J. bio-sci. 15: 165-168, 2007 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index **-Short Communication**

LOAD OF *AEROMONAS SALMONICIDA* IN SWAMP WATER AND IT'S EFFECT ON TILAPIA (*OREOCHROMIS MOSSAMBICUS*)

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Swamps are one of those types of lentic water bodies, which form links between terrestrial and aquatic ecosystems. Dehadrai and Tripathi (1976) have characterized these water bodies as waterlogged, shallow water areas with a loose peaty bottom, rich in decaying organic matter retaining water either periodically or shrinking or drying summer months. There are numerous water bodies, including swamps, present in Bangladesh. But all of these water bodies are not used for scientific fish culture. To meet the increasing protein demand and to solve the unemployment problem in Bangladesh, swamps should be used for fish culture scientifically. This may also be helpful to destroy the habitat of many biological vectors such as mosquitoes.

Rahman *et al.* (1998) reported that swamps are suitable for fish culture in Bangladesh. The bacterial load in swamp water remains undetermined in Bangladesh. A wide range of bacterial flora is abundant in water and associated with fish diseases. Horseley (1973) investigated the relationship between the bacterial flora of Salmon and its environment and observed that the bacteria of skin were similar with the bacteria in water. Rahman *et al.* (2001) reported that Aeromonads are very destructive pathogens for warm water fishes.

It is recognized that *Aeromonas salmonicida* is a gram-negative non motile bacterium which causes "Furunculosis" is one of the most serious infectious diseases of fresh water fishes. In recent years the major economic impact of this disease has been found on Salmon cultivation, principally in Europe, North America and Japan, but some devastating epizootics of Furunculosis have also been recorded in wild fish populations. It has been observed that *A. salmonicida* shows high growth rate at around 25^{o}C. *A. salmonicida* populations may be high in polluted warm water bodies of Bangladesh. Study of aquatic bacteria associated with fish is very limited in Bangladesh. Tilapia is one of the very important commercial fishes and is abundant in swamps. Some times infected Tilapia are observed but the pathogens remain unidentified.

Considering the above reasons the present research was undertaken to investigate the load of *A. salmonicida* in swamp water and its effect on Tilapia fish.

Materials and Methods

Sampling: Four swamps situated in the Rajshahi University campus were selected for the present investigation. Water samples were collected randomly in sterilized reagent bottles from each swamp from the surface and bottom twice a week from November 2001 to October 2002.

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Determination of the load of *Aeromonas salmonicida* in swamp water sample

To investigate the load of *A. salmonicida* in swamp water, all necessary equipments such as petridishes, conical flasks, syringes, spreader, pipette, etc. were sterilized by the autoclave at 121^{o}C for 20 minutes. Then Furunculosis agar medium was prepared by using distilled water and it was sterilized. The Furunculosis agar plate was prepared in petridish and kept at 20^{o} C temperature for several hours. The sample water (0.5 ml) diluted in physiological saline (0.85% NaCl solution) through 10 fold dilution and 0.5ml of the suspension was spreaded over the agar plate. The petridishes were incubated at 20^{o}C for 48 hours at inverted position. The plates having brown diffusable colonies were counted by direct counting method

Test of the effect of *A. salmonicida* on Tilapia fish

For this the pathogenicity of *A. salmonicida* on Tilapia fish was observed. Fishes weighing 10 g were collected from a fish market. They were kept in 100 liter tanks with well-aerated water and were fed on commercial pellets. Different concentrations of the suspension of *A. salmonicida* were made in physiological saline by 10 fold dilution and then injected intraperitonially into 5 groups, each consisting 5 fishes. Injected fishes were reared for 15 days at 20^{o}C to 25^{o}C and the mortality was recorded. The infection was confirmed by isolating the bacteria from the kidney and lesions of dead fish using Furunculosis agar plate.

Isolation of *A. salmonicida*

To isolate the pathogenic bacterium from dead fish, the body surface of fish was disinfected with 70% ethyl alcohol and their abdomen was opened by aseptic dissection. The pathogens were isolated carefully from the kidney and lesions of dead fish. The samples were homogenized for preparation of suspension in physiological saline. The suspension was inoculated on Furunculosis agar plate and incubated at 20^{o}C for 48 hours and then brown pigments were observed for the growth of *A. salmonicida* on agar plate.

