35%) and *V. alginolyticus* (35%) and *V. cholerae* (30%). *V. cholerae, V. alginolyticus* contamination was also found in both *Artemia* and rotifer samples, and *V. harveyi, V. parahaemolyticus* in only rotifer sample. *Aeromonas* spp. were also detected in larval tank water and feed, in the period of the batch-1 and batch-2. There was no *Pseudomonas* spp. infection detected in the larval production units.

The crablet feed had the TBC of 1.6 x 10^4 - 2.2x10^4 CFU/g. It is of note that the crablet unit had no infection with *Vibrio, Aeromonas and Pseudomonas* which were detected in brood and larval rearing units. Continuous changing of tank water, use of probiotics and antibiotics might reduce pathogenic load gradually in the later stages of mud crab larvae.

In this study, feed sample collected in the wet season had *Vibrio* contamination which is an important source of microbial pathogens entry to the rearing system. Usually, broods are fed with minced tilapia which were washed thoroughly to remove dirt and pathogens. Hence the chance of broods to be infected with pathogenic bacteria by feed stuff is very low. However, in wet season sources of water might be contaminated in various ways. Therefore, precautions are needed to use water in the hatchery unit to avoid any undesired contamination.

The present study demonstrated that the TBC ranged between 5.6 x 10^5 – 2.2 x 10^7 cfu/gm in soil, 9.0x10^5 – 9.2x10^6 cfu/gm in water samples and 1.2x10^5 – 2.5x10^5 cfu/gm collected from Satkhira; 2.2 x 10^6 – 6.5 x 10^8 cfu/gm in soil, 2.2x10^6 – 4.5x10^7 cfu/gm in water and 4.2x10^5 – 1.5x10^6 in feed samples collected from Khulna; 3.1 x 10^5 – 1.2 x 10^7 cfu/gm in soil, 2.5x10^7 – 1.8x10^8 cfu/gm in water and 9.2x$10^{\4}$ – 1.3x10^5 in feed samples collected from Bagerhat areas. The load of specific bacteria in water, soil and feed samples collected from mud crabs farms of Satkhira, Khulna and Bagerhat districts (Table 38, 39, 40). *Vibrio* spp. were detected in water, soil and feed samples from the three locations; some samples from Satkhira were contaminated with *Aeromonas* spp.; but *Pseudomonas* spp. were rarely detected in the water, soil and feed samples. Such results indicate that feed can be a source of *Vibrio* spp. entry into the farms.

Table 38. Specific bacterial load (CFU/gm) in soil collected from farms

Bacteria	Satkhira	Khulna	Bagerhat
Vibrio spp.	4.5 x 10^3 – 5.2 x 10^4	2.8 x 10^4 to 4.6 x 10^4	2.2 x 10^4 to 1.9 x 10^5
Aeromonas	5x10^2	ND	ND
Escherichia coli	2.8x10^3 - 2.5x10^4	4.0 x 10^4 to 5.0 x 10^4	6x10^3
Enterococcus	2.0x10^2 – 2.4x10^3	1.3x10^4	4.3x10^3
Enterobacter and Klebsiella	2x10^2 - 2x10^3	1.2 x 10^4 to 4.0 x 10^4	1.3-1.5x10^4
Salmonella, and *Shigella*	2.5x10^2 – 4x10^4	1.7 x 10^5 to 5.0 x 10^5	1.3 - 7.5x10^4
Pseudomonas	1.5 -4.2x10^3	ND	ND

ND=not detected.

Table 39. Specific bacterial load (CFU/gm) in water collected from farms

Bacteria	Satkhira	Khulna	Bagerhat
Vibrio spp.	5.4 x 10^2 - 3.2 x 10^3	2.0 x 10^4 - 2.2 x 10^5	2.6 x 10^4 - 2.8 x 10^6
Aeromonas	5x10^2	ND	ND
Escherichia coli	2.5x10^2 – 1.8x10^4	2.0 x 10^3 to 1.7 x 10^5	1.9x10^4
Enterococcus	2.5x10^2 – 2.8x10^3	3x10^4	1.6x10^4
Enterobacter and Klebsiella	3x10^2 – 1.0x10^4	9.0 x 10^3 to 5.0 x 10^4	2.7x10^4
Salmonella and *Shigella*	4.5x10^2 – 1.0X10^4	2.2x10^4 – 3.0X10^5	1.3-5.6x10^4
Pseudomonas,	ND	ND	ND

ND=not detected.

Table 40. Specific bacterial load (CFU/gm) in feed collected from farms

Bacteria	Satkhira	Khulna	Bagerhat
Vibrio spp.	7.6x 10^3 – 8.0x10^4	1.1-1.3x10^3	6.5-8.0x10^4
Aeromonas	5x10^3	ND	3-4.5x10^2
Escherichia coli	1.0-2.5x10^3	1.5x10^2	1.2x10^2
Enterococcus	4.5x10^3	2.0x10^2	1.6x10^2
Enterobacter and *Klebsiella*	2x10^3	4x10^2	4x10^2
Salmonella and *Shigella*	2.5-5.0x10^3	3x10^2	5x10^2
Pseudomonas	ND	ND	ND

Since the present study reveals that *Vibrio* spp. were the most dominant species in the mud crab production units of Bangladesh, an experimental investigation was conducted to determine the pathogenic load of *Vibrio* sp. causing vibriosis in mud crabs. For the challenge

test, of 1×10^6, 1×10^7 and 1×10^9cfu/ml *Vibrio* sp. were inoculated in the treatment groups T_1, T_2, T_3, respectively; the control group had no *Vibrio* inoculum. During the challenge period, crabs were fed with chopped Tilapia. This study reported that there was a significant difference of mortality rate ($P<0.05$) between the control and the treatment groups (Table 41). The control group had no mortality but the treatment groups had 50% cumulative mortality rate (CMR) on 6 day post challenge (Fig. 20). The first 100% CMR was observed in the T_2 group, and accordingly, the *Vibrio* load > 10^6cfu/ml was found to cause acute symptoms of Vibriosis in *S. olivacea* in a short period of time.

Table 41. Mortality (%) of the experimental mud crabs

Day After challenge	Mortality (%)				Disease symptoms
	Control	Treatment$_1$[b]	Treatment$_2$[b]	Treatment$_3$[b]	
Day 0[a]	0	0	0	0	
Day 1[a]	0	0	0	0	
Day 2[b]	0	25	25	0	No
Day 3[a]	0	0	0	0	
Day 4[a]	0	0	0	0	
Day 5[a]	0	0	0	0	
Day 6[b]	0	25	25	0	White patch on shell
Day 7[b]	0	0	25	25	Soft abdomen shell
Day 8[c]	0	25	25	25	Small black spot on the carapace
Day 9[c]	0	0	-	50	Soft abdomen shell

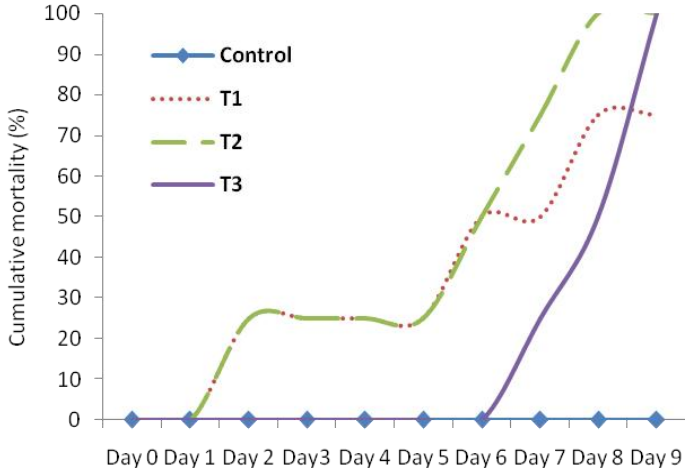


Fig. 20. Cumulative mortality % of mud crab challenged with *Vibrio* sp.

In addition, the count of *E. coli, Salmonella* and *Shigella*, which are human pathogenic, in mud crabs, water, soil feed samples from the farms indicates that there is lack of hygienic and sanitation maintenance, sound post-harvest practices (i.e. mishandling) along the production

channel of mud crab. The load of these bacteria also address another concern, the quality of water being entered in the farms form the surrounding water sources.
In the present study, the water temperature, dissolved oxygen (DO), pH and salinity of mud crab farms located in Satkhira, Khulna and Bagerhat districts were recorded (Table 42.). The lowest salinity range was found in mud crab farms of Bagerhat. Besides, higher mortality occurred in the Bagerhat areas (Fig. 16).

Table 42. Temperature, pH and Salinity (range)

Water parameters	Satkhira	Khulna	Bagerhat
Temperature ^{0}C	24.6-25.1	24.6-26	25.2-26.1
DO ppm	6.8-8.9	7.6-8.2	7.8-8.6
pH	7.2-8.2	7.7-8.7	8.3-8.7
Salinity ppt	10-14	0-11	0-6

Accordingly, a study was conducted to evaluate the effect of salinity and feeding regime on the survival of mud crab *(Scylla Olivacea)*. Mature but lean ("empty") female mud crabs were fatted in cages feeding with chopped tilapia fish twice a day at a rate 8% of the total body weight; 30% in the morning and 70% in the afternoon. In this experiment, the stocking density was 5/m^2 , river water (0 and 3 ppt) and daily feeding and one-day interval feeding strategies were used; accordingly there were four treatments named, S_3d_f (3ppt and daily feeding), S_3d_{if} (3 ppt and 1 day interval feeding), (0 ppt and daily feeding) and S_0d_{if} (0 ppt and 1 day interval feeding) with four replicates for each treatment group. The fattening period was 21 days.

At the end of the experiment, the highest survival was high in the pond of salinity (3ppt) with daily feeding and the lowest survival in the pond of low salinity (0 ppt) with 1 day interval feeding (Fig. 21). In the present study it was found that there was significant effect ($p< 0.05$) of salinity on survival but there was no significant ($p> 0.05$) effect of feeding regime on survival.

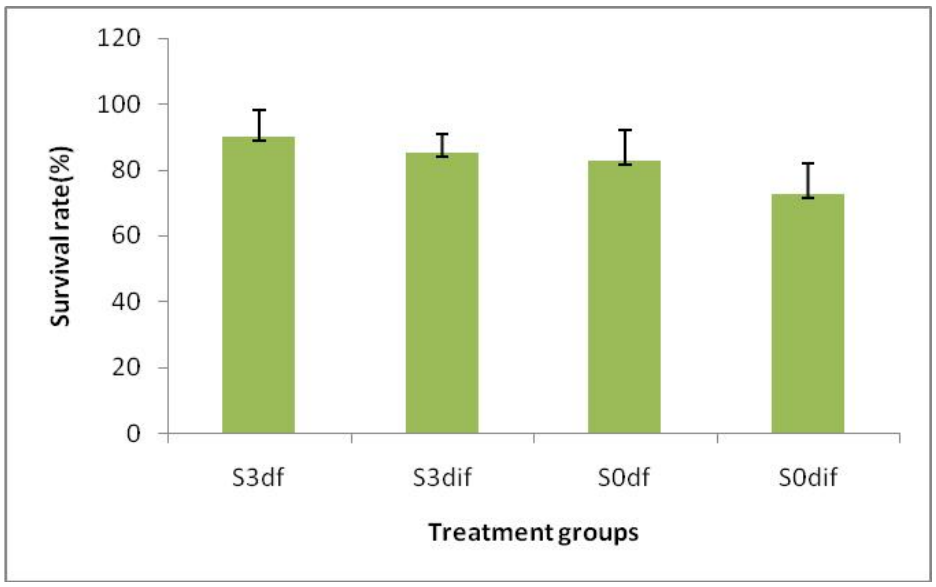


Fig. 21. Survival rate (%) of mud crab (*S. olivacea*) in different treatments

So, the present study has determined that the water quality, particularly salinity affects the survival of mud crabs in farms. Moreover, regarding occurrence of diseases and mortality in farms, a number of issues/factors which are linked to mud crab mortality occurrence in the hard-shell and soft-shell farms was revealed through FGD in the present study (Table 43). Specifically, crabs collected from forest died substantially once released in farms, particularly low saline farms; such mortality is likely linked e to the combined effect of salinity stress and physical stress due to long distance as well as transportation hazard. Farmers also highlighted temperature is an important factor causing high mortality; high to moderate mortality was observed in hard-shell and soft-shell farms from summer to early autumn. The soft-shell farms had higher mortality in the month of May and June when temperature was very high (Fig. 16). It is of note that the cages of the soft-shell farms are kept in the surface water, and the height of the cage is very low (15 cm); therefore, during hot season, crabs in the soft-shell cages were highly stressed due to higher water temperature. In rainy season, mud crabs in the farms are also stressed due to fall in salinity and variation in pH; so crabs becomes more prone to opportunistic pathogens, and thus diseases occur in mud crab population. Another issue, stocking density, is matter of concern; in all types of culture systems more than 95 percent farmers stocked crabs all the year round, but, the highest rate of stocking was reported from late April to May when a lot of wild small mud crabs are available, and from October to December when after demand is high in the export market in January – March, particularly for new year festival in China.

