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CLIMATIC STRESS ON CULTURED *SCYLLA* SP.: IMPACT ON LIVELIHOOD AND PUBLIC HEALTH IN WEST BENGAL.

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ABSTRACT:

Scylla sp. is the one of the most demandable aquacultural sp. in throughout the India due to its export value, large size, high quantity meat content and good source of protein and calcium ions. In West Bengal coastal region there are estuaries, creeks and tidal mud flat, mangrove swamp that are the natural habitat of aquacultural shellfish *Scylla* sp. Mudcrab (*Scylla* sp.) is an important biotic decapod member that has important role to maintain the ecological balance of Sundarbans Biosphere Reserve. In West Bengal capture and aquaculture both type fishery activities are performed. A large portion of people of West Bengal involve in crab fishery for their livelihood by wild seed capture and fattening since late 1990s. In West Bengal different districts have brackish water fishery for direct marketing after fatting. Smaller sized mud crabs from capture fisheries contribute to the domestic market whereas crab fatting grow-out system aim for export market. According to Global Climate Risk Index, 2019 India obtained 7th position in World. Water and soil physico-chemical quality are most important factors for surviving and production of *Scylla* sp. Climate fluctuations have impact on mudcrab natural habitat due to increases salinity, pH and temperature. The availability of commercially-important crablets and their stock in brackishwater has been gradually decline due to high salinity, temperature, pH. Such climatic as well as bio-unsafe environment also helps pathogens like bacteria to invade as opportunistic pathogens (e.g. Vibrio sp.) and create diseases frequently. Mass mortality of *Scylla* sp. is very common in West Bengal due to pathogenic infection (Bacteria, Virus, fungi etc) and Vibrio bacteria infected crabs may potentially transmit toxic genes as zoonotic strains in other organism including human through consumption. Fever, Chills, nausea, hypotensive septic shock, lesion, infection, Gastroenteritis and Diarrhea are common diseases. So stressors could affect mudcrab, public health as well as economy of State. The main goal of this study is to screen the responses of *Scylla* sp. against climatic stress and suggest. So proper environment management can secure the environment, animal, food safety and livelihood of people of West Bengal.

Key words: *Scylla* sp., Aquaculture, livelihood, Climatic stress, Diseases, Public health

INTRODUCTION:

Fishery in India based on capture and aquacultural activities and about 465 cultivatable aquatic organisms, belonging to 107 families of animals were screened by Jhingran and Gopala- Krishnan in 1974. Due to high Productivity, high protein and minerals content, integrated farming, earning foreign exchange aquaculture is now fasting growing industry in India and according to report (2018) of Fishery ministry of Govt. of India, India possesses 3rd position in world (china 1st and Indonesia 2nd) for sharing percentage of aquacultural production. Indian coastal region surrounded and protected by the marine water and such regions are used as EEZ for aquacultural fish and shellfish (i.e. prawn, crab and shrimp). In west Bengal coastal region consists

of large numbers of estuaries, creeks, tidal mud flat, bheries, mangrove swamp that supports a good number of aquacultural shellfish which is economically (foreign exchange), nutritionally (protein and minerals rich flesh of muscle), ecologically (estuarine detritus feeder) important (Nandi and Ghatak, 1985). Aquacultural mudcrab, prawn and shrimp, edible form of aquatic species and considered as an important member of Biosphere Reserve (Fig. 1). Among crabs *Scylla* sp are considered as the most valuable species crab species for culture in India (Mahapatra *et al.*, 1996; Dev Roy and Nandi, 2019) (Fig. 2). According to Global Climate Risk Index (2019) out of the 10 most affected countries, India obtained 7th position in World after Afghanistan (GLOBAL CLIMATE RISK INDEX 2021). So climate changes has a significant effect on extreme weather events, increasing their frequency, intensity and duration. Global Climate Risk report is showing that climatic fluctuation immensely alter temperature, pH and salinity of soil and water in affected countries. High temperature, pH and salinity can modulate as well as reduce biological activities which can decline the adaptively and also prone to infect frequently the species in its natural habitat that can destroy the population in near future (Das and Nandi, 1999) (Fig. 5 and 7). Controlled water and soil physico-chemical quality are most important factors for aquaculture and modulation may collapse the entire system by affect the life cycle of aquacultural species as well as cultured farm (incomplete moulting, pathogenic infection, hindrance of growth and development etc. (Heasman and Fielder, 1983; Mandal *et al.*, 2018) (Fig. 3). By consumption of infected mudcrab, human may suffer with various health problems including Fever, Chills, nausea, hypotensive septic shock, secondary lesion, infection, Gastroenteritis and Diarrhea etc. (Sarijito *et al.*, 2018). Very less information on climatic stress on aquacultural *Scylla* sp. and impact on livelihood, Public health in West Bengal is available in literature (Saha, 2012; Saha and Roy, 2014; Saha and Biswas, 2015) (Fig.6). The aim of the present study is to screen the responses of mudcrab under climatic stress and effect on livelihood, economy and public health also. Different responses of mudcrab can be used as early detecting signals of environmental stress and bio indicators before whole organism become decline.

