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LOAD OF *AEROMONAS SOBRIA* IN SWAMP WATER AND ITS PATHOGENICITY TO KOI, *ANABAS TESTUDINEUS* (BLOCH)

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Among the fishery resources, swamps are very important for various fish species such as *Oreochromis nilotica, Oreochromis mossambica, Anabas testudineus, Channa punctatus* and *Heteropneustes fossilis*. Swamps require shorter time and small investment for fish culture (Rahman *et al*. 1998). However, diseases are major problems in fish production both in culture system and wild condition in Bangladesh (Rahman and Chowdhury 1996). Bacteria, especially *Aeromonas* sp. is one of the causative agents of fish diseases. Bangladesh is situated in the tropical zone. Temperature is one of the important factors which affect the growth of pathogenic bacteria (Ljungh and Wadstrom 1982). *Aeromonas sobria* increases and grows at 17-25°C (Rahman and Chowdhury 1996). *Aeromonas* sp. is the causative agent of various kinds of ulcerative disease of fishes (Karunasagar and Sugumar 1995). Yesmin *et al*. (2004) and Chowdhury and Baqui (1997) reported that *Aeromonas* is a very common pathogen in carps and live fishes. Hossain *et al*. (2006) worked on the bacterial load, *Pseudomonas aeruginosa* and their artificial infection to *Oreochromis niloticus*. The present study was undertaken to investigate the bacterial load in swamp water and the pathogenicity of *A. sobria* in *A. testudineus.*

Study areas: For the present investigation five swamps situated at the Rajshahi University Campus were selected. The location and areas of five swamps are as follows-

Swamp no.	Location	Total area (m^2)	Water area (m^3)
1	Northern side of third science building, Rajshahi University	32.63	23.71
2	Southern side of Mother Box Hall, Rajshahi University	37.18	18.67
3	In front of Rabindra Bhaban, Rajshahi University	69.65	41.73
4	In front of Monnujan Hall, Rajshahi University	92.89	67.93
5	Opposite side of Tapashi Rabeya Hall, Rajshahi University	33.83	17.25

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Determination of the Bacterial Load of Swamp Water Sample: Water was collected from each swamp twice a week to investigate the bacterial load. Water was collected from the bottom of the swamps in sterilized bottles. All necessary instruments were autoclaved for sterilization at 121^0 C for 20 minutes. Water was autoclaved at the same temperature for the same duration. Water was diluted in physiological 10-fold saline. The bacterium was inoculated into agar plate. Sample water (0.1 ml) was pipetted at the centre of the petridish using a sterile pipette. For each dilution (1 ml sample + 9 ml physiological saline) the content was spread as quickly as possible. Inoculated materials were incubated at the room temperature (25^0C) for 48 hours. Colony was counted by direct counting method.

Identification: Identification of *A. sobria* was made according to the Bergey's manual.

Pathogenicity Test:

Isolation: *A. sobria* was collected from diseased fish from swamps. Bacteria were isolated from kidney, spleen, lesion, liver and blood in agar plates.

Preparation of Bacterial Samples: A pure colony of A. *sobria* was reisolated into agar medium and then cultured at 25^0C for 24 hours. Fishes weighing 5-10 gm were kept in 100L tanks with well-aerated water. The fish were fed on commercial pellets. Different concentrations of bacteria were made by 10-fold dilution and were then injected intraperitonially into five fish groups, each consisting of 10 fishes. Injected fishes were reared for 15 days at temperatures of 20-25^0C, and their mortality was recorded. Infection was confirmed by reisolating the bacteria from the kidney of dead fish using agar.

Results

Bacterial load: Monthly variations in the load of *A. sobria* in the study swamps were investigated. The range of the load of A. *sobria* of study period was from 2.2×10^6 to 3.8×10^6 CFU/ml. The yearly mean of five swamps was 3.2×10^6 CFU/ml (Table 1).

Pathogenicity: The mortality of *A. testudineus* were 20, 30, 50 and 80% at 3.2×10^5, 3.2×10^6, 3.2×10^7, and 3.2×10^8 CFU/ml/fish respectively. No mortality occurred at 3.2×10^4 CFU/ml (Figure 1).

Table 1. Monthly variations in the load of *Aeromonas sobria* in study swamps from Nov., 2001 to Oct. 2002.