Table 43. Major issues linked to mud crab mortality addressed by farmers in South-west coastal region of Bangladesh

Determinants, Factors/issues	Practices/experiences	Level of mortality		Level of disease incidence (%)	
		Hard-shell farms	Soft-shell farms	Hard-shell farms	Soft-shell farms
Culture systems	Fattening	Few; occasionally	-	1-2%; shell perforation, leg broken	-
	Monoculture	Low	-	1-3%; shell perforation, leg broken; gill fouling; green color mats on gills	-
	Mixed culture	Low; sometimes moderate	-	3-4%; shell perforation, leg broken; gill fouling; green color mats on gills; insects (~parasites) in gills	-
	Cage culture	Few; occasionally	High	Leg broken, sudden death	10-30%;
Sources of crabs					
	Adjacent rivers	Very low	Moderate	-	-
	Ghers	Very low	Moderate	-	-
	Forests	Moderate; sometimes high	High	-	-
Quality of crabs					
	Soft	High	-	-	-

Determinants, Factors/issues	Practices/experiences	Level of mortality		Level of disease incidence (%)	
		Hard-shell farms	Soft-shell farms	Hard-shell farms	Soft-shell farms
	Broken legs	Low	-	-	-
	Carapace shell discoloration	No	-	-	-
	Shell shed	-	Just before and/or during shell shedding , high mortality	-	-
	Shell perforation	Low	-	-	-
Stocking density	High	Moderate	High	Moderate	High
Transportation	Longer; crabs from distant places	Moderate; sometimes high	High	low	High
Feed type		Fewer cases	No	No	No
Feeding regime		No	No	No	No
Water quality					
	Salinity	No experience	No experience	No experience	No experience
	Temperature	High	High	High	High
Soil quality		Low	Moderate	No experience	No experience
Season					
	Summer	High;	High	Shell perforation, leg broken; gill fouling; green color mats on gills	No experience
	Rainy	High	High	3-4%; shell perforation, leg broken; insects (~parasites) in gills	No experience
	Autumn	Moderate-high	Moderate-high	shell perforation, leg broken; insects (~parasites) in gills	No experience
	Late Autumn	low	low	shell perforation, leg broken	No
	Winter	low	low	shell perforation, leg broken	No
	Spring	low	low	shell perforation, leg broken; green color mats on gills;	No

12. Research highlights (Title of the sub-project, background, objectives, methodology, key findings and key words):

Title of the sub-project: Adoption of Innovative Technology: Seed production to Fattening of Mud crab (Scylla olivacea) and Health Management in Bangladesh Condition

12.1. Component 3 (BFRI-BS)
12.1.1 Research highlight-01
Background

Traditionally, mud crab aquaculture in Bangladesh started before 100 years by few enthusiastic farmers with fattening of lean crabs to develop gonad in the body cavity to make export quality to obtain premium price. Farming practice changed as time passed. Besides, number of farms, depots and production has been changed simultaneously. However, sufficient documented information is therefore, scares on mud crab resources. Before starting the project trials, collection of sufficient information on present status of mud crab resource was obvious. Thus, this survey aimed to collect sufficient multidimensional information on current status of mud crab resources in the coastal region of Bangladesh.

***Objective*:** Uunderstanding and documentation of present status of mud crab resource in Bangladesh.

Methodology
Data collected through questionnaire survey, FGD and literature review.

Key findings
Satkhira, Khulna and Bagerhat district produces majority of the crabs. Crab production farms and depots have been increased with time passed. Recently production of mud crab gradually decreasing in Bangladesh. Average size of crab also decreased dramatically. Recent interventions of soft shell farms caused indiscriminate harvest of both adult and juveniles limiting the recruitment in natural stocks. Most of the soft shell farms are not getting adequate small crabs for year round operation. Some farms already closed and rest operated partially. Farmers are interested on hatchery produced crablets.

Keywords: Mud crab, Status, *Depot*, Farms, Production

12.1.2 Research highlight-02

Background
One of the remarkable obstacles for crab seed production in hatchery condition is the unavailability of quality berried brood crab. Potential female broods migrate to the deep sea in quest of suitable environmental conditions for spawning, embryonic development and larvae survival. Therefore, collection of broods from the deep sea is laborious, hardy, risky and expensive also. Very often, collected broods remain infected with various diseases and some broods did not provide good quality larvae. Hence, development of qualitative and quantitative berried broods in captive condition is the best solution for smooth hatchery operations.

***Objective*:** To produce qualitative and quantitative berried broods for smooth hatchery operation.

Methodology
Development of suitable environment likely the natural breeding places for spawning. Providing good spawning vessel, sand bed bottom, aeration, 27-30 ppt sea water and shedding with orchid net for providing darkened. Superior and intact gravid broods were collected from a clean water sources to avoid fouling. Transportation was done in wet condition with source water and aeration. Broods were acclimatized with hatchery conditions. Eyestalk of the broods were unilaterally ablated and transferred in the spawning tanks. At least 30-40% water were exchanged 3-4 times in a week. Feeding was done with a weekly schedule of various feed items like brackishwater/marine water fish, mussel meat, blood cockles, pollychaets, squid, etc. and were supplied twice a day @10-15% of biomass. Berried broods may spawn within 3-60 days of collection. Berried broods were taken out from spawning tanks and transferred into the hatching tanks prepared with 27-30 ppt seawater and aeration. Berried broods were fed for 1st 3 days then seized the feeding. Uneaten feed were siphoned after 1 hour of feed supply. Hatching usually starts in the very early morning and ends within 30 minutes to 1 hour. The broods were transferred from the hatching tank after completion hatching. An adult crab is able to lay eggs twice more within 1 to 4 months without molting and further matting.

Key findings
Possible to produce berried broods in captive conditions providing facilities similar to the natural habitats. Berried brood production success was 35-61% with superior quality zoea.

***Keywords*:** Berried brood, Performance, Hatchery condition.

12.1.3 Research highlight-03

Background
Mud crab farming in Bangladesh exclusively relies on the wild for small juvenile crabs to adults and the main revealed issues provoking wild exploitation due to unavailability of hatchery produced seeds. Therefore, the development of proper hatchery protocol is prerequisite to support the development of the mud crab aquaculture industry. Larvae rearing techniques, disease outbreak and nutritional composition of larvae feed are the major three areas of research which support the commercial production of crustacean larvae. Thus, these interconnected areas should be addressed and optimized properly to establish a complete hatchery protocol. Many technical improvements were developed over the past years those could be useful for mud crab larvae rearing with several modifications. Moreover, the upgraded strategies like, feeding, water treatment strategy, skills and disease mitigation strategy should employed depending on available local resources to maximize the larval survival rate.

***Objective*:** To develop larvae rearing protocol of mud crab to maximize survival rate.

Methodology
To enhance the survival rate of mud crab at crablet stage consecutive trials were conducted on feeding and water treatment strategies to set a protocol in mud crab larvae rearing. The 1st experiment was conducted with 3 feeding treatments *viz*, T1: larvae fed with live feed only (rotifer+Artemia); T2: larvae fed with liquid diet (liquid rotifer+liquid Artemia); and T3:

larvae fed with live feed+liquid diet. The 2nd experiment was done on water treatment plans with 3 treatments viz., T1 (water treated with prebiotic @ 10g/ton), T2 (water was treated with Fish probiotic @ 1g/ton) and T3 (water was treated with both Prebiotic (5g/ton) and probiotics (0.5g/ton) of culture water). The 3rd trial was based on water treatment with some modifications of experiment 2. In this experiment three water treatment plans was viz., T1 (treated with both Prebiotic (5g/ton) and probiotics (0.5g/ton) of culture water), T2 (water was treated with prophylaxis @0.3 ppm) and for T3 (water was treated with both Prebiotic (5g/ton) and probiotics (0.5g/ton) up to 10 days and with prophylaxis @0.3 ppm from day 14 onwards). Stocking density of larvae was 50 zoea/l. Feeding was done with Rotifer + Liquid rotifer for Z1 to Z2 stage followed by enriched Artemia + Liquid Artemia for Z3-megalopa stage (best feed obtained from 1st year experiment). Duration of each larvae rearing experiment was 25-30 days as long as larvae metamorphosis to crablet stage.

Key findings
Feed size, density, nutritional quality and proper feeding scheme enhanced larvae survival. Application of both pre-biotic, probiotic and prophylaxis improved the water quality, reduced the disease risk, shortened metamorphosis duration and enhanced the survival up to 7%.

Keywords**:** Larvae rearing, Prebiotic, Probiotic, Prophylaxis, Survival

12.1.4 Research highlight-04

Background
Crustaceans like mud crab grew bigger through molting and molting is therefore crucial stage in life cycle due to vulnerability to the predators. During and after molting the outer shell remain very soft and easily affected by the predators. mud crab is a cannibalistic species and soft shell crabs are thus eaten by the hard shell crabs. Hence, development of adequate nursery protocol is mandatory to promote survival in juvenile stage. Nursery, a concept was coined as a shelter of juvenile that can contribute greater than other habitats used by juveniles of a particular species. Specific criteria for the habitat of mud crab nursery pond are required for a successful and sustainable farming of juveniles. In case of nursery in confined water body, optimum stocking density is also a key factor for the survival and growth of orange mud crab. Thus, developing nursery rearing strategies for mud crabs is very essential to bridge between the gaps of hatcheries and grow out farms. Appropriate nursery techniques should be tailored for Mud crab in terms of specific environmental requirements, modification of pond habitats and stocking density to promote the survival rate.

Objective**:** To optimize stocking density and development of suitable pond atmosphere for enhancement of survival and growth of juvenile mud crab in nursery rearing.

Methodology
Two consecutive trials were conducted to optimize the pond habitat and stocking density in nursery phase. The 1st experiment was conducted with three stocking densities viz, (T1) was implemented with 30 crablet/m^2, (T2) was with 50 crablet/m^2 and treatment (T3) was at a density of 70 crablet/m^2 in the brackishwater earthen ponds. The 2nd experiment was done on shelters with three treatments viz, (T1) was executed in the pond bottom without shelter and (T2) was implemented with 40 micro-meshed *hapa* nets and the size of each *hapa* was 1×1×1.5 m^3. Besides, (T3) was set as nursery in pond bottom with different shelters like hanging and sinking nets as well as plastic pipes. Stocking density of 30 crablet/m^2 were

maintained for all the treatments under this experiment. Trash fish like small tilapia was chopped into pieces and used as a feed for the crablets in the nursery ponds at the rate of 10 % body weight. Feeding was administered twice daily in the morning (07:00 am) and evening (07:00 pm). Each of the experiment was conducted for 30 days, and then harvested and data was collected.

Key findings
A stocking density of 30 crablet/m^2 was found suitable for better survival and intactness. Meanwhile, nursery in soil touch (pond bottom) with sufficient shelters improved the survival and intactness.

***Keywords*:** Nursery, Density, Shelter, Survival, Intactness, Juvenile, mud crab

12.1.5 Research highlight-05
Background
Mud crab aquaculture is solely dependent on natural feeds like, trash fish (Tilapia) and mud eel. Price of these items is not fixed and in the lean season the price hikes and reached out of purchase capacity**.** Moreover, natural feeds are perishable; needed deep fridge for preservation and for commercial aquaculture huge amount required feeds are not possible to store in fridge. Meanwhile, commercial feeds are not available for species like Mud crab. In these situation, found out of suitable feeds is necessary for mud crab grow out culture.