1. Mudcrab (*Scylla* sp.) aquaculture and alternative livelihood in West Bengal:

Out of 15 species, only 10 sp. of crabs are consumed in West Bengal and are categorized as edible. They are found in considerable quantities in ponds, beels, baors and other aquatic bodies throughout the Gangetic plains of southern West Bengal (Das and Nandi, 1999; Ray and Saha, 2011). West Bengal is the only state in India, where fishes have been cultivated in all types of water bodies', i.e., sweet water, brackish water, sewage water, and marine water, etc. The total productions of inland fish and marine fish in WB are 15.30 Lac ton and 2 Lac ton, respectively. These are mainly consumed in the state and rest spare for Delhi, Uttar Pradesh, Madhya Pradesh, Bihar, and other adjoining states. Export of marine fish earned handsome revenue of Rs.700 crore in the year 2009-2010. Foreign exchange through mud crab in India (2000) was US$ 5.5 million. West Bengal occupies the 4th position in the country regarding export of sea-food products (Dev Roy and Nandi, 2019). Fishes are exported primarily to Japan, Vietnam, and China. Out of the total exports, 90% are shrimps and the rest includes ornamental fish, crab, fresh water prawns (Food Processing Industries Survey, West Bengal, 2012-13. According to the Red List published by IUCN (2008), approximately 89 species of crabs and copepods are under threatened and major caucuses for threats to

crustacean specially crabs are habitat destruction, over exploitation, intensive aquacultural practices without appropriate planning and pollution due to land for human settlement, industrial development and used of insecticides in agricultural fields, climatic change etc. (Saha and Biswas. 2015).

In West Bengal capture (adult mudcrabs collection and sale in market) and aquaculture (crablets or juvenile mudcrabs collection and grow-out in culture farm) both type fishery activities are performed for livelihood among the small holders (Fig. 4). Mudcrab fattening predominates farming practices in West Bengal since late 1990s where mudcrabs are held individually in the containers or cells for the culture of soft shell mud crabs in this system small crabs (200 gm) with soft shells are held in isolation within box until shell harden with until marketable weight gained (200 to 400 gm for *Scylla olivacea* and 300 to 900 for *Scylla serrata* for 4 to 6 months) (Mandal *et al.*, 2018) (Fig. 4). In West Bengal, three districts have brackish water fishery (North 24 Parganas, South 24 Parganas and Purba Midnapore) for direct marketing as well as aquaculturing. Smaller sized mud crabs from capture fisheries contribute to the domestic market whereas crab fattening or grow out systems aim for the export market (Fig. 4). Through wild seed collection, purchasing crablets, nursery culture in hapa, grow-out in pond as polyculture, box culture, supply local market and export market, a large number of people of rural and urban are involved in alternative livelihood as mudcrab fishery in West Bengal (Lalramchhani *et al.*, 2019, Ghosh, 2019) (Fig. 4). Aquacultured farmers generally purchase seeds or crablets of mud crab from wild seed collectors (0.10 USD / 20 gm. size) and crab fattening is carried out with such feed in pond (seasonal or dependent upon availability fish, molluscan) according to body weight either directly or pen or cage systems for a period of 20-30 days approximately until the mature gonad developed as marketable good price. For crab feeding a group of people involve to feed different low cost foods (by catch fish, molluscan meat) according to size and weight and availability (Lalramchhani *et al.*, 2019, Ghosh, 2019). At present intensive cage or pen culture method of mud crab fattening is a secure commercial business in West Bengal among mud crab farmers. Most of the farmers directly sell their product to exporters. In box cage systems, fattening is largely carried out although grow-out is also done in a lesser scale.