Load of *A. salmonicida* in studied swamp water

During the study period, the load of *A. salmonicida* in the four studied swamps varied from 1.9 ? 10^{6} CFU/ml (January, 2002 in swamp-1) to 4.1? 10^{6} CFU/ml (September, 2002 in swamp-4) (Table 1). In swamp-1 the maximum load of *A. salmonicida* was observed as 3.8 ? 10^{6} CFU/ml in October, 2002 and the minimum was observed as 1.9? 10^{6} CFU/ml in January, 2002. The annual mean load of *A. salmonicida* in swamp-1 was recorded as 3? 10^{6} CFU/ml. The maximum load of *A. salmonicida* in swamp-2 was recorded as 3.9? 10^{6} CFU/ml in October, 2002 and the minimum as 2.1? 10^{6} CFU/ml in January, 2002. The mean load of *A. salmonicida* in this swamp water round the year was calculated as 3.1? 10^{6} CFU/ml. In swamp-3 the maximum load of *A. salmonicida* was recorded as 3.8? 10^{6} CFU/ml in October, 2002 and the minimum as 3.1? 10^{6} CFU/ml in May, 2002. The annual mean load of bacterium in swamp-3 was calculated as 3.5? 10^{6} CFU/ml.

Table 1. Monthly variation in total load of *Aeromonas salmonicida* in studied swamp water samples from November 2001 to October 2002.

Date of sampling	Bacterial load in swamp water (CFU/ml)				Annual average bacterial load in 4 swamps (CFU/ml)
	Swamp-1	Swamp-2	Swamp-3	Swamp-4	
Nov, 01	3.3? 10^6	3.2? 10^6	3.3? 10^6	3.4? 10^6	
Dec, 01	2.5? 10^6	2.5? 10^6	ND	ND	
Jan, 02	1.9? 10^6	2.1? 10^6	ND	ND	
Feb, 02	2.5? 10^6	2.6? 10^6	ND	ND	
Mar, 02	3.2? 10^6	3.2? 10^6	3.3? 10^6	ND	
Apr, 02	3.5? 10^6	3.5? 10^6	3.6? 10^6	ND	
May, 02	3.1? 10^6	3.1? 10^6	3.1? 10^6	3? 10^6	3.3? 10^6
June, 02	2.9? 10^6	3.1? 10^6	3.2? 10^6	3.4? 10^6	
July, 02	3? 10^6	3.2? 10^6	3.3? 10^6	3.5? 10^6	
Aug, 02	3.5? 10^6	3.4? 10^6	3.5? 10^6	3.8? 10^6	
Sept, 02	3.7? 10^6	3.6? 10^6	3.7? 10^6	4.1? 10^6	
Oct, 02	3.8? 10^6	3.9? 10^6	3.8? 10^6	3.9? 10^6	
Mean	3? 10^6	3.1? 10^6	3.5? 10^6	3.6? 10^6	

ND - Not determined (Because swamps was dried)

The minimum load of *A. salmonicida* in swamp-4 was observed as 3? 10^6 CFU/ml in May, 2002 and the maximum was found as 4.1? 10^6 CFU/ml in September, 2002. Yearly mean load of *A. salmonicida* in this swamp was calculated as 3.6? 10^6 CFU/ml. The annual average load of *A. salmonicida* in four swamps studied herein was calculated as 3.3? 10^6 CFU/ml.

Effect of *A. salmonicida* on Tilapia fish

The highest mortality (80%) by *A. salmonicida* on Tilapia fish was observed at 3.3? 10^8 CFU/g and the lowest (20%) was observed at 3.3? 10^6 CFU/g respectively. No mortality was observed at 3.3? 10^5 and 3.3? 10^4 CFU/g/fish (Fig.1).