***Objective*:** To find out suitable feeds for mud crab in grow out culture

Methodology
Two consecutive trials were conducted to found out suitable feeds for mud crab in grow out system. The first experiment was conducted to evaluate the effect of different feeds on growth; survival and intactness of mud crab in grow out phase in the earthen ponds. The experiment had three treatments depending on feeding variations *viz,* T1: feeding with chopped trash fish; T2: feeding with chopped mud eel and T3: feeding with chopped trash fish + chopped mud eel. The second trial was conducted with some modification employing five treatments *viz*, T1= Chopped trash fish; T2= Chopped mud eel; T3= Chopped trash fish + chopped mud eel (1:1); T4= Chopped trash fish+ commercial diet (1:1); and T5= 100% commercial diet. Each of both the trial treatment had 3 replications. Stocking was done with juvenile crabs at a density of 4/m^2 and feeding was done at the rate of 5-7% of body weight as per experimental design. The ponds periphery was encircled with bana and nylon net and the experiment was continued for a period of 60 and 105 days, respectively.

Key findings
Better body weight gain, survival rate and intactness was found in co-feeding of natural feeds. Commercial diet provided poor performance in terms of weight gain, survival and intactness. However, natural item (trash fish and mud eel) is therefore, proffered feed for mud crab.
***Keywords*:** Feed preference, **N**atural, mud crab, Grow out

12.1.6 Research highlight-06
Background
Soft shell shedding is new addition in mud crab aquaculture. The sub-sector showed rapid growth due to increased demand and preference of consumption in contrast to the hard shell crabs. The sector and farm owners are facing various problems including high mortality and

low rate of shedding. It is assumed that several physical, physiological as well as environmental factors are regulating the shedding performance of mud crab. However, found out of factors affecting the soft shell shedding performance and mortality of mud crab is therefore warranted.

***Objective*:**
To find out factors affecting the soft shell shedding of mud crab

Methodology
To found out the regulatory factors influencing the overall soft shell crab production, three consecutive trials were conducted in the cemented cisterns (7 m^3 each) in the hatchery complex of Brackishwater Station. The first experiment was conducted to found out the effect of prophylaxis on soft shell shedding. There have two treatments, T_1: pre-moulty treated with prophylaxis, and T_2: pre-moulty directly stocked without disinfection. Each of the treatment had 3 set of replications with 20 crabs in each replica. The second experiment was conducted to observe the effect of aeration on soft shell shedding. This trial had also two treatments, T_1: shedding water aerated with bottom line aeration, and T_2: shedding water not aerated. Each of the treatment had 3 set of replications with 20 crabs in each replica. Each crab was stocked in separate plastic boxes floated on a plastic pipe made frame. The last experiment (Trial 3) was done to observe the seasonal variation on soft shell shedding, viz, S_1: winter season, S_2: summer season, and in S_3: rainy season. Each of the seasons had 3 replicated set of boxes with 60 crabs in each set. All the experiment was conducted for a period of 45 days each.

Key findings
Premoult crabs treated with prophylaxis showed better survival and shedding performance. Likelihood of prophylaxis treatment, setting up of aeration provided similar results in the case of survival and moulting efficiency. Besides, rainy season enhanced the survival of crabs rather than winter and summer season.

***Keywords*:** Prophylaxis, Aeration, Seasons, Shedding, Soft shell

12.2 Component 4 (KU)
12.2.1 Research highlight-01
Background
Mud Crabs (*Scylla* spp.) has become a key artisanal coastal fisheries resource in many tropical and subtropical Asian countries. In Bangladesh, mud crab has been exploited commercially since early 1980's around the coastal belt (Hasanuzzaman et al. 2014). It is traditionally harvested by fishermen and provides a basic source of income for a number of coastal fishing communities. But, this resource of economic importance is at risk from diseases; particularly shell disease predominately caused by chitinolytic bacteria (Cook and Lofton, 1973; Joseph and Ravichandran, 2012).

***Objective*:** To make a profile of bacteria mostly linked to chitinolysis of mud crab shell.

Methodology
Mud crabs were collected from the waters in and around the Sundarbans areas. Samples were preserved following standard approaches, and bacteria isolated were identified by standard microbial plate using selective media. Biochemical tests have been being performed to confirm the identification of isolates.

Key findings
Vibrio spp.*, Aeromonas* spp.*, Pseudomonas* spp. were enumerated from the wild mud crab samples. Among these chitinolytic bacteria, *Vibrio* spp. were detected as most dominant in wild mud crabs.
***Keywords*:** Mud crab, Wild, Chitinolytic bacteria, Shell disease.

12.2.2 Research highlight-02
Background
Mud crab (*Scylla* spp*)*, an uncommon export oriented aquaculture species, has been exploited commercially in Bangladesh since early 1980's around the coastal belt (Hasanuzzaman et al. 2014). Despite the potential role in the national economy and livelihood improvement(Kamal, 2002; Zafar, 2004; Zafar and Hossain, 2008), mud crab aquaculture is not well established in Bangladesh except fattening (Begum et al. 2010). The exported crabs are being caught from natural sources, causing intense pressure on the natural stock. For sustainable aquaculture of the species, seed production in hatchery condition is an effective alternate option. BFRI-BS successfully produced the seed in hatchery condition during 2015-2017, but the survival was low which is likely due to microbial infection.

***Objective*:** To identify pathogenic bacterial infection and factors linked to infection in the mud crab hatchery.

Methodology
Mud crab brood, larvae, crablet, water, sand, soil, feed samples were collected from the hatchery. Samples were preserved following standard approaches, and bacteria isolated were identified by standard microbial plate using selective media. Biochemical tests have been performed to confirm the identification of isolates.

Key findings
Vibrio spp.*,* were identified in brood and larval rearing units; *Aeromonas* spp.*, Pseudomonas* spp. were also found in some samples. Larval live feed has been found as a crucial source of *Vibrio* infection in the mud crab hatchery.

***Keywords*:** Mud crab, Hatchery, Larvae, Larval feed, *Vibrio*.

12.2.3 Research highlight-03
Background
In Bangladesh, mud crab aquaculture was developed in late 1980s in the form of fattening wild lean crab harvested from the forests and rivers around the Sundarbans and the Chakaria Sundarban in Bangladesh. In the recent past, crab farmers in Bangladesh have addressed mortality problems in their farms; specifically the occurrence of mud crab mortality has been severely evident in the farms at Rampal Upazila of Bagerhat district, where water salinity of the farms is low.

***Objective*:** To determine the association of salinity variation with the mortality occurrence in mud crab farms.

Methodology
Mud crabs collected from the Sundarbans rivers of salinity >9 ppt were fattened in the cages installed in the farms of Paikgacha of which water salinity was in range between 0 and 3 ppt. There were four treatments named, S_3d_f (3ppt and daily feeding), S_3d_{if} (3 ppt and 1 day interval feeding), (0 ppt and daily feeding) and S_0d_{if} (0 ppt and 1 day interval feeding).

Key findings
There was significant effect ($p < 0.05$) of salinity but no significant effect ($p > 0.05$) of feeding regime on mud crab survival. So, salinity variation between the fattening farms and the waters from where mud crabs are caught has been found linked to the sudden mortality of mud crabs in the farms.

Keywords**:** Mud crab, Salinity, Mortality, Feeding regime.

12.2.4 Research highlight-04

Background
In Bangladesh, mud crab aquaculture was developed in late 1980s in the form of fattening wild lean crab harvested from the forests and rivers around the Sundarbans and the Chakaria Sundarban in Bangladesh. Being an important livelihood option for coastal fisher-folks, mud crab farming is increasingly practised, even recently intensified with advances in management. Such intensification may result in occurrences of diseases; there have been increasing reports on disease incidence in mud crabs across the world; now-a-days crab fatteners in Bangladesh also have pointed out the disease-associated problems in their farms.

Objective**:** To record bacterial disease incidence in mud crab farms.

Methodology
Mud crabs apparently unhealthy through gross observations (i.e. external signs, discolored patches, soft and black exoskeleton, orange or brown spot on shell) and healthy crabs were collected from the same ponds/pens. Bacterial load (cfu/g) was determined by standard plate count method. For isolation of specific bacteria, selective media were used. Biochemical tests have been being performed to confirm the identification of isolates.

Key findings
Vibrio, Aeromonas, Pseudomonas bacteria which cause shell disease were detected in crabs from the farms. More than75% unhealthy samples were infected *with V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, and *V. vulnificus* where as < 40% healthy samples were infected with at least one or more of these pathogens. Compared with healthy crabs, the count *of V. alginolyticus*, *V. harveyi* and *V. parahaemolyticus* were found more than 10 times in unhealthy crabs.

Keywords**:** Mud crab farms, Mortality, Disease, *Vibrio*, *Aeromonas*.

12.2.5 Research highlight-05

Background
In Bangladesh, mud crab aquaculture was developed in late 1980s in the form of fattening wild lean crab harvested from the forests and rivers around the Sundarbans and the Chakaria Sundarban in Bangladesh. Being an important livelihood option for coastal fisher-folks, mud crab farming is increasingly practised, even recently intensified with advances in management. Such intensification may result in occurrences of diseases; there have been increasing reports on disease incidence in mud crabs across the world (Lavilla-Pitogo et al., 2001; Poornima et al., 2008; Jithendran et al., 2010); in the recent years, crab farmers in Bangladesh also have addressed mortality and disease-associated problems in their farms. *Vibrio* spp. are pathogenic bacteria, and cause shell diseases of mud crabs. There is dearth of information on pathogenic load of bacteria causing vibriosis disease symptoms in *S. olivacea*.

Objective: To determine *Vibrio* load causing mortality and disease symptoms in mud crab *S. olivacea*.

Methodology

Fresh and active mud crabs of 75-110 g were collected from depot of Khulna city, and the experiment was conducted in the laboratories of Fisheries and Marine resource Technology Discipline, Khulna University. The crabs were acclimatized in the experimental tanks for 3 days, and then the challenge experiment with 1×10^6, 1×10^7 and 1×10^9 cfu/ml *Vibrio* inoculums for treatment group T_1, T_2, T_3 was conducted for 8 days (till 100% mortality occurred in one of the treatments). *Vibrio* sp. were isolated from apparently diseased (i.e. discolored patches on shell, black exoskeleton) mud crabs collected from wild sources.

Key findings

This study reported that there was a significant difference of mortality rate ($P<0.05$) between the control and the treatment groups. The control group had no mortality but the treatment groups had 50% cumulative mortality rate (CMR) on 6 day-post challenge (dpc). The first 100% CMR was observed in the T_2 group, and accordingly, the *Vibrio* load > 10^6cfu/ml was found to cause acute disease symptoms in *S. olivacea* in a short period of time. This is the first investigation determining the *Vibrio* load causing mortality of *S. olivacea* in response to *in vivo Vibrio* challenge.

***Keywords*:** Mud crab, *Vibrio*, Pathogenic load, Mortality.

B. Implementation status

1. Procurement (Component wise)

i. Coordination component (BFRI) : Not applicable

ii. Component 2 (BARC) : Not applicable

iii. Component 3 (BFRI-BS)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	Laptop (1)	60000	Laptop (1)	63000	Procurement done under revised budget
	Digital Camera (1)	25000	Digital Camera (1)	32000	
	UPS (1)	6000	UPS (1)	6000	
	IPS (1)	40000	IPS (1)	37000	
	Desktop computer (1)	60000	Desktop computer (1)	51000	
(b) Lab & field equipment	Magnetic Aerator (ACO-006) , 1 No	8100	Magnetic Aerator (ACO-006) , 1 No	8300	
	Magnetic Aerator (ACO-003) , 3 Nos	15900	Magnetic Aerator (ACO-003) , 3 Nos	16500	

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
	Air Switch (200 pcs)	3400	Air Switch (200 pcs)	3400	Procurement done under revised budget
	Filter Bag (4 pcs)	5700	Filter Bag (4 pcs)	5600	
	Air stones (10 inch), 50 Nos	6500	Air stones (10 inch), 50 Nos	6500	
	Aeration pipe (10 kg)	5300	Aeration pipe (10 kg)	5200	
	Air blower (1.5 HP, 1 Nos	25000	Air blower (1.5 HP, 1 Nos	24850	
	Air pump (ACO-006, 80 Wat), 3 Nos	30000	Air pump (ACO-006, 80 Wat), 3 Nos	24600	
(c) Other capital items					

1V. Component 4 (KU)

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	Desktop Computer (1)	60000	Desktop Computer (1)	66300	Procurement done under revised budget
	Laser Printer (1)	20000	Laser Printer (1)	18700	
	Scanner (1)	10000	Scanner (1)	6500	
	UPS (1)	10000	UPS (1)	7500	
	File Cabinet (1)	20000	File Cabinet (1)	19470	
	Computer Table (1)	5000	Computer Table (1)	4530	
	Computer Chair (1)	3500	Computer Chair (1)	5890	
	Visitor Chair (4)	16000	Visitor Chair (4)	14480	
(b) Lab &field equipment	Paraffin embedding bath (1)	140000	Paraffin embedding bath (1)	130000	
	Rotary microtome (1)	860000	Rotary microtome (1)	745000	
	Thermo Cycler PCR (1)	600000	Thermo Cycler PCR (1)	590000	
(c) Other capital items					