2. Environmental stress on mudcrab (*Scylla* sp.) aquaculture:

In nature we are less aware of mudcrab disease problems because sick animals are quickly removed from the population by predators. In addition, mudcrab are much less crowded in nature than in captivity. Parasites and bacteria may be of minimal significance under natural conditions, but can cause substantial problems when animals are crowded and stressed under environmental conditions. Disease is rarely a simple association between a pathogen and a host mudcrab. Usually other circumstances must be present for active disease to develop in a population and disease rarely results from simple contact between the mudcrab and a potential pathogen. Environmental problems, such as poor water quality or other stressors (high temperature, salinity, pH etc.) often contribute to the outbreak of disease (Fig. 6). Mudcrab farming in West Bengal depends on wild seed collection and environmental crisis through climatic change pose a threat to wild population. Fluctuating climatic condition increases the salinity, pH and temperature of Sundarbans environment (Saha and Biswas, 2015, Mandal *et al.*, 2018). The availability of commercially-important crab and their stock in

brackish water has been gradually decline due to high salinity, temperature, pH etc. Such climatic as well as environmental stress helps bacteria to invade as opportunistic pathogens within animal (e.g. *Vibrio* sp.) and create diseases (Ray and Saha, 2011, Jithendran *et al.*, 2010) (Fig. 5 and 7). Pathogen like Vibrio sp. grow naturally in estuarine and marine environment and rapidly grow in contaminated water with high salinity (Igbinosa and Okoh, 2008). Mass mortality in *Scylla* sp. is very common in India due to pathogenic infection (bacteria, fungus, virus, protozoan etc.) and pathogen infected crabs may potentially transmit toxic genes as zoonotic strains in other organism including human (Ausin, 2010; Batabyal *et al.*, 2014). So climatic stressors (high temperature, salinity) could affect directly as *Scylla* sp. death or through inhibiting immunity thus allowing pathogen invasion and indirectly affect public health also (Fig. 5). However, despite its ecological and economic relevance, there is no data available in *S. serrata* regarding climatic stress. High temperature and salinity can modulate as well as reduce biological activities which can decline the adaptively and also prone to infect frequently that can destroy the population. Therefore, climate change impacts may significant from global change to the community or district level. In 2001, the IPCC stated that "new and stronger evidence that most of the warming over the last 50 yrs. is attribute to human activities" and also suggested that the way in which the climate change during 21st century will be a result of both natural changes and the response of the climate system. So proper scientific management or plans can secure the environment, animal, food safety and livelihood of people of West Bengal.

3. Diseases of aquacultural mudcrab (*Scylla* sp.):

Deficit or alteration of immunocompetence response towards altered environment, mudcrab become susceptible with various pathogens that causes serious fatal diseases (white spot disease - WSSV, bacterial shell disease – Vibrio sp., shell decolouration – low pH and iron precipitation, incomplete molting – low temperature and nutrition, blackened ovary – microbial injury and melanization etc) (Fig. 5 and 7). The bacterial diseases was reported as a main problem in the edible mud crab culture and bacteria infected crabs may potentially transmit pathogens to various rearing facilities, adjoining farms, natural environment and mass mortality of farmed mud crab stocks have been reported frequently (Gunasekaran *et. al.*, 2017). In India, mud crab culture mainly depends on fattening of soft-shelled or water crabs and disease in cultured crab populations and seed production facilities need to receive a serious attention. The main problem in hatchery technology of *Scylla* still seems to be related to bacterial infection that can spread quickly within mudcrab when crabs are immunologically weak. Immunological defense depends on health status of mudcrab that may decline by environmental crisis (Batabyal *et. al.*, 2014). The common bacterial disease observed in larvae and adults which is termed as 'black spot', 'brown spot', 'burnt spot', 'shell disease' or chitinolytic bacterial disease etc. (Fig. 5, 6, 7 and 8). These are some bacterial diseases in mudcrab was caused by bactericemia, such as. *Vibrio, Aeromonas* and a Rhodobacteriales-like organisme as chitinoclastic bacteria. Several chitinolytic bacteria (Gram negative rods) included *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., and *Spirillium* spp. were reported in mud crab. *V. ordalii* and *V. harrveyi; V. vulnificus, V. splendidus,* and *V. Orientalis; V. ginolyticus* and *V.cholerae; V. parahaemolyticus, V. campbelli, V. nereis and V. fischeri* (Jithendran *et al.*, 2010) (Fig. 6). The carapace degradation of crab species in the infection of chitin outer layer the exoskeleton can be associated with melanin synthesis of the affected

region. The numerous shell disease reports on the blue crabs and other crustaceans with comprehensive description of the shell lesion pathology (Gunasekaran *et. al.*, 2017). Filamentous bacteria such as *Leucothrix mucor, Thriothrix* spp. and *Flexibacter* spp. cause mortalities by discolouration of gills and associated secondary infections and Luminescent bacterial disease is a severe, economically important bacterial infection caused by members of the genus *Vibrio* and other related genera (Jithendran *et. al.*, 2017). Currently, 12 species of *Vibrio* are known to be associated with human infections acquired by consumption of contaminated foods and water. These 12 different so described which have zoonotic potential and non-pathogenic strains. Some opportunistic *Vibrio* bacteria (*V. vulnificus, V. cholera, V. damsela*) show lethal effect for human as pathogen and human infections (Sarijito *et al.*, 2018) (Fig. 6). These bacteria are part of the natural flora of marine environments worldwide and has been isolated from water, sediments and consumption of seafood like *Scylla* sp. containing pathogenic *Vibrio* sp. can result is a severe health problems in human (fever, Chills, nausea, hypotensive septic shock, secondary lesion, infection etc.) as zoonotic diseases (contact or foodborne) especially in immunocompromised mudcrab.