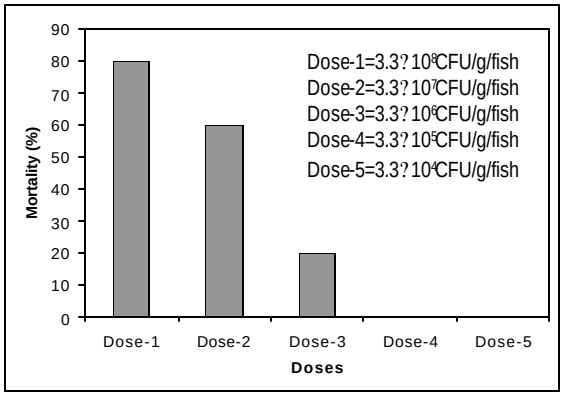

Fig 1. The mortality of Tilapia fish (*Orechromis mossambicus*) at different doses of *A. salmonicida.*

Microbial studies on aquatic environment and fishes are very limited in Bangladesh. No works have been carried out on the load of *A. salmonicida* in swamp water and its impact on Tilapia fish. The highest bacterial load was found in swamp-1, swamp-2 and swamp-3 in October 2002 and in swamp-4 in September 2002. The lowest bacterial load was found in swamp-1 and swamp-2 in January 2002, in swamp-3 and swamp-4 in May 2002 (Table 1). The annual average load of *A. salmonicida* in the studied four swamps was calculated as $3.3? 10^6$ CFU/ml. Fish mortality was recorded highest 80% ($3.3? 10^8$ CFU/g) and lowest 20% ($3.3? 10^6$ CFU/g).

In Bangladesh, Banu *et al.* (2001) investigated the bacterial load in pond water. They observed that the mean bacterial load in surface water varied from $1.39? 10^5$ (July 94) to $3.11? 10^7$ CFU/ml (September 93), while that of bottom water ranged from $1.0? 10^6$ (May 94) to $5.90? 10^7$ CFU/ml (October 93). Romanenko (1971) reported that the bacterial number in reservoir water was 1.43-0.18? 10^6 ml of water. Tewary and Mishra (1985) reported that fresh water bacteria varied from 1 to $3.00? 10^3$ CFU/ml in the lake water. Araki and Kitamikadi (1978) pointed out that the population density of bacteria ranged from 0.0 to $1.8? 10^5$ cell/ml of water in some river and pond water of Japan.

Rahman *et al.* (2001) observed the virulence of viable but non culturable state (VBNC) of *Aeromonas hydrophila* in a carp fish *Carassius auratus* and recorded the LD_{50} values of $10^{6.18}$ CFU/fish, $10^{8.45}$ CFU/fish and $>10^{9.11}$ cell/fish in VBNS cells. A perusal of the data shows that the natural average bacterial load of $3.3? 10^6$ CFU/ml or below do not produce any significant mortality in *Oreochromis mossambicus.* Much more comprehensive studies are needed in this area in Bangladesh.

Acknowledgements

The authors are grateful to Professor Dr M A Bari Miah, Institute of Biological Science, University of Rajshahi, Bangladesh, for his kind permission to use the Microbiology laboratory. They are also thankful to Professor Dr Md Altaf Hossain and Dr Mahatab Ali, Department of Zoology, Rajshahi University, Bangladesh, for providing the necessary research facilities.

References

Araki T and Kitamikadi M (1978) Distribution of common degrading bacteria in aquatic environment. *Bull. Jap. Soc. Sci. Fish* **44(10):** 1135-1139.

Banu A N H, Islam M A and Chowdhury M B R (2001) Bacterial load in pond water and different organs of a Indian major carp *Cirrhinus mrigala* Ham. *Bangladesh J. Fish Res.* **5(1):** 53-58.

Dehadrai P W and Tripathi S D (1976) Environment and ecology of freshwater air-breathing teleosts In: *Respiration of Amphibious vertebrates* (ed. G M Hughes). Academic Press, New York, 39-72 PP.

Horseley R W (1973) The bacterial flora of the Atlantic salmon (*Salmo salar L.*) in relation to its environment. *J. Appl. Bacteriology* **36:** 377-386.

Rahman M H, Hussain M A, Hossain M A and Kawai K (1998) Potentiality of swamp for fish culture in Bangladesh. *Japan Aquaculture Society, SUISANZOSHOKU* **46 (4):** 481-486.

Rahman M H, Suzuki S and Kawai K (2001) Formation of viable but non culturable state (VBNC) of *Aeromonas hydrophila* and its virulence in gold fish *Carassius auratus. Microbiol. Res.* **156:** 103-106.

Romanenko V I (1971) Total bacterial number in Rybinsk reservoir. *Microbiol,* **40:** 707-713.

Tewari B K and Mishra R R (1985) Population and productivity of heterotrophic bacteria in fish water lake. *Acta. Bot. India* **13 (1):** 51-56.