2. Establishment/renovation facilities

i. Coordination component (BFRI) : *Not applicable*
ii. Component 2 (BARC) : *Not applicable*
iii. Component 3 BFRI-BS)

Description of facilities	Newly Establishment		Upgraded/refurbished		Remark
	PP Target	Achievement	PP Target	Achievement	
Nursery ponds excavation	6 Nos	6 Nos	-	-	Achieved 100%

1V. Component 4 (KU)

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
Lab Repair & renovation	N/A	N/A	01	01	Achieved 100%

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

i. Coordination component (BFRI): *Not applicable*
ii. Component 2 (BARC)

Description	Number of participants			Duration (Days)	Remarks
	Male	Female	Total		
Inception Workshop (1)	56	7	63	1 day	All workshops held at the Conference room of BARC as per schedule of activity of the respective component
Half yearly Research Prog. Review Workshop	65+ 62	9+8	144	1+1 = 2 days	
Annual Research Prog. Review Workshop (2)	60+63	7+8	138	1+2 =3 days	
Project Completion Report Review Workshop (1 no)	45	6	52	1 day	

iii. Component 3 (BFRI-BS): *Not applicable*

iv. Component 4 (KU)

Description	Number of participant			Duration	Remarks
	Male	Female	Total		
(a) Training	N/A	N/A	N/A	N/A	N/A
(b) Workshop	32	2		Day long	
(c) Others (if any)	N/A	N/A	N/A	N/A	N/A

N/A = Not applicable

C. Financial and physical progress (Combined & component wise)

i. Combined progress

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	4750622	4687265	4654502	32763	97.67	Not applicable
b. Field research/lab expenses and supplies	8628811	8561301	8561301	0	100	
c. Operating expenses	1907418	1831946	1826883	5063	99.57	
d. Vehicle hire and fuel, oil & maintenance	892717	770145	755145	15000	92.79	
e. Training/workshop/ seminar etc.	323600	301750	151750	150000	66.67	
f. Publications and printing	452950	408002	403656	0	94.23	
g. Miscellaneous	405680	803555	379174	16379	96.59	
h. Capital expenses	1894570	1889520	1889520	0	100	
Total	**19256368**	**18676082**	**18626278**	**49805**	**98.49**	

ii. Coordination component (BFRI, Comp-1)

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	413862	350505	317742	32763	90.65	Not applicable
b. Field research/lab expenses and supplies	0	0	0	0	0	
c. Operating expenses	361957	302079	297016	32653	98.32	
d. Vehicle hire and fuel, oil & maintenance	137000	52000	37000	15000	71.15	
e. Training/workshop/ seminar etc.	0	0	0	0	0	
f. Publications and printing	25000	19400	15054	0	77.60	
g. Miscellaneous	122181	120000	103621	16379	86.35	
h. Capital expenses	0	0	0	0	0	
Total	**1060000**	**824584**	**774779**	**96795**	**93.96**	

iii. Component 2 (BARC)

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	185000	185000	185000	0	100.00	Not applicable
b. Field research/lab expenses and supplies	0	0	0	0	0	
c. Operating expenses	140276	140221	140221	0	99.96	
d. Vehicle hire and fuel, oil & maintenance	159577	159577	159577	0	100.00	
e. Training/workshop/ seminar etc.	123600	123600	123600	0	100.00	
f. Publications and printing	300000	298000	298000	0	99.33	
g. Miscellaneous	51547	51540	51540	0	99.99	
h. Capital expenses	0	0	0	0	0	
Total	**960000**	**957938**	**957938**	**0**	**99.79**	

iv. Component 3 (BFRI-BS)

In Taka

Items of expenditure/ activities	Total approved budge	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
(a) Contractual staff salary	1616733	1616733	1616733	**0**	100	**Not applicable**
(b) Field research/lab expenses and supplies	5842264	5835429	5835429	**6835**	99.88	
(c) Operating expenses	834244	832850	832850	**1394**	99.83	
(d) Vehicle hire and fuel, oil & maintenance	255869	251549	251549	**4320**	98.31	
(e) Training/workshop/ seminar etc.	150000	0	0	**150000**	0	
(f) Publications and printing	102950	77950	77950	**25000**	75.72	
(g) Miscellaneous	120308	120270	120270	**38**	99.97	
(h) Capital expenses	289000	283950	283950	5050	98.25	
Total	**9211368**	**9018731**	**9018731**	**192637**	**95.99**	

v. Component 4(KU)

In Taka

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2535027	2535027	2535027	0	100.00	**Not applicable**
b. Field research/lab expenses and supplies	2786547	2725872	2725872	0	100.00	
c. Operating expenses	570941	556796	556796	0	100.00	
d. Vehicle hire and fuel, oil & maintenance	340271	307019	307019	0	100.00	
e. Training/workshop/ seminar etc.	50000	28150	28150	0	100.00	
f. Publications and printing	25000	12652	12652	0	100.00	
g. Miscellaneous	111644	103743	103743	0	100.00	
h. Capital expenses	1605570	1605570	1605570	0	100.00	
Total	**8025000**	**7874829**	**7874829**	**0**	**100.00**	

D. Achievement of sub-project by objectives (Tangible form): Technology generated/developed

i. Component 3 (BFRI-BS)

General/specific objectives of the sub\project	Major technical activities performed in respect of the set objects	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To domesticate bloodstock and development of breeding, larvae rearing and nursery management protocol for sustainable seed production of mud crab, *S. olivacea*.	i. Breeding environment like natural breeding place has been created in the hatchery by installation of sand bed, aeration and water purification through auto-filter and biofilter. ii. Larvae rearing protocol has been developed by a series of trials covering handling of larvae, feeding schedule, water treatment measures. iii. Nursery has been completed on stocking density and different shelters under various conditions like *hapa* and earthen pond bottom.	i. Successfully domesticated brood stock and produced 33-61% berried broods. Collection of broodstock from nearest location minimized the stress and enhanced the performance. ii. Rotifer, liquid rotifer and Artemia have been found out for suitable as feeding to the larvae. Prebiotics, probiotics and prophylaxis have been detected as suitable for water treatment minimizing disease infection. A survival of 7% has been achieved at crablet stage. iii. In nursery, lower stocking density of 30 ind/m^2 has been found out suitable minimizing cannibalism and increased survival. Addition of diversified shelter like, plastic pipes, hanging net, earthen pots and sand bed have been detected to be enhanced the survival both in megalopa and nursery phase.	Production of 33-61% berried broods in hatchery condition obviously mitigated the challenge and cost of brood collection from the sea. The 7% survival at crablet stage seemed lower in comparison to fecundity, but very much compatible to the survival that achieved Internationally. Off course the locally available inputs like pre-biotic, probiotic, prophylaxis and shelter materials used in this study is therefore new interventions and pioneer work in Bangladesh. The techniques and materials can thereby used as it is or with modifications for further development of Mud crab brood and seed production.
To investigate the effect of different rations of natural and commercial diets on grow out and fattening of mud crab, *S. olivacea*.	i. Different type of natural feed like chopped tilapia, chopped mud eel and commercial feeds like shrimp feed and their combinations has been tested in Mud crab grow out culture.	i. Natural feed has been observed as still suitable for mud crab grow out culture with highest weight gain of 176 g, survival of 62% and intactness of 67% was achieved.	As ready feed for mud crab aquaculture has not been developed yet, thus, feeding with natural feeds will be the option for mud crab; besides preparation of ready should be emphasized for future.
To adopt and demonstrate	i. Fattening of mud crab was done with stocking of crab	i. Highest production of fattened crab obtained	Integrated multitrophic aquaculture is

General/specific objectives of the sub\project	Major technical activities performed in respect of the set objects	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
innovative mud crab (*S. olivacea*) fattening techniques in different aqua-eco regions of south-west coast.	in floating boxes (1 crab/box), GIFT tilapia in water column (2/m^2) and crab in pond bottom (2/m^2); it was executed in Satkhira, Khulna and Bagerhat districts. Feeding was done with trash feed of 5-8% body weight..	in cages (3.04 kg/m^2) and survival was 97%. For that of pond bottom highest production achieved 0.65 kg/m^2 and survival obtained 79%. GIFT contributed a production of 0.58 kg/m^2. Overall BCR in this integrated system was found 1:1.3.	therefore, proven technology in fisheries and it implies for the mud crab as well. The production per unit area has been increased from this practice but the BCR value seemed unsatisfactory due to COVID-19. However, simultaneous fattening of crabs in pond bottom and floating cages; and integration of salt tolerant suitable species should be practiced to promote the production and economic return.
To find out the regulatory factors on soft shell shedding of mud crab (*S. olivacea*) for sustainable production.	i. To found out regulatory factors of soft shell shedding, premoulty was treated with Prophylaxis @0.3-0.5 ppm for 30 minutes; moulting tank water was continuously aerated; and various seasons (winter, summer and rainy/wet) were tested for moulting efficiency and survival of crabs..	i. Prophylaxis treatment increased the survival 23.57% and moulting efficiency of 11.11% than non treated; aeration improved the survival 37.44% and moulting rate 42.93%. Among the seasons, pre-rainy to rainy season provided better performance in terms of survival and moulting.	Though many factors regulated the survival and moulting of mud crab, but a few have been tested. The combination of these 3 factors might provide better results for the improvement of soft shell shedding. However, the study was conducted in closed conditions and very few factors have been chosen. Testing of other factors in broader scale culture might open new and tuned findings for further development.

ii. Component 4 (KU)

General/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 quantify bacterial load and type/infection level in mud crab hatchery, farms and in wild mud crab, *S. olivacea*.	Mud crab samples from the mud crab hatchery, farms and rivers adjacent to the Sundarbans were collected; total bacterial count, specific bacterial identification and count were performed.	A leaflet focusing measures to control bacterial infection in mud crab *Scylla olivacea* hatchery. Suggestions for good aquaculture practice in Mud crab aquaculture ventures have been addressed.	⇒ The contents of this leaflet can be effectively used in the hatchery. ⇒ The information can be effective for ensuring sustainable mud crab aquaculture.

To record disease incidence in mud crab (*S. olivacea*) population in the south-west coastal region of Bangladesh.	The mud crab hard-shell and soft-shell farms have been visited and the healthy and few unhealthy mud crab samples were collected and analyzed.	12-16% Shell disease, 15-20% Vibriosis, 6-8% brown spot diseases were identified.	The information of causative agents and/or factors are likely helpful for developing prophylactics and strategies to control diseases.
To determine the association of other driving factors (i.e. feed, soil and water quality) with infection/diseases occurrence in mud crab, *S. olivacea*	Feed, water, and soil samples from mud crab hatchery and farms were collected, and bacterial analyses were performed.	Suggestions for practicing axenic culture of live food e.g. *Artemia* in the hatchery was provided and it worked. Suggestions for practicing mud crab fattening in saline waters; restricting entry of cattle e.g. cows, goats, and poultry e,g, chickens, ducks in the farms	The information will be substantially applied in the development of bio-security measurements for minimizing and/or controlling microbial contamination and consequent diseases occurrences in the production units.