4. Mudcrab (*Scylla* sp.) and Public Health:

Bacterial pathogens in water and seafood are major sources of human infection and disease globally including West Bengal, India (Sarijito *et al.*, 2018). Fecal or "enteric" pathogens are associated with human and animal fecal contamination and indigenous marine pathogens basically *Vibrio* sp. are found naturally in estuarine and coastal environments (Batabyal *et al.*, 2014). Among *Vibrio* sp., *V. vulnificus*, *V. parahaemolyticus*, and *V. cholera* being of greatest concern, especially considering rates of infections by these organisms are increasing globally day by day due to climate change (warmer waters) (Austin, 2010). *Vibrio* infections are some of the most deadly and costly of food and water-borne diseases and asymptomatic infection are also reported as an important reservoir where Vibrio disease is endemic (Igbinosa and Okoh, 2008). These pathogens are generally opportunistic nature and found in nature freely when they are chance to assess weak immunocompromised body then invade from water or soil and adverse climatic change accelerate such pathogenic invasion and finally enter human through collection, handling and consumption (Davis and Sizemore, 1982; Gunasekaran *et al.*, 2017). Both indigenous and exogenous pathogens may cause diseases from coastal and marine environment and their occurrence depends on climatic condition (Igbinosa and Okoh, 2008). We can easily irradiate the pathogenic infection in mudcrab and contamination to human some extent by follow some control measure and awareness on public health:

a. Routinely monitor the pathogen indicator organisms to assess the risk of pathogen (**Surveillance**).

b. Rapid, reliable, low priced, commercial molecular diagnostics for pathogen quantification in marine waters as well as seafood (**Diagnosis**).

c. Waste water treatment (water filtration) is one of the most important way to prevent and stop exogenous source of pathogens (**prevention**).

d. Mudcrab should be taken for quarantine before culture or market (**prevention**).

e. Improve the understanding (public awareness) of the Public Health Risk within common people (**prevention**).

f) Finally restore the nature and natural resources to maintain ecological balance and minimise climatic

stress (eco-environmental approach) through control anthropogenic activities (**Treatment**).

5. Experiment to assess the environmental stress:

a) Oxidative stress:

i) Estimation of NO:

Generation of NO is assayed spectrophotometrically at 550nm following the principle of Griess reaction. Reaction process involve optical measurement of pink colored nitrite, stable and non-volatile breakdown product of NO. This reaction system is capable of generating nitrite in biological materials including cell suspension. The molar concentration of nitrite in the sample is determined by using sodium nitrite as standard. Activity of NO generation was expressed as mM nitrite/(min 106 cells) (Green *et al.*, 1982; Saha and Ray, 2014).

ii) Estimation of Super oxide anion (O_2^-) :

Generation of superoxide anion (SOA) is estimated spectrophotometrically by nitro blue tetrazolium (NBT) reduction reaction. Blue colored formazan is measured spectrophotometrically at 630nm and activity of superoxide anion was expressed as optical density (OD)/(min x 10^6 cells) (Song and Hsieh, 1994; Saha and Ray, 2014).

iii) Estimation of Phenoloxidase (PO):

Determination of activity of phenoloxidase is carried out spectrophotometrically by estimating the rate of formation of dopachrome from L-3, 4 DOPA modification after **Sahoo *et al.* (2005)**. The rate of formation of dopachrome is screened by recording the optical density at 475 nm in 5 minutes of duration. Activity of PO is determined as an average of increment of OD at one minute of interval for a total span of 5 minutes. Activity of enzyme is expressed as Unit / mg protein / minute (Tanner *et al.*, 2006; Saha and Ray, 2014).

b) Antioxidant stress:

i) Estimation of Super oxide dismutase (SOD):

The principle of estimation of SOD activity is based on 50% inhibition of NBT reduction. Inhibition of NBT reduction is measured spectrophotometrically at 560nm. One unit of SOD activity is defined as the amount that inhibits of NBT photoreduction by 50%. Activity of SOD is expressed as U/(mg protein min) (Krishnan *et al.*, 2002; Saha and Ray, 2014).