Month	Swamp no. 1 CFU/ml	Swamp no. 2 CFU/ml	Swamp no. 3 CFU/ml	Swamp no. 4 CFU/ml	Swamp no. 5 CFU/ml	Yearly mean of five swamps CFU/ml
Nov '01	2.8×10^6	2.9×10^6	2.8×10^6	3.0×10^6	3.1×10^6	
Dec	2.7×10^6	2.8×10^6	2.6×10^6	2.9×10^6	-	
Jan '02	2.5×10^6	2.6×10^6	2.4×10^6	2.7×10^6	-	
Feb	2.2×10^6	-	2.2×10^6	2.4×10^6	-	
Mar	2.4×10^6	-	2.5×10^6	-	-	
Apr	2.8×10^6	-	2.9×10^6	-	-	3.2×10^6
May	3.0×10^6	3.2×10^6	2.9×10^6	3.4×10^6	3.2×10^6	
Jun	3.3×10^6	3.3×10^6	3.2×10^6	3.4×10^6	3.6×10^6	
Jul	3.6×10^6	3.6×10^6	3.6×10^6	3.6×10^6	3.8×10^6	
Aug	3.7×10^6	3.7×10^6	3.6×10^6	3.8×10^6	3.8×10^6	
Sep	3.4×10^6	3.5×10^6	3.3×10^6	3.6×10^6	3.8×10^6	
Oct	3.1×10^6	3.4×10^6	3.2×10^6	3.4×10^6	3.3×10^6	
Mean	2.9×10^6	3.2×10^6	2.9×10^6	3.2×10^6	3.5×10^6	

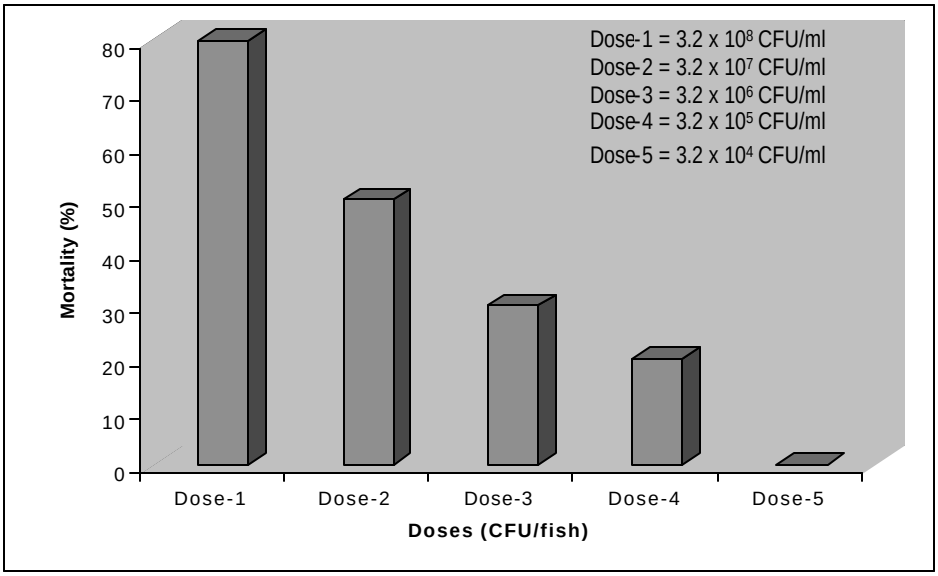

Figure 1. Mortality of *A. testudineus* by artificial infection with *A. sobria*.

The highest bacterial load (*A. sobria*) in swamp no. 1, 2, 4 and 5 were in August and in swamp no. 3 in July and August, and in swamp no. 5 in July, August and September. The lowest bacterial load in all the swamps was in January'02 and February'02 and March'02. Banu *et al*. (1995) found that bacterial load was high in August and September and low in May, December and March.

A mortality of 80% at 3.2? 10^8 CFU/ml/fish of *A. sobria* was observed in *A. testudines*. Chowdhury (1998) found 75-80 % mortality in carp and cat fish with 3.5? 10^8 CFU/ml/fish of *A. hydrophila* experimentally. Recently, Yesmin *et al.* (2004) studied the load of *A. hydrophila* in swamps and artificial infection of the snakehead, *Channa punctatus* with the bacterium. The load of the bacterium varied from 1.16? 10^7 to 4.90? 10^7 CFU/ml. Fish mortality was recorded as 12.5, 25, 75 and 100% at 3.42? 10^6, 3.42? 10^7, 3.42? 10^8 and 3.42? 10^9CFU/ml/fish respectively.

The yearly mean bacterial (*A. sobria*) load of five swamps was 3.2? 10^6 CFU/ml/fish and the mortality rate of *A. testudineus* was 30% at that dose. It was observed that the load of *A. sobria* of the studied swamps caused a little harm in fish culture. If proper and scientific fish culture methods are undertaken, conditions of swamps will improve, and the load of bacteria will decrease. This will lead to higher fish production.

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