E. Information/knowledge generated/policy generated

i. Component 3 (BFRI-BS)

General/specific objectives of the sub\project	Major technical activities performed in respect of the set objects	Output	Outcome (short term effect of the research)
To domesticate bloodstock and development of breeding, larvae rearing and nursery management protocol for sustainable seed production of mud crab, *S. olivacea*.	1. Berried broods has been successfully produced providing natural environment in the hatchery.	1. 33-61% broods turned into berried.	1. Following this technique, hatchery owner will be capable to produce berried broods in captive condition; a new value chain will be developed in crab aquaculture.
	2. Larvae rearing protocol has been optimized minimizing feeds, water treatment and disease incidence.	2. Larvae survival at crablet stage has been enhanced up to 7%.	2. Hatchery managers will be benefitted following the developed techniques, and will be capable to minimize mortality of larvae and production of quality seeds for crab aquaculture.
	3. Nursery technique of crablet has been opted by optimizing stocking density and shelter for hiding.	3. Lower stocking density of 30 cralet/m^2 and pond bottom with sufficient shelter was found for better survival in nursery.	3. Crab nursery farmers will be directly benefitted from this nursery technique. Another value chain might be added in crab aquaculture.
To investigate the effect of different rations of natural and commercial diets on grow out and fattening of mud crab, *S. olivacea*.	1. Natural feeds (trash fish and mud eel) along with commercial diet has been tested in crab grow out system.	1. Combined feeding with two natural feeds (trash fish+ mud eel) enhanced the survival and intactness of mud crab.	1. Crab farmers should use natural feeds as long as suitable commercial diet is not established especially for Mud crab.
To adopt and demonstrate innovative mud crab (*S. olivacea*) fattening techniques in	1. Simultaneous mud crab fattening in pond bottom and floating cages along with integration of GIFT has been	1. Simultaneous fattening of mud crab enhanced the total farm output and	1. Farmers might be benefitted from this technique and able to produce more fattened

General/specific objectives of the sub\project	Major technical activities performed in respect of the set objects	Output	Outcome (short term effect of the research)
different aqua-eco regions of south-west coast.	tried in different aqua-ecological areas of coastal belt.	BCR. Whereas, integration of GIFT augmented additional yield for house hold use and economic return.	crab from same piece of land. GIFT can be used for nutritional requirement.
To find out the regulatory factors on soft shell shedding of mud crab (*S. olivacea*) for sustainable production.	1. Premoulties were treated with 0.3-0.5 ppm prohpylaxis; shedding tank water was aerated and shedding was tested in different seasons.	1. Both initial disinfection of premoulty with prophylaxis and aeration enhanced the survival and shedding rate of Mud crab. Rainy season was observed suitable for shedding of mud crab in congenial environmental factors at that period.	1. Soft shell shedding farms might try intial disinfection with prophylaxis to reduce microbial loads and might set aeration system for keeping the shedding environment favorable. Implication of these two in the rainy season might enhance the shedding rate reducing the mortality. Economic returns might be increased by adoption of the technique.

ii. Component 4 (KU)

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
To quantify bacterial load and type/infection level in mud crab hatchery, farms and in wild mud crab, *S. olivacea*.	Mud crab samples were collected from wild, farms and hatchery units; total bacterial count, specific bacterial identification and count were performed.	The mud crab samples from hatchery, farms and wild had infection with *Vibrio, Aeromonas, Pseudomon*as species. Among these species, *Vibrio* spp. were detected as most dominant in infected crabs.	⇒Based on these findings, suggestions of using probiotics, antibiotics with caution, and nutritionally enriched *Artemia* from axenic culture in the hatchery have been provided; it worked as there was increased survival of larvae after using probiotics. ⇒Farmers have been provided with suggestions of using clean food and feed.
To record disease incidence in mud crab (*S. olivacea*) population in the south-west coastal region of Bangladesh.	As per the activity plan, this was the work of 3rd year; due to COVID-19 pandemic; most of the Mud crab hard-shell farms have not been in operation; very few crab samples were found, which was not enough for true representation of the data.	The unhealthy samples had infection with higher count of bacteria and specific pathogenic bacteria such as *Vibrio* spp., *Aeromonas* spp.; among *Vibrio* spp., most of them were *V. parahaemolyticus*, *V.*	The obtained data in relation to mud crab disease incidence can be effectively used: -for surveillance of mud crab diseases in farms, -to mitigate and control disease-

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
	However, healthy and few unhealthy samples were comparatively analyzed for bacterial count and isolation.	*alginolyticus* and *V. harveyi*.	associated mortality and consequent production-loss in the mud crab farms
To determine the association of other driving factors (i.e. feed, soil and water quality) with infection/diseases occurrence in mud crab, *S. olivacea*	Feed, water, and soil samples from mud crab hatchery and farms were collected, and bacterial analyses were performed.	The samples were found with contamination with *Vibrio*, *Aeromonas*, *Pseudomonas*, *E. coli*, S*almonella*, *Shigella*, *Enterobacter and Klebsiella*. Larval live feed was found as a crucial source of *Vibrio* infection in the mud crab hatchery.	⇒Axenic culture of live food e.g. *Artemia* in the hatchery was suggested, and it worked. ⇒Farmers have been provided with suggestions of using clean food (i.e. food and feed is to be washed several times with clean water ,tube-well water), practicing fattening in saline (at least moderate, > 8 ppt) waters, restricting entry of cattle e.g. cows, goats, and poultry e,g, chickens, ducks .

F. Material development/publication made under the sub-project
i.Component 3 (BFRI-BS)

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.	1	1	1. Seed Production and Fattening of Mud crab; The 100 Agro-technologies ATLAS, BARC, 2020 2. Broodstock Development of Mud Crab under Captive Conditions for Sustainable Hatchery Operation (under validation)
Journal publication	2	2	1. Larval rearing of orange mud crab, *Scylla olivacea*: improving survival rate and shortening metamorphosis period (Bangladesh Journal of Fisheries)

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
			2. Nursery rearing of mud crab, *Scylla olivacea* (Herbst, 1896): Optimizing stocking density and pond habitat (Journal of Bangladesh Agricultural University)
Video clip/TV program	1	1	BTV Mati o Manush
News paper/Popular article	19	19	21.04.2019; The Prothom Alo 25.04.21; The Daily Purbanchol 26.12.2019 The Daily Somajer Khotha The Daily Lok Somaj The Daily Nowapara The Daily Purbanchol The Daily Somoyer Khobor The Daily Sonjog Bangladesh The Daily Prothom Alo The Daily Somajer Kagoj The Dainik Probah The Dainik Kollan The Daily Gramer Kagoj The Daily Jugantor The Daily Amar Ekush 27.12.2019 The Daily Drishtipat The Daily Bangladesher Khobor 14.09.2020 The Daily Kallyan The Daily Nawapara
Other publications, (if any)			

ii.Component 4 (KU)			
Technology bulletin/ booklet/**leaflet**/flyer etc.	01	01	1. শীলা কাঁকড়া হ্যাচারিতে ব্যাকটেরিয়ার সংক্রমণ ও দমনে বায়োসিকিউরিটি বিধানসমূহ In Bangla by Prof. Dr. A.F.M Hassanuzzaman. Dept. of FMRTD; KU.  2. Good Aquaculture Practice for Mud crab Farms in Bangladesh. (Draft under Preparation)
Journal publication	3	1	Title: Determination of survival and haemocyte response of mud crab *Scylla olivaceain vivo* challenged with *Vibrio*; Bangladesh Journal of Fisheries. (2021), 33(1): 109-118]; https://doi.org/10.52168/bjf.2021.33.13
Video clip/TV program			
News Paper/Popular Article		01 	Prothom alo.com, 19 September 2021, page-06

Other publications, if Any: Project Web page on Khulna University website		 দিনব্যাপী কর্মশালায় খুবি উপাচার্য **কাঁকড়া চাষ সুন্দরবনের উপর নির্ভরশীল জনগোষ্ঠির বিকল্প কর্মসংস্থান হতে পারে** স্টাফ রিপোর্টার : খুলনা বিশ্ববিদ্যালয়ের বিশারিয়ন এন্ড মেরিন রিসোর্স টেকনোলজি (এফএমআরটি) ডিসিপ্লিনের উদ্যোগে গতকাল শনিবার সকাল সাড়ে ১০টায় 'Are Bacteria A Threat to Sustainable Mud Crab Aquaculture of Bangladesh? (বাংলাদেশে টেকসই কাঁকড়া চাষে ব্যাকটেরিয়া কি হুমকি) শীর্ষক কর্মশালা অনলাইনে অনুষ্ঠিত হয়। প্রধান অতিথি হিসেবে এ কর্মশালার উদ্বোধন করেন বিশ্ববিদ্যালয়ের উপাচার্য প্রফেসর ড. মাহফুজ হোসেন। প্রধান অতিথির বক্তব্যে তিনি বলেন, সুন্দরবনের পরিবেশ ও প্রতিবেশ রক্ষার ক্ষেত্রে কাঁকড়ার ভূমিকা রয়েছে। প্রাকৃতিক উৎস থেকে কাঁকড়ার পোনা আহরণের ফলে সুন্দরবনসহ উপকূলীয় এলাকার জীববৈচিত্র্যের ক্ষতি হচ্ছে। হ্যাচারিতে পোনা উৎপাদনের মাধ্যমে এই ক্ষতি কমিয়ে আনা সম্ভব। হ্যাচারিতে পোনা উৎপাদন ও সহজলভ্য করা গেলে কাঁকড়া চাষ অর্থ-সামাজিক ক্ষেত্রে অবদান রাখতে পারবে। চিংড়ি চাষের পাশাপাশি কাঁকড়া চাষের মাধ্যমে আয়ও বৈচিত্র্যকরণ করা সম্ভব। তিনি বলেন, ইতোমধ্যে কাঁকড়া চাষ করে চীন, ভিয়েতনাম ও থাইল্যান্ড সফলতা পেয়েছে। আমাদের দেশের কাঁকড়া চাষ নিয়ে আরও বেশি গবেষণার প্রয়োজন। সুন্দরবনে কি পরিমাণ কাঁকড়া রয়েছে তা নিয়ের এটি জরিপের প্রয়োজন। উপাচার্য বলেন, কাঁকড়া চাষ সুন্দরবনের উপর নির্ভরশীল জনগোষ্ঠির অন্যতম বিকল্প কর্মসংস্থান সৃষ্টি করতে পারে। কাঁকড়া চাষের উপর গবেষণা অব্যাহত রাখতে হবে এবং গবেষণালব্ধ ফলাফল মাঠ পর্যায়ে নিয়ে যেতে হবে। আর এই গবেষণা কার্যক্রমের সঙ্গে খুলনা বিশ্ববিদ্যালয়ের সুনাম বৃদ্ধি পাবে এবং শিক্ষার্থী-গবেষকরা উপকৃত হবেন। তিনি এফএমআরটি ডিসিপ্লিনের শিক্ষা ও গবেষণা কার্যক্রমে শিক্ষকদের সক্ষমতা ও নিয়োগ প্রক্রিয়ার কথাও উল্লেখ করেন। একই সাথে তিনি বিশ্ববিদ্যালয়ের গবেষণা কাজে প্রশাসনের পক্ষ থেকে সম্ভব সহায়তার আশ্বাস দেন। উদ্বোধনী অনুষ্ঠানে বিশেষ অতিথির বক্তব্য রাখেন বিশ্ববিদ্যালয়ের উপ-উপাচার্য প্রফেসর ড. মোনাজ্জম হোসেন আরা, জীববিজ্ঞান স্কুলের ডিন প্রফেসর ধান গোপাল কুন্ডুস, বাংলাদেশ কৃষি গবেষণা কাউন্সিলের সদস্য-পরিচালক (ফিশারিজ) ড. মোঃ মতিনুল ইসলাম, বাংলাদেশ মৎস্য গবেষণা ইনস্টিটিউটের প্রধান বৈজ্ঞানিক কর্মকর্তা ও সাব-প্রোজেক্ট ওয়ান এর কো-অর্ডিনেটর ড. মোঃ জুলফিকার আলী। উদ্বোধনী অনুষ্ঠানে সভাপতিত্ব করেন এফএমআরটি ডিসিপ্লিনের প্রধান প্রফেসর ড. মুহাম্মদ আব্দুর রউফ। স্বাগত বক্তব্য প্রদান প্রকল্পের সহকারী মুখ্য গবেষক এফএমআরটি ডিসিপ্লিনের প্রফেসর ড. গাউছিয়াতুর রেজা বাবু। অনুষ্ঠান সঞ্চালনা করেন প্রকল্পের মুখ্য গবেষক এফএমআরটি ডিসিপ্লিনের প্রফেসর ড. এএফএম হালা-মুজ্জামান পপে টেকনিক্যাল সেশন অনুষ্ঠিত হয়। সেখানে প্রকল্পের ফলাফল উপস্থাপন করেন সিএইচডি গবেষক এফএমআরটি ডিসিপ্লিনের সহযোগী অধ্যাপক মোঃ রাশেদুল ইসলাম। এপ্রোটিপ-২ শীর্ষক এ গবেষণা প্রকল্পটি বিএমআরসি বাস্তবায়ন করছে যার অর্থায়ন করছে জিওবি, বিশ্বব্যাংক ও ইফাদ। **নগরীতে ইয়াবা ও গাঁজাসহ গ্রেপ্তার ২** স্টাফ রিপোর্টার ঃ নগর পুলিশের মাদক বিরোধী অভিযানে দু'জনকে গ্রেপ্তার করা হয়েছে। গ্রেপ্তারকৃতরা হলেন, খালিশপুর থানা এলাকার মোঃ মাহাবুবুর রহমান বাবু (৩১) ও খুলনা থানা এলাকার মোঃ মিজানুর রহমান বাবু (৩০)। এসময় তাদের কাছ থেকে ৫০পিস ইয়াবা ট্যাবলেট এবং ১শ' গ্রাম গাঁজা উদ্ধার করা হয়। এ ব্যাপারে সংশ্লিষ্ট থানায় ৩টি মাদক মামলা দায়ের করা হয়েছে। কেএমপির এক বিজ্ঞপ্তিতে এ তথ্য জানানো হয়েছে।	KU website **https://epaper.purbanchal.com/** epaper/edition/1287/%E0%A6%86%E0%A6%9C%E0%A6%95%E0%A7%87%E0%A6%B0-%E0%A6%AA%E0%A6%A4%E0%A7%8D%E0%A6%B0%E0%A6%BF%E0%A6%95%E0%A6%BE/page/4