ii) Estimation of Catalase (CAT):

Activity of CAT is estimated following the principle of reduction of H_2O_2 added into the reaction mixture. Gradual decrease in absorbance was monitored spectrophotometrically at 240nm for 30 s. The difference in absorbance per unit time is considered as measure of CAT activity. The enzyme unit is defined as the amount of enzyme that catalyzes the oxidation of 1 mol hydrogen peroxide/min (Prakash and Rao, 1995**;** Saha and Ray, 2014).

c) Bacterial stress:

i) Diagnosis of vibrio load:

From collected crabs, withdrawal hemolymph from the base legs using a syringe fitted with 16 gauge needle to examine bacterial contamination. Collected hemolymph is taken and cultured in TCBS culture media by spread plate method (Davis and Sizemore, 1982). Observe for bacterial colonies after 24 hr. incubation at room temperature (37^0C) within bacteriological incubator. The presence of green and yellow bacterial colonies (droplets) on plate's indicate bacterial contamination of hemolymph with *Vibrio* sp. (Aftabuddin *et al.*, 2013). After 24 hours, the incubated plates are observed by naked eye for characteristic and count Vibrio colonies. The counts of colonies are considered as per ml and are calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution factor. The calculated results are expressed as colony forming units (cfu) per ml of sample. Each colony, separate by colour and size, is counted separately as

Colony Forming Unit per 100 µl of crab hemolymph (CFU / 100µ) thrice and averaged. Then, discrete representatives of each colony type were randomly selected for purification and characterization (Eyisi *et al.*, 2013) (Fig. 6). N number of colony present in 100 µl of hemolymph spread on TCBS N x 10^2 CFU in 100 µl = N x 10^3 CFU in 1000 µl / 1 ml So, N x 10^3 *Vibrio* sp. CFU present in 1ml of *Scylla serrata* blood (hemolymph).

ii) Histopathology of Carapace:

For histopathological analysis of carapace or exoskeleton is dissected out from infected and non-infected crabs. Dissected parts are prepared and staining through a series histological steps. After proper histological preparation, sections are passed through xylene and graded ethanol and stained with hematoxylin and eosin. Stained sections of tissues are microscopically observed under light microscope and images are recorded digitally using a camera fitted with microscope (Ray and Saha, 2011) (Fig. 8).

CONCLUSION:

Aquaculture (Fish, prawn, crab etc.) represents one of the fastest growing food producing sectors of the world. It accounts for around 17% of global animal protein serves as normal diet and support the livelihoods of 12% of world's population. Aquacultural practices of mudcrab at present are an emerging fishery farming that is alternate livelihood with respect to socio-economic evaluation (attractive growth size, rich protein, good source of minerals, good taste and high-quality meat, export value, easily packed and shipped live) in India. West Bengal coastal, estuary and mangrove support natural habitat of mudcrab and natural seeds are cultured in ponds for maturity (fattening) (Fig. 1). A good number of crab fishers are involved in this trade to earn their livelihood. In different way we are still polluting the environment and destroying its resources and disturb the ecological balance of aquatic and terrestrial environment. Such environmental alteration give birth the environmental crisis and multitude climate aberrations are the most effective result of pollution. The effects of climate change are extreme weather events causing water shortages and agricultural crop failures, ocean warming and acidification, sea level rise, adverse changes in water quality, sediment loading, or rapid fluctuations in salinity (Muralidhar *et al.*, 2012). So climate change is an immediate and future threat to global food security due to continue changes in physical properties such as temperature, salinity, sea level rise, and increased storm activity, thereby affecting animal growth and survival which may also increase their susceptibility to diseases and infections (Muralidhar *et al.*, 2012). Due to the temperature and water rises, the progressive loss and disappearance of coastal biological diversity and species (mudcrabs) that inhabit intertidal zones or mudflat. Generally pathogen like bacteria present in soil and water environment and alter environment due to climatic change helps them to replicate discriminately (Vibrio reproduce greatly by increasing salinity) (Muralidhar *et al.*, 2012). Mudcrab complete its different phases of life cycle through water and soil with standard physico-chemical nature of both environment and alteration of factors hamper its natural cycle (Ray and Saha, 2011) (**Figure. 4**). It is reported that Ganga delta contaminate with toxin producing enteropathogenic *Vibrio* and also its prevalence among *Scylla* sp. in West Bengal and among *Vibrio* sp., *V. vulnificus*, *V. parahaemolyticus*, and *V. cholera* being of greatest concern in respect to rates of infections by these organisms are increasing globally day by day due to climate change (warmer waters) and adverse economic impacts on mudcrab and seafood (Batabyal *et al.*, 2014). Naturally collected seeds as

well as cultured *Scylla* sp. gets frequently affected with infectious pathogen (viz. bacteria) and easily spread infection among them when fattening in cultured ponds (Fig. 4). Contaminated wild seed and adult of *S. serrata* become susceptible to diseases and physicochemical alteration of habitat is considered as a serious environmental threat for crab aquaculture.

