

## ABSTRACT

The present study entitled “Studies on some physico-chemical parameters of water bodies and microbial fish diseases in Eastern Nepal” consisting of the experiment has been presented in three broad sections. First section deals with physico-chemical parameters of water bodies for two years (November 2008-October 2010) emphasizing with disease prone area. Second section deals with isolation, characterization and identification of bacteria and third section includes histopathology of naturally EUS infected fishes, other commonly occurred microbial fish diseases and pathogenicity of isolated bacteria and fungus from EUS affected fishes.

Different physico-chemical factors of water of six water bodies; four ponds [three artificial ponds Baidya Fish Farm, Tankisinwari, Morang (S1), Babiya Birta Fish Farm, Morang (S2) and Tarahara Fish Farm, Sunsari (S3) and one natural pond, Betana Wetland, Morang (S4)] and two rivers Singhia river, Morang (S5) and Budhi river, Duhabi, Sunsari (S6) of eastern Nepal were studied for two years. Temperature, pH, turbidity, DO, BOD, free CO<sub>2</sub>, Chloride (Cl<sup>-</sup>), TA and TH were determined following standard methods. Correlation, t-test, two ways ANOVA among sites and seasons, and standard deviation were calculated using SPSS-V20 and Microsoft excel statistical function of computer software respectively.

Out of six water bodies EUS outbreaks were recorded in three fish farms S1, S2 and S3 from 2008-2015. Generally the outbreak of EUS takes place in the month of December and persists up to March. No outbreak of the disease was found in three water bodies S4, S5 and S6. Results showed that prior to the outbreak of the disease there were significant ( $p < 0.01$ ,  $p < 0.05$ ) declining trends of two factors e.g. total alkalinity and total hardness of water of the three fish farms and the factors remained low for few months. Declining trends of total alkalinity and total hardness were also noticed in other water bodies where no outbreak of disease was found.

But, TA and TH values of corresponding months during first and second year were insignificantly different ( $P < 0.05$ ) in all sites at 5% level of significance. ANOVA results of air temperature, water temperature and pH showed significant different at 1% level among seasons. CO<sub>2</sub> was significantly different at 5% level among sites and DO at 1% level among seasons. TA

was significantly different at 1% level among sites and seasons but TH was significantly different at 1% among sites and 5% levels among seasons. Cl<sup>-</sup> was significantly different at 1% among sites. High fluctuation of CO<sub>2</sub> was due to the rotten vegetation especially in Site 4.

Fall of surface water temperature was noticed in all water bodies studied during the one set of the disease. Water temperature remained below 30°C most of the time of the year and only for a short period of time it goes above 30°C. It appeared that this condition is favourable for the growth of bacteria and fungus.

Altogether 444 naturally EUS affected fishes were collected and examined. The order of susceptibility was about 60% *Cirrhinus mrigala*, 30% *Labeo bata* / *L. rohita*, 7% *Catla catla*, 3% *Channa striatus*, *Puntius* sp., *Heteropneustes fossilis*, *Mystus tengara*, *Clarias batrachus* and *Lepidocephalichthys guntea* (rarely) among 444 affected fishes.

Fungus (A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, and A<sub>4</sub>) were isolated from the ulcers of naturally infected *Cirrhinus mrigala*, *Catla catla*, *Labeo bata* and *Puntius* sp.. The isolated aseptate fungus was identified as *Aphanomyces* sp. by its characteristics sporangia which were not wider than the hyphae and the ball of sporangia at the tip of sporangium was developed. The single row of primary zoospores was found within the zoosporangia. The slender fungal hyphae were grown in fungal media but isolated fungus did not grow at 37°C. Zoospores (A<sub>1</sub>) of isolated fungus *Aphanomyces* sp. were pathogenic to other fishes when inoculated in experimental fish *H. fossilis*.

Histopathology of naturally and experimentally infected fish clearly showed the *Aphanomyces* sp. filaments from ulcer as a primary pathogen of EUS. When isolated bacteria and zoospores of *Aphanomyces* were inoculated separately into the experimental fish *Heteropneustes fossilis*, lesions were developed. So it is proved that the causative agent is *Aphanomyces* sp.. However, bacteria might have played the secondary role to develop the skin lesions. Other diseases prominently seen were bacterial fin rots; abdominal dropsy, gas bubble diseases and bacterial haemorrhagic septicemia.

Bacteria were isolated from the ulcers of naturally infected *Cirrhinus mrigala*, *Catla catla*, *Channa striatus*, *Puntius* sp., *Mystus tengara* and *Labeo bata*. Among 23 isolates, 2 belong to *Pseudomonas* sp. from Lb<sub>1</sub> and Cc<sub>4</sub>, 2 belong to *Micrococcus* sp. from Cm<sub>2</sub> and P<sub>4</sub>, 14 bacteria were of *Aeromonas hydrophila* isolated from Cm<sub>1</sub>, Cm<sub>3</sub>, Cs<sub>1</sub>, P<sub>1</sub>, P<sub>3</sub>, Lb<sub>2</sub>, Lb<sub>3</sub>, Cc<sub>1</sub>, Cc<sub>2</sub>, Cc<sub>3</sub>, Mt<sub>1</sub>,

Mt<sub>2</sub>, Mt<sub>3</sub> and Mt<sub>4</sub>. 1 belongs to *Moraxella* sp. from P<sub>2</sub>, 1 belongs to *Aeromonas veroni biovar sobria* from Lb<sub>4</sub> and 3 belong to *Aeromonas caviae* from Cm<sub>4</sub>, Cs<sub>2</sub> and Cs<sub>3</sub>. All together 20 bacterial isolates were pathogenic and 3 were non- pathogenic (Table 5.42).

Although lowering of water temperature, total alkalinity and total hardness was supposed to be helpful to outbreak microbial diseases including the EUS by weakening the immune system of fish, it does always not happen so and it is still unclear because in similar physico-chemical conditions, outbreak of the disease occurred in some areas (S1, S2 and S3) but not occurred in other areas (S4, S5 and S6).

From the above discussion and results of the experiments (Figs.5.1,5.2,5.8,5.9 5.11-5.13,5.15, 5.21,5.22,5.24, 5.26, 5.28, 5.34, 5.35, 5.37- 5.39, 5.41,5.47,5.48, 5.50-5.52, 5.54, 5.61,5.62,5.64-5.66,5.68,5.75,5.76,5.78-5.80; Figs. 5.81a and b to 5.88a and b ; Tables 5.36 to 5.42), it can be concluded that both bacteria and fungus *Aphanomyces* sp. play important roles when temperature remains low in winter months in the appearance of EUS (Epizootic Ulcerative Syndrome) and it is the major fish disease but other fish diseases (abdominal dropsy, haemorrhagic septicaemia of carps, fin rot and gas bubble disease) are minor fish diseases in eastern Nepal.