G. Description of generated technology/knowledge/policy:

i) **Technology fact sheet**

(*Title, introduction, description, suitable location/ ecosystem, benefits, name and contact address of author)*

Component 3 (BFRI-BS)

Title of the technology-01

Broodstock Development of mud Crab under Captive Conditions for Sustainable Hatchery Operation

Introduction

Mud crab is a popular mariculture species, usually exploited from the natural source. The population is decreasing gradually due to over exploitation. Thus, crab seedling production in hatchery condition is important to support the crab aquaculture and conserving the bio-diversity. One of the remarkable obstacles for seed production in hatchery condition is the unavailability of quality berried brood crab. Mud crab is a migratory species, potential female broods migrate to the deep sea in quest of suitable environmental conditions for spawning, embryonic development and larvae survival. Therefore, collection of broods from the deep sea is laborious, hardy, risky and expensive. Hence, development of sufficient quantity of berried broods in captive condition is the best solution for smooth hatchery operations.

Description of the technology

Development of suitable environment for spawning

A cemented house, rectangular/circular fiber glass tank or plastic drum with a height of 72 cm is suitable as spawning vessel. A set of interconnected multiporous plastic pipe to be fixed at the bottom of the tank, covering with plastic bana/pata and nylon net. The other edge of the pipe should be set up with the water level and connected with aerator. Then, the bottom of the vessel will be partially filled with 12-15 cm height of clean sand. The sand layer ensures hiding or resting shelter of the broods and also helps as spawning substrate making a small hole that minimizes the spreading of spawned eggs. Half of the tank (50 cm depth) to be filled with 27-30 ppt sea water and the rest half remain blank to restrict the crabs from escaping through climbing. The tank should be covered with orchid net for providing shed to the broods as their preference.

Selection and transportation of potential gravid broods

Brood selection and transportation feature

Broods are to be collected from a clean water sources to reduce bacterial, fungal and protozoan loads; carapace and body shell needs to be harder, clean and all the appendages must be intact; weight should be within 250-450 g and carapace width of 9.5-11.5 cm; selected broods must be fully gravid (body cavity full with yellow color gonad); chelipods to be tied to restrict from fighting; body surface of crab should be in wet condition with source water during transportation; an aerated Styrofoam cartoon/plastic bucket/plastic box could be used for brood transportation; color of berried brood must be light grey (ensured fertilized); in case of berried brood transportation, a soft layer of wet cloth or foam needs to be set to reduce stress on egg mass; some water samples from brood source to be collected to know the salinity of origin and subsequent acclimatization in transport way and in the hatchery as well.

Water and feed management

Setting up of a bio-filter is necessary where adequate seawater is in scares. If broodstock tank has no bio-filter system, at least 30-40% water must be exchanged 3-4 times in a week. For recirculation system, the water of broodstock tanks is to be partially exchanged (30-50%) after 15 days to 1 month intervals. Water temperature ranged of 27-30°C, pH 7.5-8.5, salinity 27-30 ppt, dissolved oxygen of >5 ppm and ammonia are <0.1 ppm should be maintained properly. For communal rearing, limb lost crabs and shell infected/shell fouling crabs are to be discarded if any. A weekly schedule of various feed items like brackishwater/marine water fish, mussel meat, blood cockles, pollychaets, squid, etc. can give better performance. Feed is to be supplied twice a day @10-15% of biomass of which 25-30% in the morning and the rest at evening. Feeding tray could be used for proper management of feed and removal of uneaten feeds. The feeding tray should be cleaned once a week and the bottom sand must be agitated and cleaned weekly.

Spawning of broods

Berried broods may spawn within 3-60 days depending on appropriate selection of its maturity stage. Brood must be gently taken out from spawning tanks and repeatedly washed with clean sea water prior to transfer to the hatching tanks. Eggs are yellowish-orange, orange or reddish orange in color during spawning.

Management of berried broods and hatching

The hatching tank is to be filled with 27-30 ppt sterilized sea water and facilitated with aeration. Three-forth portions of the tank need to be covered with black polythene sheet or orchid net for providing darkness and to hold a stable temperature. Berried broods are to be disinfected with 100 ppm formalin solution for 30 minutes, washed repeatedly with clean seawater and transferred to the hatching tank. For 1st 3 days, the aforesaid feed to be supplied @ 10-15% per day. After one hour, uneaten feed should be siphoned out. When the egg mass turned into dark orange or light brown color, the food supply should be stopped. Aborted eggs and feces should be siphoned out before water exchange. About 30-40% of water should be exchanged every day. In order to examine the embryonic development and bio-fouling, it is necessary to investigate a small portion of egg under microscope in every 3-4 days during incubation period. In case of infestation of eggs by fungus or protozoa, Teflon (44% trifuralin) @ 0.1 ppm may be applied in water every 2-3 days for prevention. Hatching (production of Zoea-1) may occur after 10-14 days under 27-30 ppt salinity and at a temperature of 27-30°C. Hatching usually starts in the very early morning and ends within 30 minutes to 1 hour. The hatched crab will be transferred from the hatching tank after completion of all eggs release. An adult crab is able to lay eggs twice more within 1 to 4 months without molting and further matting.

Suitable location/ecosystem
Coastal regions (Satkhira, Khulna, Bagerhat, Chattogram and Cox's Bazar) of BAngladesh

Benefits
- This technique can produce 40-69% berried brood with 84-94% hatchling success.
- Application of the technology would be helpful for sufficient berried brood supply to the hatchery for uninterrupted seed production.
- A new value chain for mud crab aquaculture would be developed through supplying cultivable seedlings to the fattening and soft shell farms.
- Adoption of the technology might be helpful for enhancement of crab production and remittance earning as well as conservation and management of the natural stock.

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Title of the technology-02
Improved larvae rearing techniques and seed production of mud crab

Introduction
Mud crab farming was curiosity started in the coastal areas of Bangladesh during 1980's through fattening. Over time, 100% of these activities depend on exploitation of crabs from natural sources. As a result, the availability of crab resources and biodiversity in the coastal areas (Khulna, Satkhira, Bagerhat, Patuakhali, Cox's Bazar) is gradually getting threatened. With increasing the demand in the international market, cultivation area is increasing gradually. As a result, there is a growing tendency to exploit mother crabs as well as juveniles and immature small crabs indiscriminately. In order to develop crab farming in a long-term and sustainable manner, hatchery production of crab seed and development of nursery techniques are essential.

Description

Live food production for mud crab hatchery management

Mud crabs larvae prefer rotifer especially *Brachionus sp* as their first food. Rotifer rapidly propagates by grazing a variety of unicellular green algae such as *Tetracelmis*, *Nanochloram*, *Nanochloroposis* and other green algae. Production of live feed (green algae and rotifers) should be increased as gravid (mother full of ovary) is being collected in the hatchery. So that there is adequate food availability as soon as the eggs are hatched. The live feed production process takes 3-6 days for the maximum concentration of *Nanochloram* and *Brachionus* cultivation. Therefore, it is essential to keep three sets of tanks for each species of live feed culture. So that, as one set is harvested then the next set is useful. A volume of 20% starter should be added for initial inoculums.

Materials and water purification for larval rearing and tank preparation

All items needed in hatchery especially, tanks and all utensils, especially beaker, mug, bowl, bucket, filter bag, hose pipe, are treated with 10-20 ppm calcium hypochloride (Bleaching) solution and additional chlorine must be neutralized by washing with sodium thiosulphate solution. Water used in the hatchery (algae culture, broodstock rearing, artemia hatching and larvae rearing) must be disinfected by applying 10-20 ppm calcium hypochloride (bleaching) with strong aeration for 1-2 hours. Then the aeration should be stopped and left for 12-24 hours. Additional chlorine should be deactivated by adding required sodium thiosulfate and strong aeration for 5-6 hours. A fibre glass or cemented smooth circular tank with a capacity 500-1000 liters is suitable for larvae rearing. The tanks should be filled with 30 ppt seawater. The tank water should be treated with 50 ppm bleaching and left for 24-38 hours with aeration, then left for further 36-48 hours to settle down. Purified water should be taken in larvae rearing tank passing through sand filter followed by 0.5 micron bag filter and finally by UV (ultraviolet) filter. Water should be prepared by applying prophylaxis at the rate of 0.3 ppm on the day before larvae stocking.

Collection and management of larvae

Berried brood production and hatching (zoea production) of mud crab has been discussed in the previous technology. It is best to collect freshly hatched zoea as soon as possible to protect them from microbial infection. One side of the hatching tank lid should be opened and the healthy swam in the surface water should be collected periodically with the help of clean glass beaker and kept in a bucket or half filled with saline water at the same temperature (26-30 °C) and light air circulation. The quantity of larvae should be determined by counting and sub-sample of 100 ml larvae from the bucket.

Crab larvae stocking process

It is better to stock the larvae just after larvae collection completed. The initial stocking density of larvae should be 50-100 per liter. Prior to stocking in the rearing tank, the water temperature of the tank and the larvae containing bucket should be measured. If there is any discrepancy, larvae should be stocked with proper acclimatization. The larvae containing bowl should be floated on rearing tank with light aeration 10-20 minutes. Then 1 liter of water from the rearing tank should be slowly added to the bowl after every 5-10 minutes. In this way, acclimatization should be completed until the temperature is equalized, and releasing of zoea should be done very carefully.

Feed management in larval rearing

First feeding to the newly stocked zoea (Zoea-1) should be done immediate after stocking. Good results are obtained by using rotifer (20-30 ind/ml) and decapsulated *Artemia* (1 no/ml)

from zoea-1 to zoea -2 as first food. Later on, *Artemia* nauplii (3-5 nos/ml) should be provided as feed from zoea-3 to Megalopa. It is better to apply the feed 4-6 times a day without applying it once. In order to maintain the nutritional quality and mobility of the rotifer, it is advisable to apply green algae at the rate of 0.5 x 10^8 per ml in the larvae tank. Also, commercial shrimp larvae feed available in the market can be applied 2-4 times a day @1 g/ton of water. Good results are obtained by applying liquid feed (EZ larvae-1) from Zoea-1 to Zoea -2 @2.5-3.0 m/ton of water 3-4 times a day.

Water quality and management

In order to reduce the incidence of larval disease, prebiotics at the rate of 5 ppm and probiotics at the rate of 0.5 ppm should be applied in the larvae tank at every three days after changing of water for a period of first 10 days. Then prophylaxis should be applied at the rate of 0.3 ppm at three days from 14 days to megalopa. Water quality (salinity, temperature, pH, dissolved oxygen, ammonia and nitrite) of larvae rearing tank must be monitored daily and maintained in congenial level. It should be better to add 10-25% clean water for first 1-2 days without changing. Subsequently 25-40% water should be siphoned out after every 1-2 days and equal volume of clean needed to be added. Suitable salinity of larvae rearing tank should be: 28-31 ppt; Temperature: 26-31°C; PH: 7.5-8.5; Dissolved oxygen: 4-6 ppm; Ammonia: 0.05-0.1 p.m. Nitrite: <0.01 ppm. In this way, zoea may be metamorphos into megalopa within 18-19 days.

Megalopa food application and management

At the megalopa stage, setting of adequate shelter is required with hanging nets, sand bottom, pieces of rock, dry tree branches or pieces of plastic pipes. After the first 3-4 days of swimming, megalopa takes shelter at the bottom. At this time 5-6 days old Artemia and fish meat, squid, oyster meat is stuffed with bender and applied twice a day (morning and afternoon) as feed. About 40-60% water should be changed every 2 days. At this time the salinity of water should be gradually reduced by 0.5 ppt each time of water change and fixed at 25-26 ppt. In this management process, the megalopa may transform into crab inster (Cablet-1) within 5-6 days under 28-30 °C temperature.