The aim of the paper is to propose a base line information and diagnosis process on mudcrab status in West Bengal and assess the status of the wild crablets stock populations in its natural habitat under climatic changes. The climatic stress will be quantify through measurement of oxidative stress, antioxidative stress, bacterial stress by collection of blood and hepatopancreas sample of mudcrab. The farmer may adapt small changes of environment but they are not prepared for rapid changes or continuous climate change. So the farmer should be assisted by scientific management that they easily predict and detect the climatic change problem and try to solve primarily as a first aid treatment. Both Central Govt. and State Govt. should introduce fruitful and sustainable policies on climate change with a focus on the adaptability of all the stakeholders involved in the crab farming sector.

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phenoloxidase activity in the atlantic blue crab, *Callinectes sapidus*. *Comp. Biochem. Physiol.*, **A144:** 218 – 223.

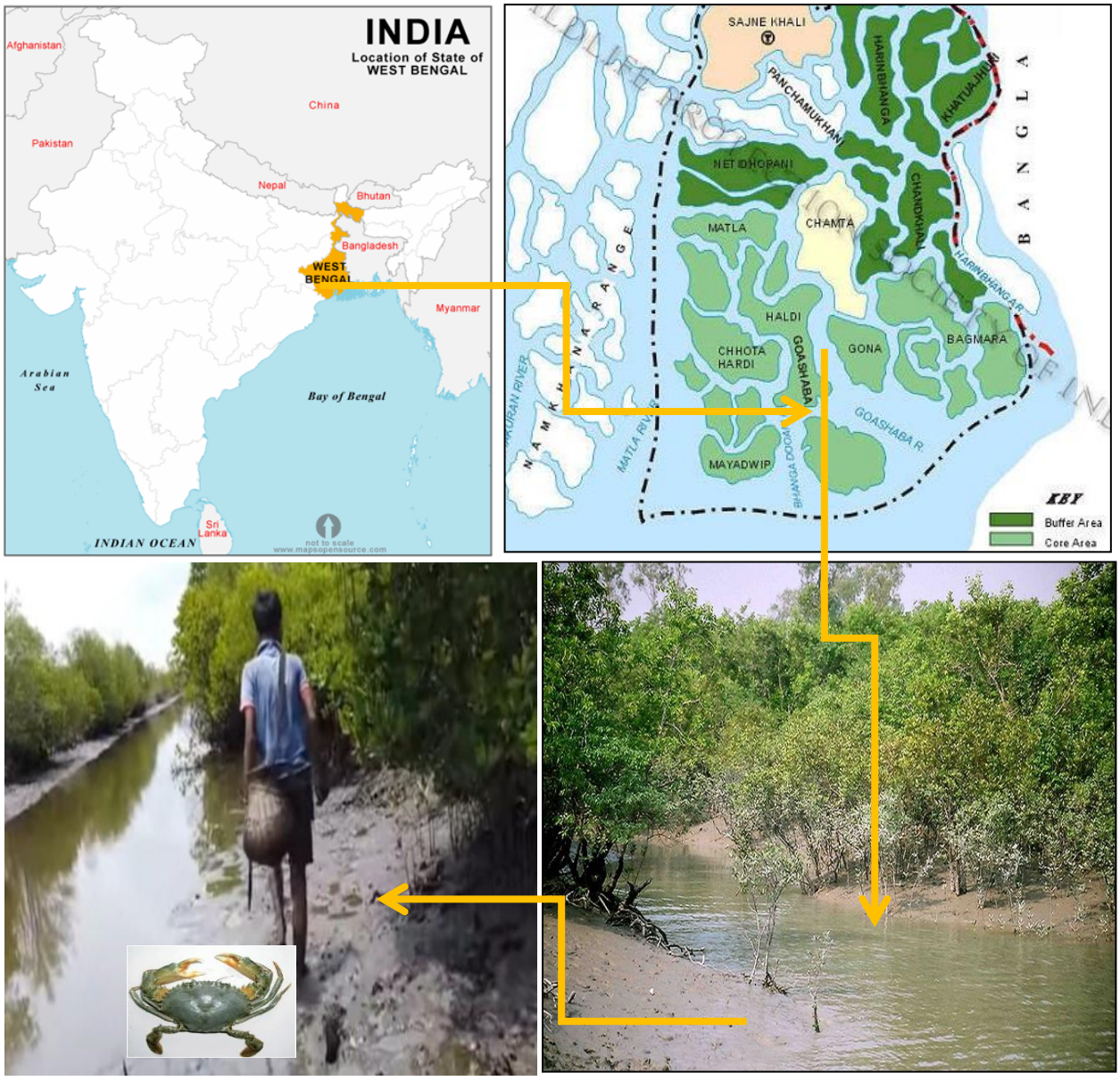

Figure: 1. Natural habitat of mud crab (Estuaries, creeks, tidal mud flat) in West Bengal