Feed management for crab Instar (C1)

Likewise of megalopa, adequate shelter is also needed to be installed for crab instar. Feeding should be done with chopped tilapia fish or trash fish and oysters meat should be applied twice a day (morning and afternoon) @40-50% of the total body weight. The bottom of the tank should be cleaned by siphoning every day and 25-50% water should be exchanged. At this tim, the salinity of water may drop down by 0.5 ppt each time of water exchange, which should be fixed at 20-22 ppt.

Survival rate of crab larvae

Zoea may metamorphos to megalopa within 18-19 days with the survival of 14%, meanwhile megalopa turned into crab instars within 24-27 days from hatching with a survival rate 7%.

Suitable place or region for technology

In the crab hatcheries and modified Bagda shrimp hatcheries of the coastal areas of Satkhira, Khulna, Bagrehat, Chittagong and Cox's Bazar.

Technology benefits

Partial supply of crab seeds for nursery, soft shell shedding and crab fattening farms. Creating of new entrepreneurs for crab hatchery set up and value chains in the crab aquaculture industry. The technology will be helpful for conservation of the declining natural resources of mud crab as well as protection of biodiversity.

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Component 4 (KU)

Title of the technology-01
Measures to control bacterial infection in mud crab Scylla olivacea hatchery

Introduction
Mud Crabs (*Scylla* spp.), a key artisanal coastal fisheries resource in many tropical and subtropical Asian countries, are important export commodity with its high demand in sea food market. Bangladesh exports mud crabs to Taiwan, China, Hong Kong, Thailand, Korea, Singapore, Japan, USA and EU. Mud crab from Bangladesh is being exported mostly hard-shell crabs in live forms, and in the last 6-7 years, soft-shell crabs in frozen forms.

Despite the potential role in the national economy and livelihood improvement, mud crab aquaculture is not scientifically well prevailed in Bangladesh; mud crab fattening and recently soft-shell mud crab production are substantially practiced, nevertheless there is no established hatchery from where crablets will be produced and supplied. About cent percent of the crabs are being caught from natural sources, which is putting natural stock at the risk of depletion; particularly a large quantity of small crabs harvested from natural sources are being stocked in soft-shell farms, and a significant mortality (10-30%; during very hot weather, >60%) occurred. So, Seed production in hatchery condition and development of nursery management are very crucial options for sustainable aquaculture of the species. Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI-BS) successfully produced the seed in hatchery condition during 2015-2017, where the survival was low. Very recently, BFRI-BS PBRG sub-project conducted research on "Adoption of Innovative Technology: Seed to fattening of Mud crab (*Scylla olivacea*) and health management in Bangladesh condition" with the aim to increase survival of mud crab seed in the hatchery condition.

There have been increasing reports on infection and/or disease incidence in mud crabs; particularly in hatchery and farms, which is a global concern. Vibriosis caused by *Vibrio* spp. has been greatly responsible for mortality in mud crab hatchery; *V. harveyi* and *V. alginolyticus* have been detected as causative agent for loss of appetite, reduced growth, dark hepatopancreas and morality of mud crabs in the hatchery.

Being component 4, Fisheries and Marine Resource Technology Discipline, Khulna University has been conducting research work on bacterial infection and/or disease occurrence in mud crab hatchery, farms and wild as well as determination of factors associated with bacterial infection and/or disease occurrence.

Description

Identification of bacteria in mud crab Hatchery

Vibrio spp. (e.g. *V. alginoolyticus, V. parahaemolyticus*), *Aeromonas* spp. and *Pseudomonas* spp. was detected in the mud crab broods in the early summer; only *Vibrio* spp. in the wet season and only *Aeromonas* sp. in the winter season. *Vibrio* spp. are identified in the brood feed. The Mud crab larvae are infected with *Vibrio* spp. (e.g. *V. alginolyticus, V. cholera*, *V. fluvialis, V. herveyi, V. parahaemolyticus*) and *Aeromonas* spp. The water of the tank may be contaminated with *Vibrio* spp. (e.g. *V. cholera*, *V. alginolyticus*) and *Aeromonas* spp. *Vibro* (e.g. *V. cholerae, V. alginolyticus, V. harveyi, V. parahaemolyticus*) and *Aeromonas* bacteria are also detected in the larval feed samples (*Artemia* and rotifer).

It is implied that mud crab hatchery is likely infected with bacteria, particularly *Vibrio* spp. and *Aeromonas* spp. Among larval stages, the zoea stage is mostly threatened with pathogen infection. To reduce infection as well as increase survival in the hatchery, the management of feed and water quality is crux; some important bio-security considerations are provided below as guidelines.

Standard practice

- Sufficient aeration must be maintained in the larval tank.
- The temperature of the larval tank is to be usually 28-30 ^{0}C; but 25 ^{0}C is recommended if there is any sort of *Vibrio* infection.
- No water change in the larval tank during the first 2-3 days, and 10% water can be added.
- About 30% of water is replaced daily on day 2–3 and up to 80% as the larvae grow bigger.
- Gentle water circulation so to keep larvae suspended singly within the water column, and constant water temperature as well.
- Excess food, moulted exoskeletons are to be removed immediately.
- Avoid high organic matter accumulation in the tank.
- No more over feeding.

Specific measurements

Disinfection and antibiotic treatment

- The water for holding the broodstock and larvae are to be treated (i.e. chlorinated, ultraviolet-irradiated); if chlorinated and then de- chlorinated with sodium thiosulfate.
- Though routine application of antibiotics is not desirable, antibiotics (e.g. oxytetracycline, Penicillin, Polymixin) and fungicide are still used throughout the world to control egg loss, zoea larvae mortality due to bacterial (e.g. *Vibrio* spp.), fungal infection and ciliate infestation; particularly during live food feeding, antibiotics can be used very carefully.

Probiotics as alternative to antibiotics

- Commercially available potential probiotics (e.g. *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp.) can be used in the larval rearing units.

Feed management

- Live feed e.g. algae, rotifer, *Artemia* are to be cultivated in axenic condition.
- Rotifer and *Artemia* can be fortified with EPA and DHA rich microalgae (e.g. *Chlorella* spp., *Nannochloropsis*) by applying bioencapsulation process.
- Hormone ecdysoncan and cholesterol can be supplemented.
- Bioflock technology can be used.

Suitable area/location: Coastal mud crab hatcheries of Bangladesh

Benefit
The occurrence of bacterial infection in the mud crab hatchery in the Bangladesh condition can be reduced and/or controlled through practicing this bio-security guideline.

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ii) **Effectiveness in policy support (if applicable)**

Component 3 (BFRI-BS)

- Harvest of mud crab from natural source has been banned on by regulations during the month of January and February of each year. However, the technology developed for domestication of brood stock in captive condition and subsequent production of berried crabs might provide qualitative and quantitative berried broods supply during the banned period for continuous and smooth hatchery operations. Thus, natural brood stock might be preserved and recruitment might be uninterrupted.
- The hatchery operation protocol and seed production technology that has been upgraded from this research might influence the hatchery entrepreneurs towards establishing new crab hatchery for supply of seeds in nursery, culture, soft shell culture and fattening. Hence, the harvest size regulations (no < than 80g) might be implemented easily.
- Overall generated knowledge, information and technologies from this research might establish new value chain like, brood stock developer, seed producer, nurseries rather than only fattening and soft shell producing.
- Mass implementation of technology might enhance crab production and foreign currency earning.

Component 4 (KU)

- Useful for developing guidelines of bio-security for mud crab hatchery. This point is related to the clauses 4 (1), (2B,F, schedule-2,9) of Fish hatchery rules 2011/12; clauses 8,9, 13,14, 17,18 of fisheries quarantine act 2018.
- Provided with the data obtained, this project may have contribution to good aquaculture practice (GAP) in mud crab farms in Bangladesh. The point GAP is linked to the clauses 10.10 (Policy related to fisheries environment) of National Fisheries Policy 1998; clause 11(schedule-3) of Fish hatchery rules 2011/12; clauses 3-7 of the fish and fish products (inspection and quality control) ordinance 1983; Clauses 3(1),8,13, 14, 15, 16,18, 22, 24,25,27, 28 of Bangladesh Gazette (26/11/2020); 18 of Fisheries Quarantine Act 2018; 6 of Marine Fisheries Ordinance 1983.
- The data is valuable for applying HACCP system along the mud crab marketing channel; traceability and quality products are related to the clauses 10.1(Establishment of hygienic fish landing centers), 10.2 (Transportation and marketing); 10.3 (Fish Processing -and Quality-Control); 10.4, 11.4 (fish export, export); of National Fisheries Policy 1998; clauses 3-7 of the fish and fish products (inspection and quality control) ordinance 1983'; 18 of Fisheries Quarantine Act 2018; 18,22, 24,25,27, 28,43 of Bangladesh Gazette (26/11/2020), and clauses 3.4.9, 5.12, 5.13,5.24,5.28.16, 6.5 the Export Policy 2018.

H. Technology/knowledge/generation/policy support (as applied)

i) Immediate impact on generated technology (commodity & non-commodity)

1. Broodstock can be developed under captive conditions instead of relying on natural sources.
2. Seed production technology will be helpful for formulating hatchery guidelines and policy making for the protection of juveniles.
3. Integrated mud crab aquaculture will be helpful for increasing per unit production.
4. Suggestive measures developed to control bacterial infection will be helpful for future mud crab hatchery operations as well as controlling bacterial diseases at farm levels.

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

1. Initial success in mud crab brood production technology will be helpful for developing future advance technology for brood development in tidal waters of Bangladesh.
2. Crablet survival under the present research has attained at highest 7% level that requires sustainability. Thus, for sustainable output, future continuation of the research in various dimensions is highly emphasized.
3. Current findings of the research on pro & pre biotics will encourages to produce locally new pro-biotics and pre-biotics applicable for mud crab hatchery and aquaculture avoiding present importation of commercial ones.
4. Research on increasing soft shell shedding rate by reducing mud crab mortality should be considered.

5. Integrated aquaculture and biological crab production should be considered.
6. The availability of new data on bacterial infection in mud crab hatchery, farms and in wild mud crab *S. olivacea* in Bangladesh., and the association of feed and water with bacterial infection in the mud crab production systems will effectively used in:
    - development of probiotic and prebiotic which can be applied in mud crab hatchery and farms.
    - application of sustainable mud crab farming techniques.
    - Development of bio-security management tools applicable in mud crab aquaculture channel.

iii) Technology transferred that help increased agricultural productivity and farmer's income

1. Overall developed technology on broodstock development, larvae rearing, nursery management and knowledge generated on fattening and soft shell shedding could create space for employment and increase aquaculture productivity, foreign exchange earnings and farmers income.

2. Based on disease history and existing pathogenic bacterial data of particular water body, effective application of selective probiotics can offer increased production of larvae/PL.

iv) **Policy support**

1. The results and findings of the project might be helpful for formulating policy on mud crab hatchery establishment and natural stock conservation of mud crab by minimizing the juvenile harvest (< than 80 g) and implications of banned period during January to February.