Figure. 2: Cultured mudcrab in West Bengal

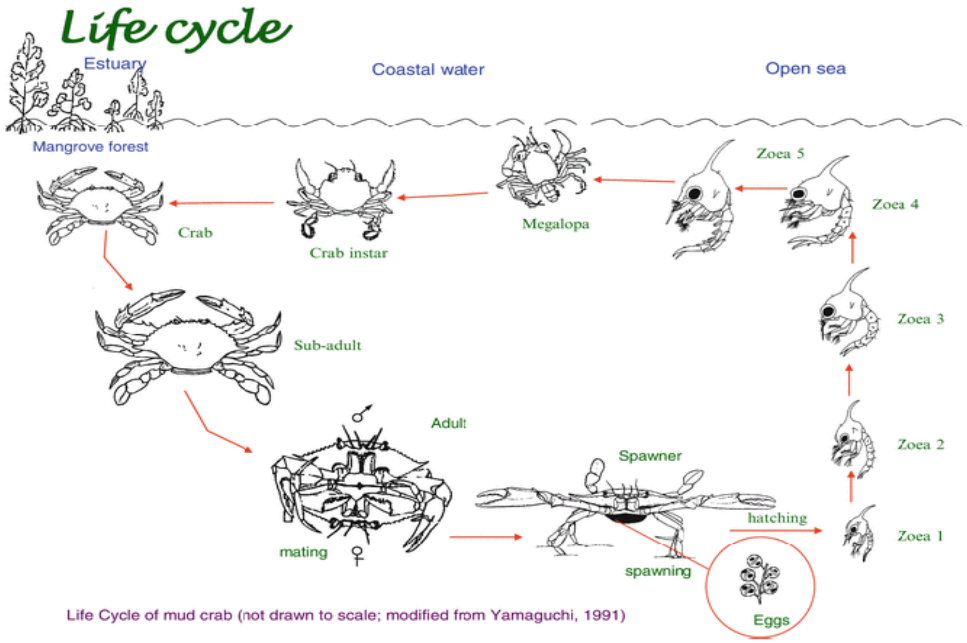

Figure. 3: Life cycle of mud crab

Figure: 4. Importance of Mudcrab

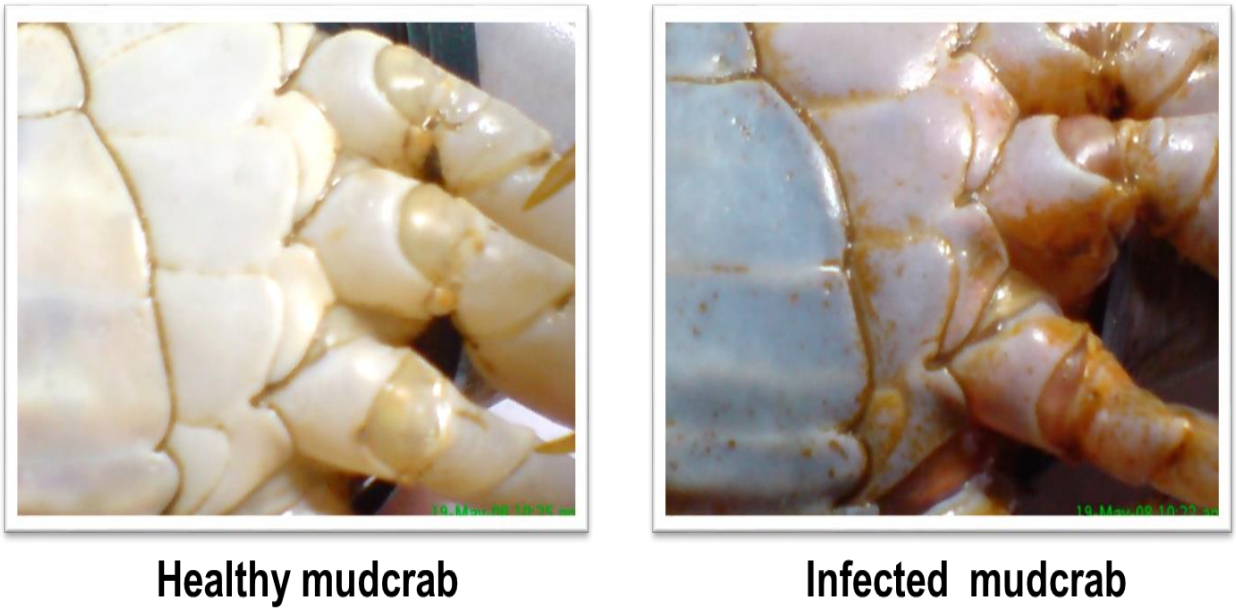

Figure: 5. Normal and Rust spot or pathogen infected mudcrab