I. Information regarding desk and field monitoring

i. Desk Monitoring (description & output of consultation meeting, monitoring workshops /seminars etc.):

Date of the programs	Program descriptions	Organizing Unit	Activity done/output
31May, 2018	Signing of the Letter of Agreement (LoA)	PIU-BARC, NATP-2	Duly signed as per the terms and condition of LoA
06 November, 2018	Inception workshop	Fisheries Division, BARC	Learned about the recruitment of manpower according to PPR 2008, procurement of inputs as per budget and refinement of proposal
23-29 Nov., 2018 01 Sep., 2019	Financial Management Training	PIU-BARC, NATP-2	Learned about procurement rules and audit system
13 Feb., 2019	Progress presentation	BFRI, HQ	Presented the research progress and got suggestions from BFRI authorities and expert members

Date of the programs	Program descriptions	Organizing Unit	Activity done/output
!8 June 2019 03 July 2019	Annual progress review workshop	PIU-BARC, NATP-2	Presented the annual research progress and got suggestions on strengthening of research according to set objectives
23 July, 2019	Financial management workshop	PIU-BARC, NATP-2	Learned about financial rules and audit system
January, 2020	Half yearly progress review workshop	Fisheries Division, BARC	Presented the half yearly research progress and got suggestions on formulating good presentation and achieving the goals
7 September, 2020	Virtual Meeting on Progress Monitoring	PIU-BARC, NATP-2	Presented the progress
24 Nov.,, 2020	Annual progress review workshop	PIU-BARC, NATP-2	Presented the 2nd annual progress and received suggestions for next work
29 December, 2020	Preparation process of PCR of PBRG sub-project of Fisheries	Nutrition Unit, BARC	Oriented about guidelines of PCR development
16 June, 2021	Virtual Meeting on Progress Monitoring of PBRG Sub-projects	PIU, NATP-2, BARC	Got guidelines on formulating and submission of technology fact
23 Nov., 2021	Annual progress review workshop	PIU-BARC, NATP-2	Presented the 3rd annual progress and received suggestions for next work

ii) Field monitoring (date & no. of visit, name and addresses of team visit and output)

SL	Date(s) of visit	Monitoring team	Total visit (No.)	Output
Component 3 (BFRI, BS)				
01	14.05.2019 12.06.2019 26.06.2019	Technical division/ BARC	03	Visited the hatchery, laboratory and evaluated the progress and records
02	18.07.2020 25.08.2020 13.09.2020 06.06.2021 11.06.2021	PIU-BARC, NATP-2	05	Visited the hatchery, laboratory and evaluated the progress and records; suggested for further improvement

03	20.05.2019 06.06.2021	Coordination component, BFRI	02	Monitored the progress and advised for the improvement and speed up the activities
04	13.09.2020	Others visitors (EC, BARC)	01	Visited the research and progress; distributed crab seeds among farmers
Component 4 (KU)				
01	29.08.2020	Technical division/ BARC	1	Advances in sampling strategies; budget revision needed.
02	16/06/2019 25/08/2020 13/11/2021	PIU-BARC, NATP-2	03	Replacement of out of order instruments with new ones; budget revision needed; timely approval of procurement plan and fund disbursement; steps taken to collect diseased samples immediately after COVID-19 lockdown withdrawn; finalizing draft PCR.
02	14.11.2019 08.06.2021	Coordination component, BFRI	02	Progress in procurements; budget revision possibility; finalizing disease samples collection, histopathology analysis and advised for the improvement and speed up the activities

Monitoring of sub project activities by various team members

iii) **Weather data, flood/salinity/drought level (if applicable) and natural calamities:**

Both the research components (component 3 and 4) are located in Khulna district and all the research activities have been carried out in Khulna division. The following table is presenting last three years (2019-2022) average weather information collected from Khulna regional office.

Parameters	Seasons						Remarks
	Pre-Monson (January–April)		Monson (May – August)		Post Monson (Sept – December)		
	Max	Min	Max	Min	Max	Min	
Av. Rainfall (mm)	136	1	300	146	187	5	
Av. Temperature (°C)	34.43	12.63	35.04	25.63	33.67	15.1	
Av. Humidity (%)	77.67	70.67	86	78	84.33	78.33	
Flood (year & category)	-	-	-	1	-	-	
Av. Salinity (ppt)	18.50	2	19.00	5	5	0	
Natural calamity (Frequency & category)					Bulbul (category 3) Amphan (5) Yaas (category 1) Cyclone Jawad		

(Source: Regional Metrological Office, Khulna)

J. Sub-project auditing (covers all types of audits performed)

i. BFRI Coordination component (Component 1))

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 20.11.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	173661.00	Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams	Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 17.11.20 for the year 2019-2020	No objection raised, found all relevant documents updated as per guideline	190532.00		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 28.10.21 for the year 2020-2021	No objection raised, found all relevant documents updated as per guideline	224976.00		Financial management & project performance found satisfactory

ii. Component 2 (BARC)

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 30.10.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	209840.00	Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams	Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 09.12.20 for the year 2019-2021	No objection raised, found all relevant documents updated as per guideline	175779.00		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 11.10.21 for the year 2020-2021	No objection raised, found all relevant documents updated as per guideline	230074.00		Financial management & project performance found satisfactory

iii. Component 3 (BFRI-BS)

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 20.11.19 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	3075674.00	Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams	Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 30.06.20 for the year 2019-2020	No objection raised, found all relevant documents updated as per guideline	1428499.00		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 28.10.21 for the year 2020-2021	No objection raised, found all relevant documents updated as per guideline	2631047.00		Financial management & project performance found satisfactory

iv. Component 4 (KU)

Types of audit	Major observation/ issues/ objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial & Performance Audit by FAPAD on 14.11.2019 for the year 2018-2019	No objection raised, found all relevant documents updated as per guideline	3445179.00	Financial management of the component found running smoothly till the end of the project. No query or objection raised at any stage of operation by the audit teams	Financial management & project performance found satisfactory
Financial & Performance audit by FAPAD on 07.12.2020 for the year 2019-2020	No objection raised, found all relevant documents updated as per guideline	1217671.00		Financial management & project performance found satisfactory
Financial & Performance Audit by FAPAD on 14.10.2021 for the year 2020-2021	No objection raised, found all relevant documents updated as per guideline	2417767.00		Financial management & project performance found satisfactory

K. Lessons learned

The total sub-project activities allowed learning of the following lessons:

1. Knowledge about overall status of crab fishery in the southwest coastal part of Bangladesh.
2. Identifying the potential broods and domestication protocol for breeding
3. Techniques for berried brood production under captive condition have been explored.
4. Understanding the factors affecting the embryonic development and larvae quality
5. Crucial stages of larvae and subsequent nutritional requirement and water management technique
6. Overall techniques for hatchery operations and bio-security management.
7. Scenario of the prevalence of most pathogenic bacteria in wild, farm and hatchery condition and ooccurrences of disease of mud crab, causative factors and linked factors those accelerate the chances of mud crabs to be infected with pathogens.
8. Information for policy makers to strengthen policies for sustainable development of mud crab fishery sector.
9. Occurrence of disease of mud crab, causative factors and linked factors those accelerate the chances of mud crabs to be infected with pathogens.

L. Challenges (if any)

1. Shortage of skilled manpower for hatchery and nursery operation.
2. Lack of sources for sufficient seawater for hatchery operation and establishment of bio-security measures.
3. Gap of seasonal information on wild harvest due to two times ban period
4. Non-structured farms and non-scientific farming practice, and no record keeping by the stakeholders limiting data collection process.

M. Suggestions for future planning (if any)

1. Manpower development through domestic and international training
2. Mud crab hatchery should be established near to the resources, research should be directed with new dimensions for further development
3. Besides seed production, future research directions should be focused on value added product development
4. Assessment of stock status, breeding and nursery ground should be considered.
5. Training for mud crab farmers to adopt improved culture including GAP and HACCP.
6. Selection of appropriate site for crab farming i.e. zonation/cluster farming based on environmental suitability for Mud crab farming techniques.
7. Proper monitoring to stop ill fishing practice in the Sundarbans area.
8. The ban period should be revised through identifying proper breeding season of mud crab.

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Signature of the Coordinator	**Counter signature of the Head of the organization/authorized representative**
(Dr. Md. Zulfikar Ali) Date: 25.04.22 ***Chief Scientific Officer*** ***Bangladesh Fisheries Research Institute***	***(Dr. Yahia Mahmud)*** Date: 25.04.22 ***Director General*** ***Bangladesh Fisheries Research Institute***

Annexures

Coordination component

Annexure – 1 (*BFRI Coordination component*)

Recommendation of the coordination meetings

Meeting No.	Recommendations	Action taken
01	The meeting emphasizes the development of a joint activities sub-plan for establishing coordination mechanism within BFRI-BS and KU components so that a smooth and regular works of the components (particularly on water quality parameters, season, culture practice, bacteria sample collection, stage of development of the animals etc.) can be continued. In this respect, the two PI of the mentioned components will jointly develop the workplan within a shortest time period.	Implied as per meeting decision. Joint activity plan was developed by March'19 and implemented accordingly throughout the research period
02	Baseline study of both the components (BFRI-BS & KU) should be preferably done in farms of common fields/areas and hatcheries (as per objective of the survey) so that a complete picture of the status of crab culture and development activities, type of disease causative agents and intensity of disease occurrence can be reflected.	Based on the nature of activities of each individual component, most of the attempts taken as per recommendation.
03	In addition to various water quality parameters, KU component should also be taken into consideration the stage of development of crabs (wild and hatchery produced sources) while collecting samples from BFRI-BS component. BFRI-BS will extend necessary cooperation in this regard. KU component will transmit the sample analysis findings to BFRI-BS component time to time for necessary precaution.	Recommendation followed as and where necessary
04	The meeting reviewed the implementation status of all the previous recommendations/comments of different half yearly and annual progress review workshops held at BARC. The Meeting also emphasized immediate possible implementation attempts for necessary action against so far unimplemented recommendations.	Necessary action taken as per recommendation.

BARC component

Annexure –2.A

Recommendation of the Inception Workshop and status of action taken

Recommendations	Action Taken
General recommendation	
• Revision of the title of the Sub-project suggested	Title revised as follows: "Adoption of Innovative Technology: Seed to Fattening of Mud crab (*Scylla olivacea*) and Health Management in Bangladesh".
BFRI-BS component	
• Key problem in crab aquaculture need to be focused in the background justification of the sub-project;	Incorporate accordingly
• Difference in production of crabs between tradition practices and the practices under research attempts should be reflected specifically (attempt wise) in the reports;	Followed during the respective repot preparation
• Composition of the liquid feed used in larval rearing practices should be mentioned;	Stated as per suggestion
KU component	
• Use of the word "*quantify*" instead of "*enumerate*" under specific objective "1" suggested to make the sentence more catchy;	Necessary change done
• Under specific objective "3", name of another driving factor "feed" suggested to add along with soil and water"	Complied as per suggestion

Annexure -2.B

Recommendation of the Half Yearly Workshops

Recommendations of the First Half Yearly Workshop	Action taken
General comments	
• BFRI-BS and KU components should pay special attention for collection and gathering of base line information of the respective areas without further delay	Advice complied
• In addition to season and water quality, stage of development and sex should also be considered in case of bacterial load study and treatments etc.	Suggestions followed where necessary
Recommendations of the Second Half yearly Workshop	**Action taken**
BFRI-BS component	
• More emphasize to high up the level of survival rate of crab larvae should be given in the next part of the work;	Emphasize give. Crab larvae survival rate attained to 7% from previous 5%.
• More emphasize suggested for mitigating cannibalism among the cultured crabs;	Special attention paid
KU component	
• Result should also indicate the possible causes for mass mortality of crab larvae along with suggested precautionary measures to reduce/control mortality level due to pathogens;	Findings with justifications included in the next reports

Annexure – 2.C

Recommendation of the Annual Workshops

Recommendations of the First Annual Workshop	**Action taken**
BFRI-BS component	
• No baseline information was presented;	Completed survey report was presented in the next workshop/meeting
• Causes and justification must be expressed in case of better performance of hatching related to distance of brood collection, stress and degree of gravidness of the female crabs;	Specifically described in the next reports
• A relation should be discussed between the brood management practices and hatching performance of the gravid crab;	Complied accordingly
• More emphasize suggested for mitigating cannibalism among the cultured crabs;	Complied accordingly
KU Component)	
It is suggested to discard the literature review part and other information related with international/foreign research activities from the baseline findings. Rather this should only contain the prevailing native situation of the respective area of research;	Revision done
Study should also be focused on pathogen transmission pattern i.e. horizontal or vertical and role of crab (culture or wild) as transfer media of S*higella* and S*almonella* to the ultimate consumer;	Due to unavailability of additional funding support and time limitation this attempt was not undertaken
Collection and study of crab samples from a mixed population of crab-shrimp-fish of the Sunder bans area for pathogenicity emphasized;	Sample collection and study done accordingly
Recommendations of the Second Annual Workshop	**Action taken**
BFRI-BS component	
• More trials under different treatments (considering effecting factors) are advised for scientifically establish the higher survival performance (7%) of crab seed;	Complied as per suggestions
• Thrust on study to find out the regulatory factors on soft shell shading suggested. Effect of seasonal change should be taken into consideration because temperature as great effecting factor is expected to be started reducing from the mid of November;	Vital factor identifies and recorded
KU Component	
• Development of guidelines to minimize/reduce infection possibility and disease in mud crab ponds through control of pathogens;	Extension guidelines developed
• Necessary precautions for monitoring of environmental parameters to control growth of microbes in crab body particularly in early stages (Zoe 1, 2 and 3) may be hopefully to increase the survival rate of larvae;	Observation done results came up with positive views