Figure: 6. Vibrio sp in TCBS culture media of mudcrab (hemolymph)

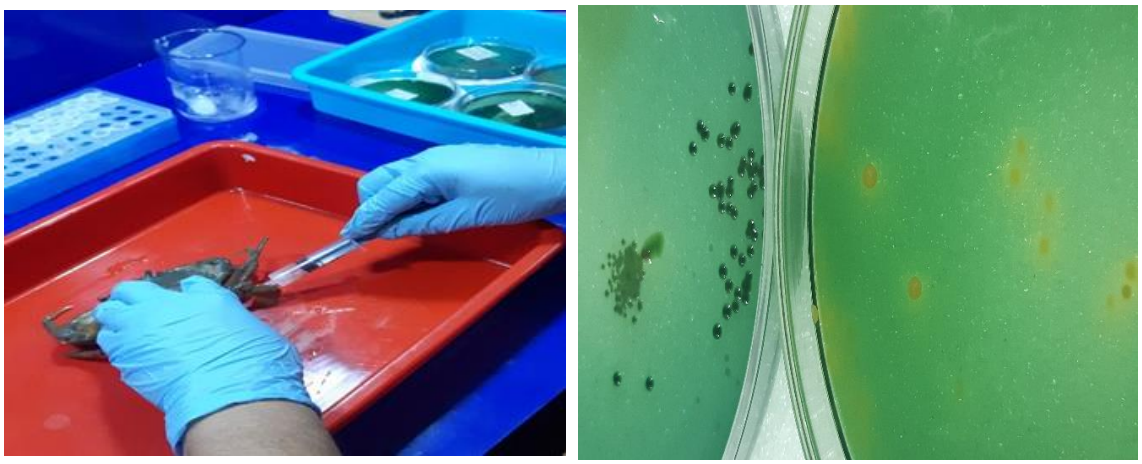

Figure: 7. Carapace lesions in mudcrab

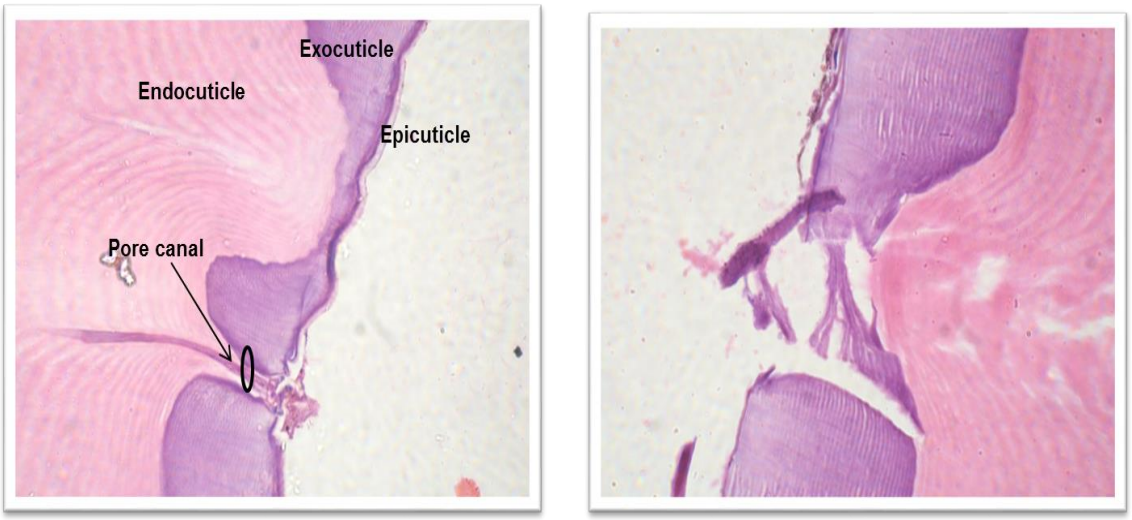

Figure: 8. Histological section of Carapace lesion in mudcrab